5th Conference on Experimental and Translational Oncology

Kranjska gora, Slovenia,
March, 26-30, 2008

BOOK OF ABSTRACTS

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Tyrosine kinase inhibitors increase the anti-tumour effect of chemotherapy drugs in malignant gliomas

Paula Kinsella, Martin Clynes and Verena Amberger Murphy

National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland

Despite major improvements in the surgical management the prognosis for patients bearing malignant gliomas is still dismal. Traditional chemotherapy was unable to offer extended survival gain for a long period of time, until radiation and concurrent Temozolomide followed by adjuvant Temozolomide became the standard of care for newly diagnosed glioblastoma multiforme. The new understanding of cellular and molecular mechanisms of tumours has led to the development of targeted agents, which are aimed at the tumour-specific microenvironment and/or molecules.

Taxanes are a group of chemotherapeutic drugs, which have proven to be very effective in the treatment of a variety of cancer types; however, were ruled out for the treatment of brain tumours, because of their inability to cross the blood brain barrier. New application techniques, like convection enhanced delivery, allow surpassing the blood brain barrier and delivering the drug directly to the tumour area.

Therefore, we tested the effects of taxanes (Taxotere and Taxol) in combination with a tyrosine kinase inhibitor on proliferation and invasion activity of brain tumour cell lines. Taxotere or Taxol alone at nanomolar concentrations strongly inhibited proliferation and invasion of one astrocytoma grade III and 3 glioblastoma cell lines *in vitro*. However, after removing the drug, invasion activity resumed suggesting a reversible effect on the tumour cells. The combination of Imatinib, a tyrosine kinase inhibitor for e.g. platelet derived growth factor, c-kit and c-able, together with Taxotere inhibited tumour cell proliferation in a synergistic manner, which was even increased when drug application was scheduled. Invasion was also strongly inhibited by the drug combination and this effect was irreversible. We also tested this drug combination on newly developed cell cultures from biopsy tissue and found similar effects. These results demonstrate that, compared to single drug application, the combination of tyrosine kinase inhibitors and classical chemotherapy drugs strongly increases the anti-tumour effects *in vitro*, which could have implications for the treatment of malignant gliomas *in vivo*.

Plasma TIMP-1 and CEA in detection of primary colorectal cancer: a prospective, population based study of 4990 persons

Ib Jarle Christensen[1], *H. Nils Brünner*[2] *and Hans Jørgen Nielsen*[3]

[1]The Finsen Laboratory, Copenhagen Biocenter, Copenhagen, Denmark; [2]Institute of Life Sciences, University of Copenhagen, Frederiksberg, Denmark; [3]Dept. of Surgical Gastroenterology, Hvidovre University Hospital, Hvidovre, Denmark

Previous results have suggested that plasma TIMP-1 may be a useful biomarker for the early detection of colorectal cancer (CRC), and in particular colon cancer (CC). These results also demonstrated that CEA with plasma TIMP-1 independently improved the detection of CRC. A prospective, multi-center and population based study was carried out to test these hypotheses.

Persons referred to endoscopy due to symptoms of CRC were accrued. After informed consent, blood samples were collected and baseline data including life style variables, current medication and relevant co-morbidity were recorded. The findings of the examination by sigmoidoscopy and/or colonoscopy were recorded. For patients having CRC, localization and TNM stage were registered. For patients with adenomas, type, size, number and dysplasia were recorded. All findings were registered using ICD-10 codes. Plasma levels of TIMP-1 and CEA were determined using the Abbott ARCHITECT ® i2000 automated immunoassay system.

Four-thousand nine hundred and ninety persons were included in the study. The examination was sigmoidoscopy in 34% and colonoscopy in 64% of the population (2% other). CC was detected in 189 (3.8%) persons and rectal cancer (RC) in 115 (2.3%). Other findings were 10 patients with a cancer other than CRC, 923 patients with adenomas and 1,217 with another finding, primarily diverticulosis. Plasma TIMP-1 and CEA are significantly elevated in CRC patients compared to subjects without cancer ($p<0.0001$). In addition, both biomarkers were showed higher levels in patients with comorbidity compared to age and gender matched subjects with none. Analysis of the primary endpoint CRC demonstrated TIMP-1 to be a significant predictor of CRC ($p<0.0001$, AUC = 0.71, OR = 18.36, 95% CI: 7.37-45.74). A similar result was seen for CEA ($p<0.0001$, AUC = 0.73, OR = 12.53, 95% CI: 7.32-21.46). A multivariable analysis of each of the biomarkers in relation to the primary endpoint of CRC and including confounding covariates demonstrates that both are significant and independent (TIMP-1: $p<0.0001$, OR = 5.08, 95% CI: 1.95-13.23; CEA: $p<0.0001$, OR = 7.36, 95% CI: 4.206-12.89) with an AUC of 0.80. Restricting the endpoint to CC and colonoscopy results in an AUC of 0.83 with both biomarkers being independently significant (TIMP-1: $p<0.0001$, OR = 4.16, 95% CI: 1.36-12.7; CEA: $p<0.0001$, OR = 6.90, 95% CI:2.62-18.16). Subgroup analyses showed that TIMP-1 and CEA could detect CC with a sensitivity of 60% and 46%, respectively at 90% specificity.

These results confirm that TIMP-1 and CEA may be useful in the detection of colorectal cancer and in particular colon cancer.

Cancer gene therapy by electrotransfer of nucleic acids into tissues

Maja Čemažar

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Effective and safe delivery of genes to target tissues still remains the main obstacle toward successful gene therapy. Among non-viral methods, electroporation is gaining a lot of interest. Electroporation is a physical method for delivery of various molecules into the cells by transiently increasing permeability of cell membrane, using application of controlled external electrical field to the cells or tissues. Due to the easiness of the preparation of large quantities of endotoxin free plasmid DNA, control and reproducibility of the method and the development of electric pulse generators approved for the clinical use, gene electrotransfer holds a great potential for the clinical treatment of various diseases; most of the work in currently focused on treatment of cancer and infectious diseases.

Tumors represent histologically heterogeneous group and these different properties are most probably the reason for lower transfection efficiencies obtained after electrotransfer of tumors compared to other tissues. Therefore, our studies concentrate in one hand on the optimization of current electrotransfer protocols for delivery of plasmid DNA to muscle and tumors in mice and dogs, aiming at elucidation of the role of physiological parameters that affect transfection efficiency. On the other hand, different therapeutic genes, such as p53 and IL-12 delivered by electroporation for local or systemic expression and distribution are tested for their antitumor effectiveness in murine tumor models.

In electrogene therapy of cancer, the first electrogene therapy using IL-12 is underway; therefore it can be presumed that electrotransfer of therapeutic genes into tissues will soon form a validated alternative to viral delivery systems in clinical settings.

The financial support from the state budget of the Slovenian Research Agency (program No. P3-0003; project No. J3-7044) is greatly acknowledged.

Markers predicting clinical outcome in breast cancer patients treated with anti-estrogen therapy: towards the identification of biologically and clinically relevant information

Maria Grazia Daidone, Danila Cordini, Valeria Musella, Loris De Cecco, Maya Fedeli, Manuela Gariboldi, Patrizia Miodini, Vera Cappelletti, Marco Pierotti

Foundation IRCCS – National Institute of Tumors, Milano, Italy

In estrogen receptor positive (ER+) breast cancer patients, the identification of molecular markers predictive of early relapse following anti-estrogen treatment still represents an investigational open question. We evaluated the expression of RERG (Ras-related, estrogen-regulated and growth-inhibitor, a unique estrogen-dependent regulated protein belonging to the Ras superfamily of GTP-binding proteins) by quantitative real-time-PCR in 113 ER+ primary breast cancers from patients subjected to surgery and adjuvant tamoxifen, and investigated its relationship with some patho-biological factors (including tumor size, grade and histology, lymph node involvement, ER and progesterone receptor [PgR] levels) and biomarkers indicative of a typical luminal phenotype (GATA3) or known to be associated with tamoxifen resistance (HER-2/neu and PTEN). RERG expression was significantly associated with hormone-related factors: in fact, it was positively correlated with both mRNA and the corresponding protein level of ER and PgR and with GATA3, which codes for a transcription factor recently shown to be involved in the positive regulation of the expression of ERS1 gene in a cross-regulatory feedback loop. Conversely, no association was found between RERG and PTEN or HER-2 mRNA expression, thus indicating an independence of molecular mechanisms of cell response to hormones and anti-hormones involving these genes.

Follow-up analysis indicates that reduced RERG mRNA expression was present in tumors from women that will develop relapse compared to those long-term disease-free. The association of RERG expression with patients outcome was independent of the most important patho-biologic features of prognostic relevance following anti-estrogen treatment: lymph nodal involvement, PgR status and ER content. In a multivariate Cox's proportional hazard regression analysis a low RERG expression was a significant predictor of relapse occurrence (hazard ratio [HR] for low versus high 2.084, 95% confidence interval [CI] 1.092-3.979, P=0.026), similarly to GATA3 expression (HR for low versus high 2.570, 95% CI 1.301-5.077, P=0.0066). Conversely, HER-2 and PTEN failed to provide prognostic information. Low expression of RERG associated with low GATA3 in PgR-negative tumors identified patients with the worse prognosis, even after adjustment for lymph node involvement and ER content.

RERG contribution to anti-estrogen cell response was also supported by *in vitro* data. In fact, in MDA-MB361 and MCF7 cells, two ER-positive cell lines responsive to the antiproliferative effect of anti-estrogens, the growth inhibition by 4-OH-tamoxifen was paralleled by an increase in RERG mRNA expression while the lack of proliferative effect observed in ZR-75.1 was accomplished by a progressive down-regulation of the gene expression suggesting that RERG may be involved in mediating the growth-inhibitory

effect of tamoxifen.
From a clinical point of view, notwithstanding these data need to be further validated, the finding that the simultaneous down-regulation of RERG, PGR1 and GATA3 genes (all involved in hormone-dependent mammary cell growth and differentiation) is paralleled by low ER protein concentration (a characteristic of subtype B of luminal phenotype) and associated to an unfavorable prognosis is of particular interest because indicative for the presence of aggressive tumors also putatively refractory to tamoxifen treatment, as suggested by *in vitro* results.

Plasma MMP1, MMP8 and MMP13 expression in breast cancer: protective role of MMP8 against lymph node metastasis

Julie Decock[1,2], Wouter Hendrickx[1,3], Ulla Vanleeuw[1], Vanya Van Belle[4], Sabine Van Huffel[4], Marie Rose Christiaens[3], Dylan Edwards[2], Shu Ye[5], Robert Paridaens[1,3]

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Elevated levels of matrix metalloproteinases have been found to associate with poor prognosis in various carcinomas. This study aimed at evaluating plasma levels of MMP1, MMP8 and MMP13 as diagnostic and prognostic markers of breast cancer.

A total of 208 breast cancer patients, of which 21 with inflammatory breast cancer, and 42 healthy controls were included in the study. Plasma levels of MMP1, MMP8 and MMP13 were measured using ELISA and correlated with clinicopathological characteristics.

Median plasma MMP1 levels were significantly higher in controls (3.45 ng/ml) than in breast cancer patients (2.01 ng/ml), while no difference was found in MMP8 levels (10.74 vs. 10.49 ng/ml). ROC curve analysis revealed an AUC of 0.67, a sensitivity of 80% and specificity of 24% at a cut-off value of 4.24 ng/ml. Plasma MMP13 levels could not be detected. No correlation was found between MMP1 and MMP8 levels. We found a trend of lower MMP1 levels with increasing tumour size (p=0.07); and of higher MMP8 levels with premenopausal status (p=0.06) and NPI (p=0.04). A 2-fold decrease in MMP1 (p=0.02) and MMP8 (p=0.007) levels was observed in inflammatory breast cancer patients. Intriguingly, plasma MMP8 levels were positively associated with lymph node involvement but showed a negative correlation with the risk of distant metastasis. Both healthy controls and patients without lymph node involvement (pN0) had lower MMP8 levels than patients with moderate lymph node involvement (pN1, pN2) (p=0.001), and showed a trend for higher MMP8 levels as compared to those in patients with extensive lymph node involvement (pN3) and a strong predisposition to distant metastasis (p=0.11). Based on the hypothesis that blood and tissue protein levels are in reverse association, these results suggest that MMP8 in the tumour may have a protective effect against lymph node metastasis.

In summary, we observed differences in MMP1 and MMP8 plasma levels between healthy controls and breast cancer patients as well as between breast cancer patients. Interestingly, our results suggest that MMP8 may affect the metastatic behaviour of breast cancer cells through protection against lymph node metastasis, underlining the importance of anti-target identification in drug development.

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Cox-2 is a target gene of Rho GDP dissociation inhibitor β in breast cancer cells

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Rho-GDIβ, an inhibitor of Rho-GTPases, is primarily expressed by haematopoietic cells, but also found in epithelial cancer cells. Recently, we have identified Rho-GDIβ as a target of the transcription factor Ets1. Here we show that, in breast cancer cells, Ets1 regulates Rho-GDIβexpression on the RNA and protein level and binds to the upstream region of the Rho-GDIβgene. Furthermore, in primary breast cancer, Rho-GDIβ is co-expressed with Ets1. Studying the function of Rho-GDIβ in breast cancer, we found that a Rho-GDIβ-specific siRNA increased cellular migration, but also decreased the expression of Cox-2 oncogene as demonstrated by microarray, Q-RT-PCR and Westernblot analyses. Further studies revealed that Rho-GDIβ regulates Cox-2 gene at least partly on the transcriptional level most likely by activating NFAT-1. Vav-1, an interaction partner of Rho-GDIβ, was also found to interfere with Cox-2 expression and NFAT-1 cellular distribution suggesting a cooperative action of Rho-GDIβ and Vav-1 on Cox-2 expression. To explore the importance of Rho-GDIβ for the survival of breast cancer patients, two cohorts including 263 and 117 patients were analyzed for clinical outcome in relation to Rho-GDIβ RNA and protein levels, respectively. Expression of Rho-GDIβ was not associated with either disease-free or overall survival in the two patient population. Our data suggest that the expression of Rho-GDIβ in breast cancer is neither beneficial nor disadvantageous to the patient. This may be the net effect of two opposing activities of Rho-GDIβ, one that suppresses tumor progression by inhibiting migration and the other that stimulates it by enhancing Cox-2 expression.

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ADAM (a disintegrin and metalloproteinase) proteases as potential cancer therapeutic targets

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Adamalysins (a disintegrin and metalloproteinase, ADAMs) are a family of cell surface transmembrane proteins that have broad biological functions encompassing proteolysis, adhesion and cell signal regulation. Several function as "ectodomain sheddases" that are responsible for the proteolytic release or activation of membrane associated ligands and receptors. In particular, ADAM-17 (tumour necrosis factor-α converting enzyme, TACE) is involved in the activation of pro-TNF-α and has an essential developmental role in regulating the biological functions of ligands of the epidermal growth factor receptor (EGFR). ADAM-10 also has a prominent role in Notch signaling. I will review current knowledge of the roles of ADAM proteins in cancer biology, and focus on recent work from our group that has linked ADAM-15 with clinical aggressiveness in breast cancer. The potential for selective targeting of ADAM proteins will also be discussed.

Cancer preventive potential of xanthohumol from HOP (*Humulus lupulus L.*)

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Xanthohumol (XN) is the major prenylated flavonoid present in the hop plant, *Humulus lupulus* L. (Cannabinaceae) and also a common ingredient of beer. It gained considerable interest as a cancer preventive agent as several studies showed that it exerts chemopreventive activities relevant to suppression of cancer development at the initiation, promotion and progression phases. Xanthohumol has been reported to modulate the activities of enzymes involved in carcinogen metabolism, have antioxidant and free radical scavenging activity, acts as an anti-inflammatory agent by inhibiting cyclooxygenases -1 and –2, inhibits DNA synthesis and induces cell cycle arrest, apoptosis and cell differentiation.

We studied the protective effect of xanthohumol against the genotoxicity of two most common procarcinogens benzo(a)pyrene (BaP) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ). *In vitro* experiments were performed with human hepatoma HepG2 cells and precision cut rat liver slices. The advantage of these two liver models is that they represent intact cells, in which metabolic enzymes are expressed in an inducible form and therefore they enable detection of protective mechanisms that are present in liver but not in other *in vitro* models. Xanthohumol by itself was neither cytotoxic nor genotoxic at concentrations below 10 μM, while nearly complete prevention of BaP or IQ induced DNA damage was observed at 10 nM xanthohumol in both models. In accordance with previous reports xanthohumol inhibited CYP1A activity in rat liver microsomes, but to our surprise neither in rat liver slices nor in HepG2 cells the activity of CYP1A was reduced. Xanthohumol also did not affect mRNA expression of the CYP1A1, 1A2 enzymes.

In rats that received xanthohumol (30 μg/kg/day which corresponds to human consumption of half a liter/day/70 kg of xanthohumol enriched beer (Xan) with 4 mg/l of xanthohumol) together with IQ we found significant prevention of the formation of liver, but not colon aberrant crypt foci (i.e. aberrant crypt foci in colon and enzyme altered GSTp+ foci in hepatic tissue). However as in rat liver slices also in rats we did not detect any effect of xanthohumol on the activity of metabolic enzymes.

In contrast to previous suggestions that are based on the observations of xanthohumol-mediated inhibition of CYP1A enzymes in cell-free experimental systems (microsomes), our results indicate that in intact cells, tissue or whole organism, inhibition of metabolic activation of pro-carcinogens by CYP1A is not likely to be the mechanism of its antigenotoxic action. Although the mechanism of the protective effect of XN seems to be more complex than previously suggested, our results provide additional evidence for the cancer preventive potential of xanthohumol.

New inhibitors of steroid metabolizing enzymes as potential anticancer agents

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Androgens and estrogens increase the number of cell divisions and the opportunity for random genetic errors and are thus involved in carcinogenesis of hormone related cancers. Pre-receptor regulatory enzymes interconvert the active forms of hormones with high affinities to corresponding receptors with their less active forms with very low affinities (1). These enzymes represent interesting targets for development of new drugs for prevention and treatment of conditions caused by disturbed hormone action. We focused our attention to hydroxysteroid dehydrogenases (HSD), which are enzymes that act as molecular switches (2). We study human recombinant AKR1C1, AKR1C3 and 17beta-HSD type 1. AKR1C1 reduces a potent progesterone to a weak 20alpha-hydroxyprogesterone, while AKR1C3 reduces a weak androgen androstenedione to a potent testosterone. Both AKR1C3 and 17beta-HSD type 1 convert weak estrogen estrone to a potent estradiol (3, 4). The recombinant enzymes AKR1C1 and AKR1C3 were isolated and our in-house bank of compounds was screened for potential inhibitors. Automated docking was used to explain the possible binding orientation of best inhibitors within the active site. Three groups of compounds were shown to potently inhibit AKR1C1 and AKR1C3: phytoestrogens (5,6), cinnamic acids (7) and nonsteroidal anti-inflammatory drugs and their analogues (8). To search for potential inhibitors of 17beta-HSD type 1 we used the easily available model enzyme 17beta-HSD from the fungus *Cochliobolus lunatus*. We found that this enzyme is inhibited by phytoestrogens, cinnamic acid esters and cinnamamides (9). In addition to screening and analogue-based approach, structure-based virtual high-throughput screening is performed to discover new small molecule inhibitors of all enzymes under investigation.

References:
1. Henderson, B.E.; Feigelson, H.S. Carcinogenesis, 2000, 21, 427-433.
2. Nobel, S.; Abrahmsen, L.; Oppermann, U. Eur. J. Biochem., 2001, 268, 4113-4125.
3. Penning, T. M.; Burczynski, M.E.; Jez, M.E.; Hung, C.F.; Lin, H.K.; Ma, H.; Moore, M.; Palackal, N.; Ratnam K. Biochem. J., 2000, 351, 67-77.
4. Penning, T.M. Endocr. Rev., 1997, 18, 281-305.
5. Brožič, P.; Šmuc, T.; Gobec, S.; Lanišnik Rižner, T. Mol. Cell. Endocrinol., 2006, 259, 30-42.
6. Brožič, P.; Golob, B.; Gomboc, N.; Lanišnik Rižner, T.; Gobec, S. Mol. Cell. Endocrinol., 2006, 248, 233-235.
7. Brožič, P.; Šmuc, T.; Gobec, S.; Lanišnik Rižner, T. Enzymology and molecular biology of carbonyl metabolism 13. Purdue: Purdue University, 2007, 252-262.
8. Gobec, S.; Brožič, P.; Lanišnik Rižner, T. Bioorg. Med. Chem. Lett., 2005, 15, 5170-5175.
9. Sova, M.; Perdih, A.; Kotnik, M.; Kristan, K.; Lanišnik-Rižner, T.; Šolmajer, T.; Gobec, S. Bioorg. Med. Chem..2006, 14, 7404-7418.

Gene expression regulation by siRNA electrotransfer

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RNA interference (RNAi)-mediated gene silencing approaches appear very promising for therapies based on the targeted inhibition of disease-relevant genes. The major hurdle to the therapeutic development of RNAi strategies remains however the efficient delivery of the RNAi-inducing molecules, the short interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs), to the target tissue. SiRNA gene silencing could be obtained *in vivo* on reporter as well as endogenous genes. The demonstration in 1998 of drug and plasmid electrotransfer and gene expression in tumours [1] led to the proposal that *in vivo* electropulsation was a promising tool for exogenous agent delivery.

In this study we have investigated the contribution of electrically-mediated delivery of siRNA and/or shRNA into muscles or tumours stably expressing a green fluorescent protein (EGFP) target reporter gene [2,3]. The silencing of EGFP gene expression was quantified over time by fluorescence imaging in the living animal. Indeed, exogenous gene expression of fluorescent reporter proteins such as GFP can be accurately followed of the same animal as a function of time with no adverse effects either on the reporter gene product or on the animal itself. Our study indicate that electric field can be used as an efficient method for RNAi delivery and associated gene silencing into cells of solid tumours *in vivo*.

For example, it has successfully been used by others to silence the Mitf gene in mice tumours leading to reduction in the outgrowth of subcutaneous melanoma [4].

References:

1. *In vivo* electrically mediated protein and gene transfer in murine melanoma . Nature Biotechnology, 1998; 16, 168-171. M.P. Rols, C. Delteil, M. Golzio, P. Dumont, S. Cros and J. Teissié.

2. Inhibition of gene expression in mice muscle by *in vivo* electrically mediated siRNA delivery. Gene Therapy, 2005; 12, 246-251. Golzio M, Mazzolini L, Moller P, Rols MP, Teissié J.

3. *In vivo* gene silencing in solid tumors by targeted electrically mediated siRNA delivery. Golzio M, Mazzolini L, Ledoux A, Paganin A, Izard M, Hellaudais L, Bieth A, Pillaire MJ, Cazaux C, Hoffmann JS, Couderc B, Teissie J.Gene Therapy 2007; 14(9):752-9.

4. Therapeutic RNA interference of malignant melanoma by electrotransfer of small interfering RNA targeting Mitf. Nakai N, Kishida T, Shin-Ya M, Imanishi J, Ueda Y, Kishimoto S, Mazda O. Gene Therapy, 2007;14(4):357-65.

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Stem cells in toxicology

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Rapidly increasing knowledge of human stem cells offers the unique possibility to establish *in vitro* toxicological methods in many different areas in which currently only primary cell cultures, cell lines derived from animals or immortalized cells are used. Stem cells of different sources can be used as "cell factory" for human cell based systems. However, the relevance and reliability of such *in vitro* test systems is strongly correlated to the quality of the source, i.e. the stem cells used, standardized differentiation protocols, the purification rate of stem cells and their derivates, selected toxicological endpoints as well as the developed prediction models. Hematopoiesis is a fascinating system in which pluripotent hematopoietic stem cells (PHSCs) differentiate into many highly specialised circulating blood cells. At least 95% of hematopoietic cells fall into morphologically recognizable cell lineages. Dormant PHSCs are recruited into the cell cycle by many cytokines as IL-1, IL-3, IL-4, IL-6, IL-11, IL-12, SCF (stem cell factor), G-CSF (granulocyte-colony stimulating factor), M-CSF (macrophage-colony stimulating factor), Epo (erythropoietin), LIF (leukemia inhibitory factor), FLk2/FLT3 (tyrosine kinase receptor) ligand, TPO (thrombopoietin).
Lineage-specific factors support the survival, proliferation and maturation of progenitors that are committed through hypothetical stochastic expression of specific groups of differentiation genes. The foetal stem cells, for their plasticity, can generate a perpetual supply of healthy, normal human cells for disease modeling, drug discovery, and toxicology, because they can potentially generate suitable models for cardiotoxicity, hepatotoxicity, genotoxicity/epigenetic and reproductive toxicology. Furthermore, gender-differences in drug sensitivity can be evaluated using human hematopoietic stem cells from different donors.

Aptamer-based capture molecular as a novel method to isolate porcine mesenchymal stem cells

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Mesenchymal stem cells (MSC) are a stem cell population present in bone marrow that can be isolated and expanded in culture and characterized *in vitro*. The potential of MSCs to replicate undifferentiated and to mature into various mesenchymal tissue cells makes it an attractive source for bone tissue engineering. Cell-scaffold constructs promise to be better candidates for transplantable bone substitutes than the conventional bone grafts. To improve the cell seeding, we tested a new coating method for scaffolds. We used high specific binding nucleic acids, called aptamers, generated by combinatorial chemistry with an *in vitro* technology named systematic evolution of exponential enrichment (SELEX). Porcine MSCs were used as a target of the SELEX procedure. After 12 rounds of selection, the binding sequences were cloned and sequenced to get single aptamers. The selection procedure was screened by flow cytometry. The binding affinity was detected by a cell sorting assay with streptavidin-coated magnetic microbeads. Additionally we use tissue culture plates immobilized with aptamers to fish out MSCs from the cell solution. Then we incubated the aptamers with the whole bone marrow blood to identify the capture ability of the aptamers. The immobilized MSCs were cultured with osteogenesis-promoting medium. ALP assay and Von Kossa staining were performed to evaluate the differentiation character of MSCs. We generated aptamers which can capture pig MSCs out of bone marrow blood with high affinity and specificity. By immobilizing aptamers on solid surfaces, MSCs can be fished out in a short time, which can facilitate MSCs adhesion and enrichment directly on a scaffold. Induced by growth factors, the immobilized MSCs can differentiate into osteoblasts as shown by ALP assay and Von Kossa staining.Aptamers, as novel coating substances for scaffolds, can bind MSCs directly from whole bone marrow efficiently. We suppose that this could allow short time incubation between the scaffold and bone marrow blood to fish out MSCs rapidly. The potential clinical application of aptamers in tissue engineering will bring new visions to the traditional field of bone repair.

Clinical use of cleaved forms of the urokinase receptor

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It is well established that the urokinase plasminogen activator, uPA and its cellular receptor uPAR are involved in cancer invasion and metastasis and the tumor tissue and circulating levels correlate with cancer patient survival. In addition to the cellular binding, uPA cleaves uPAR in the linker region between domains I and II and thereby inactivates uPAR´s binding potential. Cleavage of uPAR thus reflects the activity of uPA and possibly the aggressiveness of the tumor. uPA in physiological relevant concentrations cleaves glycolipid-anchored uPAR but not soluble uPAR (suPAR). To elucidate the mechanism of uPA-mediated uPAR cleavage and identify the different uPAR forms we have used PMA–stimulated U937 cells. On the cell surface as well as inside the cell, both intact uPAR(I-III) and cleaved uPAR(II-III) are present, whereas in the media only intact suPAR and uPAR(I) are detected. Treatment of the cells with Pi-PLC releases both uPAR(I-III) and uPAR(II-III) from the cell surface. Biotinylation of cell surface proteins showed that when uPAR cleavage was inhibited by incubation with a neutralizing anti-uPA antibody, uPAR(II-III) was not found on the cell surface. The intracellular pattern of uPAR(I-III) and uPAR(II-III) was not affected by inhibiting the cell surface cleavage. However, in the media from cells grown in the presence of the neutralizing anti-uPA antibody, both suPAR(I-III) and suPAR(II-III) were present. This indicates that uPAR(II-III) is shed from the cell surface when uPA-mediated cleavage of uPAR is blocked.

