

Role of telomerase in cellular ageing and malignant transformation.

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Telomeres are the repetitive non-coding sequences found at the end of all eucariotic chromosomes. Telomere shortening with each cell division has been proposed as molecular clock of cell senescence. There are many observations indicating that telomere shortening parallels cellular ageing, however its causative role is questionable. Telomerase, a ribonucleoprotein complex allows cells to grow indefinitely and it is believed that reactivation of telomerase plays an important role in cell immortalization and carcinogenesis. Cell immortalization is almost always accompanied by the expression of telomerase, which stabilizes telomere length and is likely necessary for the continued growth of cancer cells. Telomerase is activated in most malignant tumors but is usually inactive in normal somatic cells. Telomerase is a ribonucleoprotein complex consisting of reverse transcriptase (hTERT), proteins (TP1) and RNA template for telomeric DNA synthesis (TR). Despite the questionable role of telomerase in cell senescence and carcinogenesis, telomerase itself may serve as diagnostic marker for tumor development. Several studies have demonstrated that the presence of telomerase activity can be used to distinguish malignant from normal tissue of various organs. In this study we analyzed both telomerase activity and expression of three components of telomerase complex (hTERT, hTP1 and hTR) along with telomere length of normal somatic and neoplastic cells. The objective of this study was to see whether there is any relationship between telomerase activity and expression and telomere length in normal proliferating cells as well as cancer cells. Expression of hTERT, hTR and TP1 has been studied by reverse transcriptase PCR technique. Telomerase repeat amplification protocol-TRAP and PCR-ELISA was used for analysis telomerase activity. FISH technique was used to detect possible TERT gene amplification. The all telomerase components were consistently expressed in cancer cells. Neoplastic RNA produced consistently very strong amplification signals either for hTR hTERT and TP1. The expression of hTR was observed in RNA isolated from all normal bone marrow cells and from peripheral blood lymphocytes. The expression of TP1 and hTERT has been found in the majority of normal cells, however the amplification signals produced were usually much weaker than in malignant cells. The limiting dilution experiments indicated that the cancer cells have at least 100-fold higher telomerase activity and at least 25-fold higher TP1 and hTERT expression in comparison to normal cells. FISH analysis revealed amplification of TERT and hTR genes in malignant cells. It can be concluded that all cancer cells tested have higher telomerase expression and activity, as compared to normal cells. The high expression and activity of telomerase in cancer cells can be explained by amplified TERT and hTR genes. Therefore telomerase can be a good cancer marker provided quantitative analysis is carried out. Chemiluminescent detection of terminal restriction fragments (TRF) from DNA isolated from malignant cells showed variable pattern of telomere length. The various neoplastic cells appeared to have both long and short telomere lengths, in contrast to normal lymphocytes producing limited pattern of TRF (short telomeres). Some malignant cells produced short telomere pattern despite high telomerase activity and expression. High telomerase activity and expression in neoplastic cells not always correlate with telomere length (TRF pattern). Generally in normal cells telomere length shorten in elders as compared to children.