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Research Article

Expression of Telomerase in Breast Cancer and its Correlation with Clinicopathological Parameters

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Abstract

Background: Telomerase is expressed in more than 85% of human tumors and in over 90% of breast carcinomas whereas in normal tissues it is not active or detectable. Several recent studies have proven that high telomerase activity is associated with poor prognosis of breast cancer.

Objective: We investigatedthe telomerase activity by immunehistochemistry and its expression in tumor and non-tumor breast tissue and its clincopathological correlation with other established prognostic markers.

Methods: Immunohistochemistry (IHC) was used to detect the expression of Human Telomerase Reverse Transcriptase (hTERT) in the tissues of 20 cases of human breast cancer and 20 cases of benign lesions and its correlation with other established prognostic factor like ER, PR and her-new status.

Results: Nuclear expression of telomerase by IHC was found in 7 out of 20 breast cancer patients (35%). None of the 20 benign breast tissue samples stained for telomerase. The variation of hTERT expression as per T stage, N stage, ER, PR and her 2 neu statusin breast cancer was not significant.hTERT expression was similar in triple negative and non triple negative breast cancer patients.

Conclusion: hTERT expression needs to be correlated with response to chemotherapy by further studies and may emerge as a useful tool in selecting most appropriate chemotherapy protocol for a given patients.

Keywords: Breast cancer; Telomerase; Immunohistochemistry

Introduction

Breast cancer is one of the most common female malignancies and is the second leading cause of cancer-related death all around the world [1]. Telomerase is now applied as a tool for diagnosis and prognosis of all cancers. Telomerase activity is 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. It has been shown that telomerase is highly expressed in more than 85 % of human tumors and in over 90 % of breast carcinomas whereas in normal tissues it is not active or detectable [2,3]. Several recent studies have proven that high telomerase activity was associated with poor prognosis of breast cancer [4]. The aim of the present study is to investigate the telomerase activity by immune-histochemistry and its expression in tumoral and non tumoral breast tissue and its clinco-pathological correlation with other established prognostic markers.

Methods

Immunohistochemistry Method (IHC) was used to detect the

expression of hTERT in the tissues of 20 cases of human breast cancer and 20 cases of benign lesions in breast admitted to S.S. Hospital between 2013 and 2015. The clinicopathological findings (age, hTERT, tumor size, clinical staging, lymph node metastasis and family history) were evaluated in the patient population. Histological grade was scored using the Nottingham system. Estrogen Receptor (ER), Progesterone Receptor (PR), and Her-2 statuses were determined on the basis of Immunohistochemical(IHC) staining. Hormone Receptors (ER and PR) were considered positive if at least 10 % of tumor cells nuclei were stained. Tumors were considered Her-2 positive if they were scored as 3+ using IHC.

Immunohistochemical Assay

All breast tissue samples were fixed, dehydrated, dipped, and wax-embedded into paraffin blocks. The paraffin was sliced to about 4 μm. Immunohistochemistry was carried out by streptavidin-biotin complex method. Antigen retrieval was performed with a steamer for 15 min. Then the sections were blocked with 5-10 % normal goat serum; 10 min later, the sections were incubated with first antibodies for 1-2 h at 37°C. After, the sections were incubated with biotinylated secondary antibody (1:800) for 10-30 min in 37°C. Then sections were incubated with streptavidin-alkaline phosphatase for 10-30 min in 37°C. Using 3,3′-diaminobenzidine (DAB) as the chromogenic agent, the section was developed for 3-10 min then washed with distilled water for 3-5 min; hematoxylin counter staining and dehydration were done, then the samples were cleared and sealed. Phosphate-Buffered Solution (PBS) was used to wash the sections for 5 min per step for three times.

IHC staining was scored according to the following criteria: three fields were randomly selected at high magnification (×400) of each slice; 100 tumor cells were counted in each field; when the number of positive cells is less than 20 %, the sample was consider to be negative; when the number of positive cells is more than 20 %, the sample was positive.