In tumor tissue and body fluids both intact and cleaved forms of uPAR have been identified using Western blotting and immunoassays quantifying the individual uPAR forms. In tumor extracts from patients with non-small cell lung cancer uPAR(I) is a stronger prognostic marker then the total amount of all uPAR forms. Also in serum from patients with non-small cell lung cancer the level of uPAR(I) correlates with overall survival. The concentrations of uPAR(I) as well as suPAR(II-III) are significantly elevated in serum samples from patients with prostate cancer compared to the concentrations in serum from men with benign prostatic conditions. Furthermore, specific measurements of uPAR(I) were found to improve specificity of prostate cancer detection. Similarly high concentration of plasma uPAR(I) is an independent preoperative marker of poor prognosis in patients with ovarian cancer. The combination of plasma suPAR(I-III)+suPAR(II-III) and CA125 discriminates between malignant and benign ovarian tumors with an AUC of 0.94. We will verify our findings in a larger collection of plasma/serum samples from prostate, ovarian and non-small cell lung cancer patients and investigate the prognostic significance and possible diagnostic utility of cleaved uPAR variants in other forms of cancer.

The expression pattern of the urokinase plasminogen activation system in human colon cancer is recapitulated in liver metastases with desmoplastic encapsulation

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Metastatic growth and invasion by colon cancer cells in the liver requires the ability of the cancer cells to interact with the new tissue environment. This interaction does not lead to one consistent histological invasion pattern but in two major distinct patterns: those with desmoplastic encapsulation and those with solid growth. The potent matrix degrading protease, plasmin(ogen), is activated on cell surfaces by urokinase plasminogen activator (uPA), which activity is regulated by uPA receptor (uPAR) and type-1 plasminogen activator inhibitor (PAI-1).

To compare the expression patterns of uPA, uPAR and PAI-1 in primary colon adenocarcinomas with that in colon cancer liver metastases, we have analysed matched samples from 14 patients of which 6 had liver metastasis with desmoplastic growth pattern and 8 had metastasis with solid growth. In the primary tumours, we found focal upregulation of uPAR-immunoreactivity and uPA mRNA expression in stromal cells at the invasive front. Similarly we found focal upregulation of uPAR at the metastasis periphery in all 6 liver metastases with desmoplastic encapsulation, whereas only one of the 8 liver metastases with solid growth pattern had focal upregulation of uPAR in the metastasis/liver parenchyma interface. uPA mRNA was also found upregulated in 5 of the 6 liver metastases with desmoplastic growth in the desmoplastic periphery and was not found upregulated in the metastases periphery in any of the 9 remaining metastases. In addition, we found invasive budding cancer cells, which are positive for Laminin-5γ2 (LN5γ2), expressing both uPAR and uPA mRNA in all primary tumours and in the liver metastases with desmoplastic growth pattern. PAI-1 was found in myofibroblasts located within the desmoplastic capsule of liver metastases in a comparable pattern as found in the primary tumours. Interestingly, PAI-1 was also found in some hepatocytes located close to the metastases independent of the metastasis growth pattern. We conclude that metastasis-induced desmoplasia with recruitment of macrophages and myofibroblasts upregulates the expression of uPAR, uPA and PAI-1 in a pattern similar to that in the primary tumours. Our findings suggest that the molecular expression patterns can be partly recapitulated in metastases and that alternative invasion mechanisms in metastases remains to be characterised.

Cathepsin X controls migration and invasiveness of T lymphocytes

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Cathepsin X is a lysosomal cysteine protease exhibiting carboxypeptidase activity. Its expression is high in the cells of immune system and its function has been related to the processes of inflammatory and immune responses. It regulates processes such as adhesion, T lymphocyte activation and phagocytosis through interaction with β_2 integrins. To investigate the role of cathepsin X in the migration of T lymphocytes, Jurkat T lymphocytes were stably transfected with the pcDNA3 expression vector containing cathepsin X cDNA. The cathepsin X up-regulated T lymphocytes exhibited polarized migration-associated morphology, enhanced migration on 2-D and 3-D models using intercellular adhesion molecule – 1 (ICAM-1) and Matrigel coated surfaces and increased homotypic aggregation. The increased invasiveness of cathepsin X up-regulated cells does not involve proteolytic degradation of extracellular matrix. Confocal microscopy showed that the active mature form of cathepsin X was co-localized in migrating cells together with lymphocyte function-associated antigen 1 (LFA-1). The co-localization was particularly evident at the trailing edge protrusion, the uropod, which plays an important role in T lymphocyte migration and cell-cell interactions. We propose that cathepsin X causes cytoskeletal rearrangements and stimulates T lymphocyte migration by modulating the activity of β_2 integrin receptor LFA-1.

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Tumour vascular disrupting agents: mechanism of action

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Tumour blood vessels are both structurally and functionally different from those of normal tissues and these characteristics make them good targets for cancer therapy. Two distinct but related tumour vascular targeting approaches are currently under development. These are the *anti-angiogenic* strategies, which hinder the development of new blood vessels from pre-existing ones, and approaches using *vascular disrupting agents* (VDAs) that damage pre-existing blood vessel networks. Effective VDAs achieve selective and prolonged vascular damage, which restricts the blood flow to the tumour, and indirectly causes necrosis of the tumour tissue. Tubulin-binding agents comprise a major group of low molecular weight drugs, with tumour vascular disrupting properties. Depending on scheduling of administration, tubulin-binding VDAs are also likely to interfere with angiogenesis. Significant progress has been made into introducing several VDAs into the clinical testing phase, although their mechanism of action and the reasons why they are selective for tumour blood vessels remain unclear.

We have focused our investigations on elucidating the mechanism of action of tubulin binding VDAs using both *in vitro* and *in vivo* models, and Combretastatin A-4-phosphate (CA-4-P) as a model drug. We found that rapid responses of cultured endothelial cells to CA-4-P were mediated by cross talk signaling between interphase microtubules and the actin cytoskeleton. CA-4-P signaling was through Rho-GTPase/Rho kinase and mitogen-activated protein kinases (MAPKs) and was associated with altered endothelial morphology, increased contractility, disruption of VE-cadherin cell-to-cell junctions and a rise in monolayer permeability. These effects were modulated by cAMP and cGMP elevating agents and by hypoxia, which is a common characteristic of the tumour microenvironment. A rise in tumour vascular permeability also occurred *in vivo* at early times after CA-4-P and is strongly implicated in the tumour vascular shut-down which occurs within minutes of treatment with the drug. Inhibition of Rho kinase using Y27632 attenuated CA-4-P-mediated vascular shut-down in a SW1222 human colorectal carcinoma xenograft in SCID mice, which suggests that Rho signaling is involved in early tumour vascular effects of CA-4-P *in vivo*. CA-4-P also inhibited angiogenic responses, including migration and morphogenesis of endothelial cells into capillary-like structures in matrigel *in vitro*, and both processes were dependent on activation of the Rho/Rho kinase signaling pathway.

These results further our understanding of molecular mechanisms responsible for the vascular disrupting effects of CA-4-P. Elucidation of the mechanisms of VDA action is important for improving their efficacy and identifying new targets for drug development.

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Protein microarray approach in tumour biology investigation

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The completion of the Human Genome Project has led to intensive development of genetics-related technologies that are finding their way into all areas of biological research. Functional genomics and systems biology are based on post-genomic philosophy considering a cell as a system of simultaneous events of hundreds or thousands interplaying molecules constituting a complex network of a cell's life, replying its present physiological condition. High-throughput genomics and proteomics techniques that allow a global description of molecular contents of a cell or biological sample have been quickly adapted to develop tools for medical research, to identify new biomarkers and molecular mechanisms of disease, and to develop new approaches for clinical applications. As proteins are the "real players" of life, proteomics is the best way, in particular in combination with metabolomics, to look for the most relevant disease biomarkers typical for disease proteomes or appearing in the way of disease development. However, "classical" differential proteomic approach seems be quite static, allowing comparison of disease and normal proteomes at selected fragments of time that are limited by frequency, due to the complex and time consuming technology. We are developing a whole-proteome microarray based on multichannel plasmon resonance imagery in order to allow a "real-time" insight into cancer proteome during development of a tumour. We believe that this, simpler and more accurate technique will contribute to improved knowledge on disease biomarkers and will also allow to return back, i.e. to develop more relevant and simpler-to-handle transcriptomic approaches for molecular diagnostics and for studying systemic cellular responses in targeting drug development.

Matrix metalloproteinases, their tissue inhibitors and progression of head and neck squamous carcinoma

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Matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) have become of interest with regard to their role in the cancer development. MMPs are able to degrade connectiv tissue, among other substrates the basement membrane collagen, which seems to be of critical importance in tumor invasion and metastasis. MMPs are inductor of transition from benign tumour to a malignant and metaststic one due to cleavage of E-cadherin and disruption of cadherin-mediated cell-cell contacts. During formation of metastases MMPs are required for cells to invade, intravasate and migrate. TIMPs inhibit the catalytic activity of MMPs and they are also able to regulate tumour growth and angiogenesis.

While some previous studies have associated MMPs and TIMPs with survival in various cancer types, there are no findings in the possibility to use these parametres for prognosis of radiotherapy efficacy. In this study, the role of MMP-2,3,9, and TIMP-1,2 has been explored in the progression of head and neck squamous sell carcinoma (HNSCC), including tumor growth, formation of metastases and tumour responce on radiotherapy. Immunohistochemical investigation showed significant decrease of TIMP-1 expression in cells of metastatic carcinomas than in nonmetastatic. Expression of MMP-1,2,9 in stroma correlated with expression of extracellular matrix metalloproteinase inducer (EMMPRIN) in tumor cells. Probably carcinoma cells expressed EMMPRIN for increase of MMPs production by surrounding cells. Also the increase of TIMP-1 level was determined in blood serum of patients with metastasis in regional lymph nodes in comparison with level serum of patients without metastasis. The blood level of MMP-9 correlated with tumor size. Blood levels of TIMP-1 and TIMP-2 might be the possible criteria of radiotherapy efficacy with the specificity of 66,6% and 87,5%, sensitivity of 72,7% and 60%, respectively.

In conclusion, MMP-9 and TIMP-1 play a important role in development of head and neck squamous sell carcinoma and TIMP-1 level in blood serum and cancer tissues is connected with first grade of regional lymph node metastasis. TIMP-1 and TIMP-2 blood levels are associated with tumor response on radiotherapy of HNSCC patients and might help in development of new treatment modalities.

Cooperation of urokinase-type plasminogen activator and tissue inhibitor of metalloproteinases-1 in the creation of a pro-metastatic niche *via* hepatocyte growth factor-signaling in the liver

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Metastasis of tumor cells relies not only on their metastatic potential, but also on the susceptibility of a target organ to invading tumor cells. We recently demonstrated that elevated host expression of tissue inhibitor of metalloproteinases-1 (TIMP-1), which correlates with poor prognosis in cancers, leads to promotion of metastasis and tumor cell scattering in the liver. This was associated with induction of hepatocyte growth factor (HGF)-signaling and up-regulation of urokinase-type plasminogen activator (uPA). Yet, the impact of uPA in this TIMP-1-induced promotion of metastasis and the functional link between these molecules is unknown.

Metastasis of *lacZ*-tagged murine T-cell lymphoma cells was compared in uPA knock out mice and their wild-type controls, both expressing elevated TIMP-1 levels by adenoviral transduction of TIMP-1. uPA-deficiency significantly inhibited TIMP-1-induced liver metastasis and scattering of tumor cells. In presence of uPA, elevated levels of TIMP-1 led to increased total metastasis burden in the liver by 2.5-fold as compared to the virus-control. Lack of host cell-derived uPA abrogated this increase. In absence of host uPA, the TIMP-1-associated activation of pro-HGF was markedly reduced. Also, host uPA-deficiency suppressed TIMP-1-induced activation of HGF-signaling via the receptor cMet by 5.7-fold.

TIMP-1-induced host cell-derived uPA is necessary for the pro-metastatic effect of TIMP-1, by participating in the activation of the HGF/cMet-signaling pathway. Cooperation of a protease with an inhibitor of a different protease family in signaling demonstrates the complexity of the proteolytic network in the modulation of a pro-metastatic niche.

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Altered expression of cysteine cathepsins and matrix metallo-proteases (MMP2 and MMP9) in brain tumors differentially affect glioma progression

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Gliomas are the most abundant of a great variety of histological subtypes of brain tumours. Grade IV glioma (glioblastoma) is highly malignant, with a survival rate for the patients of about 12 months. Proteases play significant but diverse roles in various steps in glioma progression, being expressed both in tumour and stromal cells (particularly in macrophages and vascular endothelial cells). Here, we discuss the differential role of lysosomal cathepsins B and L and matrix-metalloproteases MMP2 and MMP9 (gelatinases) in glioma progression *in vitro*, using U87 cell cultures and their co-cultures with macrophages and endothelial cells.

We have confirmed that cathepsin B, along with MMP2 and 9, is mostly responsible for glioblastoma cell invasion *in vivo*. Complementary data from clinical studies on (87) glioma patients have led to the conclusion that cathepsin B, a marker for invasiveness, is also highly prognostic for patient's survival, although its role in angiogenesis may also contribute to its prognostic impact (1). In addition, cathepsin B and MMP9 expression is also involved in a cross-talk between glioblastoma (GBM) cells with macrophages and endothelial cells, which is mediated by cytokines. *In vitro* studies of cathepsin L, which is not prognostic, suggest that higher levels of this protease in malignant gliomas are not related to cell invasiveness, but rather to resistance to therapy. These data support thus the role of CatL as a modulator of the response of glioma cells to various apoptosis - inducing signals, as already described by our group (2,3).

In conclusion, our experiments using various methods for knocking out and silencing cathepsin genes in normal and glioma cells, have clearly revealed differential expression and functions of cysteine cathepsins B and L as well as MMP2 and MMP9. Co-culture studies reveal that the signaling pathways initiated and mediated by these proteinases may effect gene regulation in the same and/or surrounding cells, changing their behavior.

References:

1.) Strojnik T, Rbsland GP, Sakariassen PO, KavalarR. Lah TT.(2007) Neural stem cell markers, nestin, and musashi proteins in the progression of human glioma: correlation of nestin with prognosis of patient survival. Surgical Neurology, vol. 68, no. 2.133-143.

2. Levičar N, Dewey RA, Daley E, Bates TE, Davies D, Kos J, Pilkington GJ, Lah TT. (2003). Selective suppression of cathepsin L by antisense cDNA impairs human brain tumor cell invasion *in vitro* and promotes apoptosis. Cancer Gene Ther. 10: 141-151.

3. Zajc I, Hreljac I, Lah T. (2006). Cathepsin L affects apoptosis of glioblastoma cells: a potential implication in the design of cancer therapeutics. Anticancer Res. 26: 3357-64.

Monomerisation of cystatin F: who's in charge?

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Cystatin F is a cysteine protease inhibitor expressed mainly in cells important for the immune system. Glycosylation, predominant lysosomal localisation and 6 cysteines instead of four make cystatin F different from other members in the type II cystatin family. As a monomer cystatin F is a potent inhibitor of C1 family of lysosomal cysteine proteases. Inhibitory potential is abrogated in disulfide bonded dimers, which are predominantly found in the cells. Cystatin F was proposed to regulate proteolytic activity in lysosome – like organelles and hence the antigen presentation on MHC II molecules.

It is not known, whether active monomeric cystatin F is present in the lysosomes. Monomeric cystatin F could be a transient form before dimerisation in the endoplasmic reticulum (ER), or in the lysosomes after transportation of the monomers from the ER. Alternatively, it may be created de novo by reduction of dimers in the reducing lysosomal environment, or by assistance of GILT, the only known lysosomal reductase. Monomerisation of cystatin F could also be performed by a specific protease, cleaving in the N-terminal region after cys26. Cys26 is involved in the intermolecular disulfide bond with cys44 from another molecule.

U937 promonocytic cells were subjected to ultracentrifugation experiment. We showed, that monomeric form of cystatin F is present in the lysosomal fractions as well. The ratio between the monomers and dimers changes in favour of the monomers upon activation or differentiation of U937 cells. Disruption of the lysosomal pH effectively prevents or delays this process, suggesting that acidic environment or an enzyme active in acidic pH is involved in the process of monomerisation. Co-transfection of cystatin F and GILT reductase into HEK293 cell line did not increase the ratio of the monomeric form, suggesting that dimeric cystatin F is not an endogenous substrate for GILT. N-terminal sequence of immunoprecipitated dimeric and monomeric cystatin F from transfected HEK293 cells was as expected (GPSP), suggesting that a protease is not involved in the generation of monomers. In contrast, monomers in U937 cells had a shorter N-terminus. On the basis of cellular experiments we conclude, that a U937 cell line specific protease acts downstream on removing the N-terminus of monomeric cystatin F, but it is not necessary for the monomerisation process. We suggest, that lysosomal reductive environment alone is responsible for the monomerisation of cystatin F, since we were able to achieve monomerisation of recombinant dimeric cystatin F in buffers mimicking lysosomal conditions (pH, redox potential).

Adult stem cells: from bench to bedside

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Recent advances in understanding of stem cell biology have raised expectations for using stem cells as new cellular therapeutics in regenerative medicine. Although stem cell therapy is not considered classically within the realm of clinical medicine, this technology is becoming increasingly important in clinical arena. Potential strategies to replace repair and restore the function of the damaged tissues or organs include adult stem cell transplantation and it is believed that novel cellular therapeutics could perform better than any medical device, recombinant protein or chemical compound.

We have isolated, from mobilised and leukapheresed blood, a morphologically and phenotypically homogeneous subpopulation of CD34+ cells (~1%) that exhibits the necessary properties. These cells are able to differentiate into several lineages and express genes corresponding to hepatic differentiation (albumin, alpha-feto protein, alpha-1-antitrypsin) and pancreatic differentiation (Pdx-1, Pax-4, CK19). Animal experiments showed that they can help regenerating liver damage. Moreover, when transplanted into mice with streptozotocin induced diabetes they significantly reduced blood glucose, suggesting their possible use in cell therapy. We performed a phase I clinical study in patients with liver insufficiency, where cells were injected into the portal vein or hepatic artery. Patients were followed up for 18 months. All patients tolerated the procedure well and there were no treatment-related side effects or toxicities observed. Majority of the patients showed improvement in serum bilirubin and albumin. Our study showed considerable promise for using adult stem cell therapy in the future the clinical applications.

Proteases in cancer: progression or protection?

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Proteolytic enzymes have long been associated with cancer progression because of their ability to degrade extracellular matrix components, which facilitates tumour invasion and metastasis. These facts provided the rationale for clinical trials with protease inhibitors, which, unfortunately, have not been positive in most cases. Recent studies have revealed how proteases contribute with both pro-tumour and anti-tumour activities in all tumour-progression stages, what has forced a re-evaluation of the prevailing concepts in this field. To date, more than 30 proteolytic enzymes belong to the growing group of anti-tumour proteases which mainly includes different caspases, deubiquitylases, kallikreins, and members of the MMP and ADAMTS families.

We have recently described several intracellular and extracellular proteases with anti-tumour properties. Thus, autophagin 3 (Atg4c) is an intracellular cysteine protease involved in processing events associated with autophagy. Mice lacking autophagin-3 show a high incidence of carcinogen-induced fibrosarcomas, which has been correlated with the decrease in autophagy found in $Atg4c^{-/-}$ fibroblasts. Among extracelullar proteases, we have previously described that MMP-8 reduces skin tumour susceptibility by modulating inflammatory response to chemical carcinogens. We have recently extended these results with the finding that MMP-8 expression in mice also prevents metastasis by reducing invasion capability and altering cellular adhesion. Moreover, MMP-8 levels in human breast cancer are associated with good clinical prognosis, reinforcing the idea of the protective role of this enzyme. Parallel studies on different members of the ADAMTS family have allowed us to propose novel tumour-defying functions for ADAMTS-12 and ADAMTS-15. Thus, ADAMTS-12 controls tumour progression by modulation of the Ras-dependent ERK signalling pathway. Further work has revealed that this gene is epigenetically silenced in colon carcinomas, suggesting that this mechanism may operate *in vivo* to inactivate the tumour-suppressor actions of this metalloproteinase. Finally, mutational analysis of different proteases presumably associated with cancer has shown that ADAMTS-15 is inactivated by mutation but not by epigenetic silencing, in several carcinomas. On the basis of these results, we can conclude that tumour protective roles of proteases are much more relevant than originally anticipated, thereby contributing to expand the functional complexity of the cancer degradome, which is the complete set of proteases produced by a malignant tumour.

Specific laboratory animal husbandry in oncologic research

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There are a number of specific needs in terms of husbandry when dealing with animal models for cancer research. Most of the time mice immunodepressed are selected for inoculation of solid tumors, nude, scid and transgenics are today widely distributed all over the world. Rats are another interesting rodent model required by some scientists working on gene theraphy.
The general health condition of these models under esperiment is always at high risk due to the often spontaneously or induced immunocompromised condition. A second problem relates to the use of chemicals and drugs for the induction of specific tumors or their treatment. In this situation the protection of technicians and investigators becomes a must.
A number of technical solutions were found over the years in terms of primary enclosures to achieve the goal of double protection, mainly when working with mice or single (staff) protection when dealing with rats.
An overview of the possible caging-systems solutions will be given during the talk with examples of cancer research carried out with different primary barriers.

Tumor ablation with irreversible electroporation

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We report the first successful use of irreversible electroporation for the minimally invasive treatment of aggressive cutaneous tumors implanted in mice. Irreversible electroporation is a newly developed non-thermal tissue ablation technique in which certain short duration electrical fields are used to permanently permeabilize the cell membrane, presumably through the formation of nanoscale defects in the cell membrane. Mathematical models of the electrical and thermal fields that develop during the application of the pulses were used to design an efficient treatment protocol with minimal heating of the tissue. Tumor regression was confirmed by histological studies which also revealed that it occurred as a direct result of irreversible cell membrane permeabilization. Parametric studies show that the successful outcome of the procedure is related to the applied electric field strength, the total pulse duration as well as the temporal mode of delivery of the pulses. Our best results were obtained using plate electrodes to deliver across the tumor 80 pulses of 100 μs at 0.3 Hz with an electrical field magnitude of 2500 V/cm. These conditions induced complete regression in 12 out of 13 treated tumors, (92%), in the absence of tissue heating. Immune cell recruitment during the treatment of sarcoma tumors in mice with irreversible electroporation was studied by immunohistochemistry. Employing irreversible electroporation parameters known to completely ablate the tumors without thermal effects we did not find infiltration of immune cells probably because of the destruction of infiltration routes. Irreversible electroporation is thus a new effective modality for non-thermal tumor ablation. Moreover, since immune response is not instrumental in irreversible electroporation efficacy, we propose that irreversible electroporation may be, therefore, a treatment modality of interest to immunodepressed cancer patients.

The pharmacogenetic basis for individualized thiopurine therapy

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Dose adjustment is due to narrow therapeutic range of cytotoxic drugs a critical step in therapy design and a significant predictor of overall event free survival. Patients with acute lymphoblastic leukemia (ALL) are treated during the consolidation and maintenance phase of the therapy mainly with 6-mercaptopurine (6-MP). Thiopurine S-methyltransferase (TPMT) is one of the metabolizing enzymes of 6-MP, its inactivating potential renders lower cytotoxic thioguanine nucleotides (TGN) formation. Individuals with low TPMT activity tend to have higher TGN concentrations when normal 6-MP doses are administered, resulting in the increased incidence of toxic effects. Decreased TPMT activity is caused by mutations in the TPMT gene, and also other factors, such as S-adenosylmethionine (SAM) and enzymes of folate metabolism.

Patients with homozygous mutant TPMT alleles confer very low enzyme activity and are in need of dose reductions. However, the advantage of dose modulation in heterozygous patients is less clear. To assess the influence of TPMT haploinsufficiency and MTHFR mutations on thiopurine treatment toxicity and efficiency, we have retrospectively analyzed the association of the 6-MP dose reduction, the incidence of 6-MP toxic effects and ALL relapses with TPMT and MTHFR genotypes in 320 children with ALL who had undergone their treatment at the same institution over the period of 30 years. Our results suggests that heterozygositiy at TPMT gene locus predisposes individual to hematologic toxicity and consequently 6-MP dose reduction. This is further augmented by mutations in MTHFR gene. To our knowledge this is the first retrospective correlation study, its major strengths are in blinded phenotype-genotype assessments, confirming the potency of genotypic prognostic relevance on the basis of clinical outcomes.

Inflammation, angiogenesis and tumor invasion: the role of proteolytic enzymes in the malignant progression of skin SCCs

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Tumor progression is significantly controlled by an activated tumor promoting microenvironment that is initially induced by tumor cell derived growth factors. We provide evidence that an essential contribution of these growth factors to promoting malignant progression lies in the induction of a tumor supporting inflammatory infiltrate as well as in the activation of tumor- and stroma-derived MMPs.

We have generated a growth factor driven tumor progression model of skin SCCs encompassing each step of progression. Induced expression of PDGF promotes benign tumor growth, whereas expression of VEGF, IL-6 and G-CSF induce malignant tumor growth with increasing malignancy, respectively. While benign tumor growth is associated with a transient inflammatory cell recruitment and angiogenesis, malignant progression induced by VEGF, IL-6 or G-CSF leads to a persistent recruitment of neutrophils followed by persistent angiogenesis. This is associated with a strong and persistent accumulation of MMP-9, which co-localizes with neutrophil and a low level of stromal MMP-13 that seems strictly associated with tumor invasion. Co-expression of VEGF, IL-6, G-CSF and GM-CSF induce an enhanced malignant and spontaneously metastasizing tumor phenotype, with high expression of tumor derived MMP-1 and MMP-14 as well as of stromal MMP-9 and MMP-13. Interestingly, lack of MMP-9 expressing neutrophils, achieved by neutrophil depletion, as well as lack of MMP-13 inhibit angiogenesis and tumor invasion. While neutrophils depletion seems to change the tumor supporting nature of the inflammatory cell infiltrate and inhibits already early stages of angiogenesis, lack of MMP-13 in MMP-13 knock out mice does not inhibit the initial angiogenic response that occurs in both benign and malignant tumors. Instead it inhibits the persistent angiogenesis as well as the tumor invasion that characterizes malignant tumor growth. This is associated with a reduction of VEGF expression in late stages of tumor transplantation. Thus, we hypothesize that of stromal MMP-9 as expressed by neutrophils contributes to the initial angiogenic response that accompanies both benign and malignant tumor growth. In contrast stromal MMP-13 seems essential for the maintenance of angiogenesis and the induction of tumor invasion.

