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Telomerase as a marker of tumour development – analysis of telomerase expression and activity

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Introduction. Reactivation of telomerase is believed to be an important factor of both immortalization and carcinogenesis. Despite criticisms of the etiopathological significance of telomerase in carcinogenesis, an evaluation of its expression and activity may be considered to be a potentially useful diagnostic marker.
Material and methods. Telomerase expression has been studied by RT-PCR using specific primers for telomerase reverse trancriptase. Telomerase repeat amplification protocol-TRAP was used for the analysis of telomerase activity.
Results. In a preliminary study a high expression of telomerase in gastric and colon cancer, acute lymphoblastic and myeloblastic leukaemia, as well as in myeloid line K562 has been found. Lymphocytes stimulated by PHA have also revealed high telomerase expression. Low telomerase expression has been found only in some cases of non-cancerous mucosa from corresponding patients with gastric and colorectal cancer and peripheral blood lymphocytes. Telomerase activity in 10 000 cells has been observed in all studied samples. 300-cells lysate of all cancer cells, as well as PHA stimulated lymphocytes showed telomerase activity, which is in contrary to normal cells.
Conclusions. From these results one may conclude that all cancer cells tested have higher telomerase expression, as compared to normal cells. Taking into account the clear-cut relationship between telomerase activity and cell number, it may be postulated that either quantitative or semiquantitative evaluation of telomerase activity and expression should allow to distinguish cancer from normal tissue.

Telomeraza jako marker procesu nowotworowego – analiza ekspresji i aktywności

Wstęp. Przyjmuje się, że indukcja aktywności telomerazy odgrywa ważną rolę w immortalizacji komórek i w procesie nowotworzenia. Pomimo szeregu zastrzeżeń co do znaczenia telomerazy w karcinogenezie, ocena jej ekspresji i aktywności może być przydatnym markerem procesu nowotworzenia.
Materiał i metody. Ekspresję telomerazy oceniano techniką RT-PCR, posługując się starterami swoistymi dla genu odwrotnej transkryptazy. Aktywność telomerazy analizowano reakcją TRAP (telomerase repeat amplification protocol).
Wyniki. We wstępnych badaniach stwierdzono wysoką ekspresję telomerazy w komórkach raka żołądka i jelita grubego, komórkach ostrej białaczki limfoblastycznej i mieloblastycznej oraz w komórkach linii K562. Podobnie wysoką ekspresję telomerazy wykazano w limfocytach krwi obwodowej, stymulowanych PHA. Niską ekspresję telomerazy stwierdzono w komórkach zmienionej zapalnie błony śluzowej żołądka i jelita grubego. Jedynie w części przypadków prawidłowej błony śluzowej żołądka i jelita grubego oraz niestymulowanych limfocytów krwi obwodowej zdrowych dawców udało się wykazać niską ekspresję telomerazy. Aktywność telomerazy wykazano we wszystkich badanych próbach przy izolatach z 10 000 komórek. Wszystkie komórki nowotworowe i stymulowane PHA limfocyty wykazywały aktywność telomerazy w lizacie z 300 i nawet z 30 komórek. W lizacie z 300 komórek prawidłowych (błony śluzowej żołądka i jelita grubego oraz limfocytach) nie stwierdzano aktywności telomerazy.
Podsumowanie. Na podstawie przeprowadzonych badań można stwierdzić, że badane nowotwory wykazują wyższą ekspresję telomerazy w porównaniu z tkankami i komórkami prawidłowymi. Wykazana w pracy zależność aktywności telomera-

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zy od liczby badanych komórek wskazuje na konieczność zastosowania ilościowej lub półilościowej oceny aktywności i ekspresji telomerazy w celu odróżnienia tkanki nowotworowej od prawidłowej.

Key word: telomerase expression, telomerase activity, gastric cancer, colon cancer, acute lymphoblastic leukaemia, acute myeloblastic leukaemia
Słowa kluczowe: ekspresja telomerazy, aktywność telomerazy, rak żołądka, rak jelita grubego, ostra białaczka limfoblastyczna, ostra białaczka mieloblastyczna

Introduction

Telomeres, the stretches of DNA cup chromosomes, posses short repeated units of conservative sequence TTAGGG [1, 2]. In human telomere lengths are estimated to be 5–15 thousand base pair, and in mice about 30 thousand.