Statistical Analysis

The associations between hTERT expression levels, and clinicopathological parameters were evaluated using Chi-squared test. The level of significance was set at $P<0.05$. All statistical tests were performed using the Software Package SPSS, Version 20.0, Chicago, IL, USA.

Results

The age range of the patients of breast carcinoma ranged from 26-70 yr. with mean age of 48.73 years. Patient demographics, clinical and pathological information are listed in Table: 1.

Variables	
Age	48.7±12.116
Age at menarche	12.40±.598
Age first birth	20.60±1.142
Histological grade	
Grade I	3 (15.0)
Grade II	10 (50.0)
Grade III	7 (35.0)
T-stage	
T_x	4 (20.0)
T_2	5 (25.0)
T_3	8 (40.0)
T_{4a}	0 (0)
T_{4b}	3 (15.0)
N-stage	
N0	7 (35.0)
N1	13 (65.0)
ER	
Negative	15 (75.0)
Positive	5 (25.0)
PR	
Negative	18 (90.0)
Positive	2 (10.0)
HER 2 neu	
Negative	11 (55.0)
Positive	9 (45.0)
Nuclear telomerase expression	
Negative	13 (65.0)
Positive	7 (35.0)

Table 1: Patient characteristics.

All the breast cancer and fibroadenoma patients had a breast lump on presentation. Palpable lymph nodes in the axilla were found in 17 (85%) out of 20 patients of breast cancer patients. Ulcreation was found in 3 (15%) out of 20breast cancer patients. None of the breast cancer patients was nulliparous while 16 (80%) out of 20 of benign breast disease patients were nulliparous. History of OCP intake was present in 3 (15%) out of 20 of breast cancer patients.

On clinical staging of diseases predominance was seen in this study for T3 (40%) fallowed by T2 (25%), T4a (20%) and T4b (15%). Similarly in N staging them was predominance of N stage (65%). There was no clinical or radiographic evidence of distant metastasis. Infiltrating ductal carcinoma was found in 90% of cases on FNAC. On histological evaluation Grade I carcinoma found in 3 (15%), Grade II in 10 (50%) and Grade III in 7 (35%) out of 20 patients. Estrogen Receptor (ER) positive (Figure 1) status was positive in 5 (25%) out of 20 patients, progesterone Receptor (PR) positive (Figure 2) status in 2 (10%) out of 20 patients & Her2neu positive (Figure 3) in 9 (45%) out of 20 patients. Nine patients (45%) were

classified as triple negative breast cancer.

We found that 7/20 (35%) of breast cancer patients were hTERT positive (Figure 4) while none of the 20 controls subject exhibited hTERT positivity. On correlation with the T-stage of the tumor, no significant difference in hTERT expression between different stages could be found. Similarly the correlation of hTERT status with Estrogen Receptor (ER), progestrone receptor (PR) and her 2 neu status was not significant. The hTERT status was also almost similar in triple negative breast cancer and non triple negative breast cancer patients ($p>0.423$) in our study group (Table 2).

	hTERT negative	hTERT positive	p-value
T-staging			0.3
T_x	1 (7.7)	2 (28.6)	
T_2	4 (30.8)	1 (14.3)	
T_3	6 (46.2)	3 (42.9)	
T_{4a}	0 (0)	0 (0)	
T_{4b}	2 (15.4)	1 (14.3)	
N-staging			0.044
N0	7 (53.8)	0 (0)	
N1	6 (46.2)	7 (100)	
ER status			0.417
Negative	9 (69.2)	6 (85.7)	
Positive	4 (30.8)	1 (14.3)	
PR status			0.521
Negative	11 (84.6)	7 (100)	
Positive	2 (15.4)	0 (0)	
Her-2neu status			0.423
Negative	8 (61.5)	3 (42.9)	
Positive	5 (38.5)	4 (57.1)	
Histological grading			0.522
Grade I	3 (23.1)	0 (0)	
Grade II	6 (46.2)	4 (57.1)	
Grade III	4 (30.8)	3 (42.9)	
TNBC and non TNBC			0.423
TNBC(9)	5(55%)	4(45%)	
Non TNBC (11)	8(72%)	3(28%)	

Table 2: Correlation between hTERT positivity.