Mechanisms in metastasis

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Many proteins have now been identified whose expression in tumor cells results in altered metastatic potential. Matrix metalloproteinase is one of those proteins. We undertook a comprehensive proteomic screen to identify MMP-9 substrates that might affect metastasis. We compared the peptides in conditioned medium from PC-3ML cells to the same cells with MMP-9 knocked down by RNAi. Using a label-free quantitative proteomics approach based on UPLC-ESI-MS/MS to determine the levels of specific peptides in the conditioned medium. From more than 200 proteins identified, 69 showed significant alteration in levels at the absence of the protease (> +/- 2-fold) including both previously identified substrates and new candidates for substrates. To validate the screen, we compared the amounts of 6 of the candidate proteins in the conditioned medium by Western blotting and confirmed that they differed in amounts corresponding to those found by UPLC-ESI-MS/MS. The strong correlation between results by UPLC-ESI-MS/MS and by immunoblotting gave credence to the approach as a way to compare peptide amounts under different conditions without reliance on stable isotope tags even with relatively small differences (as little as 2 fold). It further has allowed us to identify protein nexin-1 as an MMP-9 substrate that potentially affects the metastatic process.

In another approach to determine how metastasis proceeds and to identify cellular interactions required, we have characterized the alterations in the liver microvasculature in response to metastatic colonization using two-photon microscopy The liver is a common site for metastasis. After intrasplenic injection, the tumour cells extravasated as single cells over the first 3 days. As they proliferated the host vessels were disrupted and the microvascular networks destroyed. This resembled the angiopoietin-2 dependent regression of retinal neovascularization. We tested the hypothesis that ang-2 dependent vascular co-optation was involved in live metastasis. First we demonstrated that angiopoietin-2 (Ang-2) was induced on the surface of the host vessels adjacent to the tumor cells. Second the vascular destruction did not occur in ang-2 deficient mice. Lastly we found that colony growth was more extensive in the livers from the angiopoietin-2 deficient mice. The tumour cells tracked along the vessels in the ang-2 deficient livers in contrast to the more spherical pattern seen in the wild type. We conclude that angiopoietin-2 dependent vascular destruction but not angiogenesis occurs during tumour metastasis in the liver Ang-2 itself appears as an impediment to tumour growth. The interactions of the tumour cell and the host vasculature differ depending on the metastatic site arguing that therapy should may need to be tailored to the involved organs.

Kinetics of tumour growth and of vascular perfusion flow in electrochemotherapy

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It is shown that experimental tumour growth and tumour perfusion flows in electroporation-based clinical treatments like electrochemotherapy (ECT) can be quantitatively analyzed in terms of nonlinear flow relationships, using the concept of time-dependent flow coefficients (reflecting time-dependences of flow cross sections, for instance of vascular blood flow).

This novel, simple and powerful, approach of minimum complexity yields, for instance, characteristic perfusion flow coefficients, stationary recovery levels indicating efficiency of perfusion blocks by ECT, and characteristic time constants for the recovery of electrically treated tumour cells and blood vessels under different pulse conditions (from staining the tumours as a function of time (t/h) during the recovery phase after drug application and electric pulses).

The characteristic parameters of the integrated tumour growth equations are in terms of up to 20 days and more.

The new method for the quantitative analysis of the results of electroporation data obtained with pulse trains rationalizes, for instance, that a pulse train of 8 pulses and a repetition frequency 1 Hz (8 s total train time) is electroporatively more efficient than the shorter pulse train of 5 kHz (1.6 ms total train time).

The reason for the efficiency of longer time intervals between the individual pulses of a train is the well documented longevity of electroporation-induced structural changes, leading to long-lived permeability increase of membranes for drugs, and finally also to constrictively blocking tumour blood vessels.

The lymphatic ring assay: a new *in vitro* model of lymphangiogenesis

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The detection of sentinel lymph node helps physicians to diagnose and select a therapy for patients with diverse types of cancer. This clinical approach is reflecting a lymphatic bed invasion by tumoral cells leading to metastatic dissemination. Study on lympangiogenesis, the recruitment of new lymphatic vessels in tumor is hampered by the lack of reproductible ex vivo or *in vitro* model. In order to overcome the lack of specific lymphatic vessel culture system to study lymphatic vessels, we have developed a new and original *in vitro* model. We successfully transposed the three-dimensional aorta ring assay to a mouse lymphatic thoracic duct assay. Fragments of thoracic duct isolated from mice are embedded into a collagen gel and cultured for two weeks. By immunochemistry and transmission electron microscopy, we characterized the outgrowing cells as being lymphatic cells that organize into microvessels containing a lumen. A computer-assisted quantification system has been developed to quantify lymphatic cell outgrowth. Lymphatic cell spreading is stimulated by well-known lymphangiogenic activators such as VEGF-C and PDGB-BB and is inhibited by sVEGF-R3. We are currently investigating the implication of proteases or their inhibitors in the outgrowth of lymphatic vessels by using synthetic inhibitors and mice deficient for one gene or the other. In conclusion, we have set up a new three-dimensional reproducible model of lymphatic cells. This model will permit to test activators and inhibitors of lympangiogenesis as well as unravelling molecular mechanisms of lymphangiogenesis by using transgenic KO mice.

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A glioblastoma-specific kinase expression profile

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Specific kinase inhibition induces clinically relevant responses in kinase driven human cancers. The best kinases to target by small molecular inhibitors are often unknown. We address the issue of the best treatment targets for glioblastoma in relation to other human malignancies, such as lung, breast, colon, renal and prostate cancer, based on differential gene expression compared to normal tissues. For this purpose, we retrieved array expression data from 34 publicly available datasets of a wide variety of tumor types, including two glioma datasets, and extracted the differential gene expression data for the kinase gene family consisting of 518 protein kinases and 33 lipid kinases. Kinases were ordered based on levels and frequencies of overexpression in the patient population. The upper 5% of percentile fold changes was considered to be substantial overexpression and expression in more than 20% of the patient population was considered as frequent overexpression. In both glioma datasets, 9 kinases appeared to be substantially and frequently overexpressed, MAPK7, DDR2, EGFR, WEE1, TTK, AURKA, CDC2, MELK and BUB1. Two of these 9 selected kinases, EGFR and AURKA, have been described previously as overexpressed in human glioblastoma samples, confirming the plausibility of the glioblastoma-specific kinase profile. Moreover, selective inhibition of EGFR has shown response in a subgroup of glioma patients. We also confirmed overexpression of the 9 kinase genes with quantitative RT-PCR in samples of glioblastoma and glioma cell lines compared to normal brain from our institution's Brain Tumor Bank. Four of the 9 kinases were exclusively overexpressed in glioblastoma, MAPK7, DDR2, EGFR and WEE1, whereas MELK, TTK, AURKA, CDC2 and BUB1 were frequently overexpressed in various cancer types. The four glioblastoma specific overexpressed kinases are putative treatment targets because these were overexpressed early in gliomagenesis and therefore, may be causal.

The use of immuno-nanoparticles for impairement of intracellular proteolytic activity in invasive breast tumor cells

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Tumour associated proteases represent promising targets for anti-tumour therapy. However, the delivery of anti-tumour agents, in particular protein molecules, to tumour proteases is rather difficult without using delivery systems, such as polymeric nanoparticles. Specific delivery of drug-incorporated nanoparticles can be achieved via active targeting by introducing ligands that are recognised uniquely by receptors or certain other proteins expressed on tumour cells. Nanoparticle system is especially efficient for targeting of intracellular lysosomal proteins, including cathepsins, which are known to be involved in processes of tumour progression.

To inactivate lysosomal cysteine protease cathepsin B in tumour cells, we developed poly(lactide-co-glycolide) PLGA nanoparticles, incorporating active cysteine protease inhibitor cystatin and labelled with monoclonal antibody, specific for cytokeratins expressed on the surface of breast epithelial tumour cells. First, cystatin was incorporated into PLGA polymeric matrix and then, the antibody was adsorbed onto the cystatin loaded nanoparticles. The new delivery system was tested on co-cultures of invasive breast epithelial MCF-10A neoT cells, enterocytic Caco-2 cells or differentiated monocyte/macrophage U-937 cells. By using fluorescent microscopy and flow cytometry we showed that the antibody labeled cystatin-loaded nanoparticles solely bound to MCF-10A neoT cells. Moreover, they were rapidly internalized and reduced proteolytical activity of cathepsin B.

Our results show that our new nanoparticulate delivery system enables active targeting of breast cancer tumour cells and efficient inhibition of tumour associated proteolytic activity.

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Immunohistochemistry in oncology: a tool for diagnosis, prognosis and treatment

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Immunohistochemistry enables the visualization of specific antigens tissue distribution. It localizes protein targets of interest by applying specific monoclonal or polyclonal antibodies to tissue surfaces in a process called antibody incubation. This technique was developed since the 1940's, when Coons and colleagues from Harvard Medical School described the identification of tissue antigens by using a direct fluorescence protocol. Detection of reliable fluorescent signals was initiated by pharmaceutical chemists, such as Riggs, in 1961, who replaced the toxic and unstable compound isocyanate by a more stable dye, fluorescein isothiocyanate (FITC). The first applications of this method were bacteriology and the diagnosis of autoimmune diseases. The availability of numerous specific monoclonal antibodies in the 1970's provided major advances in pathology, internal medicine and oncology. Immunohistoenzymology coupled with transmission microscopy (vs immunofluorescence), is the elective method for many surgical pathologists since it makes it possible to visualise at once signals and adjacent structures.

In the field of oncology, histological markers (which are not always correlated to seric markers) allowed major advances in basic research and clinical science: for example estrogen receptors detection on histological slides in breast cancer is a validated routine tool for therapy. In fundamental research, visualisation of growth factors, cytokines, apoptosis inhibitors, oncogenic proteins, hypoxia markers, endothelial cells and other cell types involved in angiogenesis, combined with various methods, such as those derived from molecular biology is a very valuable tool for the understanding of cancerogenesis mechanisms.

However, interpretation of immunohistochemistry has to take into account the pitfalls of this method: poor specificity of some antibodies, discrepancies between hospitals often related to the heterogeneity of fixation procedures and, during many years, the drawback of an exclusively morphological approach. Early adepts of quantification of these signals spent longs hours counting manually histological structures such as blood vessels, with a poor intra-observer and inter-observer reproducibility.

Since 2004, quantification of stained signals is performed at Gustave Roussy Institute in Villejuif with a system combining a dedicated slide scanner and a computer-assisted image analysis: PIXCYT is a software package designed by the Groupe Régional d'Etudes sur le Cancer, Centre François Baclesse, Caen) and represents a revolutionary approach of markers interpretation on histological slides for experimental pathology applications, such as the count of immunostained microvessels, nuclear or cytoplasmic stainings in tumor cells.

Automation with a dedicated script implemented to PIXCYT proved to be very helpful for measurement of lung metastases surface in a model of B16F10 tumors xenografted in nude mice. This software is also currently used to assess tumor microvessel density

after antiangiogenic strategies, such as a combined treatment with radioiodide therapy and adenovirus-mediated delivery of an angiogenic inhibitor, Canstatin, stimulating direct tumor cell apoptosis in MDA-MB231 breast tumor model. Anti-proliferative effect of restoration of the BRCA1 gene by adenovirus-mediated transfer in lung carcinoma xenografts is assessed by the dramatic reduction of the number of nuclei marked by Ki67 in treated animals vs controls.

Pathological findings coupled to immunohistochemistry represent a significant achievement in the evaluation of many anti-cancer therapies : gene transfer, electrotransfer of recombinant plasmids, immunotherapies administered alone or in combination with chemo- and radiotherapy and contribute to diagnostic and treatment strategies in clinical oncology.

Reproducibility of Her-/2neu analysis: analytical and clinical implications

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The clinical interest in HER-2/neu is related to Trastuzumab, a drug used to treat patients with invasive breast carcinoma overexpressing the HER-2/neu protein. As suggested by the Food and Drug Administration (FDA) guidelines, the eligibility of breast cancer patients for treatment with Trastuzumab is based on the accuracy of the assessment of the HER-2/neu status of the tumor (Lidgren, 2007). However, the specificity of Her-2/neu immunohistochemical analysis remains still unsatisfactory. Several attempts have been done to improve the performances of the assay.

Recently, the using of higher cut-off for the percentage of positive cells has been proposed with interesting results (Hameed, 2007). However, the quality of the assessment of the HER-2/neu status has been stressed as a crucial point to correctly identify patients who may benefit from Trastuzumab.

The results of several Quality Control (QC) programs for IHC determination of HER-2/neu have been published and among them, the programs of UK-NEQAS (United Kingdom National External Quality Assessment Service) are perhaps the longest lasting and involving the largest number of laboratories. In particular, the UK-NEQAS program focused its attention on pre-analytical aspects of the IHC assay. Conversely, the INQAT Group (Italian Network for Quality Assessment of Tumour Biomarkers) developed a QC program concerning the analytical phase of HER-2/neu determination in Italy. The INQAT program investigated inter-intra laboratory reproducibility of the optical microscope analysis on a set of IHC slides.

The combined analysis of these two QC programs permit to make interesting comments: the need for each laboratory to have an overall picture of the quality of the whole process of the biomarker determination and a better understanding of the possible reasons of its questionable performance during the different phases of the biomarker determination; the need to implement quality control programs focused on the whole process of biomarker determination moving from standardization of reagents and techniques to reproducibility of biomarker analysis at optical microscope. These items are relevant in clinical practice where the treatment choice is based on categories defined by this assay, suggesting the need of adopting educational programs and/or new reference materials to improve the assay performance.

The role of integrative genomics/proteomics in the detection and treatment of metastatic breast cancer

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The sequencing of human genome has provided the first look at all genes. The next steps however require powerful high-throughput techniques. Medicine is entering a new era of global approaches in scientific techniques. These approaches, named –omics represents a global, systematic and comprehensive way for identifying and describing the processes and pathways involved in normal as well as abnormal states. Omics are global methodologies to characterization of all, or most members belonging to a certain family of molecules, in one single analysis. It is characterized by high-throughput or large-scale experimental methodologies combined with statistical and computational analyses of the results. The fundamental strategy of omics approach is aimed to expand the scope of biological investigation from studying single entities (genes/proteins) to studying all possible parameters collectively in a coordinated and systematic fashion. In general, these studies have emphasized the potential of technology for biomarker discovery, as well as for addressing the issue of cancer heterogeneity, new classification, early diagnosis and new therapeutic targets. Two important omics approaches – transcriptomics and proteomics have become a powerful tools for deciphering the complex signaling pathways in tumor biology. Cancer proteomics have already identified proteins of potential clinical interest. There are two major foci for the use of omics in oncology. The first is to discover new molecular markers for the profiling of tumors and the second is to decipher pathways that lead to cancer cell development. Such data are beginning to provide a knowledge base for the identification of possible therapies for the disease and subsequent development of innovative strategies. Unquestionably, cancer is the disease that has received most attention and the generated data address mainly aspects of clinical oncology. However, functional genomic methods are new and highly dependent on bioinformatics and statistics, which require additional improvement, since the meaning and importance of the results derived from such large-scale experiments are not widely appreciated. The goal of my presentation is to discuss the possible applications of these technologies in breast cancer, since they already have great impact on the management of cancer: in the discovery of new drug targets, development of new molecular tools for diagnosis and prognosis as well as in improving treatments by taking into account the molecular characteristics of a given tumor. Breast cancer proteomics have already identified proteins of potential clinical interest. Practical consequences for treatment derived from a better understanding of the molecular basis of cancer cell growth are now emerging, as evidenced by the development of therapeutic strategies. One of the most exciting challenges today is to move cancer omics from the "benchside" to the "bedside".

Numerical modeling in electroporation-based biomedical applications

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In general, cell membrane is impermeable for larger molecules; however, the application of adequately high electric pulses to cells, either in suspension or tissue, causes the electroporation of cell membrane, increasing its permeability and making it possible for larger molecules, such as drug molecules or DNA, to enter the cell. Electropermeabilization of the cell membrane, when used properly, can be used on different types of cells, does not affect cell survival and does not disrupt cell functions. Therefore, it can be used for wide range of applications, the most advanced being electrochemotherapy, gene electro transfer and transdermal drug delivery.

Analytical methods used to be the only option for theoretic studying of the effects of electromagnetic fields on cells and tissues. They are rather complicated and are only feasible for use on problems where the geometry, material properties and boundary conditions can be described in a defined coordinate system (like Cartesian, cylindrical, polar...). In the last decades, however, increased computer capabilities and speed led to the development of powerful commercial numerical methods software packages based on finite elements method that can easily be used to model intricate biological systems. The principle of the finite elements method is the discretization of the geometry into smaller elements where the quantity to be determined is approximated with a function or is assumed to be constant throughout the element. Tissue inhomogeneities and anisotropies can also be modeled and different excitations and boundary conditions can be applied easily. When constructed, the model consists of a system of equations that can be solved by an appropriate numerical method.

Various parameters (current and voltage amplitude, field strength and orientation, electrode geometries...) can thus be evaluated by means of numerical modeling. In such models, the excitations can be changed easily, being that it only involves changing the boundary conditions on the same model. Once built, a good model in agreement with experimental results can be a powerful tool and can offer useful insight into the understanding of biological processes modeled and helps us to plan future *in vivo* experiments. Also, experimenting with such models is easier and sometimes the only possible or ethically acceptable alternative to experimenting on real biological systems. Modeling of electric current and electric field distribution during cell and tissue electroporation proved to be useful for describing different aspects of the process, allowing us to design electrode geometries and electroporation protocols as a part of treatment planning.

Effect of the tumour microenvironment on targeted therapies

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Radioimmunotherapy (RIT), the use of systemically administered antibodies against tumour-associated antigens labeled with therapeutic radionuclides, has the ability to selectively target solid tumours, increasing the specificity of treatment and reducing systemic toxicity. RIT has produced responses in pre-clinical and clinical studies, but the tumours have a tendency to regrow. This can be explained by the heterogeneous pathophysiology of solid tumours, which determines the distribution of administered radiolabeled antibody *in vivo*, and produces a regional response to therapy.

In order to understand the patterns of antibody distribution within the tumour mass we have investigated, quantitatively, the effect of the tumour microenvironment on the uptake and distribution of radiolabeled anti-CEA antibody in colorectal tumours, and its subsequent effect on therapy. The heterogeneity of localization was investigated in both subcutaneous xenografts and the more clinically relevant orthotopic liver metastasis model. For high resolution information the antibody was also fluorescently labeled, and digital multifluorescence microscopy was used to study the distribution of antibody over time in relation to a range of tumour pathophysiology biomarkers (eg blood vessel distribution and perfusion, hypoxia).

Both radio- and fluorescently-labeled antibody studies demonstrated the key role of the tumour microenvironment in determining regional distribution of antibody, and the ultimate efficacy of RIT. Future work will incorporate other biomarkers, eg for radiosensitivity, proliferation rates and DNA repair, in order to provide a fuller picture of factors affecting tumour response. This work illustrates the importance of studying intratumour antibody distribution by multiple microscopy systems, and demonstrates the necessity for a combined therapeutic approach to effectively treat the whole tumour mass.

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Development of three-dimensional (3D) all-human, *in vitro* models for study of the biology of primary and metastatic brain tumours

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Animal models for the study of local invasive behaviour and metastasis of brain tumour poorly reflect the situation in human counterpart neoplasms. Moreover, 3D *in vitro* invasion models for invasion generally utilise non-human (rat/mouse) glioma cells and/or rat brain/chick heart fragments as a "target" for invasion. Similarly, *in vitro* models of the B-BB generally utilise porcine or murine brain endothelium and rat astrocytes. In addition, these models are grown in foetal calf serum supplemented conditions which modify growth rates and cell adhesive properties. Our aims were, therefore, to develop 3D *in vitro* models from human brain-derived cells for the study of: a) brain cell:glioma cell interaction and invasive behaviour and b) passage of metastatic somatic cancer cells across the cellular blood-brain barrier (B-BB).

For the invasion model we have used human glioma biopsy-derived cells and temporal lobe epilepsy resection (TLER) brain maintained as spheroids through an adaptation of the hanging drop method and juxtaposed in 3D confrontation culture under human serum (HS) supplementation. For the B-BB model we have used astrocyte-rich human cultures from TELR brain in combination with human cerebral microvascular endothelial cells (CMECs) immortalised with hTERT/SV40LargeT, under HS supplementation. All cells have been characterised with appropriate immuno markers using flow cytometry and immunocytochemistry while growth curves and adhesion properties have been established.

Growth curves, spheroid growth rates, scanning electron microscopy, confocal microscopy, antigenic expression, adhesive properties and growth on Transwells have been established for each model and for the individual component cells.

We are currently assessing the models using a combination of live cell imaging, WetSEM, TIRF microscopy and confocal microscopy in studies where putative inhibitors of invasion/metastasis are under investigation.

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Addressing the angiogenic switch by studying tumor-host interactions in an animal model of glioblastoma multiforme

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In solid tumor growth, a crucial turning point is the transition from the avascular to the vascular phase, leading to a more aggressive tumor growth. Anti-angiogenesis therapy is a powerful approach aimed at blocking/reducing tumor growth. The identification of novel angiogenesis-specific proteins is crucial for the development of new anti-angiogenic therapies, and such proteins are potential new biomarkers in cancer. A xenograft animal model of human glioblastoma multiforme (GBM) has been developed in our lab. The model includes 4 generations of animals with the GBM tumor phenotype varying from very invasive, non-angiogenic in the first to less invasive, fully angiogenic in the last generation. The objective of our study was to explain the molecular background of the phenotypic change – the angiogenic switch – at the protein level. For this purpose we used quantitative proteomics and bioinformatics on a membrane enriched fractions of the tumors. In four generations of tumors, we identified 1170 non-redundant, host or tumor specific proteins (C.I. ≥ 95%). Bioinformatic analyses over four tumor phenotypes revealed distinct groups of proteins with specific expression profiles that may be involved in the angiogenic switch and tumor angiogenesis. It appears from these profiles that the regulated host proteins are strongly involved in the angiogenic switch, whereas the tumor proteins interact and/or support the process of angiogenesis. The expression of particular proteins representing these profiles is being validated by non-proteomic methods (e.g. GBM tissue arrays) as well as by functional assays using a novel GFP-expressing NOD/Scid mouse model, recently developed in our laboratory. Functional studies using this model will allow us to confirm the tumor/host interactions involved in the angiogenic switch.

Photosensitizer-loaded nanoparticles for improving the efficacy and selectivity of photodynamic therapy of tumours

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Photodynamic therapy (PDT) is an innovative effective and minimally invasive therapy against cancer and several non-oncological diseases. PDT is based on the concept that some photosensitising molecules or photosensitisers (PSs) localise and are retained preferentially in malignant tissues. Following activation with appropriate wavelengths of red light, the PS generates reactive oxygen species (ROS) which cause oxidative damage to various cellular components therefore leading to cell death. PDT induced cell death occur through necrosis or apoptosis; the preferred mechanism is determined by the physico-chemical properties of the PS, the dose of PS and light, the characteristics of the tumour cells. In addition to the direct damage to tumour cells, PDT causes alterations of the tumour vascularization, with the consequent deprivation of oxygen and nutrients for cancer cells, and stimulation of inflammatory and immune responses against the tumour. The relative contribution of these pathways to the therapeutic effect depends on the distribution of PS in the tumour compartments which is determined by its pharmacokinetic properties and can be modulated by the PS administration and light illumination interval. The first PS approved for clinical PDT is Photofrin® (hematoporphyrin derivative) whose performances are far from optimal but is still the most largely used PS. More recently, Foscan® (tetra-hydroxyphenyl chlorin, mTHPC) has been introduced in the clinical practice for the palliative treatment of head and neck advanced carcinomas. However for widening the use of PDT to the treatment of various types of early stage localised tumours, it appears of outmost importance to improve the efficacy and selectivity of PDT by increasing the selectivity of the PS localisation. The entrapment of the PSs in nanosystems of different nature is being investigated as a strategy to fully exploit the advantages that PDT offers over conventional therapies. Therefore, liposomes and several biodegradable polymeric nanoparticles have been used for the delivery of various types of PSs to tumour cells. In addition, nanoparticles of non-biodegradable polymers have also been considered in spite of some concerns about their safety. In all cases, most of the investigations with the PS-loaded nanoparticles have been carried with tumour cell lines *in vitro* while only a few *in vivo* studies are reported.

The more recent and significant findings on the PS delivery with nanoparticles will be discussed with the aim to critically evaluate the potential advantages offered by the emerging nanomedicine for improving the efficacy and selectivity of PDT.

Genetic predisposition on breast cancer – clinical perspectives

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Breast cancer is a multi-factorial disease with a significant genetic predisposition. Despite significant advances in the identification of 'high penetrant' genes and their routine clinical application to individuals with a 'strong' family history, many genetic variants making a mild to moderate contribution to risk remain uncharacterised. It is well recognised that a substantial contribution to the risk of many common multi-factorial genes is from commonly occurring genetic variants which interact with each other or with environmental factors. International collaborations such as the 'Breast Cancer Association Consortium' (BCAC) are underway to pool efforts and resources in an attempt to unravel the tangled web of genetic, gene-gene and gene-environment interactions that contribute to disease predisposition.

This talk will discuss the evolution of a large population association genetic epidemiology project in Sheffield (Sheffield Breast Cancer Study), which has now become part of the Breast Cancer Association Consortium (BCAC). The key results including the identification of several gene polymorphisms as 'low to moderate' susceptibility genes and the relevance of these findings to the breast cancer clinician will be discussed. The limitations to this approach include the risk of false positive results and the difficulty in immediate application to clinical practice. However, the ability to improve risk stratification and the increased insight into the molecular pathways that may yield further targets for anti-cancer treatment is promising. Close collaboration between clinicians and scientists are vital to the formulation of specific clinical questions, design and conduct of such studies and the translation of findings to the bedside.

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Interaction of human adult mesenchymal stem cells and malignant gliomas

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We evaluated the role of adult human mesenchymal stem cells (hMSC) as potential contributors to tumor growth and neoangiogenesis in malignant gliomas. First, we could isolate hMSC from human glioblastomas demonstrating the potential relevance of this cell type. Experimentally, hMSC showed to be actively integrated into tumor vessels of human gliomas grown in immune-deficient rats (RH-rnu-rnu). The use of the endothelial specific Tie2 promoter/enhancer in engineered hMSC demonstrated the selective activation of a reporter gene in the context of hMSC differentiation while being recruited into the neoangiogenetic vasculature of the glioma. *In vitro*, hMSC were found to be readily incorporated into growing endothelial tubes in tube formation assays using endothelial cells derived from human malignant gliomas (GB-EC). By the use of intense FACS analysis, we found the cells isolated by our protocol to express typical surface markers of hMSCs including CXCR4, the receptor of SDF-1. Differentiation assays showed that the cells, although derived from malignant glioma tissue, exhibit differentiation potential into mesenchymal cell lines, producing fat or bone. Directed invasion of hMSC *in vitro* was increased in presence of a SDF-1 gradient. Malignant glioma tissue showed to overexpress SDF-1, compared to normal brain tissue, obtained from patients with epileptic surgery. Serum-SDF-1 levels were elevated in patients with gliomas compared to healthy volunteers.

Glioma tissue contains a progenitor cell type, resembling mesenchymal stem cell (hMSC) characteristics, which contributes to the tumor`s neoangiogenesis. This indicates active recruitment of these cells from the circulation by gliomas. As a possible mechanism for recruitment of hMSCs from bone marrow we identified the SDF-1 / CXCR4 system.

