

## Telomerase in lung cancer diagnostics

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**Background.** Telomerase is a ribonucleoprotein that looks after the telomeric cap of the linear chromosomes maintaining its length. It is over expressed in tumour tissues, but not in normal somatic cells. Therefore the aim of this study was to determine the telomerase activity in lung cancer patients as novel marker for lung cancer detection evaluating the influence of tissue/cell obtaining technique.

**Material and methods.** Using the TRAP (telomeric repeat amplification protocol), telomerase activity was determined in material obtained from bronchobiopsy (60 lung cancer patients compared with 20 controls) and washings from transthoracic fine needle aspiration biopsy performed in 10 patients with peripheral lung tumours.

**Results.** Telomerase activity was detected in 75% of the lung cancer bronchobypsies, and in 100% in transthoracic needle washings.

**Conclusions.** Measurement of telomerase activity can contribute in fulfilling the diagnosis of lung masses and nodules suspected for lung cancer.

*Key words:* lung neoplasms - diagnosis; telomerase; bronchoscopy

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### Introduction

Lung malignancies represent a repeated problem in the respiratory clinics constantly arousing attention due to the rapid evolution, extremely bad prognosis and frightening number of new patients every following year. Lung cancer is the most frequent pathology among all malignancies with more than

1000 000/year, and the main cause of death in 29% in this pathology. The distribution shows dominance in the developing countries with 61%, mainly due to the early presence of smoking habits.<sup>1,2</sup> The 5-year survival is the lowest one, compared to the most frequent cancers: (colon 63%, breast 83%, prostate cancer 93%).<sup>2,3</sup> It remains alarmatic low: from 8% in 1960 to 14% 2001. On the other hand there is a dramatic difference in the 5-year survival, related to the stadium of the disease. The localized form of the disease shows survival up to 40% (IA=67% IB=57%, IIA=55%, IIB=39%, IIIA=23%), compared to the extensive form of the disease -only 14%. The emergency need to deal with this highly fatal disease pointed out

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that the main interest in this field has to be: developing and using methods for early detection of lung cancer such as sputum cytology, native chest radiogram, spiral chest CT, fluorescence bronchoscopy and molecular markers for malignancy.<sup>2,3</sup>

One of the most important factors in establishing the cellular integrity are the telomeres, specialized structures of the chromosomal end in all eucariotic cells build of repetitive short DNA sequences (TTAGGG) and associated combining proteins. They serve as puffer zones against the chromosomal spending in the aging process and protectors in the degradation and recombinant process during the chromosomal junctions in the mitosis. The shortening of the telomere is the signal for stopping the cell division.<sup>4-6</sup> The enzyme telomerase is a ribonucleic protein with function of resyntetising the telomeric DNA of the chromosomal ends. It maintains the telomeric length giving the cell the opportunity for uncontrolled cellular division. Its quantity is carefully regulated, but genetic mutation and DNA damage can cause its activation or deactivation. The main characteristics of the tumour growth are avoidance of normal proliferative control so the renewal of telomeric repeats by activating the enzyme telomerase may be a path for the tumour cells to avoid senescence and death. This enzyme is normally detected only in reproductive cells and cells with self renewal capacity (bone marrow, lymphocytes, intestinal crypt cells, epidermal basal cells), but it is undetectable in normal somatic cell. The development of highly sensitive PCR-based commercial kit (TRAP) by Kim et al. in 1995 allows telomerase detection in various biosamples.<sup>7</sup>

The analysis of the telomerase activity in lung tumours was evaluated predominantly in surgical specimens (frozen samples of proven tumour tissues), after the diagnosis of lung cancer was already established. Therefore this study was designed to evaluate the role of telomerase in lung cancer diagnos-

tics<sup>6,8</sup> in fresh specimens obtained by routine lung cancer diagnostic sampling: bronchoscopy and transthoracic fine needle aspiration biopsy.

### Material and methods

The study involved 60 patients with central lung tumour, and 20 pts with pneumonia as a control group. All of the pts underwent bronchoscopy and in cases of endoscopic lesion, bronchobiopsy (1 mm) was obtained for TRAP analysis. The same was performed in the control group taking 1mm sample from bronchial mucosa. Another 10 patients with peripheral lung lesion were included, and transthoracic fine needle aspiration biopsy (FNAB) was performed. The mean smear was sent to citopathology lab and the needle washings were analysed for telomerase. Analysis of telomerase was performed in total of 80 samples of bronchial mucosa, 10 samples of FNAB.

