Diagnostic and prognostic value of telomerase assay in lung cancer

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Submitted: 11 June 2007
Accepted: 4 March 2008

Arch Med Sci 2008; 4, 4: 353–357

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Abstract

Lung cancer is the main cause of death related to neoplastic diseases. Because of nearly asymptomatic onset of the disease, early diagnosis of lung cancer is highly unsatisfactory and only 10-15% of patients survive 5 years. It is necessary therefore to find new molecular diagnostic and prognostic markers. Telomerase, an enzyme associated with cellular immortality, is highly expressed in the majority of cancers. Assessment of telomerase activity could be helpful in differentiation between benign and malignant peripheral tumours of lungs and could improve the sensitivity of cytological examination of few-cell aspirates, suggesting malignant aetiology of the suspected lung lesions. It is also thought that high telomerase activity in tumour cells is an unfavourable independent prognostic factor of survival. This article reviews current knowledge about the importance of telomerase in carcinogenesis and its diagnostic and prognostic value in lung cancer.

Key words: lung cancer, telomerase, non-small cell carcinoma, small cell lung cancer.

Introduction

Each year about 1 200 000 people develop lung cancer worldwide [1]. Lung cancer is the most frequent of all malignant tumours and it is the main cause of deaths related to neoplastic diseases [1]. During the last two decades a 4-fold increase of deaths from lung cancer was observed in the developed countries [1, 2]. In Poland, lung cancer is responsible for about 35% of deaths of men and 14% of deaths of women with malignant neoplastic diseases [3]. The most frequent type of lung cancer is non-small cell carcinoma (NSCLC), comprising histologically heterogeneous subtypes of cancer (squamous, adenogenic, mixed, bronchio-alveolar, large cell without neuroendocrine activity) with a similar prognosis and therapeutic approach [1, 2]. Small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC) are diagnosed in less than 20% of cases [4]. According to a new WHO classification of lung tumours carcinoids form a separate group [1].

Because of nearly asymptomatic onset of the disease and quite frequent distant dissemination of locally non-advanced lung cancer, results of treatment of lung cancer are highly unsatisfactory. It is estimated that only 10-15% of patients survive 5 years [2, 5]. Survival could be improved by quick recognition of aetiology of focal lesions located in the lung

parenchyma and finding new molecular and genetic markers for predicting cancer disease progression.

According to Hanahan and Weinberg [6], there exist six fundamental routes of carcinogenesis associated with activation of different groups of genes and signals. Those acquired capabilities of mutating cells are: self-sufficiency of cell in growth signals, insensitivity to anti-growth signals, lack of apoptosis, unlimited replication, promotion of angiogenesis and tissue invasion (Figure 1) [6]. Limitless replicative potential results first of all from augmented activity of specific DNA polymerase, called telomerase.

Telomeres and telomerase

Telomeres are present at the ends of eukaryotic chromosomes. Nucleotide sequences of telomeres are very similar in different kinds of living organisms, e.g. in the protozoan *Tetrahymena* (in which telomeres were first found) telomeres consist of 70 tandem repeats of the TTGGGG sequence, while in mammals they have 2000 TTAGGG repeats [7, 8]. Telomeres protect chromosomes from enzymatic degradation and prevent chromosome-to-chromosome fusion [9]. Because of the inability of DNA polymerase to replicate DNA at the ends of chromosomes, chromosomes lose about 50-200 nucleotides per cell division [10-12]. This shortening of chromosomes is the "mitotic clock" and is responsible for cellular senescence in somatic cells, because after a number of mitoses they become unable to further divide and die [12]. Germ-line and most neoplastic cells achieve their immortality due to the activity of telomerase – the enzyme which is able to elongate telomeres after each cell division [10, 13].

Telomerase consists of a protein component, comprising telomerase reverse transcriptase and telomerase-associated protein 1, and an RNA component [13]. Telomerase RNA is a template for the elongation of telomeric repeat sequences. The RNA component of the complex is encoded by the *TERC* gene on chromosome 3q26.3, while the telomerase reverse transcriptase component is encoded by the *TERT* gene located at chromosome 5p15 [14].