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Kallikrein-related peptidases: novel therapeutic target molecules

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The novel tumor-associated biomarkers, the kallikrein-related peptidases (tissue kallikreins, KLK) KLK4-15 have the potential to serve as novel prognostic and even predictive markers for patients afflicted with solid malignant tumors. Tissue kallikreins are a subgroup of the serine protease enzyme family. Until the mid of the 1990s, it was thought that the human tissue kallikrein gene family contained only three members: (1) tissue kallikrein [KLK1], (2) glandular kallikrein-1 [KLK2], and (3) prostate-specific antigen [PSA/KLK3]. In the past years, the expansion of the human tissue kallikrein gene family from 3 to now 15 members, all of which co-localize to chromosome 19q13.3-13.4 was reported. With the identification and characterization of all members of the tissue kallikrein gene family, accumulating reports have indicated that in addition to the established tissue kallikreins KLK1, KLK2, and KLK3, the novel tissue kallikreins KLK4 to KLK15 might also be related to hormonally regulated malignancies such as that of the prostate, testis, breast, and ovary.

Growing evidence suggests that certain KLKs are involved in cancer progression; several may even serve as novel cancer biomarkers for tumor staging and prediction of therapy response, associated with the course of disease of cancer patients. While KLKs are expressed in a variety of normal tissues, such as prostate, ovary, breast, testis, the central nervous system, and skin, their expression or downregulation in tumor tissues have only partially been revealed by immunohistochemical approaches although expression of KLKs have been investigated in malignant tissue extracts by PCR (mRNA) or ELISA (protein). Therefore, we have generated/selected a panel of highly specific antibodies to various KLKs which allow localization and assessment of protein expression in tumor tissues.

Recent advances in our laboratory allowed recombinant protein production of all of the 15 KLKs in bacteria in their pro-forms and conversion into their enzymatically active forms. For some of the KLKs, protein crystals were obtained (KLK4, KLK5, KLK6, KLK7) and the structures of these kallikrein-related peptidases solved by crystallography. Furthermore, extended substrate specificity profiling for the nonprime side and further enzyme kinetic studies have been performed. The shape and polarity of the specificity pockets of the investigated KLKs explain very well the substrate preferences of KLK4, 5, 6, and 7. KLK4 and KLK5 exhibit a trypsin-like specificity with a strong preference for P1-Arg, while KLK6 is less stringent for arginine in P1, but prefers this residue also in P2 whereas KLK7 displays a unique chymotrypsin-like specificity for tyrosine both in P1 and P2 position. Comparison of structure and substrate specificity may support the discovery of natural substrates as well as the design of efficient inhibitory compounds for targeting these KLKs *in vivo* as a novel approach of tumor therapy.

Pro-carcinogenic effects of non-genotoxic chemical carcinogens

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Chemical carcinogens play a more a major role in the causation of human cancer. These agents are generally readily detectable in appropriate short term assays. However, an increasing number of compounds has been found which have no detectable genotoxic activity but nevertheless produce tumors in animal experiments and, though rarely, also in humans. These compounds include important drug candidates and many other chemicals. Carcinogenic effects of non-genotoxic carcinogens are therefore a considerable problem as to accessing their impact on human health, and elucidation of mechanisms of carcinogenic effects is urgently needed. These mechanisms include promotion of initiated cells, which arise spontaneously or are induced through indirect effects of the compounds (e.g. production of reactive oxygen species, ROS). In addition, evidence has been provided by several groups that epigenetic mechanisms such as modification of the methylation status of the DNA can be important for initiation at least in specific cases. For many non-genotoxic carcinogens activation of nuclear receptors in epithelial cells has been identified as a key mechanism which may result in growth of preneoplastic lesions and tumor formation. However, non-parenchymal cells seem to contribute essential factors such as ROS and growth stimulatory cytokines. Cytotoxic carcinogens may induce alterations of multiple pathways in both epithelial and non-parenchymal cells reflecting acute inflammation which favour survival and growth of early initiated cells. In conclusion, expanded mechanistic insight may eventually provide additional information helpful for risk assessment of carcinogenic compounds.

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Vascular disrupting action of electroporation and electrochemotherapy

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The predominant underlying mechanism of electrochemotherapy is electroporation of cells in tissues, which facilitates access of poorly or nonpermeant molecules, including cytotoxic drugs such as bleomycin and cisplatin, into the cells. In addition to electroporation of tumor cells, electric pulses were found to modify blood flow in normal tissues and in tumors. This suggests that in addition to direct cytotoxic effect of electrochemotherapy, application of electric pulses to the tumors have also indirect cytotoxic effect by modification of tumor blood flow and vascular disrupting effect.

The phenomenon was well documented by different techniques, demonstrating reduced blood flow and oxygenation of the tumors after application of electric pulses to the tumors. The degree of tumor blood flow reduction is dependent on the amplitude of the applied electric pulses and the number of them. Reduced blood flow is observed immediately after application of electric pulses and starts to restore within 2-3 h with almost complete restoration of tumor blood flow within 24 h. This phenomenon is most probably due to increased vascular permeability inducing leakage of plasma and occlusion of vascular flow due to increased interstitial pressure. On adherent endothelial monolayer it was demonstrated that electroporation induces disruption of actin filaments and microtubule network cytoskeleton as well as loss of cell-cell contact junctions, which altogether increased endothelial cell monolayer permeability.

Application of electric pulses to the tumors permeabilizes also stromal cells, including endothelial cell lining in the vessels. Based on mathematical model it was calculated that endothelial cells are exposed to even higher electric field (~40%) than the tumor cells. Therefore, in electrochemotherapy, where endothelial cells are in direct contact with the chemotherapeutic drugs, they undergo cell death due to the increased drug uptake. Histological analysis has demonstrated endothelial cell swelling and apoptosis already after 7-8 h after electrochemotherapy. This leads to abrogation of tumor blood flow for prolonged time, inducing a cascade of tumor cell death surrounding the disrupted blood vessels, and central tumor necrosis. Physiological measurements have confirmed this, demonstrating prolonged disruption of tumor blood flow, either by electrochemotherapy with bleomycin or cisplatin. Furthermore, these changes correlated with reduced oxygenation of the tumors, and increase in hypoxic regions in the tumors.

Therefore, electrochemotherapy has besides its direct cytotoxic effect on tumor cells also vascular disrupting action by causing a rapid shutdown of tumor blood flow, leading to reduced tumor oxygenation, increased tumor hypoxia and extensive tumor necrosis.

The role of the immune proteasomes in the anticancer defence

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The immune proteasomes play an important role in the mammalian and human T cell immunity. Producing the antigenic epitops from proteins, they participate in the negative selection of thymocytes in thymus and in the activation of naive T-CD8+ lymphocytes in the secondary lymphoid organs. In any tissue or organ except the brain, the immune proteasomes switch on the signal for the elimination of abnormal cells which express altered (mutant) or foreign (viral) genes. In such cells the immune proteasomes produce or start the production of antigenic epitops from mutant or viral proteins. An antigenic epitop binds molecules of the major histocompatibility complex class I to be transported to the cell surface. This structure represents the signal for cytotoxic T lymphocytes to identify abnormal cells and induce their apoptosis. On the one hand, if the immune system is defective, numerous mutations in somatic cells can cause the malignant transformation. On the other hand, can changes in the immune proteasome pool in a cell be related to its malignant transformation? To answer this question, we investigated the peculiarities of the immune proteasome pool in the developed ascite carcinoma Krebs-II on the 7th day after transplanting to Balb/c mice. Besides, we investigated the changes in the immune proteasome pools in carcinosarcoma Walker 256 on the 7th and 14th days after transplanting to WAG and Brattleboro rats. The latter are the unique vasopressin deficient rats in which malignant tumor Walker 256 slowly grows up to the 15-18th day, then decreases and disappears up to the 30th day. In all experiments, we compared the changes in the immune proteasome pool with those of the total proteasome pool and 26S-proteasome pool by Western blotting.

We showed that the level of the immune proteasomes in all developing tumors dra-matically falls. Besides, proteasome pool is deficient in the immune subtypes in Walker 256 cells in Brattleboro rats on 7th day, and on the contrary, the level of the immune proteasomes significantly increases in this tumor up to 14th day. These data confirm that the reason of the successful development of a malignant tumor may be related to the partial or complete exclusion of the immune proteasomes from its cells. That allows these cells to avoid the immune surveillance. In contrast to the growing tumor, the in-crease of the immune proteasome level in the disappearing tumor may open its cells to the immune system and it can be the reason of the tumor regression. It is interesting that the decrease of the immune proteasome level in the growing tumors is accompanied by the increase of the 26S-proteasome pool and of the total proteasome pool levels, and on the contrary, the increase of the immune proteasome level in the regressing tumor is ac-companied by the decrease of the total proteasome pool level. Moreover, in the cells of regressing tumor in Brattleboro rats, 26S-proteasomes disappear at all up to the 14th day. It is clear that increased pool of 26S proteasomes is necessary for exact cell cycle regulation of actively proliferating tumor cells and its elimination can be the second reason of the tumor regression. Thus, the peculiarities of the proteasome expression in tumor cells define their future fate.

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Cysteine cathepsins in tumors and their microenvironment

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Cysteine cathepsins, which are endopeptidases belonging to the papain family (C1) of the CA clan of cysteine proteases, are highly upregulated in a wide variety of cancers by mechanisms ranging from gene amplification to post-transcriptional modification. There are eleven human cysteine cathepsins that function in normal tissues primarily within intracellular lysosomes. There are, however, intracellular non-lysosomal functions and extracellular functions for some cysteine cathepsins. An example of the former is the participation of cysteine cathepsins in apoptosis and of the latter is cathepsin K, which participates in bone degradation in the resorptive pits formed between osteoclasts and the underlying bone. Extracellular roles for cysteine cathepsins also occur in tumors due to the secretion of both inactive and active forms of these proteases. Secreted cysteine cathepsins associate with binding partners in membrane microdomains on the tumor cell surface where proteases of other classes are also found. For example, cathepsin B binds to S100A10, a subunit of the annexin II heterotetramer (AIIt); plasmin(ogen) and tissue plasminogen activator (tPA) bind to AIIt; urokinase plasminogen activator (uPA) binds to its receptor uPAR in association with beta1 integrin; matrix metalloproteinase (MMP)-2 binds to alphaVbeta3 integrin; and MMP-14 or membrane type 1-MMP is a transmembrane protein. Similar localizations of these proteases have been observed on endothelial cells, consistent with cell-surface proteolysis facilitating the angiogenesis required for growth of tumors. The juxtaposition on the cell surface of a cysteine cathepsin, serine proteases of the plasminogen cascade and MMPs has been shown to produce a proteolytic cascade, resulting in activation of growth factors and focal proteolysis of basement membrane. Direct proof that cysteine cathepsins play causal roles in tumor growth, migration, invasion, angiogenesis and metastasis has been shown by downregulating or ablating the expression of individual cysteine cathepsins in tumor cells and in transgenic mouse models of human cancer. The latter studies have identified roles for cysteine cathepsins in tumor-associated cells that enhance malignant progression including endothelial cells and also fibroblasts and inflammatory cells (macrophages). On the other hand, studies in transgenic mice have also identified protective roles for cysteine cathepsins in tumor-associated cells such as mast cells. The specific cysteine cathepsins that enhance or reduce malignant progression differ with tumor type. Clinically, the levels, activities and localization of cysteine cathepsins and their endogenous inhibitors have been shown to be of diagnostic and prognostic value. Given that it is active cysteine cathepsins that play causal roles in malignant progression, it is intriguing that one of the endogenous cysteine cathepsin inhibitors, i.e., cystatin M, seems to be a tumor suppressor in breast cancer and is epigenetically silenced by methylation of its promoter. To effectively target these enzymes for anti-cancer therapies, we need a more complete understanding of how cysteine cathepsins function in neoplastic

progression, e.g., to identify the cysteine cathepsins expressed in a given tumor type and stage as well as in the various constituents of the tumor microenvironment at the level of transcripts, proteins and active enzymes; to determine the relevant substrates cleaved by those cysteine cathepsins; to delineate the respective contribution of intracellular and pericellular proteolysis to malignant progression; and to determine how the cysteine cathepsins interact within tumor-associated proteolytic networks.

Cysteine cathepsins and stefins in head and neck cancer: an update of clinical studies

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To distinguish biologically more aggressive and less aggressive head and neck carcinomas within each traditional risk category, numerous new prognostic factors were evaluated on genetic, mRNA or protein levels. The recent implementation of microarray technology for biological profiling of tumors confirmed the multifactorial origin of carcinogenesis. Among the factors that promote tumor growth and invasion, several protease systems, involved in proteolytic degradation of extracellular matrix components, were studied, including papain-like lysosomal cysteine proteases, such as cathepsins B and L, and their physiological inhibitors cystatins, such as cystatin C, stefin A and stefin B.

Alterations in the expression of cystatins at mRNA and protein levels, as well as in trafficking and activity were reported in malignant and stromal cells compared to normal counterparts in a variety of tumors. These alterations were found to correlate with the malignant phenotype of the tumor *in vitro* and *in vivo*. In a clinical setting, the observed correlation with treatment efficacy and patient survival was suggestive of their predictive and/or prognostic role in some cancer types.

In our previous studies on operable head and neck carcinoma, high levels of cysteine protease inhibitors in tumor tissue homogenates appeared as prognostically advantageous. Specifically, in two independent, but smaller prospective cohorts of patients, this relationship was confirmed for stefin A, stefin B and for cystatin C. To the contrary, cysteine cathepsins failed to show any potential to predict survival in the same group of patients. Data on the immunohistochemically determined expression profile of cysteine cathepsins is scanty and available only for oral cavity tumors, but not also for pharyngeal or laryngeal carcinomas; the same finding was also arrived at in the case of their possible prognostic significance. So far, to the best of our knowledge, stefins have not been subjected to immunohistochemical evaluation in any of the studies conducted on head and neck carcinomas.

At the conference, the results from recent studies analysing the predictive and prognostic role of cysteine cathepsins B and L and stefins A and B in operable and inoperable squamous cell carcinoma of the head and neck will be presented and discussed.

Electrochemotherapy in veterinary oncology

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Electropermeabilization is a method that uses electric field pulses to induce an electrically mediated reorganization of the plasma membrane of cells. Electrochemotherapy combines local or systemic administration of chemotherapeutic drugs bleomycin or cisplatin that have poor membrane permeability, with electropermeabilization, by direct application of electric pulses to the tumors. Preclinical studies have demonstrated excellent antitumor effectiveness of electrochemotherapy on different animal models and various tumor types, minimal toxicity and safety of the procedure. Based on results of preclinical studies, clinical studies were conducted in human patients and demonstrated pronounced antitumor effectiveness of electrochemotherapy with 80 to 85% objective responses of the treated cutaneous and subcutaneous tumors. Clinical studies in veterinary oncology have demonstrated that electrochemotherapy is very effective in treatment of cutaneous and subcutaneous tumors of different histologies in cats, dogs and horses. Results of these studies have also demonstrated ~ 80% long lasting objective responses of the electrochemotherapy treated tumors. Predominantly primary tumors of different histologies were treated.
Electrochemotherapy in veterinary oncology has premises for the future to be highly effective treatment, which could be used as a curative treatment for primary or recurrent solitary or multiple cutaneous and subcutaneous tumors of different histologies or as an adjuvant treatment to surgery.

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Time dependence of electric field effects on cell membranes

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Electropulsation is one of the non-viral methods successfully used to transfer drugs and genes into living cells *in vitro* as *in vivo*. This approach shows promise in field of gene and cellular therapies. This presentation first describes on the factors controlling electropermeabilization to small molecules (< 4kDa) and then the processes supporting DNA transfer *in vitro*. The description of *in vitro* events brings our attention on the processes occurring before (s), during (ms) and after electropulsation (ms to hours) of DNA and cells. They all appear to be multistep events with well defined kinetics. They cannot be described as just punching holes in a lipid matrix in a two states process. The faster events (may be starting on the ns time scale) appear to be under the control of the external field while slower events are linked to the cell metabolism. Investigating the associated collective molecular reorganization by fast kinetics methods and molecular dynamics simulation will help in their safe developments for the *in vivo* processes and their present and potential clinical applications.

Supports from the Proteus Slovenian French program, the region Midi Pyrénées and the AFM should be acknowledged.

IL-12 gene therapy of murine sarcoma tumors and metastases combined with radiotherapy

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Interleukin-12 (IL-12) has immunomodulatory effects on tumors. Unfortunately it's associated with toxicity when delivered systemically as a recombinant protein. An alternative to recombinant protein therapy is delivery of IL-12 by gene therapy. Especially attractive is IL-12 electrotransfer to skeletal muscle which results in prolonged expression and secretion and therefore systemic distribution of therapeutic protein. Gene therapy can be additionally improved when combined with radiation, as IL-12 may have a radiosensitizing effect.

The aim of our study was to evaluate the antitumor efficacy of intramuscular IL-12 electrotransfer alone or combined with radiation on subcutaneous tumors and induced lung metastases of two murine sarcomas, LPB and SA-1.

Lung metastases and subcutaneous tumors were induced by intravenous and subcutaneous injection of tumor cells, respectively. IL-12 electrotransfer was performed by intramuscular injection of 20 μg of plasmid DNA encoding IL-12 followed by local application of 1 high voltage (600 V/cm, 100 μs, 1Hz) and 4 low voltage (80 V/cm, 100 ms, 1Hz) square-wave electric pulses. Mice bearing subcutaneous tumors were treated with IL-12 electrotransfer 3-times every second day, starting 24h before tumor irradiation (10 Gy). Treatment effectiveness was evaluated by tumor growth delay assay. Mice with induced lung metastases were treated 4-times every second day, starting 1 day before injection of tumor cells. Eight (SA-1 tumor) or 16 (LPB tumor) days after induction of metastases, mice were euthanized, their lungs were excised, fixed and colonies counted. In addition, total IL-12 in serum from the treated mice was determined by ELISA at different times post-treatment. The growth of subcutaneous tumors treated by IL-12 electrotransfer alone was delayed for ~24 days in SA-1 and ~11 days in LPB tumors. Furthermore, 30% of SA-1 tumors responded by complete tumor eradication, which lasted for at least 100 days. In LPB tumors the complete response was observed in 13%. The number of lung metastases was significantly reduced after IL-12 electrotransfer; for ~90 % in SA-1 and ~85 % in LPB tumors. The serum IL-12 levels increased for ~30-times after IL-12 electrotransfer and peaked on 7 day post-treatment. When IL-12 electrotransfer was combined with irradiation, 45% of SA-1 tumors responded with complete eradication. Combined therapy of LPB tumors resulted in 100% of complete responses. Thus, gene therapy with IL-12 showed radiosensitizing effect in both tumors. This study demonstrates that systemic delivery of IL-12 by intramuscular electrotransfer has a pronounced antitumor effect on solid subcutaneous tumors as well as on lung metastases of murine sarcomas. Results of combined therapy indicated on synergistic action of therapies in both LPB and SA-1 tumors.

Melanoma genomics and molecular targeting

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With the invent of DNA microarray technology several attempts have been made recently to identify a melanoma-specific gene signature. Comparison of the major studies published in this area indicated that only a dozen of melanoma genes can be collected, the expression of which is repeatedly found in the literature and the majority is confirmed at protein level and functional data are also available. These genes include those of the transcription factor NOTCH2, WNT5A, proliferation-associated genes TOPO2A and CDC2, membrane receptors FGFR and EphA3, adhesion molecules N-cadherin, β3 integrin and syndecan-4, and the cell surface antigen CD59/protectin, and MIA. We have used three genetically unrelated human melanoma cell lines grown as sc. xenografts in SCID mice to reveal a metastatic melanoma gene signature. To identify relevant genes, we have exploited the manipulation of the host to promote or inhibit spontaneous lung metastasis (newborn-metastatic versus adult-nonmetastatic mice). With the murine stroma it is possible to separate the melanoma- and stromal genes involved in metastatic potential. Expression profiles of primary human melanomas (3 cell lines) were obtained by the 41k Agilent Whole Human Genome Oligo Microarray. The gene set significantly different in all the three cell lines (min. 2 fold difference) was further validated by qPCR using Taqman card of 96 genes (Applied Biosystem). Using this model, we have identified a 832 metastatic melanoma gene signature ($p<0.05$), from which we were able to derive a 39-gene set validated by qPCR. This melanoma metastasis initiating gene signature contained 9 previously reported members including transmembrane heparan sulphate proteoglycan, β3 integrin, AKT and TOPO2A. The largest group of metastasis genes belongs to those involved in the regulation of apoptosis (8) and those involved in developmental regulation (NOTCH1, FOXD3, FZD9, HOXC13). Since AKT is known to be upregulated and functionally active in human melanoma, we have looked further for potential cause of this signaling feature.

EGFR expression of human melanoma is known for decades but was not used as prognosticator or target for therapy. We have used 8 human melanoma cell lines of different genetic origin to study EGFR expression. We have found that all of these cell lines express EGFR at mRNA and protein levels. We have sequenced the tyrosine kinase and the C-terminal domains of EGFR in human melanoma cell lines but beside SNPs at codon 775, 787, 903 and 994 EGFR was of the wild-type. Since none of the anti-EGFR antibodies recognizing the extracellular domain was positive on these human melanoma cell lines, we have sequenced the entire extracellular domain of EGFR in all the 8 melanoma lines using 7 overlapping primer pairs. Wild-type sequences were found in 25% of cell lines while 2/8 cell lines we have detected the EGFRvIII splice variant. We have found complete deletion of the extracellular domain in 25% of cell lines while partial deletion or mutations were found in another 25% of them. The frequency of EGFR extracellular domain mutations was also tested on 28 human skin melanoma samples (none of them belonging to the non-

UV induced rare forms) using RT-PCR. Alternative/mutant EGFR extracellular domains were found with high frequency: 15 out of the 28 samples contained low molecular weight PCR products. We have postulated that the mutant EGFR in human melanoma may serve a target for EGFR TK inhibitors. We have used two clinically available EGFR inhibitors, Gefitinib (Iressa, ZD1839) and Erlotinib (Tarceva, OSI-774) to test the sensitivity of human melanoma cell lines. Melanoma cells were proved to be resistant to OSI-774 while ZD1839 exhibited >50% inhibition of proliferation at 10 μM. Furthermore, in 6 out of the 8 human melanoma cell lines ZD1839 induced significant increase in apoptotic rates at a concentration of 25 μM. Next we have pretreated HT168M1 cells with ZD1839 for 48 hr and the surviving tumor cells were tested for their migratory potential in a Boyden chamber assay. We found that ZD1839-pretreated cells lost their migratory potential, which started at 0.1 μM drug concentration and became highly significant at 10 μM. Phosphoproteome analysis of WM983B human melanoma cells indicated that ZD1839 inhibited constitutive phosphorylation of MAPK/ERK1, CDC2 and JNK at 5 and 30 min exposures. Ultimately, we have tested the antitumoral effects of ZD1839 *in vivo* using a spleen-liver melanoma (WM983B) metastasis model in SCID mice. Tumor cells were inoculated into the spleen at a dose of 106 cells/animal and daily i.p. drug treatment was started on day 7th (0.1-20 mg/kg) for two weeks. When the weight loss of animals in the control group exceeded 20%, the experiment was terminated. Analysis of the primary spleen tumors revealed no effect of ZD1839. However, ZD1839 significantly inhibited liver metastasis formation of human melanoma cells by 62% (2 mg/kg) and 86% (20 mg/kg). Our data suggest that human melanoma may frequently carry extracellular domain mutations in the EGFR and these aberrations may render it sensitive to EGFR tyrosine kinase inhibitors.

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Response to VEGF receptor inhibition and vascular disrupting therapy of mouse tumours expressing only single isoforms of VEGF-A

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Mouse fibrosarcomas expressing only single VEGF-A isoforms (120, 164 or 188), or all isoforms (w/t) under endogenous promoter control have been developed. Subcutaneous VEGF120 tumours transplanted into SCID mice have poor vessel wall development, with very few α-smooth muscle actin or desmin staining cells, indicative of lack of pericytes. This is associated with susceptibility to vessel breakdown following treatment with the prototype tumour vascular disrupting agent, combretastatin A4-phosphate (CA-4-P). VEGF188 tumours have more uniform vascular networks and better-developed vessel walls (positive staining for α-smooth muscle actin and desmin) than VEGF120 tumours and are less responsive to CA-4-P. We tested the effect of chronic treatment with a VEGF receptor inhibitor, SU5416, on angiogenesis in the different tumour types and subsequent response to CA-4-P.

Measurements of angiogenesis and vascular response to CA-4-P in VEGF120, 164, 188 and w/t tumours were studied in SCID mice using dorsal skin flap window chambers. Subcutaneously implanted tumours were studied for growth measurements and to perform immunohistochemistry. In subcutaneous tumours, SU5416 (50 mgkg-1, i.p, twice weekly) did not affect growth rates in any tumour line. There were higher levels of α-smooth muscle actin staining in SU5416-treated VEGF120 tumours than untreated tumours, implying greater vascular maturity, in terms of pericyte recruitment. Less effect of SU5416 on pericyte staining was observed in the other tumour types. In the window chamber, angiogenesis was inhibited in all VEGF-isoform tumours treated with SU5416, as measured by total vessel length density versus time after transplantation. In VEGF120 tumours pre-treated with SU5416 (50 mgkg-1, i.p, twice weekly), CA-4-P (30 mgkg-1, i.v) decreased red blood cell velocity after 1hr, but there was complete recovery by 24hrs. In contrast, in VEGF120 tumours either pre-treated with vehicle (twice weekly), or untreated tumours, CA-4-P caused rapid vascular shutdown and haemorrhage with no recovery after 24hrs. SU5416 had less effect on response to CA-4-P treatment in the other tumour types, in keeping with the results for pericyte staining.

The VEGF receptor inhibitor SU5416 caused a significant 'normalization' of the vasculature in VEGF120 tumours, involving an increased proportion of tumour vessels that are stabilised by pericytes, and resulting in a poor response to CA-4-P. These results suggest that response to vascular disrupting agents is strongly influenced by pericyte investment and that vascular normalisation may be an undesirable effect of anti-angiogenic agents such as SU5416.

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Lysosomal protease-mediated killing of cells: possible use in cancer therapy?

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Cancer cells are known to evade clearance by apoptosis. There are numerous mechanism utilized by cancerous cells to escape apoptosis, including p53 mutations and/or degradation, upregulation of antiapoptotic Bcl2 homologs and upregulation of IAPs. Although seemingly different, caspase activation is much less efficient in all of them. There is mounting evidence that in addition to caspases cysteine cathepsins play an important role in apoptosis. Lysosomal destabilization, accompanied by the release of cathepsins into cytosol, was often found to be an early event in apoptotic cascade, preceding other hallmarks of apoptosis including mitochondrial outer membrane permeabilization and caspase activation. Bid and other members of Bcl2 family were found to be important messengers of the signal, whereas other cathepsin substrates, which could explain potential role of cathepsins in caspase-independent cell death were not identified as yet. Using lysosomotropic compound LeuLeuOMe as a model, we were able to show that apoptotic cell death is almost completely abolished in the absence of mitochondrial membrane permeabilization with cathepsins B and L being the major players. This suggests that cysteine cathepsins predominantly trigger apoptosis through MMP and subsequent caspase activation and not through caspase-independent pathways, which could provide a potential for clearance of cancer cells, where Bcl-2 homologs and/or IAPs are upregulated.