#### *Telomerase assay*

Telomerase activity was qualitatively evaluated using the TRAP (Telomerase Repeat Amplification Protocol) of Boehringer Mannheim. Practically it is a four step process: Telomerase, if present, adds multiple 6-nucleotide telomeric repeats to a biotinylated synthetic primer. Then the telomerase reaction product is amplified by PCR, using a biotinylated primer. Denaturation follows and the PCR product hybridizes to a digoxigenin-labeled probe specific for the telomeric repeat. The last step is binding of the DNA hybrid to a streptavidin-coated microtiter plate, and anti-digoxigenin-peroxidase so TMB substrate generates a coloured product measurable with a microplate reader.<sup>9-11</sup> Statistical analysis of the data included method of clinical test evaluation (Bayesian analysis).

## Results

Telomerase positively was detected in 45 out of 60 (75%) of the positive lung cancer biopsies, compared with the controls of normal mucosa 2/20 (10%). Statistical analysis for telomerase in lung cancer biopsies showed accuracy of 80% (Ac), sensitivity of 75% (Sn), specificity of 90% (Sp) positive predictive value of 95.7% (PPV) and negative predictive value of 40.4% (NPV).

Histology analysis showed predominance of telomerase positivity in small cell lung cancer (SCLC), and the lowest activity in metastatic deposits (Table 1). Telomerase activity was analyzed in needle washings after FNAB was performed in patients with peripheral lung tumour. Telomerase was detected in all 10 (100%) samples, compared to cytology where malignancy was confirmed in 8 out of 10 (80%) samples.

## Discussion

This study design was performed in order to find out if the novel molecular marker of malignancy telomerase can be used in diagnosis of lung cancer. This question revealed three new points: how can we use telomerase, in what way and what type of samples in lung cancer diagnostics. The routine lung cancer investigation usually starts with bron-

choscopy as a basic method for obtaining samples for histopathology. On the other hand up to 90% of the endobronchial lesions are confirmed by histopathological analysis of bronchobiopsy material, but sometimes we have to repeat the procedure in order to obtain sufficient sample for diagnosis. In our study telomerase positivity was detected in 75% in lung cancer bronchobiopsies compared to non-malignant tissue (10%). Statistical analysis showed highly significant value of  $\chi^2=93.25$  and  $p<0.0001$  that proved this method to be significant in separating malignant of non-malignant tissue. Several authors such as Hiyama et al.1995<sup>12</sup>, Yang et al. 1998<sup>13</sup>, Lee et al.<sup>9</sup> 1998 Shay et al.1999<sup>14-16</sup> etc reported very high telomerase activity in lung cancer tissue 75-85%.<sup>17</sup> These results, including those from our study impose the conclusion that telomerase is one of the leading factors in development of lung cancer. Most of the telomerase studies on lung cancer were conducted on material obtained by surgical resection or lung cancer cell lines. Statistical analysis pointed out that telomerase activity is slightly higher in such material (increasing the Sn, Sp, Ac), but the PPV is the same (Table 2). This data suggests that in the clinical practice in cases where modest endobronchial changes lack histopathological conformation, and the telomerase activity is detected, the diagnostic procedure should be persistent and directed in establishing the malignant disease.

**Table 1.** Histology of lung cancer and telomerase activity

Type (Total 60)	Samples	Telomerase/+/		Sn=TP/TP+FN
NSCLC	34	26	76.5%	76.5%
SCLS	15	12	80%	80%
MS deposits	11	7	63.6%	63.6%

**Table 2.** Telomerase activity in lung cancer: surgical sampling increases the Sn, Sp, and Ac of the method compared with bronchobiopsy sampling

Sn	Sp	Ac	PPV	NPV	
Hiyama et al./1998 <sup>12</sup>	80%	94%	96%	96%	89%
Sen et al./1999 <sup>18</sup>	87%	100%	88%	96%	84%
Kovkarova et al./2002	75%	90%	81%	96%	40%

In the analysis of peripheral lung lesions, transthoracic fine needle aspiration biopsy is a basic diagnostic method for tissue sampling. Telomerase activity was determined in needle washing after the preparation of material for cytology analysis. Telomerase positivity was found in all of the samples (10/10), compared to cytology were due to massive necrosis malignancy was confirmed in 8 out of 10 samples. Sen et al.<sup>18</sup> and Naritoku et al.<sup>11</sup> established telomerase sensitivity higher than the cytology (Table 3). Naritoku stresses the value of telomerase debating that this molecular marker finally determines the blurred cytological report of rare atypical cells that often confuses the pulmonologist.<sup>12,18</sup>

Second objective to the validation of the telomerase activity was to establish any connection with the histology type of the lung cancer (Table 1). This analysis showed no link to telomerase positivity in primary lung cancers, but the lowest rate of telomerase activity was detected in the metastatic type.

Some authors like Strovel<sup>19</sup> and Allbanell<sup>20</sup> the telomerase activity can be associated with the tumour proliferation rate, response to therapy and final outcome. Quantification of these markers according to these authors can be used as valid prognostic marker of lung cancer.