In healthy mature organisms, very low telomerase activity is detectable in fast-dividing cells, such as germ cells, epithelial cells, lymphocytes or activated fibroblasts [12, 13]. Sporadic cases of high telomerase expression in a fatal course of pneumonia and cystic fibrosis have also been described [15]. It is thought that in normal, fast-renewing tissues telomerase activity is definitely lower than in malignant neoplastic tissues. For example, it has been shown that the activity of telomerase in oesophageal cancer

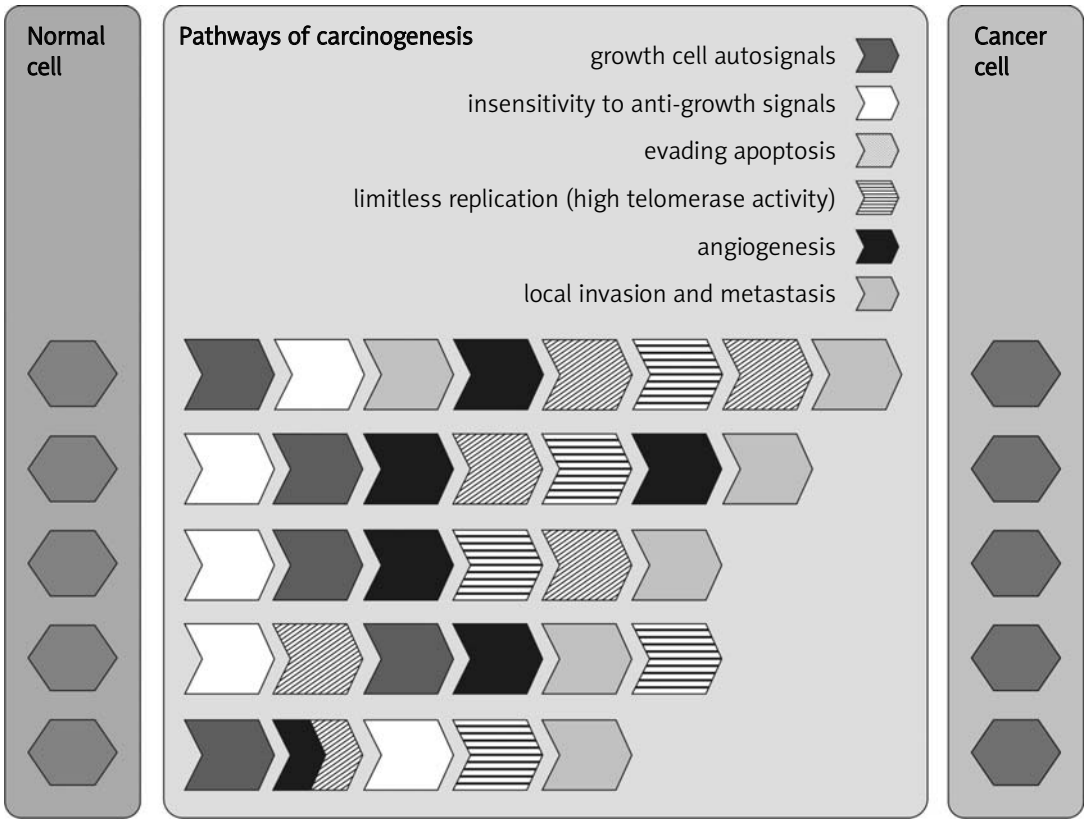
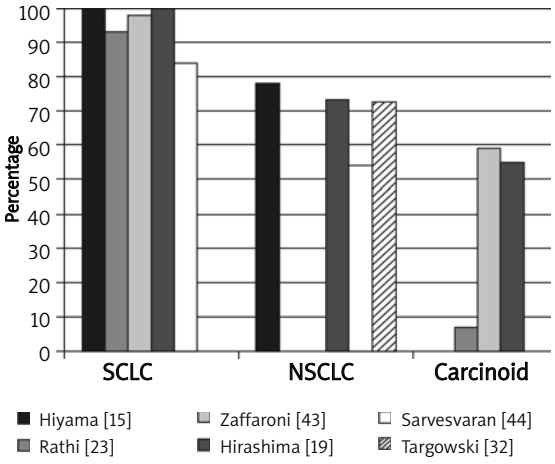


Figure 1. Different pathways of carcinogenesis and place of telomerase in these processes (acc. to [6])

cells is 600-fold higher than in diploid fibroblasts [16]. High telomerase activity is characteristic first of all for cells of malignant tumours and contributes to their uncontrolled proliferation [13].