Chemotherapy-induced cell death and its assessment *in vivo*

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Oncologists are always concerned about whether or not chemotherapy succesfully kills the cancer cells in their patients. It is thought that the mode of cell death could be apoptosis, which is also called programmed cell death, although the other cell death modes may also be possible. One of the characteristics of apoptosis is the distruption of cytoskeleton in which cytokeratin 18 constitutes the major part. Once cells have undergone apoptosis, cytokeratin 18 is cleaved at certain positions by apoptosis-spesific proteases, also known as caspases. After the cleavage, a neo-epitope is formed. Caspase-cleaved cytokeratin 18 (M30 antigen) is released into blood stream, following apoptosis. This neo-epitope is recognized by so-called M30 antibody. Using an ELISA assay for M30 antigen, it is possible to detect the soluble M30 antigen in serum of patients prior to and after the chemotherapy, which allows to estimate the efficacy of the drugs *in vivo*. In our recent study with lung cancer patients (Ulukaya et al, 2007), we found that M30 antigen level statistically significantly increased after the application of chemotherapy. Likewise, it elevated in breast cancer patients following chemotherapy as well. The basal levels of M30 antigen may also have a potential to use it as a predictor of the prognosis of patients. M30 antigen seems to be a favorable novel serum marker in the better management of cancer patients although more clinical studies are required.

Reduced tumour cell proliferation and delayed development of high grade mammary carcinomas in cathepsin B deficient mice.

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Expression levels of the papain-like cysteine protease cathepsin B have been positively correlated with mammary tumour progression and metastasis; however, its roles in the hallmark processes of malignant growth remain poorly defined. Using cathepsin B deficient mice we investigated tumour cell differentiation, proliferation and apoptosis in the Tg(MMTV-PyMT) mouse mammary cancer model. Absence of cathepsin B significantly impaired development of high grade invasive ductal carcinomas and reduced the metastatic burden in the lungs. Mice lacking cathepsin B exhibited reduced cell proliferation in mammary carcinomas and their lung metastases. Notably, intravenous injection of primarily isolated, cathepsin B expressing tumour cells into congenic cathepsin B deficient mice revealed impaired cell proliferation in the resulting experimental lung metastases, providing evidence for the involvement of cathepsin B in paracrine regulation of cancer cell proliferation. No cathepsin B genotype-dependent difference in tumour cell death was observed *in vivo* or by treatment of isolated PyMT cancer cells with TNF-α. However, cancer cells lacking cathepsin B exhibited significantly higher resistance to apoptosis induced, via Bid cleavage, by the lysosomotropic agent Leu-Leu-OMe. Thus, our results indicate an *in vivo* role for cathepsin B in promoting cellular anaplasia in mammary cancers and proliferation in lung metastases.

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Towards high throughput, high content, fluorescence lifetime imaging

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Instrumentation to perform Fluorescence Lifetime Imaging (FLIM) at the single cell and tissue levels is now well developed and the technique is finding ever-increasing applications in the biomedical sciences. Nevertheless, some difficult issues remain to be solved. These can be grouped in two broadly overlapping 'problem' areas: how to perform signal acquisitions as fast as possible with minimal insult to the specimen and, having acquired the information, what confidence can be placed on the result. This in turn suggests observation of large fields (e.g. multi-well plates) or large numbers of tissue samples (e.g. tissue micro-arrays).

Although there are numerous applications of FLIM, its ability to quantify protein interactions, through the application of Főrster Resonance Energy Transfer (FRET) is of particular interest. Robust analysis methods to determine fluorescence lifetime components have been developed and their limitations when very low photon counts are available will be presented. Global analysis methods are particularly advantageous in many cases where the photophysics and kinetic models are well understood.

Recent and current developments in acquiring data from large areas will be discussed and advantages and limitations of various approaches will be presented. While it is always desirable to minimise instrumental limitations, biological variability very often limits the eventual 'noise'. This in turn dictates the imaging of large numbers of samples, which demands a high degree of automation of the acquisition and analysis phases of the experiment. Conventional 'microscope' platforms are not always optimal for such work and systems to overcome limitations will be outlined. Technological solutions to provide optimised imaging, and emerging parallel detection systems, specifically to overcome the challenges imposed by optical proteomics will be discussed. Potential pitfalls in the automated analyses of data acquired with automated platforms are numerous and must be carefully avoided, partially by sound biological experiment design and partially by tight quality control. The reward is worthwhile, since the reporting of fluorescence lifetime, spectral content and other photophysical parameters allows us to explore the environment of a molecule, and in the case of FRET, with a tool that provides near-field information with a far-field technique. Various bottlenecks in data flow are, in general, inevitable and means to minimise these will be explored. Directions of current and future work, specifications, requirements and possibilities afforded by novel approaches will be discussed, with the aim of setting the scene to apply FLIM in much wider areas of biological research.

Resveratrol induces fidelity control mechanisms of DNA double-strand break repair *via* activation of ATM/ATR signalling pathways

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The phytochemical resveratrol (RV) has become a focus of intense research owing to its roles in promoting longevity and in cancer prevention. As an anti-cancer agent RV has primarily been linked to growth and death regulatory pathways. So far, ambiguous observations have been published regarding its influence on genomic stability. To study RV´s effects on DNA double-strand break (DSB) repair we applied our fluorescence-based assay system on RV-treated lymphoblastoid cell lines (LCLs). We showed that, under physiological conditions, RV inhibits both, homologous recombination (HR) and non homologous end-joining (NHEJ) independently of its known growth and death regulatory functions. Using (i) isogenic cell lines, which differ in their p53 status, (ii) LCLs from patients with ataxia telangiectasia, (iii) shRNA mediated p53 knockdown, and (iv) chemical inhibition of ATM/ATR, we established an ATM-p53-dependent pathway of HR inhibition by RV. Additional use of LCLs from Nijmegen breakage syndrome (NBS) patients furthermore provided evidence for an ATM/ATR-Nbs1-dependent inhibition of microhomology-mediated NHEJ (MM-NHEJ) after RV-treatment. We propose that activation of ATM and ATR is a central effect of RV which underlies cell cycle regulation and the activation of fidelity control mechanisms in DSB repair. Repression of error-prone recombination subpathways could at least partially explain the chemopreventive effects of this natural plant constituent in animal cancer models.

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Mechanisms of proteolytic tumor invasion resulting in multicellular tumor patterning

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Invasive cell migration through tissue barriers requires pericellular remodeling of extracellular matrix (ECM) executed by cell surface proteases, particularly membrane-type 1-matrix metalloproteinase (MT1-MMP, MMP-14). Using time-resolved multimodal microscopy, we here show how invasive fibrosarcoma cells coordinate mechanotransduction and fibrillar collagen remodeling by segregating the anterior force-generating leading edge containing β1 integrin, MT1-MMP, and F-actin from a posterior proteolytic zone executing fibre breakdown. During forward movement, sterically impeding fibres are selectively realigned into microtracks of single-cell calibre, which become expanded by multiple following cells via large-scale degradation of lateral ECM interfaces, ultimately prompting transition towards *in vivo*-like collective invasion. Both ECM track widening and transition to multicellular invasion are dependent upon MT1-MMP-mediated collagenolysis, shown by broad-spectrum protease inhibition and RNA interference. The findings establish invasive migration and proteolytic ECM remodeling as interdependent mechanisms that control tissue micro- and macropatterning and, consequently, individual and collective cell migration.

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P1. *Tina Batista Napotnik:* High-voltage nanosecond electrical pulses affect plasma membrane of cultured cells

P2. *Aleš Berlec:* Mistletoe extract triggers mitochondrial pathway of apoptosis by influencing the expression of CK II, Mcl-1 and PRDX3 proteins

P3. *Ajda Biček:* Ruthenium-based anticancer agent in combination with electroporation exerts higher cytotoxicity than agent alone

P4. *Suzanne Birks:* Glioma: combating the invasive cell phenotype

P5. *Lea Bojič:* Cysteine cathepsins are not involved in Fas/CD95 signalling in primary skin fibroblasts

P6. *Anja Bubik:* Protease inhibitory and cytotoxic effects of "non-toxic" cyclic peptides from cyanobacteria

P7. *Leonora Buzanska:* Non-immortalized somatic neural stem cell line – possible implementation for developmental neurotoxicity and oncological research

P8. *Chiara Compagnin:* Delivery of meta-tetra(hydroxyphenyl)chlorine (mTHPC) in organically-modified silica (ORMOSIL) nanoparticles to cancer cells

P9. *Andrej Cör:* Increased tumour hypoxia and morphological blood vessel changes in tumours after electrochemotherapy

P10. *Maja Čemažar:* Platinum analogue *trans*-[$PtCl_2$(3-Hmpy)$_2$] has high antitumor effectiveness against different tumor types

P11. *Selma Čorović: In vivo* electroporation level detection based on propidium iodide test and magnetic resonance imaging and numerical modeling and optimization of local electric field in muscle tissue

P12. *Bojan Doljak:* Reduction of plasminogen activity in breast tumour cells by anti-cytokeratin monoclonal antibody

P13. *Petia Genova Kalou: In vitro* studies of cytotoxicity and apoptosis of tamoxifen in human estrogen-receptor positive ovarian cancer cells

P14. *Boris Gole:* Cysteine cathepsins and their endogenous inhibitors in the invading and non-invading GBM cells

P15. *Alenka Grošel:* Electrogene therapy with *p53* alone or in combination with electrochemotherapy using cisplatin reduces the survival of human colon carcinoma cells

P16. *Saša Haberl:* The influence of Mg ions on the efficiency of gene electrotransfer

P17. *Irena Hreljac:* Effects of model organophosphorous pesticides on DNA damage and proliferation of human hepatoma HepG2 cells

P18. *Signe Ingvarsen:* Dimerization of endogenous MT1-MMP is a regulatory step in the activation of the 72 kDa gelatinase, MMP-2, on fibroblasts and fibrosarcoma cells

P19. *Urška Kamenšek:* Construction of eukaryotic expression plasmid with DNA damage responsive p21 gene promoter

P20. *Nina Kočevar:* Chemical acylation improves membrane permeability of cystatin

P21. *Tatiana A. Korolenko:* Role proteases of Kupffer cells in murine tumor development and metastasing

P22. *Eva Kralj:* Comparison of the results for the JAK2 V617F mutation detection by three methods; allele specific PCR and two real-time quantitative PCR on polycythemia vera and essential thrombocythemia DNA samples

P23. *Veronika Kralj Iglič:* Plasma-mediated attractive interaction between membraneous structures as a possible suppressive mechanism of tumor progression

P24. *Simona Kranjc:* Tumor blood flow modifying changes after application of different sets of electric pulses

P25. *Matic Legiša:* Post-translational modification of 6-phosphofructo-1-kinase in cancer cells

P26. *Yan Qun Liu:* Sodium iodide method is suitable for DNA isolation from serum of colorectal cancer patients

P27. *Alenka Maček Lebar:* Increased permeability of cell membrane by sonoporation

P28. *Suzana Mesojednik:* Gene transfer into solid tumors by electroporation

P29. *Georgy Mikhaylov:* Development of novel magnetic nanoparticles based drug delivery systems for cancer treatment

P30. *Miha Milek:* Reversal of thiopurine cytotoxicity in lymphoblasts by S-adenosylmethionine

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High-voltage nanosecond electrical pulses affect plasma membrane of cultured cells

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The exposure of cells to an electric field results in poration of the plasma membrane (i.e. electroporation). The electroporation is already in use in clinical treatments in electrochemotherapy and gene electrotransfection. Typical pulses that are used for the electroporation are in micro- to millisecond time range and with electrical field strength of 100 to a few 1000 V/cm.

High-voltage nanosecond electrical pulses (with duration from 1 to 100 ns and the electric field strength up to a few 100 kV/cm) can be achieved by high-voltage generators, usually derived from a Blumlein pulse-forming network. These pulses are short enough and high enough to induce sufficiently high induced voltage across the intracellular membranes. The nanosecond pulses have greater effect on cell organelles than on the plasma membrane: they can induce pore formation in membranes of cell organelles. This can lead to elevating cell calcium, DNA damage and apoptosis. When pulse parameters (duration, voltage, and number of pulses) are augmented, also the effects on plasma membrane increase.

We have designed a modified Blumlein generator for generating high-voltage nanosecond electrical pulses of various durations (10 to 300 ns) and pulse frequency in a pulse train (up to very high frequencies of a few 100 kHz). The electrodes made of gold are deposited onto a microscope cover glass and placed under a fluorescence microscope in order to observe immediate effects on cultured cells. The gap between the two electrodes is 100 μm so very high field strength can be achieved (up to 100 kV/cm).

The B16 F1 mouse melanoma cells were exposed to high-voltage nanosecond pulses (60 ns, 44 kV/cm) of various repetitition frequency (up to 100 Hz) and number of pulses (up to 60) in a pulse train. We observed cell uptake of propidium iodide (PI) after the pulsing. We confirmed that higher number of pulses and higher frequency of pulses in a pulse train cause rise of plasma membrane permeability, therefore cells become positive for PI staining. These results show that high-voltage nanosecond pulses in higher frequencies and number of pulses in a pulse train affect plasma membrane of cultured cells.

Mistletoe extract triggers mitochondrial pathway of apoptosis by influencing the expression of CK II, Mcl-1 and PRDX3 proteins

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European mistletoe (*Viscum album*) extracts have been used as anti-cancer agents in complementary medicine for centuries. Cytotoxic and immunomodulatory effects have been reported in *in vivo* and *in vitro* studies. Their precise mode of action however remains unknown. We studied the effects of commercially available extract on two leukemic cell lines (Jurkat, Molt). Differential expression of proteins was monitored on transcriptional and translational level. After treatment with the extract we observed differential expression of proteins involved in pro-survival and apoptotic processes (CKII, Mcl-1) and several mitochondrial proteins including PRDX3. Our results indicate that mistletoe extract initiates mitochondrial pathway of apoptosis.

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Ruthenium-based anticancer agent in combination with electroporation exerts higher cytotoxicity than agent alone

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Indazolium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019) is a potential anticancer agent. It has already entered clinical trials and is together with NAMI-A the most successful representative of ruthenium-based drugs. KP1339 is a sodium salt of KP1019 with higher solubility. It also exerts moderate cytotoxicity (1).

It is already known that addition of agents KP1019 or KP1339 to tumor cells caused DNA-strand breaks and the loss of mitochondrial membrane potential depending on the concentration of the anticancer agent. Therefore it is assumed that KP1019 and KP1339 have intracellular biological targets and that cytotoxic potency of the agents is directly influenced by the cellular uptake. Electroporation can be used to facilitate delivery of agents for which membrane represents barrier into cells. We have for example already used electroporation to increase cytotoxic effect of agent NAMI-A on tumor cells *in vitro* (1).

In this work we aimed at determination whether application of electroporation modifies *in vitro* cell cytotoxicity of KP1339 and KP 1019. Cell cytotoxicity was determined in two different cell lines; B16F1 and CHO. Cells were treated either with 30 μM KP1019 or 100 μM KP1339 alone or in combination with electroporation. For electroporation a 50 μl droplet of cell suspension containing 10^6 cells was placed between stainless steel plate electrodes 2 mm apart. Pulse voltage applied during electroporation was 160 V. A train of eight square electric pulses of 100 μs duration were delivered at 1 s interval. Cell survival responding to anticancer agent's cytotoxicity was mesured by MTT assay. The cell suspension of non-pulsed cells diluted only in electroporation buffer served as the control to which results were normalized.

Our results show that the combined treatment of either KP1019 or KP 1339 with electroporation potentiates the effect of these ruthenium-based compounds, although this potentiation was not statistically significant for KP1019. Electroporation alone caused cell membrane permeabilization without seriously affecting cell viability. These results are in agreement with our previous study with NAMI-A (2).

References:

1. Hartinger CG, Zorbas-Seifried S, Jakupec MA, Kynast B, Zorbas H, Keppler BK. (2006) From bench to bedside-preclinical and early clinical development of the anticancer agent indazolium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019 or FFC14A). J Inorg Biochem. 100: 891-904.

2. Bicek A, Turel I, Kanduser M, Miklavcic D. (2007) Combined therapy of the antimetastatic compound NAMI-A and electroporation on B16F1 tumour cells *in vitro*. Bioelectrochemistry. 71: 113-7.

Glioma: combating the invasive cell phenotype

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A small subset of cells displaying stem-like properties, maintaining the ability to self-renew and sustain growth of the tumour has been identified in glioma. The trans-membrane protein, CD133, has been identified in such cells and shown to promote multi drug resistance (MDR). It has also been hypothesised that CD133 facilitates brain tumour cell invasion and that such tumour stem cells may also serve to disseminate the tumour within the brain. In addition, ganglioside GD3 plays a pivotal role in the regulation of tumour growth and invasion by facilitating tumour cell adhesion. Although in non-neoplastic cells build up of GD3 induces mitochondrially-mediated apoptosis this does not occur in tumour cells due to the acetylation of the terminal sialic acid residue on GD3 to form 9-0-acetyl GD3 (GD3A). We are using glioma biopsy-derived tissue and early passage cultures to determine a) the migratory properties of CD133+ tumour stem cells and b) the influence of deacetylation of GD3A on glioma cell apoptosis. Baculoviruses are a family of insect specific viruses which have recently been shown to effectively drive the expression of a reporter gene in both dividing and non-dividing mammalian neural cells whilst remaining safe and non-pathogenic. We will initially determine whether the baculovirus vector can be modified to target GD3 in human gliomas and give transient and stable gene delivery. CD133+ and - cells have been segregated, by autoMACS™ magnetic cell separation, from low passage biopsy-derived cells and transformed into neurospheres via the hanging drop method. All cells used were been characterised by flow cytometry and immunocytochemistry, using appropriate immuno markers. We have extracted GD3A from bovine buttermilk by solvent extraction and partitioning and ion-exchange chromatography. The purity of this is then tested and the GD3/GD3A used in motility studies. CD133 expression was seen on tumour cells, as well as a 1.43% positive expression of CD133 via flow cytometry. Cell migration and invasion in CD133+ and CD133- populations is being studied by Transwell™ Boyden Chambers and live cell imaging. An "all human" 3D-invasion model is also being used. The cultured CD133 (+) and CD133 (-) neuropsheres, from brain tumour biopsies, juxtaposed, *in vitro*, to a spheroid produced from brain resected from epileptic patients. Invasion is observed via live-cell imaging and confocal microscopy. GD3 and GD3A has been prepared from buttermilk and used to determine influence on glioma cell viability and apoptosis. Influence of CD133+ cancer stem cells within glioma may determine not only therapeutic response but promote local spread of the neoplasm within the brain. In addition, it has been shown that by enzymatically cleaving the acetyl group from GD3A restores the pro-apoptotic potential to that of GD3. We intend to use the Baculovirus transfected with the cDNA of the acetylesterase enzyme to target GD3A in glioma cells *in vitro* in attempt to remove the acetyl group and then assess apoptosis by Western blot analysis and the employment of the TUNEL and Annexin V assays.

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Cysteine cathepsins are not involved in Fas/CD95 signalling in primary skin fibroblasts

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The potential role of cysteine cathepsins, especially cathepsin B, in Fas/CD95-induced apoptosis was investigated using wild-type and cathepsin B –deficient primary skin fibroblasts. Apoptosis was induced with an anti-Fas/CD95 antibody in the presence of cycloheximide and no difference was observed between the two genotypes. Preincubation of cells with the broad spectrum cathepsin inhibitor E-64d had neither an effect on apoptosis progression. First signs of mitochondria damage were observed ~3 hours post apoptosis induction, whereas a significant number of cells with damaged mitochondria was seen after 11 hours. In contrast, lysosome damage was only seen after 15 hours with no difference between the two genotypes. In addition, Bid cleavage was found to be diminished in cathepsin B-deficient cells. These results suggest that cysteine cathepsins have no active role in Fas/CD95 apoptosis and that lysosomal damage and diminished Bid cleavage observed are probably late bystander events.

Protease inhibitory and cytotoxic effects of "non-toxic" cyclic peptides from cyanobacteria

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Toxic cyanobacterial blooms are common events also in the republic of Slovenia (1). These organisms are a rich source of various metabolites with strong biological activities (2, 3). Various "non-toxic" cyclic peptides belonging to two main groups, depsipeptides and cyclic peptides with the ureido linkage inhibit serine proteases that play an important role also in the human organism (4). We have tested three representatives isolated from the *Planktothrix rubescens* bloom (2); one depsipeptide planktopeptin BL1125 (PP BL) and two ureido linkage possessing representatives, anabaenopeptin B (AnP B) and anabaenopeptin F (AnP F). Our aims were (a) to find, which physiological important serine proteases are inhibited by PP BL, AnP B and AnP F, and (b) to investigate the possible influence of PP BL, AnP B and AnP F on the growth characteristic of one normal cell line (human astrocytes NHA) and two tumour cell lines, human glioblastoma cells (U87) and human large cell carcinoma (LCLC 103H).

Our results have proved a strong inhibition of leukocyte (K_i = 15.8 nM) and pancreatic (K_i = 5.8 nM) elastase and chymotrypsin (K_i = 2.3 nM) with PP BL and a weaker inhibition of these enzymes with AnP B and AnP F. Planktopeptin has been also effective in inhibiting the elastinolytic activity of leukocyte elastase even when the enzyme has been allowed to form a tightly bound complex with elastin. The examined cyclic peptides have demonstrated no inhibitory activity towards trypsin, urokinase, kallikrein 1 and cysteine chatepsins B, K, L and S. The cytotoxic effects of PP BL, AnP B and AnP F on all tested cell lines were not observed within 24 hours in concentration range 0.1-10 μM. They have had a significant influence on the metabolic activity of normal NHA cells after 48, 72 and 96 hour exposures, but not on any of tumour cell lines.

The selective inhibitory activity of cyclic peptides on elastase is important for a potential application in inflammatory diseases and possibly in inflammatory cancer. Differential effects of PP BL on normal and tumour cells indicate the presence of a biological target, related also to cell proliferation, which will be the subject of our future work.

References:

1. Sedmak B, Kosi G (1997): Mycrocystins in Slovene freshwaters (central Europe) – first report. Natural Toxins, 5: 64-73.
2. Grach-Pogrebinsky O, Sedmak B, Carmeli S (2003): Protease inhibitors from a Slovenian Lake Bled toxic waterbloom of the cyanobacterium Planktothrix rubescens. Tetrahedron, 59: 8329-8336.
3. Pogrebinsky O, Sedmak B, Carmeli S (2004): Seco[D-Asp3] microcystin-RR and D-Asp3, D-Glu(OMe)6] microcystin-RR, two new microcystins from a toxic water bloom of the cyanobacterium Planktothrix rubescens. Journal of Natural Products, 67: 337-342.
4. Namikoshi M, Rinehart KL (1996): Bioactive compounds produced by cyanobacteria. Journal of Industrial Microbiology, 17: 373-384.

Non-immortalized somatic neural stem cell line – possible implementation for developmental neurotoxicity and oncological research

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The promise held by neural stem cells (NSC) for the cell therapy of neurodegenerative and oncological disorders is now widely accepted and stem cell research is focused on this aspect of NSC application. In parallel, the NSC culture can be considered a model system for *in vitro* screening of putative toxic or transforming factor effects on their proliferation, viability and differentiation potential into neuronal cells. However, both the ethically controversial sources of the NSC (human embryos or foetuses) and the limited life-span of the somatic NSC cultures make these studies difficult. These two challenges were faced by our group by showing the possibility of differentiation of the stem cells isolated from Human Umbilical Cord Blood (HUCB) into neuronal cells (Buzanska et al., J.Cell Sci., 115; 2002) and further by generating the first, non-transformed, clonal and karyotypically stable somatic neural stem cell line "HUCB-NSC" (Buzanska et al., Stem Cells Dev., 15; 2006). Over six years of culturing, these cells have revealed a stable growth rate, an ability to self-renew and the maintenance of differentiating potential into neuronal, astroglial and oligodendroglial cells. The HUCB-NSC can be expanded and halted in culture at different developmental stages – from floating non-differentiated stem cells to committed progenitors and differentiated neuronal cells (Buzanska et al., Tox *In Vitro*., 19; 2005). The developmental stages of HUCB-NSC have been truly characterized by transcriptional profiling, immunocytochemistry and electrophysiological studies, thus HUCB-NSC as a non–immortalized stem cell line can serve as an alternative, human-based model for *in vitro* oncogenic transformations and developmental neurotoxicity studies.

Delivery of meta-tetra(hydroxyphenyl)chlorine (mTHPC) in organically-modified silica (ORMOSIL) nanoparticles to cancer cells

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Nanoparticles, nanotubes, quantum dots and dendrimers are objects of intense investigations due to their potential application in many areas of biology and medicine. In particular these materials are receiving considerable attention because of their potential use for bioimaging, diagnostic technology and drug or gene delivery. The benefits offered by targeted nanoscale drug carriers could be an improvement of drug bioavailability, precision of drug targeting and reduced drug toxicity. For this reason the entrapment of anticancer agents in nanoparticles is considered an innovative method for drug delivery aimed at improving the efficacy of cancer therapies.

We investigated the use of ORMOSIL nanoparticles (NPs) loaded non covalently with meta-tetra(hydroxyphenyl)chlorine (mTHPC) for its delivery to human oesophageal carcinoma cells (KYSE 510) in vitro. Silica nanoparticles are known for their compatibility in biological systems. mTHPC is a hydrophobic second generation PDT (Photodynamic Therapy) photosensitiser approved in Europe for the palliative treatment of advanced head and neck cancers and characterized by a high antitumoral activity. mTHPC loaded in NPs or delivered by the standard solvent ethanol/polyethyleneglycol 400/water (20:30:50, by vol.) was internalised by KYSE 510 cells very quickly and localized in the Golgi apparatus and endoplasmic reticulum. NP-entrapped mTHPC was taken up by the cells less efficiently than free mTHPC but this did not decrease the efficiency of cell photokilling. No cytotoxicity in the dark was observed when mTHPC was delivered in NPs with respect to free mTHPC. Irradiation with 0.12 J/cm2 of red light (600-700 nm) induced a complete loss of cellular viability following 24 h incubation with 1 μM NP-entrapped or free mTHPC.

The results show that ORMOSIL NPs can be used as nanosystems for the delivery of hydrophobic drug and may improve the selectivity of cancer therapy through their targeting with functional groups specifically recognised by receptors over-expressed in tumour cells.

Increased tumour hypoxia and morphological blood vessel changes in tumours after electrochemotherapy

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It is widely accepted that no solid tumour can grow larger than a critical size of ~ $1mm^3$ without developing a blood supply network. Tumour vascular networks are chaotic, featuring complex branching patterns and a lack of hierarchy. Vessel diameters are irregular and blood flow rates are low (1). Evidences has accumulated showing that up to 50-60% of locally advanced solid tumours may exhibit hypoxic and/or anoxic tissue areas, which are heterogeneously distributed within the tumour mass.

The underlying mechanism of electrochemotherapy is electroporation of the cell membrane, which facilitates access of poorly or non-permeable molecules into the tumour cells. Additionally to the well-established direct cytotoxic effect on tumour cells, blood flow measurements showed an immediate reduction of tumour blood flow after application of electric pulses and electrochemotherapy (2).

In our study, SA-1 solid subcutaneous sarcoma tumours in A/J mice were treated by bleomycin (BLM), the application of electric pulses (8 pulses, 1040V, 100 μs, 1Hz) or a combination of both – electrochemotherapy. At various time points after therapy, tumours were excised and analysed histochemically and immunohistochemically with antibodies against CD-31 as endothelial cell marker, Glut-1 as intrinsic hypoxic marker and active casapase-3 as apoptotic marker.

After application of electric pulses alone, as well as after electrochemotherapy, the extent of tumour hypoxia, detected by Glut-1, reached its peak after 2h and lasted up to 8h after the treatment. After the application of electric pulses, the recovery to pre-treatment level lasted 14h, whereas after electrochemotherapy the recovery to pre-treatment level did not occur for at least 48h.

