Telomerase activity in breast cancer, promising marker of disease progression

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Telomerase is the intracellular reverse transcriptase responsible for the elongation of chromosomal telomere after each division cycle of viable cells. There is progressive shortening of human telomeres with ongoing cell division to reach specific length induced cell senescence. Telomerase now reliably applied as an attractive tool for diagnosis and therapy of all human cancers, since it maintain tumor cells division keeping them survive and avoiding apoptosis. It is detected in 80-90% of intraductal breast (DCIS) lesions and in 90% of infiltrative breast cancer cells, while most normal cells are devoid of any telomerase activity. Using immunohistochemistry technique telomerase can be easily highlighted in formalin fixed human tissues. Telomerase expression showed a stepwise increase in progression of breast cancer. The prospective assessment of telomerase within breast cancer may lead to its use as a diagnostic marker and improve diagnostic accuracy.

Keywords: Telomerase; hTERT; Breast cancer; cancer progression

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Introduction

Breast cancer is one of the most common human cancer all over the world, approximately it account for 25% of all cancers in females worldwide and 27% in advanced country with the western lifestyle [1]. Breast cancers can be derived from any cell in the mammary gland. Telomerase now reliably applied as an attractive tool for diagnosis and therapy of all human cancers, since it maintain tumor cells division keeping them survive and avoiding apoptosis. Telomerase is an enzyme complex consisting of a human telomerase reverse transcriptase (hTERT) catalytic subunit and human telomerase RNA component (hTR) for adding TTAGGG repeats to the end of the chromosome to maintain the length of the telomere, which is responsible for genomic stability. There is progressive shortening of human telomeres with ongoing cell division to reach specific length induced cell senescence. Telomerase activity is required for lengthening of telomeres. Studies from university Malay showed a significant correlation between telomerase activity and tumor size, lymph node status and stage of the tumor have highlighted the diagnostic and prognostic implication of telomerase assay [2], meanwhile telomerase is an attractive therapeutic tool as telomerase inhibitors can play substantial role in cancer therapy, since it could lead to death of breast cancer cells in which the telomere length is practically shorter than normal breast cells, leaving normal tissue cells unaffected [2]. Despite of the fundamental role of telomerase in cell senescence induction and sustain cancer cells division has been well known, in addition to its main function in telomere elongation, it has been suggested that telomerase play an important roles in cancer angiogenesis and metastasis, and preserving cancer stem cells immortal [3].

Breast cancer
Breast Cancers are heterogeneous disease with overt complexity and variability within its different histological subtypes giving rise to an extremely variable clinical course, response to therapy and ultimate prognosis. The heterogeneity and complexity of breast tumor cells and its maintenance are not well understood. The possibility of every tumor cell has the capacity to proliferate independently has been hypothesized resulting in an extremely variable morphological features and histological variants having their unique particular clinical behavior and prognosis. Three novel notions have recently came out in breast cancer histogenesis; the cancer stem cells (CSC) involvement and role in tumor formation, and the association of epithelial to mesenchymal transition (EMT) in the tumor local invasion and distant metastasis along with the Telomerase role in keeping the CSC immortal, and avoiding senescence.

**Telomerase activity**

Telomerase now reliably applied as an attractive tool for diagnosis and therapy of all human cancers. Telomerase activity detected in 80-90% of intraductal breast lesions and in 90% of infiltrative breast cancer cells, while most normal cells are devoid of any telomerase activity. The diagnostic implication of telomerase in breast cancer was dominate the progress on the role of telomerase in breast cancer, now a day most of ongoing researches focus on the role of telomerase inhibitors as therapeutic agent. As telomerase inhibitors can cause continuous shortening of telomere in vivo, ultimately arrest cell growth leading to cell death resulting from DNA damage induced by telomeres shortening.[6]