In spite of high telomerase activity in neoplastic cells, telomeres of atypical cells are usually shorter than in normal somatic cells, probably because activation of telomerase begins when telomeres reach a critically small length after several cell divisions [7, 17].

In human neoplastic cells telomerase was first described in 1989 [7]. Five years later telomerase was found in ovarian carcinoma cells [18]. Soon after that highly increased telomerase activity or overexpression of its components was found in most malignant tumours [13, 18-22].



SCLC – small cell lung cancer, NSCLC – non-small cell lung cancer

Figure 2. Telomerase expression (% of cases) in different types of lung cancer

Telomerase activity and lung cancer

Diagnostic value

Increased telomerase activity was found in most preneoplastic lesions of the lung. It was observed in 70% of hyperplasia cases, 80% of squamous cell dysplasia and 100% of cases of *carcinoma in situ*. However, telomerase activity in these disorders is several times lower (at least 60-fold) than in invasive lung cancer [23]. Kawai et al. [24] found expression of human telomerase reverse transcriptase (hTERT) messenger RNA (mRNA) in 66% of atypical adenomatous hyperplasias and 97% of bronchioalveolar carcinomas.

It is thought that the activity of telomerase increases in proportion to the aggressiveness of lung cancer. Its high expression is observed most often in small cell lung carcinoma and most rarely in typical carcinoid (Figure 2). Numerous authors emphasize the usefulness of telomerase assessment in diagnosis of suspected few-cell specimens, for example those obtained from fine-needle aspiration biopsies. Hiyama et al. [25] found increased telomerase activity in 86% of breast cancers (confirmed afterwards in histological specimens obtained during surgery or open biopsy), whereas cytological examination was positive only in 70% of fine-needle biopsies. In 12 out of 21 patients with a high level of telomerase activity and a negative result of cytological examination breast cancer was finally diagnosed [25]. A several-percent improvement of diagnostic sensitivity of fine-needle biopsy of tumours with the combined use of cytological and local telomerase expression detection has also been found in other studies [26].

High telomerase activity has been observed in cytological negative pleural and visceral fluids in patients with lung and uterine malignancies, as well [27].

In patients with lung carcinoma, high levels of telomerase activity have been detected in sputum, bronchial washings and brushing specimens obtained during bronchofibroscopy [19, 28]. Some authors have emphasized the lack of a close association between hTERT expression in specimens from bronchial biopsy of lung cancer and corresponding resected tumours; however, they stipulated that those results need to be validated in larger cohorts [29].

Pasrija et al. [30] found telomerase activity in 67.6% of sputum samples which had been taken from patients with lung cancer and only 10% of sputum samples taken from patients without malignant tumours of the lung. The specificity and negative predictive value were 90 and 71%, respectively, making telomerase activity in sputum a promising candidate for a non-invasive biomarker of bronchial tree malignancy.

Owing to increased availability of radiological examinations, benign peripheral nodules of the lung are diagnosed even in more than 50% of the population screened for lung cancer [31]. It seems that assessment of telomerase activity could be a useful supplemental procedure in differentiation between malignant and benign tumours of the lung. In individuals with suspected lung cancer, detection of telomerase activity in bronchoalveolar liquid (BAL) increased the diagnostic efficacy of the method to 74% in comparison with 43% efficacy of BAL cytology alone [28]. In our studies [32] assessment of telomerase activity in aspirates from fine-needle transthoracic biopsy of peripheral lung nodules significantly improved the sensitivity of biopsy from 66.7% (cytology alone) to 89.3% (cytology with telomerase activity assessment). So, more than 20% of peripheral lung nodules qualified for biopsy, with increased telomerase activity (in spite of a negative cytology), were lung cancers.