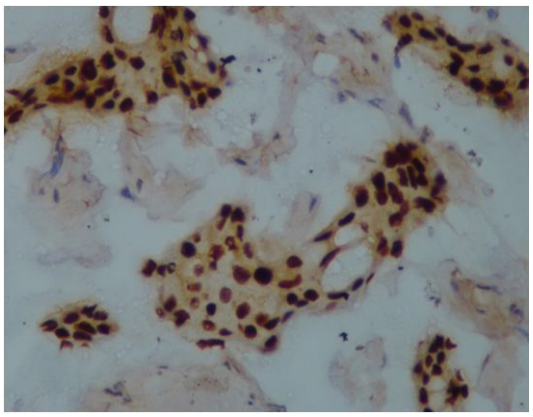

Figure 1: IHC slide (400X) showing Estrogen receptor positive breast cancer tissue.

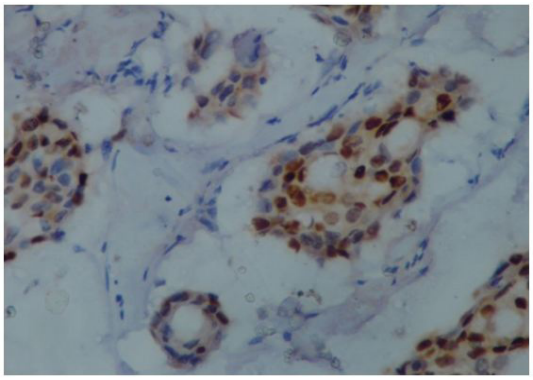

Figure 2: IHC slide (400X) progesterone receptor positive breast cancer tissue.

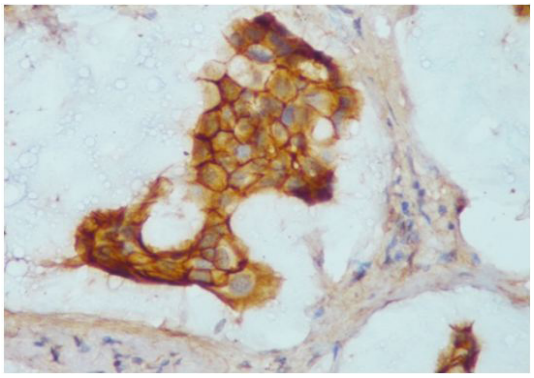

Figure 3: ISC slide(400X) showing her 2 neu positive breast cancer tissue.

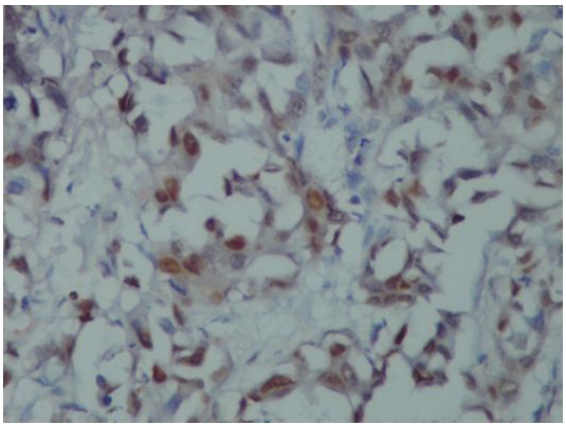

Figure 4: IHC slides (400X) showing hTERT positive nuclear staining and breast cancer tissue.