Telomerase is a specialized cellular enzyme (reverse transcriptase), ribonucleo-protein, catalyzing the synthesis of telomere, which is the terminal part of DNA, with its own RNA template; ultimately, it can compensate the lost part of telomere during each cellular division cycle. Up to date, Detectable level of the enzyme activity has been highlighted in most of human cancer cells and cancer cell lines, however all of the mature somatic cells does not show any telomerase activity, excluding stem cells, reparative cells, proliferating cells and immortal cells.[6]

Telomerase ribonucleo-protein is complex molecule comprising mainly of two molecules which are critical for the activity of the enzyme in both vivo and vitro:

1. Human Telomerase reverse transcriptase (hTERT protein). It is intracellular protein induce the catalytic function responsible for the replication of the ends of linear DNA.

2. Human telomerase RNA (hTR). It is the RNA template responsible for elongation of telomeres, consists of 51-base integral RNA convey the (TTAGGG) template responsible for the formation of the human telomeric repeat sequence[5].

One of the leading functions of telomeres is to enclose the chromosome ends preventing its degradation or fusions and maintain its stability. The telomeres get shorten after each cycle of human cell division. In the somatic cells, this shortening going to limits the proliferative capacity to 50-80 cell divisions.[6] Once the telomere gets critical short length, it will deactivate cell division cycle and induce cell senescence. As the human being grows older telomere shortening is an ultimate outcome in most of human cells[6, 7].

There is accumulating evidence suggest the role of telomere shortening to confine the human cells regenerative capacity during most of the chronic human disorders which characterized by high cellular turnover and while the human being grow older. It has been hypothesized that telomere shortening phenomena and cell senescence are atypical example of a tumor suppressor mechanism inhibiting the growth of tumor cells.[8] According to this hypothesis, any tumor cells need to trigger the telomerase activity to resume its dividing capacity to initiate the tumor, this claim supported by the existence of telomerase activity in 80-90% of human malignancies. Against this hypothesis, the risk of getting cancer is relatively increases in elderly people and those with chronic disorders having typical telomere shortening.[8]

It has been found that significant telomerase shortening coincided with activation of telomerase catalytic activity in most of human cancers, that is because telomere shortening is needed to induce the chromosomal instability to initiate the tumor, meanwhile, activation of telomerase is required to sustain the progression of the tumor. Therefore, we can conclude that telomere shortening along with activation of telomerase catalytic activity seems to have a complementary role in cancer initiation and growth. [6]

**Telomerase detection**

In normal body tissue, telomerase is detectable in proliferative germ cells, testicular seminiferous tubules, proliferating hematopoietic stem-like cells, activated lymphocytes, proliferative premenopausal endometrial cells, basal layer of cells in the epidermis, and in the crypts of the intestine. To detect telomerase activity in routine pathology practice using a tissue specimen the telomeric repeat amplification protocol (TRAP) assay is a reliable specific and sensitive practical technique, this technique cannot be
used on formalin fixed paraffin embedded tissue, but only fresh tissue specimen can be used. Moreover, TRAP technique estimate telomerase activity at the entire tissue level in contrast to immunohistochemistry which offer specific information at individual cell level by detecting telomerase expression inside each cell of tissues specimen[9].

By using TRAP technique and a highly sensitive polymerase chain reaction (PCR) based assay, telomerase activity is detected in approximately 85% to 90% of human cancer specimens and the levels of telomerase activity estimated by the TRAP assay vary among cancer tissues. These results suggest that not all human cancer cells in vivo have, or absolutely require, telomerase reactivation [9].

Telomerase activity represented by hTERT protein at the cellular level can reliably be highlighted using immunohistochemistry in formalin fixed paraffin embedded tissues, but tissue processing conditions specifically antigen retrieval method and type of antibody used are crucial. The anti hTERT protein staining is almost exclusively nuclear, accentuated in the nucleolus as a fine brown granules [10] (Figure 1). Expression of telomerase activity detected by anti hTERT protein showed significant heterogeneity, keeping in mind that part of the tumor cells with negative hTERT protein expression are undergoing apoptotic process. The sensitivity and specificity of detecting telomerase activity using immunohistochemistry is verified by RT-PCR and TRAP assay [5].