To discover the cause of the increased hypoxia in the tumours after therapy, the morphological characteristics of small tumour blood vessels were analysed. Changes in the endothelial cells' shape were observed 1h after the application of electric pulses. Endothelial cells were rounded up and swollen, and the lumens of blood vessels were narrowed. The same blood vessel morphological changes were also found after electrochemotherapy, although at later times, approximately 14h after electrochemotherapy, some endothelial cells with apoptotic characteristics and immunohistochemical active caspase-3 positivity were found. Apoptotic endothelial cells were not observed in tumours treated with electric pulses alone.

Our results demonstrate that electrochemotherapy also has a vascular disrupting action, which can greatly increase the hypoxia in the tumours.

References:

1. Tozer GM, Kanthou C, Baguley BC. Disrupting tumour blood vesels. Nat Rev Cancer 2005; 5: 423-33.
2. Sersa G, Jarm T, Kotnik T, et al. Vascular disrupting action of electroporation and electrochemotherapy with bleomycin in murine sarcoma. Br J Cancer 2008; 98: 388-98.

Platinum analogue *trans*-[$PtCl_2$(3-Hmpy)$_2$] has high antitumor effectiveness against different tumor types

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Although cisplatin is a valuable antitumor drug for treating several malignancies, it has several disadvantages including severe side effects and acquired drug resistance. The synthesis of new platinum complexes is aimed to overcome resistance to cisplatin and lower the side effects. The aim of our study was to determine the antitumor effectiveness of Pt(II) complex with 3-hydroxymethylpyridine (3-Hmpy) *trans*-[$PtCl_2$(3-Hmpy)$_2$] (S2) in comparison with cisplatin.

We studied the interactions of S2 and cisplatin with plasmid DNA by gel electrophoresis. In addition, we determined the cytotoxicity of S2 on parental and cisplatin resistant ovarian carcinoma and bladder carcinoma cells by clonogenic assay and the antitumor effectiveness of intratumoral drug administration in solid tumors in mice.

Analysis of undigested plasmid DNA electrophoretograms after treatment with S2 and cisplatin demonstrated that S2 compound caused less fast running supercoiled form and more singly nicked form band compared to cisplatin. In addition, with increasing concentrations S2 prevented BamH1 digestion of plasmid DNA indicating on change in DNA conformation, which was more pronounced than in the case of cisplatin.

Bladder carcinoma cells and ovarian carcinoma cells resistant to cisplatin were equally sensitive to complex S2 in comparison to cisplatin. The parental ovarian carcinoma cells, which are intrinsically very sensitive to cisplatin were less sensitive to complex S2 compared to cisplatin. Growth of subcutaneous tumors after treatment with S2 was less delayed than after treatment with cisplatin in both tumor types.

The results of our study demonstrate that S2 causes considerable damage to isolated DNA and is highly cytotoxic for cells, however, the antitumor effectiveness in solid tumors is less evident.

In vivo electroporation level detection based on propidium iodide test and magnetic resonance imaging and numerical modeling and optimization of local electric field in muscle tissue

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The aim of this study was to experimentally and numerically analyze local electric field distribution in anisotropic tissues to be electroporated for biomedical purposes. Indeed, *in vivo* electroporation (electropermeabilization) is an effective technique for delivery of therapeutic drugs or DNA molecules into the target tissue cells by means of local application of high voltage electric pulses via appropriate electrode configuration. Solid tumors are treated with electrochemotherapy (ECT) in routine in clinics, and several types of tissues can be transfected with foreign DNA by means of gene electrotransfer (EGT).

The level of the tissue electroporation and the distribution of the electric fields can be optimized so that the increase in cell membrane permeability can be limited to the cells of the target tissue while minimizing the damage to the surrounding tissue, which is essential in electrochemotherapy and gene electrotransfer treatment planning. The key parameter in effective tissue electroporation is local electric field distribution (E) induced by the application of short and intense electric pulses. In order to assure successful ECT and EGT outcome the entire tissue to be treated has to be optimally electroporated, which requires an adequate electric field within the treated tissue. This means that in ECT and EGT treatment planning the following requirements have to be met: 1) all the target tissue has to be subjected to the local electric field (E) inducing reversible electroporation (E between reversible and irreversible thresholds $E_{rev} < E < E_{irrev}$) while 2) the surrounding tissue should not be exposed to excessively high local electric field ($E > E_{irrev}$). The E_{rev} and E_{irrev} thresholds need to be determined experimentally.

In this study we experimentally and numerically determined the E_{rev} and E_{irrev} threshold values for muscle tissue taking into account its anisotropic electric properties. The experiments were carried out *in vivo* by means of a fluorescence test using propidium iodide, and magnetic resonance imaging detecting the electrotransfer inside the cells of an extracellular contrast agent. Electroporation of mouse anterior tibialis was examined for parallel and perpendicular directions of the electric field with respect to the muscle fibers. Based on this we determined the reversible and irreversible elelectropermeabilisation threshold values - E_{rev} and E_{irrev} - in both directions. These values were included in 3D numerical model of muscle tissue. The numerical analysis was carried using finite element method. We developed an optimization genetic algorithm for optimization of local electric field in anisotropic tissues, which can serve as an important tool in ECT and EGT treatment planning. The results of our experimental study can significantly contribute to the understanding of *in vivo* electroporation also of anisotropic tissues.

Reduction of plasminogen activity in breast tumour cells by anti-cytokeratin monoclonal antibody

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Cytokeratins (CKs) are ubiquitous structural proteins in cells of epithelial origin. Although they mainly form cytoplasmic structures, they are also localized at the plasma membrane or secreted from the cells. Some CKs are over-expressed in tumour cells and are used as diagnostic and prognostic biomarkers. Moreover, CKs have been reported to participate in cell invasion by enhancing plasminogen activation on their surface. Cell-surface activation of plasminogen leads to the activation of other proteases and finally to the degradation of the proteins of extracellular matrix, a process that is pivotal for migration, invasion and metastasis of tumour cells.

A stable hibridoma cell line producing monoclonal antibody (anti-CK MAb) was prepared after immunisation of mice with breast cancer MCF-7 cell extract. As shown by 2D-electophoresis immunoblotting and mass spectroscopy, the monoclonal antibody recognizes CK1, CK2, CK8, CK10 and CK18 in the lysate of MCF-7 cells. Using the aligned sequences of the recognized CKs, a consensus sequence of 27 AA was used to synthesize three overlapping short peptides of 12 AA. In ELISA, the anti-CK antibody expressed high affinity towards the VKIALEVEIATY dodecapeptide. The specificity of the interaction was verified by surface plasmon resonance. Plasmin formation from plasminogen was monitored in the presence of different concentrations of anti-CK MAb. In both MCF-10A neoT cells and MCF-7 cells, plasmin generation was diminished by anti-CK MAb in a dose dependent manner.

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In vitro studies of cytotoxicity and apoptosis of tamoxifen in human estrogen-receptor positive ovarian cancer cells

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Tamoxifen (TAM) is an important drug for treating breast cancer. Ovarian cancer cells are known to possess receptors for hormones, estrogen included. It has been suggested that TAM may be of some benefit. In this study, we investigated the short-term effects of this drug on estrogen-receptor-positive (ER^+) ovarian cancer cells. We performed an *in vitro* selection process by short-term treatment of one cancer cell line, CaOV with TAM. Drug effects on cell growth were determined by measurement of relative cell numbers (MTT-test), the apoptotic effects of TAM were determined by morphological observation of fluorescent DNA dies acridine orange and by DNA laddering assay. Based on the cytotoxic concentration required to inhibit cell surveillance by 50% (CC_{50}) at 48h after treatment it was found that: i) the cell surveillance depends probably of type and quantity of estrogen receptors on cell surface; ii) ovarian CaOV cancer cells was highly sensitive to TAM in a dose-depended manner. The fluorescent microscopic observation and DNA fragmentation assay indicated that: a) the absence of DNA fragmentation and apple green fluorescence of cytoplasm in control (cells incubated in compound-free medium) after short-term treated with TAM in CC_{50} values; b) morphological observation after acridine orange staining indicated yellow-green fluorescence of the cytoplasm, vesiculation in the cytoplasm, nuclei fragmentation and chromatin condensation in ovarian CaOV cancer cells (the possible availability of apoptosis) treated with the CC_{50} of TAM. The results of the gel electrophoresis of the DNA extracted from these cells show 'DNA smear' (non-specific fragmentation of genomic DNA).

p13

Cysteine cathepsins and their endogenous inhibitors in the invading and non-invading GBM cells

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Cysteine cathepsins are linked with the progression of different types of tumours, including gliomas- the most abundant type of central nervous system tumours. In the most malignant form of glioma- glioblastoma multiformae (GBM), the expression of cathepsin B was reported to be increased at the invading edges of tumours, thus linking cathepsin B with invasiveness of GBM. Another two cysteine cathepsins -L and S- were also reported to be involved in GBM invasion. The role of cathepsin L in invasion is however being challenged in some recent publications and the data on cathepsin S is still relatively scarce.

To clarify the involvement of cysteine cathepsins in GBM invasion, the presented study is focused on possible differences between the invading and non-invading GBM cell populations. We facilitate the three-dimensional *in vitro* spheroid invasion model to study expression levels of cathepsins B, L, S and their endogenous inhibitors stefins A, B and cystatin C. Analyses of the two cell populations at the mRNA, protein and protease activity level show that expression levels of the cysteine cathepsins are increased in the matrix invading cell population. Such results are in accordance with observations made for cathepsin B on histological samples from glioma patients. To complement this data, we down-regulated the endogenous activity levels of cathepsins B, L, S using specific chemical inhibitors in the three-dimensional *in vitro* invasion model. We confirmed the role of cathepsin B but not cathepsin L or cathepsin S in the invasiveness of GBM cells.

Our data add support to the idea that different cysteine cathepsins, although similarly up-regulated in the invading GBM cells, play different roles in the progression of gliomas.

p14

Electrogene therapy with *p53* alone or in combination with electrochemotherapy using cisplatin reduces the survival of human colon carcinoma cells

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Electroporation is an established and successful method for delivering either chemotherapeutic drugs, such as cisplatin and bleomycin (electrochemotherapy), or nucleic acids (gene electrotransfer) into cells *in vitro* and *in vivo*. The tumour suppressor gene *p53* plays a pivotal role in maintaining genome integrity and in regulating cell proliferation, differentiation and apoptosis. In more than 50% of human cancer, *p53* is mutated or its function is otherwise impaired.

Our study aimed to evaluate electrogene therapy with *p53* alone or combined with electrochemotherapy using cisplatin in two human colon carcinoma cell lines, HT-29 and LoVo, each with a different *p53* status.

To evaluate the cytotoxic effect of combined treatment, the cells were treated with plasmid DNA encoding wild type *p53*, cisplatin or electroporation, or combinations of these treatments. We determined the status of the *p53* gene in the cell lines immunocytochemically and by using *p53* gene sequencing. The permeabilization of cells after application of different electric pulses was measured by the propidium iodide fluorescence, and the cytotoxicity of the treatments by colony forming assay. Morphological evidence of apoptotic death was obtained by observing changes in cells stained with an acridine orange/ethidium bromide mixture up to 24 hours after treatment. The interaction between the treatments was calculated assuming that they have independent mechanisms of action.

The results of our study show that electrogene therapy with *p53* alone had a synergistic cytotoxic effect in both colon carcinoma cell lines HT-29 (*p53 mt, homozygous*) and LoVo (*p53 wt*) regardless of the *p53* status. Electrogene therapy in combination with electrochemotherapy also had a synergistic cytotoxic effect in both cell lines; The effect was more pronounced in HT-29 cells, where survival of cells after electrogene therapy combined with electrochemotherapy was significantly lower (P=0.001) than with electrogene therapy with *p53* alone. Cytological analysis of cells exposed to treatments combined with cisplatin showed a greater proportion of apoptotic cell death.

In conclusion, our study showed that electrogene therapy with *p53* alone or in combination with electrochemotherapy synergistically reduced the survival in both human colon carcinoma cell lines.

The influence of Mg ions on the efficiency of gene electrotransfer

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Gene electrotransfection is a method, that transfers DNA into living cells using electric pulses. The process is complex and is composed of several steps: binding of DNA to cell membrane, translocation of DNA across cell membrane and into the nucleus and expression of DNA.

Binding of DNA to the cell surface is the first step of the electrotransfection of cells.

Since Mg^{2+} ions where shown to act as a bridge between negatively charged DNA and cell membrane and thus increase interaction, we studied the influence of Mg^{2+} ions on gene electrotransfer and survival of cells *in vitro*.

Electropermeabilization was performed on 24 hours old Chinese hamster ovary cells grown as a monolayer culture. Before application of pulses we removed culture medium from cells and incubated them with the plasmid DNA that codes for GFP (green fluorescent protein) in a specific electroporative buffer, that had different concentrations of divalent cations (2 mM, 4 mM, 6 mM, 8 mM, 10 mM and 50 mM Mg). Trains of four rectangular pulses, duration: 200 μs, repetition frequency: 1 Hz, pulse amplitude: 60 - 280 V were applied. The distance between a pair of two parallel wire stainless steel electrodes was $d = 0.2$ cm.

After the pulsing fetal calf serum (FCS) was added (25% of sample volume). Treated cells were incubated for 5 minutes at 37° C to allow cell membrane resealing and then grown for 24h in cell culture medium at 37° C in a humidified 5% CO2 atmosphere in the incubator. Cell concentration in all media was 5×10^4 cells/ml and plasmid DNA concentration was 10 μg/ml. Efficiency of gene transfection was determined by fluorescent microscopy (Zeiss 200, Axiovert, Germany).

In general, electroporation efficiency increased with increasing electric field strength in all media tested. Our experiments suggest that within the range of $MgCl_2$ used (from mM to 8 mM) the transfection efficiency increased with increasing concentration of ions. But when we used higher concentrations (10 mM and 50 mM) of Mg ions the efficiency of gene transfection dropped significantly. The observed drop in gene transfection could be due to the fact, that divalent cations either bridge negatively charged plasmid DNA to negatively charged cell membrane with high affinity, or as it was shown in some experiments divalent ions (mostly Mg^{2+}) increase activity of DNAase, which consequently decreases the transfection rate.

Effects of model organophosphorous pesticides on DNA damage and proliferation of human hepatoma HepG2 cells

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Organophosphorous compounds (OPs) are the most commonly used pesticides. The primary mechanism of OP toxicity is the inhibition of acetylcholine esterase in the nervous system leading to a variety of acute and chronic effects. Recent studies have revealed several other targets of OPs that disturb non-cholinergic biological systems. We investigated whether low concentrations of model OPs, methyl parathion, methyl paraoxon and dimefox, induce DNA damage and/or affect cell proliferation in human hepatoma HepG2 cells. Genotoxicity of OPs was evaluated using the Comet assay. The effect on cell proliferation was tested using the MTT assay and proliferation marker Ki-67 immunocytochemistry. The effects of OPs on mRNA expression of DNA damage responsive genes: p53, p21, GADD45α and MDM2 were determined using qRT-PCR. Methyl parathion induced DNA damage at lower concentrations (1 μg/mL) than methyl paraoxon (100 μg/mL), whereas dimefox did not induce DNA damage. Methyl parathion and methyl paraoxon caused a reduction of cell proliferation at their highest concentrations (100 μg/mL) while dimefox increased cell proliferation at all concentrations used (0.01-100 μg/mL). Methyl parathion and methyl paraoxon upregulated expression of DNA damage responsive genes while dimefox upregulated expression of p53, downregulated expression of p21, and had no effect on the expression of MDM2 and GADD45α. We conclude that methyl parathion and methyl paraoxon are genotoxic, while dimefox shows mitogenic activity. The important finding of this study is that methyl parathion, which has lower acute toxicity and is more extensively used, had higher genotoxic potential than methyl paraoxon. This warrants for further investigations in order to correctly evaluate the hazard of exposure to these chemicals.

p17

Dimerization of endogenous MT1-MMP is a regulatory step in the activation of the 72 kDa gelatinase, MMP-2, on fibroblasts and fibrosarcoma cells

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Matrix metalloproteases (MMPs) are centrally engaged in the processes of extracellular matrix turnover that occur during cancer invasion. An important MMP cascade reaction is initiated by the membrane-anchored matrix metalloprotease, MT1-MMP, which serves to activate the proenzyme form of the secreted gelatinase, matrix metalloprotease-2 (MMP-2). This reaction occurs in an interplay with the matrix metalloprotease inhibitor, TIMP-2, and the proposed mechanism involves two molecules of MT1-MMP in complex with one TIMP-2 molecule. To study this, as well as other roles of MT1-MMP, we have now raised a panel of monoclonal antibodies against the protein. These antibodies have been raised in MT1-MMP knock-out mice and react against conserved epitopes in murine and human MT1-MMP. Using one of these antibodies we provide positive evidence that proMMP-2 activation is governed by dimerization of MT1-MMP on the surface of fibroblasts and fibrosarcoma cells. The antibody in question binds specifically to MT1-MMP on the cell surface, as shown by immunofluorescence experiments. It is directed against the hemopexin domain of MT1-MMP and has no effect on the catalytic activity of the protease domain. The antibody induces dimerization of the endogenous MT1-MMP on the cell surface. Through this reaction, it markedly stimulates the formation of the 62 kDa active MMP-2 and the processing into a 59 kDa product that retains gelatinolytic activity. This effect is indeed a consequence of MT1-MMP dimerization because it requires the divalent monoclonal antibody with no effect being obtained with monovalent Fab fragments. Since only a negligible level of proMMP-2 activation is obtained with MT1-MMP expressing cells in the absence of dimerization, our results identify the dimerization event as a critical level of proteolytic cascade regulation.

p18

Construction of eukaryotic expression plasmid with DNA damage responsive p21 gene promoter

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One of the problems in cancer gene therapy is uncontrolled antitumor transgene expression that can lead to normal tissue toxicity and consequently poor therapeutic index. To overcome this problem, a DNA damage responsive *p21* gene promoter can be used upstream of the transgene to restrict its expression to the radiation field only, where DNA damage appears.

To test the potential use of *p21* promoter in radiation controlled gene therapy, construction of suitable plasmids is necessary. Therefore, the aim was to construct eukaryotic expression plasmids containing p21 promoter upstream of the reporter genes GFP and DsRed and therapeutic gene *mIL-12*, and to perform preliminary testing of their expression induced by following irradiation in *in vitro* conditions.

For construction of the plasmids 5' flanking region of the *p21* gene was cut out of the plasmid wwp-Luc by *EcoRI* and *SalI* restriction enzymes, and about 2.4 bp length gene fragment containing the promoter region of *p21* gene with *p53* recognition site was obtained. A *GFP* expression plasmid was constructed by inserting the *p21* promoter into the multiple cloning site of the pEGFP vector after removal of *CMV* promoter using *NheI* and *AseI* restriction enzymes and blunt end ligation. mIL-12 encoding sequence was excised from the plasmid pORF-mIL-12 with *SalI* and *NheI* restriction enzymes and a *mIL-12* expression plasmid was constructed by cutting the p21-EGFP plasmid with compatible enzymes *SalI* and *XbaI* to replace the *GFP* encoding sequence with *mIL-12* encoding sequence. DsRed encoding sequence was cut out of the pCLRF35DsRed with NcoI and NheI and cloned to pORF-mIL-12 plasmid to obtain new restriction sites needed for subsequent cloning. DsRed encoding sequence was then cut out again, this time using SalI and XbaI restriction enzymes, and GFP encoding sequence in p21-EGFP plasmid was replaced.

For *in vitro* tests, LPB cells were transiently transfected with p21-EGFP plasmid. After 24h cells were irradiated with single 0.6 and 6 Gy irradiation doses to induce the p21 promoter and 24 h later expression of downstream reporter gene GFP was detected using Tecan fluorescence microplate reader and fluorescence microscopy. Preliminary results show upregulation of GFP expression after 6 Gy irradiation.

Eukaryotic expression plasmid with radiation inducible *p21* gene promoter p21-EGFP, p21-DsRed and p21-mIL-12 where successfully constructed and tested and are now available for further *in vitro* and *in vivo* studies.

Chemical acylation improves membrane permeability of cystatin

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The effective delivery of therapeutic proteins to the site of action is of great importance in achieving an effective therapy. Due to hydrophilicity, proteins are generally poorly transported across biological membranes. Chemical acylation with reactive fatty acid derivatives represents one of the basic methods for increasing their hydrophobicity and improving membrane permeability. Hereby, fatty acyl chains are covalently linked to the free amino residues forming a stable amide bond.

We developed a novel method for acylation of proteins using *in situ* prepared acyl chloride dispersion in aqueous acetonitrile solution, which allows protein modification under very mild conditions. Chicken cystatin, a reversible 13 kDa inhibitor of papain-like cysteine proteases, was selected as a model protein due to its high potential to inhibit intracellular cathepsins. The protein was modified using fatty acyl chlorides with 6, 8, 10, 12, 14, 16, and 18 carbon atoms.

Based on the cell culture assays, we examined the transport properties of unmodified and acylated cystatin, its efficiency to inhibit intracellular enzymes, its cytotoxicity, and immunogenicity. We demonstrated that acylated cystatin rapidly internalized into the cell and caused a complete loss of cathepsin B activity. This effect was shown to depend on the lenght of the fatty acyl chain – an increase of the chain lenght resulted in an increase of lipopfilicity and consequently, in a significantly enhanced membrane permeability. In contrast, the unmodified cystatin did not inhibit the intracellular enzymes because it was unable to enter the cells. Additionally, acylated cystatin didn't show any citotoxicity or immunogenicity.

These results strongly suggest that chemical acylation is a very effective strategy for improving cell uptake of protein molecules.

p20

Role proteases of Kupffer cells in murine tumor development and metastasing

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Activated macrophages (Mphs) play the important role in the degradation of ECM due to production of MMPs (Overall, Butler, 2007). Gadolinium chloride (GC) is a rare lanthanide which induced selective depression of liver Mphs *in vivo* has been used to abrogate Mph function to understand their role in pathology. Kupffer cells was shown to be enriched by cathepsin D, cathepsin B, as well as MMPs (MMP-2, MMP-13). The aim: to evaluate the role of proteases of Kupffer cells in tumor growth and metastazing process.

The concentrations of MMP-2 (R&D ELISA kits, USA) and TIMP-1 (BioRay ELISA kits, USA) was assayed in tumor tissue, serum and liver homogenate of CBA/C57Bl mice with Lewis lung adenocarcinoma – a solid tumor metastasing into lung. Cathepsins B, L and S activity was measured by fluorometric methods (Kirschke, Barrett, 1981) with inhibitors. Gadolinium concentration in tumor and liver tissue was assayed by adsorption spectrometer (Jober Ivon, France) after single intravenous administration of GC (10 mg/kg) to mice.

Gagolinium was shown to concentrate mainly in liver of non-parenchymal cells, and tumor Mphs were able to uptake of gadolinium (up to 5 % from the dose administered). GC pretreatment or treatment (before metastases forming, at the 4-5th days after tumor transplantation) significantly decreased the rate of tumor metastazing into lung. Electron microscopic morphometric study revealed that GC reduced the number of liver Mphs, decreased cathepsin B and cathepsin D specific activity in liver tissue. Cathepsin B activity in lung tissue increased during onset of metastazing. Increased production of TNF-α by new "phenotype" of liver Mphs, recruited after GC administration, was followed by increased TIMP-1 concentration in liver (not in serum) and decreased level of MMP-2 (ECM remodeling and increased mobility of liver Mphs).

Remodeling of ECM after abrogation of Kupffer cells *in vivo* plays the role in murine tumor development and prevention of metastazing.

Comparison of the results for the JAK2 V617F mutation detection by three methods; allele specific PCR and two real-time quantitative PCR on polycythemia vera and essential thrombocythemia DNA samples

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JAK2 V617F is a clonal acquired mutation found in the majority of patients with polycythemia vera (PV), and a significant number of patients with essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). The incidence of this mutation ranging from 65% to 97% in PRV patients, from 35 % to 70 % in ET patients and about 50 % in MMM depending on the study. These variations in percentage of patients involved is likely due to the criteria used for diagnosis and also the sensitivity of the assay used to detect this mutation. Allele specific PCR and different quantitative real-time PCR (RQ-PCR) based methods are usable techniques for its detection.

The aim of this study was to compare the results for the JAK2 V617F mutation detection on DNK of granulocytes of the PV and ET patients by three frequentlly used techniques. The results were compared to that published in the literature.

The diagnosis of ET and PV was established following World Health Organization classification. 19 patients (median age 69 years) with PV and 70 patients (median age 56 years) with ET were inculded in the study. EDTA peripheral blood was drawn and used for the isolation of the granulocytes after ficoll density centrifugation. DNA was isolated from granulocytes by QIAamp® DNA Mini Kit from Qiagen. The allele specific PCR was carried out as described in Baxter EJ *et al.* (Lancet 2005; 365:1054-1061). RQ-PCR were carried out by JAK2 MutaScreen™ Kit and JAK2 MutaQuant™ Kit (both Ipsogen).

Two patients (1 ET, 1 PV) that were negative by qualitative allele-specific PCR, were proven to be positive for the JAK2 V67F mutation by both RQ-PCR methods. However, there was also a PV patient that was negative by JAK2 MutaScreen™ Kit, but was positive by other two methods. The percentage of positivity for JAK2 V617F mutation in ET and PV patients were 63 % (44/70) and 100 % (19/19), respectively. The results were calculated by JAK2 MutaQuant™ Kit.

We obtained the best sensitivity for detection of JAK2 V617F mutation on DNA samples from granulocytes from peripheral blood by JAK2 MutaQuant™ Kit. The negative detection of JAK2 V617F by JAK2 MutaScreen™ Kit in PV patient that has been proven to be positive for the mutation by other two methods must be studied further. The percentage of positivity for JAK2 V617F mutation in ET and PV patients was similar to that published in the literature and was in the upper part of the range.

Plasma-mediated attractive interaction between membraneous structures as a possible suppressive mechanism of tumor progression

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Microvesicles shed from membranes of different cells can be considered as extracellular organelles which convey communication between distant cells. In particular, tumor-released microvesicles in cancer patients are suggested to be involved in tumor progression. It was recently shown that the plasma protein-mediated attractive interaction between phospholipid membranes could in the budding process cause adhesion of the bud to the mother membrane. Since in the *in vivo* conditions, the budding of cell membranes leads to the release of microvesicles into the circulation, a hypothesis is put forward that the ability of plasma to cause adhesion between membranes may prevent microvesiculation and thereby supresses progression of cancer. According to the hypothesis, in cancer patients with plasma which induces a more pronounced adhesion between membranes, the number of MVs in the peripheral blood is smaller. The hypothesis was tested on 5 patients with gastrointestinal cancer and 16 patients with other gastrointestinal diseases. The extent of plasma–induced adhesion between membranes was determined by assessing the average effective angle of contact between giant phospholipid vesicles created by electroformation method; larger average effective angle of contact corresponds to a more pronounced adhesion. The number of microvesicles isolated from peripheral blood was determined by flow cytometry. It was found that patients with gastrointestinal cancer had larger number of microvesicles (difference 140%, statistical significance 0.033) and smaller average effective angle of contact (difference 20%, statistical significance 0.013) compared to patients with other gastrointestinal diseases, which is in favor of the above hypothesis.

p23

Tumor blood flow modifying changes after application of different sets of electric pulses

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Electroporation is a method that by application of direct current electric pulses to the tumors induces cell permeabilization which is nowadays widely used to increase cell uptake of poorly permeant chemotherapeutic drugs and molecules such as DNA, antibodies, enzymes and dyes. Besides increased drug delivery, application of electric pulses to the tumors induces tumor blood flow modifying effect and vascular disrupting effect. The aim of this study was to determine the effects of different sets of electric pulses on tumor blood flow and their relation to antitumor effectiveness.