Discussion

Human telomeres are DNA-protein structures consisting of G-rich repeats (TTAGGG), 2-50 Kilobase Pairs (kbp) in length [5-7] with a 100-150 nucleotide 3'-end overhang [8]. Proteins such as TRF1, TRF2, PTOP (also known as TPP1, TINT1 and PIP1), POT1, RAP1 and TIN2 bind to telomeres, protecting them and assisting in the maintenance of their unique structure [9-10]. These DNA-protein complexes can form a T-loop structure, caused by the single stranded 3'-end overhang invasion of double stranded telomeric DNA on the same chromosome end [11-12]. Telomeres allow cells to distinguish natural chromosome ends from DNA breaks, thus preventing the activation of DNA damage pathways that signal cell cycle arrest, senescence, or apoptosis [13-14]. Stable telomeres also prevent chromosome fusions, which occur when telomere function is impaired. The importance of chromosome fusions to genetic stability was first observed by Barbara McClintock in the 1930s and helped laid the foundations for the field of telomere and telomerase biology [15-16]. Telomeric DNA must also be replicated or eventually telomere shortening can lead to cellular senescence [17].

Human cancer cells have been shown to maintain average telomere length over time [18] and only overexpression of hTERT and hTR together have resulted in a significant increase in telomere length [19]. Overexpression of hTR in telomerase positive cells and an extended culturing period led to a significant mean telomere length increase [20]. While mean telomere length is very predictive for the cellular lifespan of many cell types [21], it is the shortest telomeres which most critically affect cell viability [22] and they are preferentially elongated in human cells by telomerase at a high rate [23]. Human cancer cells appear to have extremely short class of telomeres, termed "T-stumps"[24], which may be important for human cancer cell viability and may thus represent a key target for preferential telomere elongation [23]. Studies have shown that telomerase is highly active in most types of human cancers including breast cancer, but remains inactive in adjacent normal tissues [25].

Although preliminary data showed 88% of all stages of breast carcinoma having positive TRAP [26], closer investigation and careful handling of initially negative samples revealed the value may be closer to 95% [27]. As reviewed by Shay and Bacchetti, 75% of breast carcinoma in situ lesions, 88% of ductal and lobular carcinomas, 5% of adjacent tissues, and none of the normal tissues were TRAP-positive [26].Yashima et al detected a progressive increase in the mean telomerase levels with the severity of histopathological change: 14% in benign breast diseases, 92% in carcinoma in situ lesions, and 94% in invasive breast cancers [28]. Expression of the hTERT mRNA can also be detected using real-time quantitative reverse transcriptase-PCR, and this assay revealed a statistical link between hTERT mRNA levels and the aggressiveness of breast tumors [29].

With the increasing number of breast cancers detected by screening procedures, a marker is needed to stratify the risk of subsequent invasive cancer. Hoos et al found a significant correlation between telomerase activity and tumor size, lymph node status, and stage [30]. A significant association between telomerase-positive infiltrating breast carcinomas and lymphovascular invasion, a fundamental step in breast cancer metastasis and a predictor of survival, has also been observed, making telomerase a useful prognostic marker [31]. Clark et al reported, in a prognostic study involving 398 patients with lymph nodepositive breast cancer, that increased telomerase activity was associated with decreased disease-free survival [32].

The present study evaluates the presence and distribution of telomere in tumoral and benign breast tissue by Immunohistochemistry. Results are compared with well established prognostic factor like estrogen and progesterone and Her-2 neu and Lymph node status. The possibility to predict which patient will respond to particular treatment modality is becoming increasingly important with increasing range of cancer therapies, the clinician must receive guidance as to which patient should be treated with which drug. Ideally, biological marker will be available for predicting whether a specific tumor will be sensitive to treatment.

We found that 7/20 (35%) of breast cancer patients were hTERT positive while none of the 20 controls subject exhibited hTERT positivity. on correlation with the T-stage of the tumor, no significant difference in hTERT expression between different stages could be found. Similarly the correlation of hTERT status with Estrogen Receptor (ER), Progestrone Receptor (PR) and her 2 neu status was not significant. The hTERT status was also almost similar in triple negative breast cancer and non triple negative breast

cancer patients (p>0.423) in our study group.