TRAP assay and Real time PCR are used regularly to estimate the telomerase activity at tissue level, these two techniques are reliably specific and sensitive assay, but they fails to reflect the exact measurement of telomerase activity at each cell within the tumor tissue, as a consequence, making the situation difficult to answer the question whether that increased telomerase activity coming from all tumor cells or originated from specific subset of tumor cells like the cancer stem cells or other proliferating tumor cells. However, recent study using IHC protocol showed that there was no association or significant correlation between the existences of breast cancer stem cells (CD44+/CD24-/low phenotype) and the detection of telomerase activity in both the invasive and lymph node metastatic lesions [10].

In situ hybridization of hTERT mRNA and hTERT RNA along with immunohistochemical detection of hTERT protein have been used to localize telomerase activity expression inside the cells. Telomerase activity detected by those techniques localized the hTERT protein within the nucleus of all positive cells, however, some researchers observed cytoplasmic staining of tumor cells, meanwhile, positive staining in normal cells also noted [10,11,12]. This may be because of using different brands of antibodies and variable methods of antigen retrieval.

In conclusion, hTERT protein can easily highlighted by IHC in formalin fixed paraffin embedded tissues, but tissue processing conditions of IHC protocol specifically antigen retrieval method and type of antibody used are decisive. The validity of hTERT immunoreactivity was verified by real time PCR and TRAP technique. Telomerase activity reliably detected in the nuclei, mainly nucleoli, and sometime seen in the cytoplasm. Expression of telomerase activity detected by anti hTERT protein showed significant heterogeneity.

**Telomerase activity and breast cancer**

The implementation of polymerase chain reaction based-assay (TRAP assay) in clinical pathology practice expand and facilitate the accurate detection of telomerase activity in various types of solid human cancer, it can detect even few positive cells expressing hTERT protein or 0.01% in mixed cell suspension.

Initial data obtained by TRAP assay showed 88% of all breast cancer exhibiting telomerase positive tumor cells, thorough examination of negative cases increased the percentage of positive samples to 95% [13], more details results showed that 75% of DCIS breast lesions, 88% of breast carcinomas, 5% of non tumoral tissues surrounding the cancer, and 0% of the normal breast lobules were TRAP-positive. Yashima et al. detected a progressive elevation in the telomerase activity levels with subsequent stage of the breast tumor; telomerase was detected in 14% of benign fibro-epithelial breast lesion, in 92% of DCIS lesions, and in 94% of infiltrative breast carcinoma [14].
Recently, Jaafar M. et al using IHC hTERT detection, in a series of 167 cases of invasive breast cancer and 63 associated lymph node lesion funded that telomerase activity was detected in 68.3% (114/167) of the cases, telomerase was expressed more frequently in DCIC lesion rather than invasive compartment, it was significantly more expressed in metastatic lymph node lesion compared to primary tumor. The proportion of tumors cells expressing telomerase ranged between a few scattered cells to more than 70% of tumor cells bulk mass [10].

Studies from the University of Malaya show findings consistent with the above trend. Moreover, it has been found that the mean telomere lengths of 3.1, 1.9, 1.0 kbp for normal breast tissues, benign lesion and infiltrative breast cancer respectively, these finding support the theory saying that activation of telomerase can be triggered by telomere shortening [2].

By using real time PCR (measuring quantity of reverse transcriptase), hTERT mRNA expression can be detected, the data revealed significant correlation between grade of breast cancer and level of hTERT mRNA, it is more prevalent in high-grade tumors [4].

Now a day, in daily clinical pathology practice, telomerase activity can be detected in Fine Needle Aspirate cytology FNAC preparation, which support and improve the diagnosis of different breast lesion. It is a well known that FNAC is a cheap, relatively accurate and having very low risk diagnostic procedure in routine clinical practice, meanwhile, using FNAC studies alone to settle the diagnosis of breast cancer is still difficult, imperfect, and unpractical. There were two studies compared the usage of telomerase assay as diagnostic tool in FNAC examination of breast lesion.