Telomeres prevent chromosomes from degradation and incorrect recombination. Cells lacking telomeres show chromosome aberration [3]. A number of aberrant chromosomes can be formed, including ring chromosome due to end fusion, dicentric and multicentric chromosomes. In somatic cells during subsequent division telomeres lose 50–100 base pair. According to the Hayflick limit, cells from various organisms undergo a limited number of possible cell divisions [2]. However, in a recent study Cristofalo and al. re-evaluated the relationship between donor age and replicative livespan of human cells in culture [4]. They found that the replicative livespan of fibroblast in vitro culture does not correlate with the donor age. Therefore the replicative livespan of normal cells in culture can't be used as a model of human cell ageing. In germ cells and in a majority of tumour cells telomeres do not shorten owing to telomerase activity synthesising 5' end of DNA chain, which is not being replicated.

Telomerase is a rybonucleoprotein complex consisting of reverse transcriptase, proteins, and RNA template for telomeric DNA synthesis [5, 6]. Each of the telomerase elements is encoded on a separate chromosome, i.e. 5, 14 and 3 respectively. It is assumed that a cell which has loost control on proliferation telomerase activity is, in fact, induced. Origibally it had been assumed that telomerase is connected with tumour development. More than 80% of tumours possess telomerase activity. Experiments with suppression of tumour development *in vivo* and *in vitro* by switching telomerase activity suggested that telomerase itself may be an ideal target for anti-cancer therapy [7, 8, 9]. Further studies have shown that some tumour cells lack telomerase activity. Moreover, a number of tumour cells have even shorter telomeres as compared to normal cells. It was even postulated that short telomeres accompanying crisis of cell division may be considered a kind of promoters of tumour development [3].

The aim of this work was to analyse the telomerase expression and activity in selected neoplasms and in myeloblasts cell line K562.

Our experiments have been designed in order to evaluate the usefulness of telomerase activity and expression as tumour markers. The study was also performed on inflammatory altered mucosa; PHA stimulated peripheral blood lymphocytes, samples of normal tissues and lymphocytes.

Material and methods

Telomerase expression and activity have been assessed in the following cells and tissue: acute lymphoblastic leukaemia (ALL) (n=10), acute non-lymphoblastic leukaemia (ANLL-M1) (n=5), myeloblastic cell line K562, gastric cancer (n=7), colon cancer (n=13), normal and inflammatory altered mucosa from the stomach and the colon of corresponding patients, and normal and PHA stimulated peripheral blood lymphocytes from healthy individuals (n=14). Solid tissue was cut with scissors, minced through a steel screen and passed through nylon mesh. The obtained single-cell suspension was washed twice with PBS, counted, and suspended to final concentration of 2 million per ml.

Telomerase expression was evaluated by RT-PCR technique using specific primers for human telomerase reverse transcriptase gene (HTERT) [5, 6, 10]. RNA has been isolated from 2 million of either normal or tumour cells. PCR products have been analysed by agarose gel electrophoresis. As a control cDNA sequence of GAPDH (human glyceraldehyde-3-phosphate dehydrogenase) has been amplified.

Telomerase activity has been analysed with TRAp-eze -Telomerase Detection Kit Assay (Oncor) [11, 12]. PCR products in polyacryloamide gel were stained with Sybr Green (Sigma) and visualised by gel documentation system CCD (Kodak).

Results

High telomerase expression has been found in all tumour cells including those from gastric and colon cancer, ALL and ANLL blasts, as well as K562. Representative results are presented in Fig. 1. Similarly high telomerase expression has been found in PHA stimulated peripheral blood lymphocytes. Inflammatory altered gastric and colon mucosa show only low telomerase expression (Fig.1). Only in some cases of normal gastric and colon mucosa and in non-stimulated lymphocytes from healthy

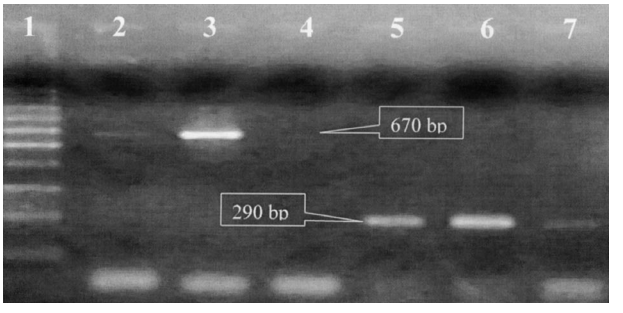

Fig. 1. Telomerase expression 1–100 bp DNA ladder (Rapidozym), 2,5-inflammatory altered gastric mucosa, 3,6-gastric cancer cells, 4,7 – normal gastric mucosa. Bands 2–4 HTERT, 5–7 GAPDH

individuals (4/14) low telomerase expression has been detected. The majority of mucosa samples and normal lymphocytes have not shown any telomerase expression.