Based on the above findings we feel that hTERT assay could be a useful parameter for the monitoring of chemotherapy in breast cancer patients, It is reasonable to assume that chemotherapy if effective should result in a decline in hTERT expression. This could help in selecting the most effective chemotherapy protocol in a given case. The drawback for this schema to be put in to practical application as per our study is that we performed the hTERT evaluation in tissue specimens which might be difficult to obtain on multiple occasion which will be acquired for response evaluation to chemotherapy. Utility of hTERT as a monitoring tool could be practicable only if its estimation could be done on FNAC or serum samples with reliable results. This will permit serial hTERT evaluation on multiple occasion.

Lu et al [33], found telomerase expression to be slightly higher in tumors with longer telomeres as well as in larger tumors or aggressive disease. Over all telomerase expression was not associated with disease outcomebut this finding may be marked by adjuvent treatment patients with high telomerase expression responded poorly to chemotherapy in terms of disease fared and overall survival but paired better if treated with endocrine therapy. They suggest that telomerase activity may be a useful factor in determining the choice of adjuvant therapy in breast cancer patients.

Hess JL et al [34]suggest that telomerase activity in easily obtained body fluids may be a useful tool for diagnosing and monitoring of cancer progression. They have estimated telomerase levels in pleural fluid, ascitic fluid and even bronchial, lavage, bladder washings and oral rinses. In all cases the TRAP assay was proved to be more sensitive than standard cytology in identifying patients with cancer. This finding would be extremely relevant if telomerase levels in blood, plasma or serum could be documented to be reliable indicator of disease presence and response to therapy.

Lanzilli G et al [35] found that the effect off resveratrol on hTERT and telomerase possesses pronounced tumor supperessor activity in line with its chemopreventive properties. This agent can be considered a promising chemoprotective, chemopreventive and chemotherapeutic compound able to play a significant role in the control of breast cancer.

Summary and Conclusion

Thus we conclude that hTERT is found to be expressed in 35% of breast cancer tissues. It can be used for monitoring and selecting the most appropriate chemotherapy regimen in patients in whom it is expressed. Agents such as resveratrol which have an antogonist effect on hTERT may be useful for therapy in hTERT expressing tumors.

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References

1. DeSantis C, Siegel R, Bandi P, Jemal A (2011) Breast cancer statistics. CA Cancer J Clin 61:409-418.
2. Hiyama E, Hiyama K, Yokoyama T, Shay JW (2001) Immunohistochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. Neoplasia 3: 17-26.
3. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, et al. (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266: 2011-2015.
4. Carey LA, Kim NW, Goodman S, Marks J, Henderson G, et al. (1999) Telomerase activity and prognosis in primary breast cancers. J ClinOncol 17: 3075-3081.
5. Allsopp RC, Chang E, Kashefi-Aazam M, Rogaev EI, Piatyszek MA, et al. (1995) Telomere shortening is associated with cell division in vitro and in vivo. Exp Cell Res 220: 194-200.
6. Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR (1997) Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. Nat Med 3: 1271-1274.
7. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR (1995) Telomere elongation in immortal human cells withoughdetectable telomerase activity. EMBO J 14: 4240-4248.
8. McElligott R, Wellinger RJ (1997) The terminal DNA structure of mammalian chromosomes. EMBO J 16: 3705-3714.
9. Liu D, Safari A, O'Connor MS, Chan DW, Laegeler A, et al. (2004) PTOP interacts with POT1 and regulates its localization to telomeres. Nat Cell Biol 6: 673.
10. Palm W, de Lange T (2008) How Shelterin Protects Mammalian Telomeres. Annual Review of Genetics 42: 301-334.
11. de Lange T (2009) How telomeres solve the end-protection problem. Science 326: 948-952.
12. Shore D, Bianchi A (2009) Telomere length regulation: coupling DNA end processing to feedback regulation of telomerase. EMBO J 28: 2309-2322.
13. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, et al. (2003)A DNA damage checkpoint response in telomere-initiated senescence. Nature 426: 194-198.
14. Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM (2004) Telomere Shortening Triggers Senescence of Human Cells through a Pathway Involving ATM, p53, and p21CIP1, but Not p16INK4a. Molecular Cell 14: 501-513.
15. McClintock B (1938) The production of homozygous deficient tissues with mutant characteristics by means of abbarent mitotic behavior of ring shaped chromosomes. Genetics 23: 315-376.
16. McClintock B (1939) The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc Natl Acad Sci 25: 405-416.