These results suggest that telomerase can be used as a complementary tool in lung cancer diagnostics especially in cases where the first line diagnosis is confused and unprecise. The studies that evaluate the telomerase activity in more simple sampling such as sputum or plasma can open a new field in early lung cancer detection especially in high risk population. On the other hand telomerase

can be used as a valid target for lung cancer treatment. The pioneer attempts are already in action, but one can not forget that the human body is very complex and therefore we have to gain more experience and knowledge in this field to achieve permanent success.

## References

- Hoffman PC, Mauer AM, Vokes EE. Lung cancer. *Lancet* 2000; **355**: 479-85.
- Landis SH, Murray T, Bolden S, Wingo PA, et al. Cancer statistics, 1999. *CA Cancer J Clin* 1999; **49**: 8-31.
- Franklin WA. Diagnosis of lung cancer: pathology of invasive and preinvasive neoplasia. *Chest* 2000; **117(Suppl 1)**: 80-9.
- Arinaga M, Shimizu S, Gotoh K, Haruki N, Takahashi T, Takahashi T, et al. Expression of human telomerase subunit gene in primary lung cancer and its clinical significance. *Ann Thorac Surg* 2000; **70**: 401-5.
- Bacchetti S, Counter C. Telomeres and telomerase in human cancer [Review]. *Int J Oncol* 1995; **7**: 423-32.
- Blasco MA, Gasser SM, Lingner J. Telomeres and telomerase. *Genes Dev* 1999; **13(18)**: 2353-9.
- Kim NW. Clinical implications of telomerase in cancer. *Eur J Cancer* 1997; **33(5)**: 781-6.
- Breslow RA, Shay JW, Gazdar AF, Srivastava S. Telomerase and early detection of cancer. *J Natl Cancer Inst* 1997; **89(9)**: 618-23.
- Lee JC, Jong HS, Yoo CG, Han SK, Shim YS, Kim YW. Telomerase activity in lung cancer cell lines and tissues. *Lung Cancer* 1998; **21**: 99-103.
- Maniwa Y, Yoshimura M, Obayashi C, Inaba M, Kiyooka K, Kanki M, et al. Association of p53 gene mutation and telomerase activity in resectable non-small cell lung cancer. *Chest* 2001; **120(2)**: 589-94.
- Naritoku WY, Datar RH, Li P, Groshen SL, Taylor CR, Imam SA. Telomerase activity: comparison between fine needle aspiration and Biopsy Specimens for detection of tumor cells. *Cancer* 1999; **87(4)**: 210-5.
- Hiyama K, Hiyama E, Ishioka S, Yamakido M, Inai K, Gazdar AF, et al. Telomerase activity in small-

**Table 3.** Telomerase activity in FNAB washings of lung cancer: superior to cytology

TTAP	Telomerase/+	Cytology
Sen et al./1999 <sup>18</sup>	35/42 84%	68.4%
Naritoku et al./1999 <sup>11</sup>	14/16 88%	68%
Kovkarova et al./2002	10/10 100%	80%

- cell and non-small-cell lung cancers. *J Natl Cancer Inst* 1995; **87(12)**: 895-902.
13. Yang CT, Lee MH, Lan RS, Chen JK. Telomerase activity in pleural effusions. Diagnostic significance. *J Clin Oncol* 1998; **16**: 567-73.
  14. Shay J, Zou Y, Hiyama E, Wright W. Telomerase and cancer. *Hum Mol Genet* 2001; **10(7)**: 677-85.
  15. Shay JW, Werbin H, Wright WE. Telomerase assays in the diagnosis and prognosis of cancer. *Ciba Found Symp* 1997; **211**: 148-55.
  16. Shay JW, Gazdar AF. Telomerase in early detection of cancer. *J Clin Pathol* 1997; **50**: 106-9.
  17. Yashima K, Litzky LA, Kaiser L, Rogers T, Lam S, Wistuba II, et al. Telomerase expression in respiratory epithelium during the multistage pathogenesis of lung carcinomas. *Cancer Res* 1997; **57(12)**: 2373-7.
  18. Sen S, Reddy VG, Khanna N, Guleria R, Kapila K, Singh N. A comparative study of telomerase activity in sputum, bronchial washing and biopsy specimens of lung cancer. *Lung Cancer* 2001; **33(1)**: 41-9.
  19. Strovel J, et al. Plasma telomerase activity predicts response and relapse in lung cancer [Abstract]. Program and Abstracts of the American Society of Clinical Oncology. 36<sup>th</sup> Annual Meeting 2000; New Orleans, Louisiana. Abstract 1899.
  20. Albanell J, Lonardo F, Rusch V, Engelhardt M, Langenfeld J, Han W, et al High telomerase activity in primary lung cancers: association with increased cell proliferation rates and advanced pathologic stage. *J Natl Cancer Inst* 1997; **89(21)**: 1609-15.