In contrary to the expression, telomerase activity appeared to be positive in all samples tested in cases where the lysate was obtained from 10 000 cells. Using 300-cell lysate resulted in revealing clear-cut differences between normal and cancer cells (Fig. 2). All tumour cells and PHA stimulated lymphocytes showed telomerase activity as detected from 300 cells. Telomerase activity in 300-

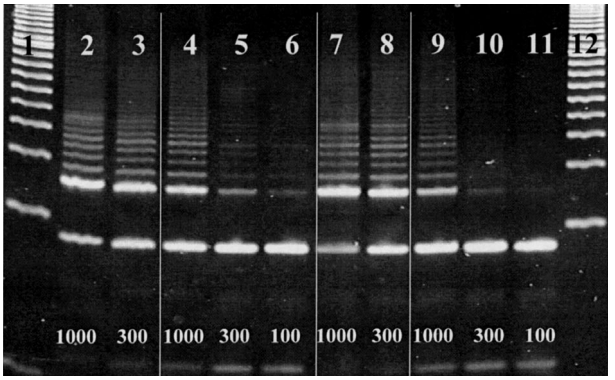

Fig. 2. Telomerase activity and cell number in a gastric cancer patient 1,12- PCR low ladder, 20bp (Sigma), 2,3,7,8 -gastric cancer, 4,5,6-inflammatory altered gastric mucosa, 9,10,11-normal gastric mucosa

-cell lysate of gastric and colon mucosa (Fig. 2) or of normal lymphocytes has not been detected (data not shown). In dose-dependent experiments telomerase activity has been found up to 30-cell lysate in gastric and colon cancer (data not shown). The highest telomerase activity revealed in leukaemia cell and K562 cells – activity has been detected in 30-cell lysate (Fig. 3) and even in 10-cell lysate (data not shown).

Discussion

On the basis of performed experiments it can be concluded that tumour cells have higher telomerase

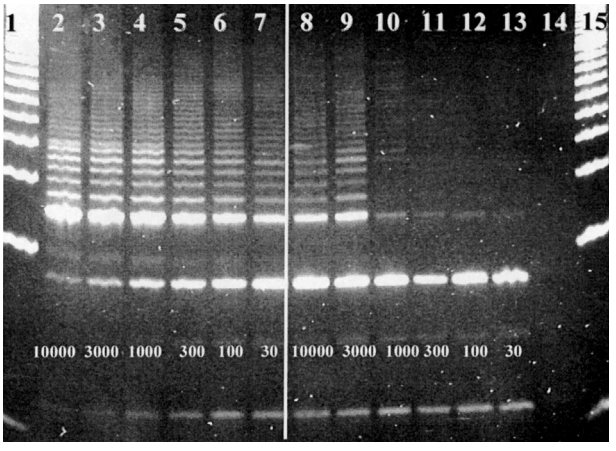

Fig. 3. Telomerase activity and cell number 1,15- PCR low ladder, 20bp (Sigma), 2-7 -ALL, 8-13 -normal lymphocytes, 14 -negative control

expression as compared to normal cells and tissue. These observations are similar with those reported in literature [10, 13].

The usefulness of telomerase activity as a tumour marker is controversial. Most of the authors assessing telomerase activity in prostate, colon and gastric cancer have attempted to show practical significance of telomerase activity as a marker which may discriminate normal tissue from neoplastically altered samples [16, 17, 18]. In our experiments we have shown that the difference between the tumour and healthy tissue is not as obvious as some authors postulate [19, 20, 21]. Our results have shown that both normal and cancer cells appeared to have telomerase activity as tested on 10 000-cell lysate. Some authors have suggested that telomerase activity is just a marker of cell proliferation, and thus not directly associated with neoplastic transformation [8].

In our opinion telomerase activity is present, to a different degree, in all normal human cells and is only a marker of cell proliferation. Various types of tissue may have different telomerase activity depending, probably, on their proliferation rate. This would explain all the results concerning telomerase activity, which we have obtained. Studies of rapidly proliferating cancer cells reveal telomerase activity comparable to PHA stimulated lymphocytes.

The most important result of our work is the obvious dependency of telomerase activity on cell quantity. These results have prompted us to suggest that quantitative evaluation of telomerase expression and activity may be useful marker to distinguish tumour tissue from healthy tissue. Combined tests expression and telomerase activity seems to be a marker of tumour development. These two markers may be considered useful for the identification of pre-cancer state, as well as for the diagnosis the cancer recurrence. Basing upon the obtained results we may postulate that telomerase is, indeed, a tumour marker, no matter whether or not it is directly involved in cancerogenesis.

Conclusions

The results of our study concerning the relationship between telomerase activity and cell number strongly argue for a need of applying quantitative or semiquantitative methods of telomerase evaluation in the discrimination between healthy tissue and tumour cells. Further studies should aim at stating whether telomerase will become a prognostic marker competitive to others presently used. This should result in the practical use of telomerase in cancer diagnostics.

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