17. Reddel RR (2010) Senescence: an antiviral defense that is tumor suppressive? Carcinogenesis 31: 19-26.

18. Klingelhutz AJ, Barber SA, Smith PP, Dyer K, McDougall JK, et al. (1994) Restoration of telomeres in human papillomavirus-immortalized human anogenital epithelial cells. Mol Cell Biol 14: 961-969.

19. Cristofari G, LingnerJ (2006) Telomere length homeostasis requires that telomerase levels are limiting. Embo J 25: 565-574.

20. Pickett HA, Cesare AJ, Neumann AA, Reddel RR (2009) Control of telomere length by a trimming mechanism that involves generation of t-circles. EMBO J 28: 799-809.

21. Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during ageing of human fibroblasts. Nature 345: 458-460.

22. Hemann MT, Strong MA, Greider CW (2001) The Shortest Telomere, Not Average Telomere Length, Is Critical for Cell Viability and Chromosome Stability. Cell 107: 67-77.

23. Britt-Compton B, Capper R, Rowson J, Baird DM (2009) Short telomeres are preferentially elongated by telomerase in human cells. FEBS Letters 583: 3076-3080.

24. Xu L, Blackburn EH (2007) Human Cancer Cells Harbor T-Stumps, a Distinct Class of Extremely Short Telomeres. Molecular Cell 28: 315.

25. Shay JW, Bacchetti S (1997) A survey of telomerase activity in human cancer. Eur J Cancer 33: 787-791.

26. Shay JW, Bacchetti S (1997) A survey of telomerase activity in human cancer. Eur J Cancer 33: 787-791.

27. Carey LA, Hedican CA, Henderson GS, Umbricht CB, Dome JS, et al. (1998) Careful histological confirmation and microdissection reveal telomerase activity in otherwise telomerase-negative breast cancers. Clin Cancer Res 4: 435-440.

28. Yashima K, Milchgrub S, Gollahon LS, Maitra A, Saboorian MH, et al. (1998) Telomerase enzyme activity and RNA expression during the multistage pathogenesis of breast carcinoma. Clin Cancer Res 4: 229-234.

29. Bieche I, Nogues C, Paradis V, Olivi R, Bedossa P, et al. (2000) Quantitation of hTERT gene expression in sporadic breast tumors with a real-time reverse transcription-polymerase chain reaction assay. Clin Cancer Res 6: 452-459.

30. Hoos A, Hepp HH, Kaul S, Ahlert T, Bastert G, et al. (1998) Telomerase activity correlates with tumor aggressiveness and reflects therapy effect in breast cancer. Int J Cancer 79: 8-12.

31. Mokbel KM, Parris CN, Ghilchik M, Amerasinghe CN, Newbold RF (2000) Telomerase activity and lymphovascular invasion in breast cancer. Eur J SurgOncol 26: 30-33.

32. Clark GM, Osborne CK, Levitt D, Wu F, Kim NW (1997) Telomerase activity and survival of patients with node-positive breast cancer. J Natl Cancer Inst 89: 1874-1881.

33. Lingeng L, Chong Z, Gongjian Z, Melinda I, Harvey R, et al. (2011) Telomerase expression and telomere length in breast cancer and their associations with adjuvant treatment and disease outcome. Breast Cancer Research 13: 1-8.

34. Hess JL, Highsmith WE Jr (2002) Telomerase detection in body fluids Clinical chemistry 48: 18-24.

35. Lanzelli E, Fuggetta MP, Tricarno M (2006) Resveratrol down regulates the growth and telomerase activity of breast cancers cells in vitro. International Journal of Oncology 28: 641-648.