Poremba et al. found that 92% of FNAC preparation of breast cancer was telomerase-positive, 94% of FNAC specimen of benign fibro-epithelial breast lesion were telomerase-negative, and there was a strong statistical link between TRAP assay result and the presence of atypical cells in the cytology preparation [15].

Hiyama et al. found that all breast lesion FNAC preparation with atypical cells and detectable telomerase activity level to be a cancer proved by histological examination after tumor excision [11]. Moreover, six out of seven telomerase negative cases found to be begin breast lesion, while 50% of telomerase positive cases, which primarily diagnosed as benign lesion, subsequently diagnosed as malignant lesion. It’s wise to say, telomerase activity detection in FNAC is reliable diagnostic tool of variable breast lesion [11], which can be used concomitantly with other diagnostic tools. Recent study found tumor-derived telomerase RNA in the serum of patient with breast cancer, which can play crucial role in breast cancer diagnosis and follow up [4].

High telomerase level in breast cancer is moreover associated with genetic aberrations in 3q (gain), 8q (gain), and 17p (deletion). These genetic abnormalities are frequently detected in breast cancers and involve the hTR (on 3q), c-myc (on 8q), and p53 (on 17p) genes, all of these inherited disorders is usually associated with telomerase regulation [16].

**Telomerase activity as a marker of breast cancer progression**

Breast cancer commonly started as DCIS lesion progressing to invasive tumor with different stages. Telomerase activity detected in the in situ carcinomas and in the invasive tumor cells, as well as in the metastatic lesion of the lymph nodes and in the normal breast tissue, when the latter were observable in the examined sections. Jaafar M. et al. detected a progressive increase in the prevalence of telomerase activity with the progression of tumor: 57.8% in DCIS lesions and 64% in infiltrative breast cancers, in addition to its higher expression in metastatic lesions compared to primary lesion [10].

Yashima et al. detected a progressive elevation in the telomerase activity levels with subsequent stage of the breast cancer; telomerase detected in 92% of DCIS lesions, and in 94% of infiltrative breast carcinoma [14]. It is wise to say, that these findings making telomerase as a marker of disease progression, associated with stepwise breast tumor progression.

**Conclusion**

Telomerase now reliably applied as an attractive tool for diagnosis and therapy of all human cancers, since it expressed in about 70-90% of breast cancer. The prospective assessment of telomerase within breast cancer may lead to its use as a diagnostic marker and improve diagnostic accuracy. Estimation the frequency of breast cancer stem cells (CD44+/CD24-/low phenotype) and telomerase activity in different variants of breast cancer, can predict the clinical course of the disease and give prognostic clues for different variants of breast cancer. The expression of telomerase activity in a wide range of breast cancers justifies the role of this nuclear enzyme in keeping cancer cell immortal and represents exciting therapeutic target by telomerase inhibitors.
Although telomerase expressed in all of malignancy, its clinical significance in routine daily practice requires more prospective clinical studies.

Therefore, the question as to whether of telomerase activity can expressing in all types of tumor cells or limited to only specific subtype of tumor cells cannot be answered. Activation of telomerase and stabilization of telomeres are consistent with the hypothesis that telomere maintenance is essential for the cancer stem cells to be immortal, and may be controlling stepwise tumor progression \[17\].

Recent study hypothesize that sub populations of CD44+/CD24−/low phenotypic cells, which show identical features and cell surface markers of normal stem cells, also express telomerase activity and exhibit telomere shortening similar to the original cancer cell line \[3\]. These results suggest that cancer stem cells responsible for tumor initiation can be initial targets for telomerase inhibitor therapy, which can be place as essential part of anti-cancer therapeutic package.

**Conflicting interests**

I have no Conflicting interests

**References**