Subcutaneous SA-1 tumors were exposed to four different sets of electric pulses; the first two were composed of 8 high voltage electric pulses, at 1300 V/cm, 100 μs, with electric pulse repetition frequencies of 1 Hz or 5 kHz; the third was the combination of 1 high (1300 V/cm) and 8 low (140 V/cm, 50 ms, 2 Hz) voltage pulses and the fourth was composed of 8 electric pulses at 600 V/cm, 5 ms and 1 Hz. The first two sets of electric pulses are usually used in drug delivery (ECT, electrochemotherapy) and the last two sets are used for delivery of DNA molecules (EGT, electrogene therapy). The changes in tumor perfusion were measured by tumor staining with Patent blue. In addition, stereological analysis on whole area of tumor sections stained with hematoxylin and eosin to estimate necrosis and caspase-3 to estimate apoptosis were performed.

Electroporation of SA-1 tumors with all sets of electric pulses induced an immediate and profound reduction in tumor perfusion (80-90%). Tumors treated with ECT pulses started to reperfuse very quickly thereafter and within 24 h reached approximately the 70% perfused tumor area compared to pretreatment value. In contrast, the kinetics of tumor reperfusion after treatment with the sets of EGT pulses with lower amplitude and longer duration resumed more slowly and tumor blood flow was 5 days after the treatment still reduced up to 47%. The decreased tumor blood flow correlated with increased rate (up to 2-times) of tumor necrosis and apoptosis after the treatment. ECT pulses induced fast changes in tumor histology observed already 24 h after the treatment, whereas the tumor histological changes caused by EGT pulses gradually increased with maximal peak 72 h after the treatment.

Electroporation of tumors with (ECT and EGT pulses) high and low voltage electric pulses induced immediate and profound reduction in tumor blood flow. The duration of tumor blood flow reduction was more pronounced after application of EGT pulses. Consequently, the lack of nutrients and oxygen supply as well as changes in tumor and endothelial cells after the electroporation lead to tumor cell killing by necrosis and apoptosis, which correlated well with antitumor effectiveness.

Post-translational modification of 6-phosphofructo-1-kinase in cancer cells

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The fact that cancer cells are able to maintain high aerobic glycolytic rates, thereby producing high levels of lactic acid, is known for decades. This phenomenon known as Warburg effect has been observed in several different tumor cell lines. In malignant cells enhanced glycolysis has been shown to correlate with cellular proliferation and disease progression. For the increased flux of glucose through Embden-Meyerhof pathway altered activities of 6-phosphofructo-1-kinase (PFK1) have been suggested. Since the cancer cells are not inherently glycolytic, some modifications of PFK1 enzyme might occur simultaneously with the transformation of normal cells to neoplastic.

Recently, two step post-translational modification of the native PFK1 enzyme was described in filamentous fungus *Aspergillus niger*. After proteolytic cleavage of 85 kDa native enzyme inactive 49 kDa fragment was formed that was activated by phosphorylation. Kinetic studies showed shorter fragment to be resistant for citrate inhibition, while specific intracellular activators, like fructose-2,6-phosphate, ammonium ions and ADP stimulated its activity to a higher lever in comparison to the native enzyme. Such post-translational modification in *Aspergillus niger* cells resulted in up-regulated glycolytic flux, causing increased level of tricarboxylic acid cycle intermediates that finally resulted in enhanced anabolic reactions.

By immunoblotting homogenates from various neoplastic cell lines, no native PFK1 enzyme could be detected, while a number of lower molecular weight fragments were found. In contrast, only the native PFK1 enzyme was observed in normal human cells using identical method. To verify post-translational modification under the *in vitro* conditions, PFK1 enzyme was isolated from the rabbit muscle. Purified PFK1 enzyme was incubated with various proteases and tested for the presence of an active shorter fragment. In a buffer containing citrate, which functions as a strong inhibitor of the native enzyme but not of the shorter fragment, PFK1 activity was detected after the cleavage with proteinaze K. The fragment formed after limited proteolysis was of about 45 kDa. A similar protein band was detected in neoplastic cell lines.

The results obtained so far suggest that post-translational modification of the native PFK1 enzyme could occur in the cancer cells leading to the formation of highly active citrate resistant shorter form of the enzyme.

Sodium iodide method is suitable for DNA isolation from serum of colorectal cancer patients

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Tumor-derived DNA has been detected in plasma or serum of a variety of cancer patients and may have potential as a tumor marker. A major limitation is that majority of these circulating DNA molecules are small DNA fragments that are usually lost substantially in the course of DNA isolation. Efficient DNA extraction is therefore essential to subsequent detection of tumor markers. In the present study, we isolated DNA in 12 pooled serum specimens from 52 colorectal cancer patients using 4 isolation approaches. These include conventional Phenol-chloroform method, commercial ZR serum DNA kit, sodium iodide method and widely used QIAamp blood Midi kit. DNA yield was calculated based on quantification by Quant-iT dsDNA High-sensitivity assay. Purity of isolated DNA was assessed using Taqman real time PCR technique with E-cadherin gene as the target gene. Quality of isolated DNA was further evaluated by analysis of bisulfite conversion of DNA isolated by two most productive isolation protocols. Bisulfite conversion was assessed by real time PCR on a fragment devoid of CpG site of β-actin gene. Although DNA yield was not performed in QIAamp blood Midi kit-isolated DNA due to utility of carrier nucleic acid, phenol-chloroform method and sodium iodide method produced significantly higher DNA yield compared to ZR method (Mean, 486.72 ng/ml; 361.3172 ng/ml; 24.32 ng/ml, respectively, $P < 0.05$ after Bonferroni correction). Interestingly, the sodium iodide method generated the highest level of E-cadherin gene copies among 4 protocols tested. It also outperformed the phenol-chloroform method in the bisulfite conversion efficiency. In addition, the sodium iodide procedure is simple, rapid and non-costly. Our data clearly demonstrated that the sodium iodide method seems to be a suitable method for circulating DNA extraction in cancer biomarker study.

p26

Increased permeability of cell membrane by sonoporation

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A cell membrane separates the interior of the cell from its exterior. The function of the cell membrane is to maintain stable cell's interior by regulating amounts and types of molecules that enter or leave the cell. Sometimes however we need to deliver molecules into the cell, which otherwise can not pass the membrane. Electroporation (application of high voltage electric pulses) is one of the most widely used techniques that allow access to the cell interior. Electroporation uses high voltage electrical pulses to make the cell membrane transiently permeable. In the past years, it has been demonstrated that ultrasound could cause similar effects. This ultrasound-mediated increase in cell membrane permeability has been termed sonoporation. Sonoporation was initially demonstrated using 20 kHz sonication.

The main purpose of this study was to evaluate if the application of megahertz frequency ultrasound leads to enhanced membrane permeabilization and molecular uptake. We used a 1-cm diameter planar 1 MHz ultrasound transducer which was leant against the chamber containing cell suspension. The transducer was driven by a function generator with the center frequency 1 MHz and power output of 10 W, 15 W and 20 W. Cell samples were exposed to continuous ultrasound for 5, 10, 15 or 20 s. The cells were suspended at a concentration of $1x10^6$ cells/ml in a medium containing a fluorescent marker (Propidium Iodide).

Cell membrane permeability was increased with increased time of cell exposure to ultrasound.

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Gene transfer into solid tumors by electroporation

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Electrogene transfer of plasmid DNA into tumors is a promising approach for the treatment of cancer. Feasibility and effectiveness of this method was already demonstrated, elaborating on pulse parameters and plasmid DNA construction. However, efficient *in vivo* use of electroporation for gene transfer into solid tumor types is still limited by low transfection efficiencies. In order to increase electrogene transfections of solid tumors some novel strategies considering tumor structural properties have to be found.

To explore the influence of histological properties of different tumor types (B16F1 melanoma, EAT carcinoma, SA-1 fibrosarcoma, LPB fibrosarcoma) on electrogene transfer, the correlation between cell density, collagen or proteoglycans content and transfection efficiencies was determined. Further, in order to increase transfection efficiency of electrogene transfer into tumors, the extracellular matrix of tumors was modulated by enzymes hyaluronidase or/and collagenase before electrotransfection.

Our results demonstrate that soft tumors with spherical and larger cell size, low proteoglycans and collagen content and low cell density are more effectively transfected (B16F1, EAT) than more rigid tumors with high proteoglycans and collagen content, small and spindle-shaped cells and high cell density (LPB and SA-1). Modulation of extracellular components with enzymes collagenase and hyaluronidase can increase electrogene delivery into rigid tumor types such as fibrosarcomas. The highest transfection efficiencies were obtained when combination of enzymes were used.

In conclusion, our study demonstrates that knowledge about histological properties of tumors is important for development of of novel strategies to deliver sufficient amounts of plasmid DNA to the tumor cells by electroporation. Namely, electrogene transfer might be improved by modulation of tumor extracellular matrix with enzymes such as collagenase and hyaluronidase.

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Development of novel magnetic nanoparticles based drug delivery systems for cancer treatment

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The development of new effective drug delivery systems for the treatment of cancer is one of the top priority trends in the biomedical technology of the last decade. Among the different methods of drug delivery, magnetic drug targeting could be a promising approach by possibility of specific delivery of chemotherapeutic agents using magnetic nanoparticles and an external magnetic field which is focused on the tumor. Recently, magnetic nanoparticles have attracted additional attention because of their potential as contrast agents for magnetic resonance imaging and heat mediators for cancer therapy. Currently there are two approaches for the use of magnetic nanoparticles in targeted drug delivery: (i) chemotherapeutic agent is coupled directly to the nanoparticles or, (ii) both the drug and the magnetic nanoparticles are encapsulated into the lipid vesicle, forming magnetoliposomes. The considerable potential advantages of liposome encapsulation are the prevention of local dilution of the drugs or contrast agents and limitation of their interactions with biological media into which they are administered.
Recently the major progress has been made in our current understanding of the role of lysosomal cysteine proteases (i.e. cathepsins) in several pathological states, such as cancer. There is increasing evidence that cysteine cathepsins contribute to the proteolytic events during tumour progression and metastasis. Thus, in our study we aim to examine the efficiency of cathepsin inhibitor therapy using magnetoliposomes based delivery system in a transgenic mouse breast cancer model.

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Reversal of thiopurine cytotoxicity in lymphoblasts by S-adenosylmethionine

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6-mercaptopurine (6-MP) is a pro-drug widely used for the treatment of acute lymphoblastic leukemia. It requires a multi-step enzymatic conversion to the active 6-thioguanine nucleotides (6-TGNs) in order to induce cell death by incorporation into DNA. The detoxification of the drug is catalyzed by thiopurine S-methyltransferase (TPMT), a polymorphic enzyme that converts 6-MP in 6-methylmercaptopurine by S-methylation. The cosubstrate S-adenosylmethionine (SAM) acts as a methyl donor in the reaction but also stabilises the 3D structure of TPMT. It has also been shown that 6-MP prevents SAM recycling in MOLT cells due to ATP depletion. The formation of methylated 6-MP metabolites (eg. 6-methyl-5'-thioinosine monophospate, MeTIMP), also catalyzed by TPMT, results in the inhibition of purine de novo synthesis and has been described as an additional thiopurine-induced cytotoxic pathway. In this study, the effect of SAM on TPMT activity was examined by its ability to modulate 6-MP-induced cytotoxicity in MOLT lymphoblasts.

Our results suggest that the addition of exogenous SAM (10-50 μM) rescues cells from cytotoxicity after 48h of induction by 6-MP (5-10 μM). Specifically, we showed by means of viability and cell proliferation assays, fluorescence microscopy and flow cytometry, that SAM restores cell proliferation, prevents DNA fragmentation and S phase arrest while promoting the progression into the G_2/M phase of cell cycle.

To investigate the molecular basis for the reversal of 6-MP-induced cytotoxicity after the addition of SAM, we measured the amount of intracellular active metabolites responsible for cell death by high-performance liquid chromatography (HPLC). We found that in the cells treated with both 6-MP and SAM, the amount of cytotoxic 6-TGNs and MeTIMP was significantly lower than in the cells treated with 6-MP alone.

Finally, in order to examine the effect of SAM on TPMT stability, we used HPLC and flow cytometry for the determination of TPMT enzyme activity and the level of immunoreactive protein, respectively, in order to correlate these results with the level of TPMT mRNA as determined by Taqman gene expression assay. The results show that the incubation with 6-MP results in lower TPMT activity and protein level than in cells treated with both 6-MP and SAM, while the amount of TPMT mRNA is unchanged. Therefore, the effect of SAM is restricted to protein stabilisation rather than the increase of TPMT expression.

In summary, we showed that SAM contributes to higher stability and hence activity of TPMT, which consequently methylates and inactivates 6-MP and reduces its devastating effect on MOLT cells. This study provides new insights into the pharmacogenetics of thiopurine drugs by indentifying the status of SAM as an important parameter of TPMT activity and, consequently, thiopurine therapy-related toxicity.

Monoclonal antibody 2A2 and its association with cathepsin B

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In our previous studies a monoclonal antibody 2A2 was prepared, which specifically binds cathepsin B and inhibits its enzymatic activity. Significant inhibition of tumour cell invasion *in vitro* and *in vivo*, internalization of the antibody into tumour cells and its ability to retain binding affinity for cathepsin B at neutral and acid pH suggest possible application in treatment of cancer and other diseases with increased cathepsin B activity. Fab fragments and chimeric human/mouse antibody were also prepared in order to enhance cell internalisation and to reduce immunogenicity during cancer therapy.

Using SPOT analysis we identified the epitope for 2A2 Mab at the occluding loop of the cathepsin B and we propose that the antibody may regulate endopeptidase/exopeptidase activity of the enzyme. Kinetics of the interaction between 2A2 Mab and cathepsin B was determined by Surface Plasmon Resonance. The results show that 2A2 preferentially binds cathepsin B/cystatin C complex compared to free enzyme. As shown by native PAGE the binding of the monoclonal antibody 2A2 or its Fab fragment to cathepsin B/cystatin C complex causes dissociation of cystatin C from the complex. Additionally, synthetic peptides, mimicking the epitope or adjacent parts of the molecule have been prepared and tested for binding to the components of the extracellular matrix.

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Quantification of viability in organotypic spheroids of human malignant glioma for drug testing

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The prognosis for patients with glioblastoma multiforme (GBM) remains poor, despite surgical resection, radiotherapy and temozolomide chemotherapy. To screen for novel therapeutics established cell lines or primary cell cultures are customarily used. However, the correlation between *in vitro* and *in vivo* responses is poor. Organotypic spheroids are grown from primary explants from surgical specimens and provide a more complex biological system than monolayer cell cultures, that maintains cell-cell interactions, extracellular matrix and cellular heterogeneity. We found that the cancer genomic profiles of 5 original GBMs and their OSs compared better than that of their primary cell culture using whole genome array comparative genetic hybridization, thus proving that the OS model is genetically more representative for GBM[1]. Accurate and reproducible quantification of therapeutic responses in the OS model has been lacking. For this purpose, lactate dehydrogenase (LDH) activity was demonstrated in cryostat sections of spheroids[2]. Calibrated digital image acquisition of the stained cryostat sections enabled demarcation of LDH-active and LDH-inactive tissue areas by thresholding at specific absorbance values. The viability index (VI) was calculated as ratio of LDH-active areas and total spheroid tissue areas. Duplicate staining and processing on the same tissue showed good correlation and therefore reproducibility. As a positive control to reduce viability, sodium azide incubation of spheroids induced reduction in VI to almost zero. However, standard therapy such as temozolomide and other positive controls such as cyanide did not show any effect on viability indicating either lack of diffusion and/or multidrug resistance of the cancer cells. We also determined sample size requirements for a valid screening strategy: how many spheroids per experimental group and how many sections per spheroid are required to detect one-third reduction in the VI after treatment? Because of the large biological variation of the VI (20%), at least 12 spheroids per group and 1 section per spheroid are required[3]. We conclude that quantification of viability in cryostat sections of OSs from GBM can be performed reliably and reproducibly with this approach. Furthermore, OSs represent GBM genetics better than primary cell cultures. Unfortunately, the large number of spheroids prevents high-throughput screening and the lack of response to treatment can either be an artefact of culture conditions or a phenomenon of glioma cells.

References:

1. De Witt Hamer PC, Van Tilborg AAG, Eijk PP, Sminia P, Troost D, Van Noorden CJF, IJlstra B and Leenstra S (2007) The genomic profile of human malignant glioma is altered early in primary cell culture and preserved in spheroids. Oncogene, publication ahead of print. doi: 10.1038/sj.onc.1210850.
2. De Witt Hamer PC, Jonker A, Leenstra S, Ruijter JM, and Van Noorden CJF (2005) Quantification of viability in organotypic multicellular spheroids of human malignant glioma using lactate dehydrogenase activity: a rapid and reliable automated assay. J. Histochem. Cytochem. 53:23-34.
3. De Witt Hamer PC, Leenstra S, Van Noorden CJF and Zwinderman AH (2008) Cancer spheroids for screening of experimental therapies: how many spheroids and sections are required? J. Microsc. in press.

Human colon cancer xenografts are sensitive to TRAIL induced apoptosis *in vivo via* death receptor upregulation

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The tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of TNF family, is particularly interesting for its unique properties to induce death of cancer cells (including colon) while sparing most normal cells. Controversial results exist about the sensitive of primary cells to TRAIL, therefore a better insight is required. Primary human cancer epithelial cells were derived from patients with colon cancer at different stages and characterised *in vitro* and *in vivo*. Both primary cell lines, PAP60 and MIH55, were found to be highly proliferative with increased transforming capability and tumourigenicity as concluded by their ability to form tumour in SCID mice. Increased apoptosis independent of p53 was observed in both primary PAP60 and MIH55 cell lines after treatment with SuperKiller TRAIL as detected by an increase in PARP cleavage and activation of caspase-3. Expression analysis of death receptors (DR) by means of RT-PCR and flow cytometry performed in the original parental tumours, the primary cultures before and after engraftment as well as the mouse xenografts, revealed a significant upregulation of both DR4 and DR5, with the latter being the most differentially expressed. DR expression pattern analysis correlated to differences in sensitivity of the cells to TRAIL induced apoptosis. Treating patient tumour xenograft/ SCID mouse models with Killer TRAIL *in vivo* for 5 consecutive days suppressed tumour growth and upregulation of DR4 and DR5 that directly correlated to its antitumour activity. This is the first demonstration of TRAIL induced apoptosis in characterised tumourigenic primary human cultures (*in vitro*) followed by the antitumour activity in xenograft models (*in vivo*).

Electrochemotherapy of cutaneous metastases of mammary carcinoma in the mammary region after radical radiotherapy

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Electrochemotherapy has proved its clinical effectiveness in treatment of various malignancies, including cutaneous recurrent mammary carcinoma. Furthermore, its clinical benefit was also demonstrated for tumor nodules that regrew in pre-treated areas, after surgery or radiotherapy. In mammary carcinoma patients, local recurrence in mammary region poses a specific problem, especially after radical irradiation. Multiple cutaneous nodules are usually dispersed, and not amenable to surgical resection. Furthermore, they are often exulcerated, and additional radiotherapy is not possible because tissue tolerance would be exceeded due to previous radical irradiation.

Among the electrochemotherapy treated patients at the Institute of Oncology Ljubljana, were also two patients with multiple cutaneous tumor nodules of mammary carcinoma in mammary region. The patients were previously treated by surgery and radiotherapy. Further treatment options were exhausted, and they agreed for electrochemotherapy. Electrochemotherapy was performed on 10 tumor nodules; their size ranging form 1 to 8 cm in diameter. Electrochemotherapy was performed in two consecutive sessions using intratumoral injection of cisplatin, and application of electric pulses to the tumor nodules using plate electrodes. The treatment was performed in local anesthesia, in ambulatory way. The same day the patients were released home without any side effects.

After two weeks the treated nodules were in partial response. The scab was formed and in the next 8 weeks in 92% treated nodules complete response was observed. Loco regional disease after electrochemotherapy did not pose any problem to these patients. Up to two years after the treatment there was no sign of local progression of the disease. However, the disease progressed in visceral organs.

The described two cases demonstrate treatment effectiveness of electrochemotherapy in patients with recurrent disease after radical radiotherapy. In such patients it is of vast importance to have another line of treatment, because the nodules are usually exulcerated and mutilating and there are no other treatment options.

References:

1. Reberšek M, Čufer T, Čemažar M, Kranjc S, Serša G. Electrochemotherapy with cisplatin of cutaneous tumor lesions in breast cancer. Anti-Cancer Drugs 2004; 15: 593-7.
2. Whelan MC, Larkin JO, Collins CG, Cashman J, Breathnach O, Soden D, O'Sullivan GC. Effective treatment of an extensive recurrent breast cancer which was refractory to multimodal therapy by multiple applications of electrochemotherapy. Eur J Cancer Suppl 2006; 4: 32-4.

Importance of electrophoretic force for successful gene electrotransfer for suboptimal plasmid concentrations

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Electroporation is a versatile biotechnology technique that among others enables gene electrotransfer, i.e. transfer of DNA into biological cells. High-voltage pulses induce structural changes in the cell membrane that thus becomes transiently permeable for ions, molecules and macromolecules. Gene electrotransfer is already an established method for gene delivery *in vitro* and *in vivo*. Currently, majority of research is focused on improving *in vivo* transfection efficiency and first clinical trials are in progress, while mechanisms involved in electrogene transfer have not been yet completely understood.

In this paper we analyze the mechanisms of gene electrotransfer by using combinations of high-voltage (HV) and low-voltage pulses (LV) *in vitro*. We applied different combinations of HV and LV pulses to CHO cells and determined the transfection efficiency using plasmid DNA coding for green fluorescent protein (GFP). Our results show that short HV pulses alone are sufficient to successfully deliver DNA into cells when optimal plasmid concentrations were used. For optimal plasmid concentration LV pulse did not contribute significantly to the transfection efficiency, in contrast to the reported results of several *in vivo* and one *in vitro* study, where combinations of HV and LV pulses markedly increased transfection efficiency compared to protocols where only HV pulses were applied. With this we demonstrated that short HV pulses are not only crucial for efficient permeabilization of the cell membrane, which enables transfection, but are alone sufficient to successfully deliver DNA into cells *in vitro* for optimal plasmid concentrations. Therefore in general it is difficult to separate the role of HV pulses as being only permeabilizing and LV pulses as being electrophoretic since both effects are usually presented in both cases.

However, for suboptimal plasmid concentrations we obtained that LV pulses which follow HV pulses increase transfection rate similarly as in *in vivo* conditions. Our results therefore suggest that low-voltage pulses are contributing to increased transfection in *in vivo* conditions due to limited mobility of plasmid DNA in the extracellular matrix resulting due to low local plasmid concentration. Namely, low-voltage pulses provide additional electrophoretic force which drags DNA toward the cell membrane and contributes to better interaction of the DNA with the membrane and better transfection efficiency, while for optimal concentrations of the plasmid (usually used *in vitro*) electrophoresis does not have important role.

Cytotoxic and genotoxic influence of nano-sized titanium dioxide particles in human hepatoma cells HepG2

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Titanium dioxide (TiO_2) is widely used in the production of cosmetics, paints, paper, plastics and welding rod-coating material because of its low toxicity. However, recent studies have shown that TiO_2 nanoparticles (<100 nm in diameter) can cause inflammation, fibrosis, pulmonary damage and also DNA damage, which generates a concern about the possible adverse effects of TiO_2 nanoparticles for exposed humans. Generally it is believed that these adverse effects are predominantly due to the generation of reactive oxygen species (ROS). However, data on potential effects of TiO_2 nanoparticles on human cells are still very scarce.

The aim of our study was to apply a test system with human hepatoma cells HepG2 to evaluate potential cytotoxic and genotoxic effects of TiO_2. Two sizes of TiO_2 particles (5 nm and 10x40 nm) were chosen and the cells were exposed to different doses (1 – 250 μg/ml) of sonicated particles for different periods of time (2, 4, 24 and 48h). The cytotoxicity of particles for HepG2 cells was determined by the MTT assay. Intracellular ROS formation was determined with 2-,7-dichlorofluorescein diacetate fluoroprobe. The genotoxicity of TiO_2 particles was measured with classical comet assay and oxidative DNA damage was determined with the modified comet assay with purified oxidative DNA damage-specific enzymes: endonuclease III (EndoIII) and formamidopyrimidine-DNA glycosylase (Fpg), which specifically recognize and digest oxidized pyrimidines and purines, respectively. Using flow cytometry we determined whether TiO_2 particles induce apoptosis by observing loss of cell membrane asymmetry with annexin V-FITC staining.

Both sizes of the particles at applied treatment conditions reduced the viability of HepG2 cells. Significant increase of intracellular ROS formation was detected only in cells exposed to 5 nm TiO_2 particles. Exposure of cells to 5 nm TiO_2 or 10x40 TiO_2 induced slight increase in % tail DNA, while oxidative DNA damage was significant. With annexin-FITC staining we saw that both sizes of TiO_2 particles have induced loss of cell membrane asymmetry in a dose-dependent manner, indicating induction of early stages of apoptosis. Taken together these results indicate that TiO_2 nanoparticles are not cytotoxic, but they induce intracellular ROS formation, oxidative DNA damage and apoptosis.

CD44 and CD155 expression on human glioma *in vitro*: a flow cytometric, immunocytochemical and TIRF microscopy study of invasion indicators

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The poliovirus receptor, CD155 (PVR) is expressed on neoplastic glia and has already been used in therapeutic targeting of glioma. Recently CD155 has been proposed as playing a key role in glioma motility and invasion. CD44 is a cell adhesion molecule, originally described as the lymphocyte homing receptor, which is has two isoforms with respective molecular weights of 80-90kDa and 150kDa. The lower molecular weight isoform mediates attachment to hyaluronic acid (HA), which is present in relatively high concentration within the brain. CD44 is over-expressed in CNS and mediates both mediates glioma cell adhesion and invasion. Moreover, CD155 resides proximal to CD44 on the cell membrane of monocytes. The interactive role of the two molecules, CD44 and CD155, therefore merits investigation in the context of brain tumour invasiveness. Our aims were, therefore, to evaluate the expression levels of, and spacial relationship between, CD44 and CD155 in cultured glioma at high and low passage.

High and low passage *in vitro* cultures of glioma were immunocytochemically stained using CD44 and CD155 antibodies and imaged by epi-fluorescence. Quantitative analysis was obtained by flow cytometry and the special relationship between the two epitopes on the cell surface was elucidated by total internal reflected fluorescence (TIRF) microscopy.

Immunocytochemistry showed both CD44 and CD155 to be expressed at high and low passage numbers of glioblastoma *in vitro*. Double staining revealed both antigens to be expressed on the cell membrane at close but distinct sites. Flow cytometry revealed a higher expression level of CD44 compared with CD155 on all cultures tested.

Having shown that these two molecules are co-expressed and are closely apposed on the cell membranes of glioma cells and are both involved in migration and invasion will are now aiming to carry out live cell imaging and Transwell™ Boyden chamber assays. In these experiments the influence of monoclonal antibody blocking and siRNA knock-down for both molecules, singularly and together will be used to establish any synergy or additive effect of CD44 and CD155 on glioma invasion.

