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Telomerase activation in neoplastic cell immortalization and tumour progression

Lai Meng LOOI *MD, FRCPath*, Min-Hwei NG *BSc, MSc* and Phaik-Leng CHEAH *MD, FRCPath*

Department of Pathology, Faculty of Medicine, University of Malaya, Malaysia

Abstract

The unique ability of tumour cells to proliferate indefinitely is crucial to neoplastic progression as it allows these cells to express the aggressive properties of cancer without the censure of physiological ageing. This is in contrast to normal somatic cells which are subject to a "mitotic clock," a phenomenon that has been linked to telomeric shortening after each round of cell replication, so that eventually the loss of genetic material reaches a critical stage and the cells undergo senescence and cell death. A study was conducted to investigate the role of telomerase, an RNA-containing enzyme that restores the telomere length, in the neoplastic cell immortalization and progression process. Fresh human tissue samples taken from excision specimens received by the Department of Pathology, University of Malaya Medical Centre, were investigated for telomerase activity using a commercial Telomerase PCR-ELISA kit (Boehringer Mannheim). Specimens comprised 33 breast lesions (10 infiltrating breast adenocarcinoma, 13 fibroadenoma and 10 non-neoplastic breast tissue), 27 colonic lesions (17 colonic adenocarcinoma and 10 non-neoplastic colonic mucosa) and 42 cervical lesions (20 cervical carcinoma and 22 non-neoplastic cervical tissues). Telomerase activity was found in 6 (60%) of 10 breast carcinomas, 6 (46%) of 13 fibroadenomas, none of the 10 non-neoplastic breast samples, 3 (17.6%) of 17 colon carcinomas and none of the 10 non-neoplastic colonic mucosal samples, 12 (60%) of 20 cervical carcinoma and 3 (13.6%) of 22 non-neoplastic cervical samples. 5/10 (50%) Stage I, 4/7 (57%) Stage II, 2/2 (100%) Stage III and 1/1 (100%) Stage IV cervical carcinomas showed telomerase activity. These findings support a contributory role for telomerase in tumourigenesis with activation occurring from neoplastic transformation and increasing with tumour progression.

Key words: telomerase, TRAP-ELISA, breast cancer, colon cancer, cervical cancer, cell immortalization

INTRODUCTION

Tumour cells have the unique ability to proliferate indefinitely, hence achieving a state of immortality. This is crucial for neoplastic progression as it allows these cells to express the aggressive properties of cancer without the censure of physiological ageing. This is in contrast to normal somatic cells which are subjected to biological ageing and programmed cell death, often referred to as the "mitotic clock," a phenomenon that has been linked to the repetitive loss of telomeric material with the mitotic cycle. In normal somatic cells, the telomeric tips of chromosomes shorten with every round of cell replication.[1] Eventually, the loss of genetic material reaches a critical level and cells enter a stage of senescence and eventual cell death. Telomerase is a ribonucleoprotein which is capable of synthesizing telomeric DNA onto chromosomal ends using a segment of its RNA component as a template.[2,3] First discovered in Tetrahymena and other eukaryotes in 1989, it has been convincingly demonstrated in humans in 1995. Recent studies indicate strong telomerase activity in germ cells (ovary and testis) and various tumours but weak or no activity in normal somatic tissues.[3,4] This has led to the notion that telomerase plays a key role in the neoplastic cell immortalization process by restoring telomere length.[5,6] This study was conducted to compare telomerase activity in benign and malignant human tumours and their non-neoplastic tissue counterparts, to gain an insight into the role of telomerase in the progression of human cancers.

Address for correspondence and reprint requests: Professor L.M. Looi, Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

MATERIALS AND METHODS

Fresh tissue were sampled from excision specimens received by the Department of Pathology, University of Malaya Medical Centre from patients of the Medical Centre, after adequate sampling for histopathological examination was completed. These were snap-frozen and stored at –80°C until batch analysis for telomerase activity by the TRAP-ELISA (Telomeric repeat amplification protocol and Enzyme Immunosorbent Assay) method using a commercial Telomerase PCR-ELISA kit (Boehringer Mannheim).[7] Based on the Telomeric Repeat Amplification Protocol (TRAP), the method incorporated photometric enzyme immunoassay detection of the generated telomeric repeats. Sample absorbance was measured using an ELISA reader at 450 nm (A_{450nm}) against blank (reference wavelength of 690 nm).