Prognostic value

An additional advantage of the evaluation of telomerase activity in tumours would be the possibility of identifying patients with a potentially unfavourable prognosis. The 5-year survival rate in the case of stage I non-small cell lung cancer (T_{1-2} N_0 M_0) ranges from 38 to 61% [5]. This means that despite the radical surgical resection of non-small cell cancer approximately 40% of patients die due to disease recurrence. According to some authors a high level of telomerase activity is an unfavourable prognostic factor in lung carcinoma, not related to tumour histological type, cancer clinical stage, patients' age, sex and smoking habit [33]. It has been shown that a high level of telomerase activity in surgically resected stage I non-small cell lung cancer (T_{1-2} N_0 M_0) is associated with significantly diminished disease-free survival and overall survival [33, 34]. Fujita et al. [35] observed that in the case of NSCLC patients with high and moderate levels of human telomerase reverse transcriptase (hTERT) mRNA expression 5-year survival rates were 46.9 and 77.9%, respectively (P=0.0001). Moreover, they proved that hTERT mRNA expression is a prognostic factor of survival similar to lymph node status, pathological TNM stage and patients' age.

It has been found that telomerase activity was more often detected in patients with non-operable NSCLC (clinical stage IIIB and IV) and patients with distant metastases (stage IV alone) [36]. It was also shown that presence of telomerase activity in specimens from NSCLC tumours is related to 7 times higher relative risk of death [RR=6.9 (CI 1.8-26.8), P<0.05] and 2.5 times higher risk of cancer recurrence after radical treatment [RR=2.5 (CI 0.3-9.3), P<0.05] [36].

Recently, a new real-time RT-PCR method has been validated for the quantification of 4 individual human telomerase reverse transcriptase (hTERT) splices. It was found that in patients with NSCLC only one (α+β+) splice expression correlates with the overall and disease-free survival [37]. However, the study was conducted on a group of 28 patients with NSCLC, so it cannot be excluded that a similar correlation with survival would have been observed for other splices of hTERT if more patients had been included.

So far, the prognostic value of telomerase in small cell lung cancer (SCLC) has not been described. However, it is known that telomerase activity in an extensive stage of SCLC, where the prognosis is poor, is at least 10-fold higher than in a limited stage of SCLC, in which there is even 22% chance of 5-year survival [38].

In conclusion, evaluation of telomerase activity in specimens derived from lung tumours could improve the sensitivity of some diagnostic techniques. Moreover, assessment of telomerase activity could supplement prognostic criteria in lung carcinoma, and in consequence could even bring about modification of cancer therapy (e.g. implementation of obligatory adjuvant chemotherapy before surgical resection in patients with stage I lung tumour and high telomerase expression). High activity of telomerase in tumours seems to be a helpful marker of clinical advancement of malignant lung tumours and an independent prognostic factor of the overall survival and period free of disease recurrence.

Anti-telomerase treatment may appear to be a promising therapeutic method in the future [39]. A trial of anti-telomerase treatment using anti-sense nucleotides complementary with nucleotide sequence of telomerase RNA component was done [40]. Such treatment blocked telomerase activity and decreased the number of cell mitoses and probability of lung cancer metastases [40]. It also appeared that intradermal administration of the telomerase reverse transcriptase peptide GV 1001 (hTERT: 611-626) in 26 patients with NSCLC was well tolerated and led to a specific cytotoxic T cell immune response against the telomerase component in about half of cases [41]. In another study, subcutaneous vaccinations with an optimized peptide of telomerase reverse transcriptase p572Y were carried out in 22 patients with advanced NSCLC [42]. It was observed that 16 patients with early proliferation of TERT572-specific CD8+ lymphocytes after vaccination had significantly longer free-of-progression and overall survival [42]. So, inhibition of the telomerase components or limitation of telomere elongation by telomerase could be a novel target for cancer treatment and chemoprevention in patients with high risk of lung cancer.

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