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Antiproliferative and adhesion-stimulating effects on human cancer cell lines elicited by lectins from mushroom *Clitocybe nebularis*

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Fungi are well known for their medicinal values and numerous bioactive compounds with pharmacological properties have been isolated from mushrooms. Lectins are a diverse and ubiquitous group of non-homologous proteins that specifically bind different types of carbohydrates. Most lectins are multivalent, containing more than one carbohydrate-binding site; therefore they can cross-link cell surface carbohydrates and agglutinate cells. Their biological activity is exerted through binding to glycoconjugates on cell surfaces, extracellular matrix and cytoplasmic or nuclear glycoproteins. Lectins elicit a variety of effects in different organisms and are suggested to have many biological functions which are still not fully understood.

In this study, several lectins were isolated from fruiting bodies of mycorrhizal basidiomycete fungus Clouded agaric (*Clitocybe nebularis*) by affinity chromatography using carbohydrates (glucose, galactose, sucrose, lactose) as ligands. Lectins were purified using HPLC and their molecular masses were determined by mass spectrometry. They were shown to be oligomeric by gel filtration and native gel electrophoresis. Hemagglutination and hemagglutination inhibition assays with human type A, B, 0, AB and bovine erythrocytes were performed to determine lectins specificity and they were shown to be pH and heat stable. Their N-terminal and internal amino acid sequences were determined by protein sequencing and degenerative PCR primers were designed to obtain genomic and cDNA nucleotide sequences using genome walking and reverse-transcription PCR as well as rapid amplification of cDNA ends methods. Deduced amino acid sequence was compared to protein sequences in databases. Effects on proliferation and adhesion were studied on human breast epithelial tumour cell line (MCF-10A NeoT), human T leukaemia cell line (Mo-T), differentiated and undifferentiated human monocytic lymphoma cell line (U-937).

From among isolated proteins, lactose-specific and galactose-specific lectins showed the most potent effects on human cancer cell lines. Galactose-specific lectins stimulated plastic adherence of differentiated U-937 cells, suggesting that they interact with integrins and activate integrin-mediated adhesion. Lactose-specific lectin exerted antiproliferative effect on human T leukaemia cell line. This lectin has a potential use in treating haematopoietic malignances so complete genomic and cDNA sequences were obtained. The gene consists of five exons and four introns. Comparing a deduced amino acid sequence to protein databases using BLASTP, SMART and Pfam tools revealed that lactose-specific lectin belongs to a ricin B-like lectin protein family.

Arsenite and the lysosomal cysteine cathepsins in cell death of U87 glioblastoma cells

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We are studying the mechanisms of action of arsenic trioxide (As_2O_3), arsenite, which is clinically approved chemotherapeutic drug for acute promielocytic leukemia, on human glioblastoma cells. Glioblastoma multiforme (GBM) is a highly resistant and recurrent form of brain tumour and arsenite therapy has also been tested in clinical trials with GBM. Lysosomal cathepsins L and B (Cat L and B) are gradually upregulated in malignant gliomas, indicating their role in the progression of the disease. The aim of this work is to investigate their possible involvement in glioblastoma cell response to cytotoxic arsenite during therapy.

We have already shown that arsenite inhibits proliferation, induces autophagic features and apoptosis in an established GBM cell line, U87. The earliest response (~5h after to arsenite treatment) was reactive oxygen species production. This was followed by induction of apoptosis, accompanied by an increased Bax/Bcl-2 ratio and transient decrease in the mitochondrial membrane potential, resulting in effector caspase 3/7 activation. Interestingly, at the same time point (24h after arsenite addition) autophagic features could be observed.

CatL has been proposed as an mediator of drug resistance and our laboratory has previously shown that CatL down-regulation increased the apoptotic response of U87 to inducers of intrinsic (staurosporine) and extrinsic (tumour necrosis factor alpha) apoptotic pathways. The same appeared to be the case after arsenite treatment, as CatL silencing (by si-RNA) significantly increased the activity of caspases 3/7. On the other hand, down-regulation of CatB had no effect on caspase 3/7 activity, but rather reduced the number of characteristic features of authophagy. The results point to a different role for both Cats during arsenite treatment of U87 cells. The increased response after CatL inhibition could be helpful in lowering the arsenite dose and thus minimising its toxic side effects.

On the other hand, arsenite treatment also induces changes in CaL and CatB expression and activity, lowering CatB and increasing CatL activity. Possible feedback mechanisms of this phenomenon on autophagy and apoptosis will be discussed.

Mushrooms as a source of antitumour substances

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For centuries, mushrooms have been valued as an edible and medical resource. They represent a vast and yet largely untapped source of powerful new pharmaceutical products. Among the most important ones are substances with immunomodulatory and antitumour properties, of which high molecular weight polysaccharides are the best known, although fungal lectins have recently gained some research attention. Our research is focused on proteolytic systems of higher basidiomycetes. We have shown that mushrooms are a rich source of diverse proteases and their inhibitors that show unique features possibly exclusive to basidiomycetes.

Proteolytic enzymes play important part in tumour progression. Four families of proteases have been implicated in the process of tumour progression and metastasis, namely matrix metalloproteases, cysteine proteases, serine proteases and aspartic proteases. Cysteine cathepsins, lysosomal cysteine proteases, have been implicated in multiple steps of tumour progression, including processes of cell transformation and differentiation, motility, adhesion, invasion, angiogenesis and metastasis. Cysteine cathepsins are promising drug targets for treating cancer, however due to complexity of their pathophysiological roles, understanding appropriate cysteine cathepsins to target at each stage of tumour development and progression is crucial for development of specific inhibitors for therapeutic use. Protein inhibitors of cysteine proteases from basidiomycetes offer unique biochemical features and inhibitory spectra unlike that of any other family of cysteine protease inhibitors. Clitocypin, cysteine protease inhibitor from the mushroom clouded agaric (*Clitocybe nebularis*), for example, inhibits cathepsins L and K, but does not inhibit cathepsins B and H.

Recently, serine proteases, namely tissue kallikreins, have also been directly linked to neoplastic progression, similarly as cathepsins showing stimulatory or inhibitory effects in many phases of tumour progression. Protein inhibitor of serine proteases, CNSPI from the clouded agaric mushroom (*Clitocybe nebularis* serine protease inhibitor) exhibits unique biochemical and inhibitory properties, as it strongly inhibits trypsin and chimotrypsin, weakly pancreatic kallikrein while elastase is not affected.

Mushroom-derived protein inhibitors of cysteine or serine proteases that show unique features of selectivity and specificity reveal mushrooms as a valuable source of protease inhibitors that could find use in research towards understanding the role of individual proteases in different stages of tumour progression as well as in drug development and design for anti-tumour or antiprotease therapy of cancer patients.

Effect of heparin on microvesiculation of membranes. A possible mechanism of treatment of Trousseau syndromme

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Trousseau syndromme in a strict sense is defined retrospectively when a thromboembolic event preceeds a diagnosis of cancer, while in a broader context it includes all hypercoagulabile states connected to cancer. Heparin was found to be a successful drug not only in preventing thromboembolic events but also in slowing down the development of some types of cancer. Other anticoagulant drugs (e.g. warfarin) did not prove successful in the latter. As microvesiculation of membranes is enhanced both in hypercoagulabile states as well as in cancer, a hypothesis of anticoagulant and anti-tumor-progression effects of heparin is put forward. According to the hypothesis, heparin mediates an attractive interaction between membranes and therefore suppresses microvesiculation by adhesion of buds to the mother membrane. The effect of heparin on membranes was tested in *in vitro* system of giant phospholipid vesicles created by electroformation and observed under the phase contrast microscope. It was found that addition of heparin into the suspension of giant phospholipid vesicles causes a time and concentration- dependent adhesion between negatively charged vesicles as well as between neutral vesicles. The above hypothesis was tested on a population consisting of 14 patients treated by low-molecular weight heparin due to orthopaedic surgery and 36 controls. Microvesicles were isolated from peripheral blood and counted by flow cytometry. It was found that the number of microvesicles was lower in the group which received low-molecular weight heparin than in the control group. The difference (32%) was statistically significant (p=0.02). These results indicate a possible additional anticoagulant and an anti-tumor-progression effect of heparin by suppression of microvesiculation.

Avoiding systemic toxicity of the TNF superfamily ligands: induction of cell death without ligands

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Tumor necrosis factor alpha (TNF-α) is a cytokine capable of inducing hemorrhagic necrosis and sometimes even full regression of tumors *in vivo*. Unfortunately, systemic administration of TNF-α as an anti-cancer drug is not possible because of its severe adverse effects. Lately, TNF-α analogues with potent antitumor activity and much lower systemic toxicity have arisen, diminishing, but not completely eliminating adverse effects of TNF-α therapy. Two other members of TNF superfamily, FAS ligand and TRAIL have also been extensively investigated over the last two decades.

Signal transduction begins with ligand binding to the receptor resulting in clustering of receptors on the extracellular side of the membrane. A cluster of at least two receptors is required for induction of a conformational change in the intracellular part of the receptor triggering the activation of the downstream cascade. To avoid ligand toxicity an interesting alternative approach would be anti-cancer therapy without ligands.

In order to achieve clustering of the receptors without ligands, we introduced histidine tags (HIS-10) into the extracellular part of the TNF-R1 molecule. Interactions of histidines with transition metal ions in biological systems have been known for a long time and they have also been successfully employed for purification of histidine rich proteins in Immobilized Metal Affinity Chromatography (IMAC). In our case, HIS-10 tags in the extracelullar parts of TNF-R1 receptors could serve for association via Zn^{2+} ions and biologically compatible chelating molecules, such as phytic acid and TETA (1,4,8,11-Tetraazaciclotetradecane-1,4,8,11-acetic acid). This could lead to clustering of receptors and consequently to activation of the downstream cascade.

HIS-10 tag has also been introduced into the truncated TRAIL-R1 molecule (in which the extracellular part of the TRAIL-R1 molecule was deleted) to prove that clustering of receptors is enough for inducing the downstream cascade and the extracellular parts of TRAIL-R1 receptors are not required for signal tranduction. This approach would be applicable for the whole superfamily of TNF receptors and also other situations where signaling depends on protein clustering.

Limb sparing treatment of bleeding melanoma recurrence by electrochemotherapy

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Electrochemotherapy is an antitumor treatment that increases intracellular drug delivery by applying electric pulses. In addition, it was also found to have a vascular disrupting effect. We report the case of limb sparing treatment of bleeding melanoma recurrence by electrochemotherapy in a patient with a bleeding melanoma recurrence. After intravenous application of bleomycin (15 000 IU/m2) fifteen runs of electric pulses were applied by hexagonal needle electrodes (1.7 cm in diameter) in the center of the lesion while additional 10 runs of electric pulses were delivered via plate electrodes (8 mm) on the rim of the tumor. Immediately after administration of electric pulses the bleeding stopped and did not reccur. The crust formation was observed and the lesion decreased in size in a matter of weeks. We conclude that electrochemotherapy should be considered as a treatment option in dealing with bleeding melanoma reccurrences as well as a limb preserving treatment.

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Advantage of nanoparticles to deliver resveratrol into the cells

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Several studies have been demonstrated that polyphenolic compound resveratrol (RSV) exerts striking cancer chemopreventive activity. What is more, investigations of resveratrol biological effects exposed out its dual action, as it has been reported to participate in prosurvival as well as prodeath cellular mechanisms, depending on cellular conditions, specific cell molecular settings and the concentration used. Additionally, low solubility and physical instability makes RSV real technological and medical challenge.

Thus, to allow RSV controlled release at the target site over a prolonged period of time and to protect it from light or other degradative processes solid lipid nanoparticles (SLN) with loaded RSV were prepared. Effects of RSV on morphology of the treated cells and the localization of nanoparticles were investigated. Parallel their metabolic activity of HEK 293 was determined and compared with results obtained with RSV alone. Ability of test dispersions to cope with stress situation (UV-B light) was also checked.

In non radiated as also in radiated cells free RSV at 10 μM was well accepted by the cells, while RSV at 100 μM cause an arrest in cell proliferation.

The effect of loaded SLN was expressed different regarding the treatment-following conditions (non radiation or radiation). Interestingly, in non radiated cells treated with RSV-loaded nanoparticles a significant increase of the metabolic activity was seen, irrespective of RSV's concentration and also elimination of the RSV's cytotoxicity at 100 μM was observed.

However, RSV in lipid nanoparticles at 10 μM lost their beneficial effect when used on radiated cells. Thus promotion of cell proliferation under stress conditions caused by UV-B light was obtained only with high amount of RSV (100 μM) loaded in nanoparticles.

Above results are supported also by fluorescence pictures that have showed any detectable alterations on cell morphology after their treatment with almost all test dispersions, exception is only RSV alone at 100 μM leading to the strange shape of nuclei surrounded with degradable or completely disappeared actin.

Ours results stressed out an importance of RSV's concentration used to achieve protective effect in normal cells, which can be transformed into cancer cells when exposed to the stress condition (radiation). Cell destruction can be prevented only with higher amount of RSV, which is unfortunately two-edged. However, loading of RSV into solid lipid nanoparticles proved to be the most cell-benefit decision. Slowly releasing of RSV from nanoparticles prevented the cells before a massive cellular distribution of RSV that can lead to its destroyable pro-oxidative effect. Amount of released RSV is just enough to cope with the oxidative stress and thus cell ability to survive is increased.

Combined effect of pulsed electric and magnetic field on CHO cells dye uptake

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The effect of pulsed electric field on biological cells such as electroporation has been a subject of many studies in the past. Electroporation as used in electrochemotherapy, increases the permeability of the cell plasma membrane due to pore creation in the membrane and membrane ruffling which in turn results in enhancing cell dye uptake (Biophysics.J. 60 (1991) 297-306). We suggest that the synergy of conventional electroporation accompanied by other means of energy like applied time-varying magnetic field (which results in induced electric filed) may increase cell dye uptake due to ruffling enhancement (Experimental Cell Research, 297 (2004) 348–362). The aim of our study was to investigate the effect of time-varying magnetic field on transmembrane molecular transport – dye uptake by CHO cells exposed to combined pulsed electric and magnetic field. In order to determine the best set of parameters like the shape, size and position of the magnetic coil under which the energy coupling can be maximized, simulation analysis was performed. Results of this simulation indicate that maximum energy coupling between magnetic coil and sample containing cells is obtained with 8 shaped coils. The coil needs to be positioned in the horizontal plane so that the sample is positioned directly under the centre of coil where the two windings meet. The smaller the radius of the windings, the greater the energy coupling. This energy coupling is higher at closer distances between the magnetic coil and the sample containing cells. Employing this simulation results, we measured in an experimental set up, the variations of dye being taken up by CHO cells using Lucifer Yellow (Biochimica et Biophysica Acta 1668 (2005) 126–137). The influence of magnetic field in our experiments is characterized for both situations in which it applies (in the range of minutes), namely before the electric field application as well as after itapplication, compared with the presence of only electric field.

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The influence of hypoosmolar medium and electric field on cell volume

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Cell electrofusion is a process where two or more cells exposed to electric field, which are in close contact, fuse. The consequence of exposure to pulses is transient and nonselective permeabilization of cell membranes, which is necessary for fusion of cell membranes. Besides optimal electrical treatment, good physical contact between cells is necessary for achieving cell fusion. In this context, it is understandable that fusion of adherent cells is common event, while it is difficult to obtain sufficient contact between cells in suspension.

Cell swelling facilitates the processes of electrofusion. For achieving contact between cells in the process of electrofusion, different methods are used, which use either electrical (in dielectrophoresis) or mechanical forces (in centrifuge, on filters...). Cells swell, when they are suspended in hypoosmolar medium. Knowledge of swelling kinetics could contribute to optimization of the methods for contact achievement.

We determined the kinetics of cell swelling for three different cell lines: Chinese hamster ovary cells CHO, Mouse melanoma cells B16F1 and Chinese hamster lung fibroblasts V79. All cells under investigation start swelling immediately after exchanging the isoosmolar medium (10 mM phosphate buffer, 1 mM $MgCl_2$, 250 mM sucrose, pH 7,2 and low conductivity 0,12 S/m) with hypoosmolar (75 mM sucrose). However, they reach their maximum size value at different time after the change (between 2 to 5 minutes) and differ in the speed of shrinking as well. We observed diameters of cells for 30 minutes after the medium exchange. In this period, cells shrunk to their original size due to regulatory volume decrease. When we kept cells in isoosmolar medium, they did not change their size.

Cell swelling is also observed after electropermeabilization. We determined the influence of combination of electropermeabilization and hypoosmolar medium on cell size. We electroporated cells 1 minute after exchanging the medium, that is before cells reach their maximum size. We used electrical parameters, which are optimal for transient permeabilization of cells in isoosmolar medium. Electropermeabilization abolished the effect of regulatory volume decrease during the 30 minutes of our observation.

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TAF12 is regulated by *ras*, to mediate the alteration of E-cadherin expression in epithelial-to-mesenchymal transition (EMT) (1)

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Activating mutations in the RAS proto-oncogene result in alteration of gene expression leading to tumorigenesis (2). The TFIID complex, composed of the TATA box-binding protein (TBP) and its associated factors (TAFs), regulates the initiation of transcription. TFIID components are altered by cellular signals to specifically redirect transcription. Notably, TBP is regulated by Ras to allow full cellular transformation. To investigate further links between TFIID and the RAS-induced carcinogenesis, we monitored the expression of TBP and TAF in human colon carcinoma cells (1). We identified TAF12 levels as being up-regulated in cell lines bearing natural RAS mutations and having some epithelial to mesenchymal transition (EMT) characteristics. TAF12 expression was further enhanced via a MEK-dependent mechanism, in an EMT-model cell line obtained after stable overexpression of a mutated RAS isoform in Caco-2 cells (2). We also showed *in vivo* and *in vitro* that the ETS1 protein was involved in the up-regulation of TAF12 expression during RAS transformation. Interestingly, reduction of TAF12 levels by siRNA enhanced E-cadherin mRNA and protein levels and reduced migration and adhesion properties of RAS transformed cells with EMT (1-3). Overall, our study indicates the importance of TAF12 in the process of Ras-induced transformation properties of human colon cells, most notably those related to increased motility, by specifically regulating expression of E-cadherin.

References:
1. Voulgari et al., under revision
2. Roberts ML, Drosopoulos KG, Vasileiou I, Stricker M, Taoufik E, Maercker C, Guialis A, Alexis MN, Pintzas A., 2006, Int J Cancer
3. Andreolas C, Kalogeropoulou M, Voulgari A, Pintzas A., 2007, Int J Cancer

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Xanthohumol may affect cancer progression with its differential cytotoxicity and apoptosis induction in normal and cancer cell lines

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Xanthohumol (XN), is the major prenylated flavonoid of the female inflorescences of the hop plant, used as a preservative and flavouring agent in beer. It has various cancer-related and antioxidant activities. Consequently, XN has been suggested as cancer chemo preventive agent (1). Resistance to apoptosis observed in cancer cells, is a hallmark of cancer progression. In our previous study, we have observed selective cytotoxicity of XN to certain cancer cells and its effect on cell apoptosis. Here, we extend our research on several normal and cancer human cell lines aiming to determine the cytotoxicity of XN and to explore its ability to induce and enhance apoptosis in cancer cell lines in contrast to normal cells. The cell lines selected for this study were NHA, HUVEC, NC-NC, MCF10A (the non-cancerous cells) and HepG2, U87, NCI H1299 and MiaPaca2, the cancer cell lines. Cytotoxicity of XN at 1-100 μM concentration was determined by MTT assay for adherent, and by MTS assay for non-adherent cells. The apoptosis was determined by the ratio of mRNAs of proapoptotic Bax and anti apoptotic Bcl2 genes, by the activity of effector 3/7caspases and by fluorescent dyeing of the cells. XN is cytotoxic for cancer cells at lower concentrations then for normal cell lines, but their LD50 ranges overlap, mainly due to different origin of the cells. However, when the cells of the same origin were compared, glioblastoma cells (U87) were more sensitive to XN then normal human astrocytes NHA (LD50 at 35μM and 60μM, respectively). Bax/Bcl2 ratio, caspase activity and differential staining show much higher apoptosis levels in cancer versus normal cells at higher XN concentration (50μM). Differential cytotoxicity of XN may therefore contribute to its selective induction of apoptosis. We conclude that XN may represent a good candidate, not only for chemoprevention at low doses (2), but also as therapeutic tool at higher concentration.

References:
1. Stevens JF and Page JE. Phytochemistry, 65: 1317-1330, 2004.
2. Plazar et al., Mutation Res. 632, 1-8, 2007.

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Optimization of electrode position and electric parameters in electrochemotherapy

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Electrochemotherapy – a combination of locally applied high voltage electric pulses and locally or systemically administered chemotherapeutics drugs – has in recent years been shown to be a very effective treatment of cutaneous and subcutaneous tumor. Successful electrochemotherapy requires that the whole tumor volume is subjected to a sufficiently high electric field, while the electric field in the surrounding healthy tissue is as low as possible to prevent damage; both can be achieved with appropriate positioning of the electrodes and appropriate choice of the treatment electric parameters. We used 3D finite element numerical models and a genetic optimization algorithm to determine the optimum electrode configuration and electric parameters for treatment of three subcutaneous tumor models of different shapes and sizes and a realistic brain tumor model that was acquired from medical images. We compared two existing parallel needle electrode arrays and a hexagonal needle electrode array and determined that parallel electrode arrays were the best choice in all four tumor cases. While the magnitude of electric field was sufficiently high in all cases, the optimum parallel electrode configurations required less electric current and caused less healthy tissue damage when compared to the hexagonal electrode arrays. Our optimization algorithm was able to determine the best electrode configuration in all four presented models and with further improvement it could be a useful tool in clinical electrochemotherapy treatment planning.

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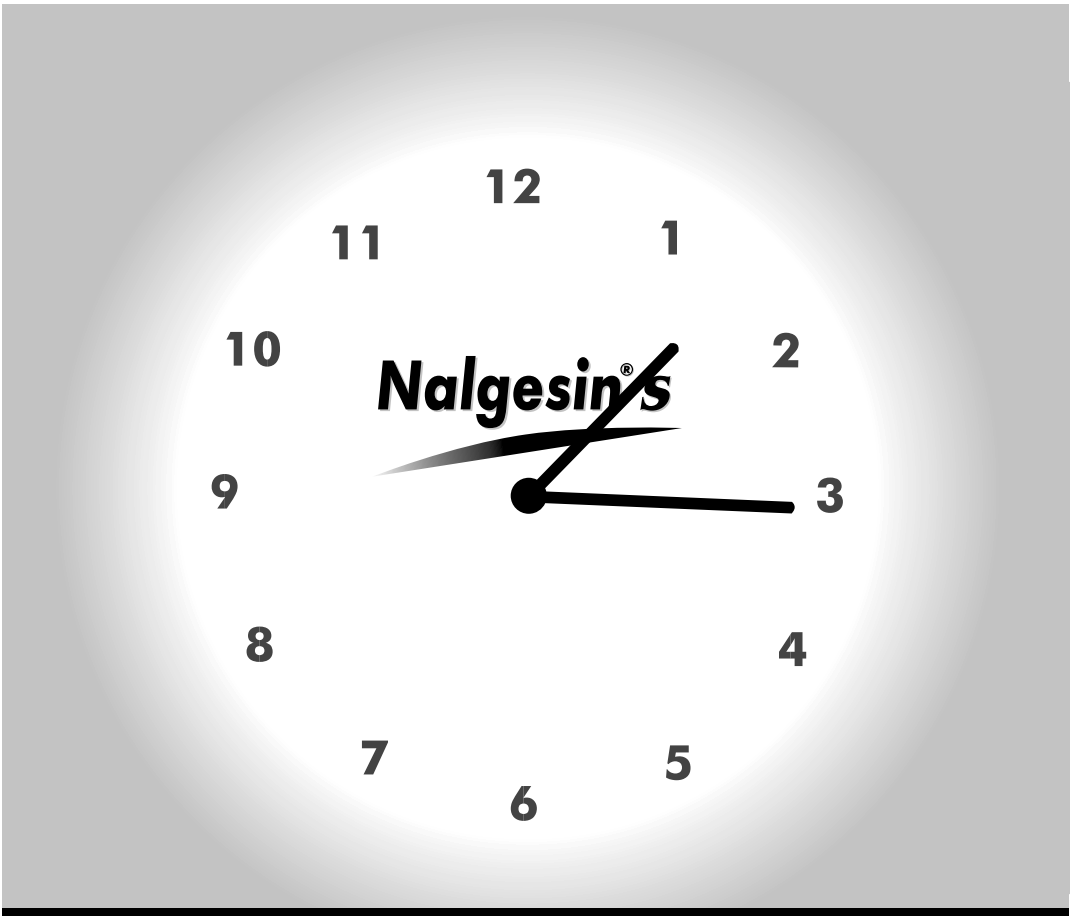

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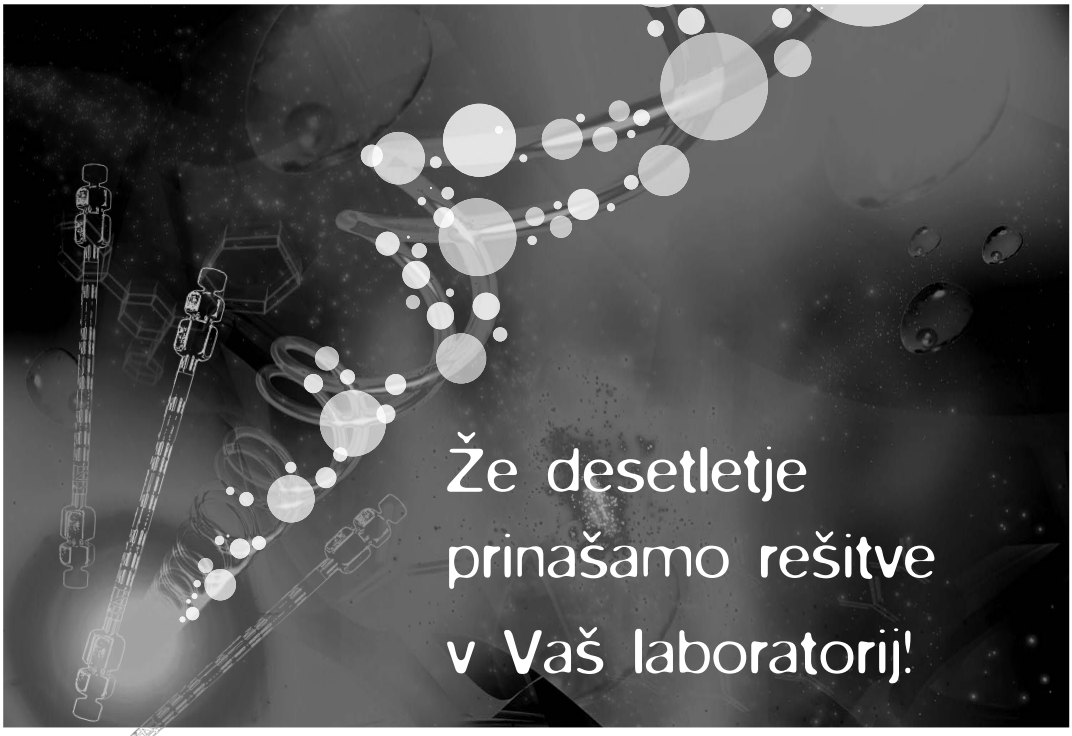

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