Specimens analysed comprised 33 breast lesions (10 infiltrating breast adenocarcinoma, 13 fibroadenoma and 10 non-neoplastic breast tissue), 27 colonic lesions (17 colonic adenocarcinoma and 10 non-neoplastic colonic mucosa) and 42 cervical lesions (20 cervical carcinoma and 22 non-neoplastic cervical tissues).

RESULTS

Table 1 shows the prevalence of telomerase activity in the various lesions investigated. Telomerase activity was detected in 18-60% of malignant tumours and 46% of fibroadenoma (benign tumour). It was not detected in non-neoplastic breast and colonic tissue. In addition, 5/10 (50%) Stage I, 4/7 (57%) Stage II, 2/2 (100%) Stage III and 1/1 (100%) Stage IV cervical carcinomas also showed telomerase activity.

DISCUSSION

Tumour persistence, invasion and metastases are ultimate factors influencing morbidity and mortality in cancer patients. Cell immortalization is an important step which allows tumour cells to persist long enough for progressive genetic mutations to occur, resulting in the acquisition of increasingly aggressive properties. Unravelling the steps in tumour progression is crucial for strategies towards improved diagnosis and assessment of cancer patients, identifying targets for anticancer therapy and design of cancer control programmes.

Recent studies indicate that telomerase, an RNA-containing enzyme that restores telomere length, may play an important role in the cell immortalization process.[3,6] Strong telomerase activity have been reported in germ cells (ovary and testis) and various tumours, whereas normal somatic tissues show weak or low activity. Furthermore, telomerase activity has been shown to be repressed in immortal cell lines at the quiescent phase or during cellular differentiation, providing the basis for a repression-derepression model for telomerase regulation.[8] Hence, it is likely that telomerase activation plays a key role in the means whereby tumour cells overcome the natural senescence process which is linked to the loss of telomeric genetic material with every cell cycle.

Our study has demonstrated telomerase activity in 20-60% of neoplastic tissue samples from breast, colon and uterine cervix, whereas non-neoplastic controls show almost no detectable telomerase activity. It is noteworthy that more malignant tumours show telomerase activity than benign tumours. Further, it has been demonstrated, in carcinoma of the cervix that, with increasing stage of the cancer, more tumours express telomerase activity. These findings support a contributory role for telomerase in tumourigenesis with activation occurring from neoplastic transformation and increasing with tumour progression.

It is noteworthy that telomerase activity was detected in 14% of non-neoplastic cervical samples. Considering that cervical malignancy

TABLE 1: Telomerase activity in tumours and non-neoplastic controls

		No. positive/ No. test (% positive)		
Tumour type	No. tested	Malignant	Benign	Non-neoplastic
Colon	27	3/17 (18%)	–	0/10
Breast	33	6/10 (60%)	6/13 (46%)	0/10
Cervix	42	12/20 (60%)	–	3/22 (14%)

undergoes a well-known evolution along a spectrum of changes from mild to severe dysplasia/cervical intraepithelial neoplasia,[9] it is likely that the 'non-neoplastic" samples are not totally normal. Although morphologically normal, these may already be tumour initiated samples. Hence the finding of telomerase activity in these samples may not be so surprising.

Variability in telomerase expression by tumours may reflect cycles in telomerase activity that is consistent with a repression-derepression mode of regulation. That the differential presence of telomerase in tumours and non-tumours may provide a potential basis for anti-neoplastic chemotherapy has generated considerable excitement and optimism.[10,11] The feasibility of using telomerase assay as an adjuvant marker of malignancy has also been mooted.[12,13] Nevertheless, the telomerase mechanism may not be ubiquitous and alternative or co-existent mechanisms for cell immortalization should be investigated.

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