Journal of Advances in Medicine (JAM)
Citation: JAM– 6(1): 27-33, June 2017
DOI: 10.5958/2319-4324.2017.00005.0

RESEARCH ARTICLE

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

Ram Niwas Meena[1], Agni Gautam Shah[2], Priya Ranjan Prasad[2], S.C.U. Patne[3], Sashi Prakash Mishra[2], Sandeep Patel[4] and Rahul Khanna[5*]

[1]Assistant Professor, Department of General Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
[2]MBBS, Junior Resident, Department of General Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
[3]Assistant Professor, Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
[4]Data Analyst, Center of Excellence, Dept. of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
[5]Professor, Department of General Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Keywords: Breast cancer, Telomerase, Immunohistochemistry

Corresponding author
Prof. Rahul Khanna
Department of General Surgery
Institute of Medical Sciences
Banaras Hindu University
Varanasi – 221005, UP,
India
Email: dr_rahul_khanna@rediffmail.com

Abstract

Background: Telomerase is an enzymes which can be expressed in approximately 90% of carcinomas and in over 90% of breast cancer whereas in normal tissues it is not detectable. Recent studies have been proved that high telomerase expression is associated with poor prognosis of breast carcinoma.
Objective: We investigated the telomerase activity by immune-histochemistry and its expression in tumor and non-tumor breast tissue and its clinco-pathological correlation with other established prognostic markers.
Methods: Immuno-histochemistry (IHC) was used to detect the expression of human telomerase reverse transcriptase (hTERT) in the tissues of 20 cases of breast carcinoma and 20 cases of benign breast lesions and its correlation with other prognostic factors like ER, PR and her2-neu 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 her2-neu status were not statistically significant in breast cancer. hTERT expression was comparable in patients with triple negative and non triple negative breast cancer.
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.

Breast cancer is most common female malignancy and second leading cause of death in all around the world[1]. In recent year telomerase is a diagnostic and prognostic marker in all malignancy. Expression of telomerase was detected in 80-90 % of breast cancer cells, while in normal cells no telomerase activity was present. Previous study has been shown that telomerase is highly expressed in approximately 90% of human carcinoma and in over 90% of breast cancer whereas in normal tissues it is not detectable[2,3]. Carey *et al.* have confirmed 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 prognostic markers.

METHODS

Immuno-histochemistry 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 clinic-opathological findings (age, hTERT, tumor size, clinical staging, lymph node metastasis and family history) were evaluated. Nottingham system was used for scoring of histological grade. IHC staining was used for determining Estrogen receptor (ER), progesterone receptor (PR), and Her-2 statuses.

Immuno-histochemical assay

Immuno-histochemical assay method was used as per study conducted by Xu *et al.* as described below. At first the sample of breast tissue were dehydrated and fixed with paraffin wax-embedded to prepare blocks for cutting. The paraffin embedded block was cut by size nearer to 4 μm. Immuno-histochemical analysis was performed by the streptavidin–biotin complex method. For 15 min antigen retrieval was carried out with a steamer and after that the section were blocked approximately 5-10 % normal goat serum and then after 10 min, the sections were incubated for 1-2 h at the temperature 37°C with the first antibodies. After that the sections was incubated for 10-30 min at the temperature 37°C with biotinylated secondary antibody and then the section was incubated for 10-30 min at the temperature 37°C with streptavidin–alkaline phosphatase. The section was developed for 3–10 min then washed with distilled water for 3–5 min by using 3,3'-diaminobenzidine (DAB) as the chromogenic agent. The samples were cleared and sealed after performing hematoxylin counter staining and dehydration. The section was washed in Phosphate-buffered solution for three times per step 5 min. The following Criteria has been adopted for IHC staining which is described as at large magnification (×400) of each slice three field was randomly selected and in each field 100 tumors cells were counted. The sample was counted as negative and positive if number of positive cells is < 20% and > 20% respectively.

Statistical analysis

The statistical analysis was done by using the Software Package SPSS, Version 16.0, Chicago, IL, USA. Chi-Square test and Fisher's exact test were performed to see the associations between hTERT expression, and clinic-opathological parameters. P-value < 0.05 was considered as statistically significant association.

Table 1: Patient characteristics

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	13 (65.0)
Negative	7 (35.0)
Positive	

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. 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 20 breast 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 (Fig.1) status was positive in 5 (25%) out of 20 patients, progesterone receptor (PR) positive (Fig. 2) status in 2 (10%) out of 20 patients & Her2neu positive (Fig. 3) in 9 (45%) out of 20 patients. Nine patients (45%) were classified as triple negative breast cancer.

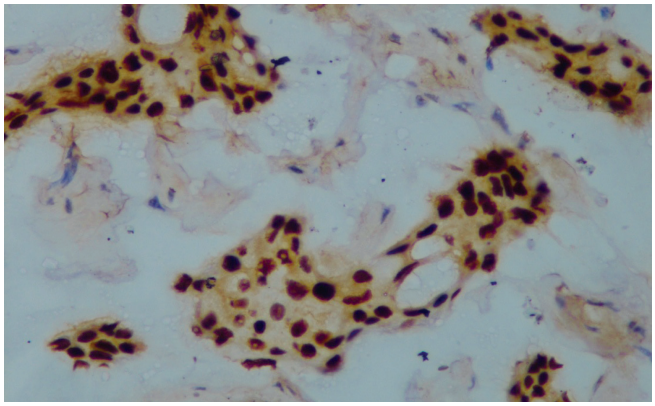

Figure 1: IHC slide (400×) showing Estrogen receptor positive breast cancer tissue.

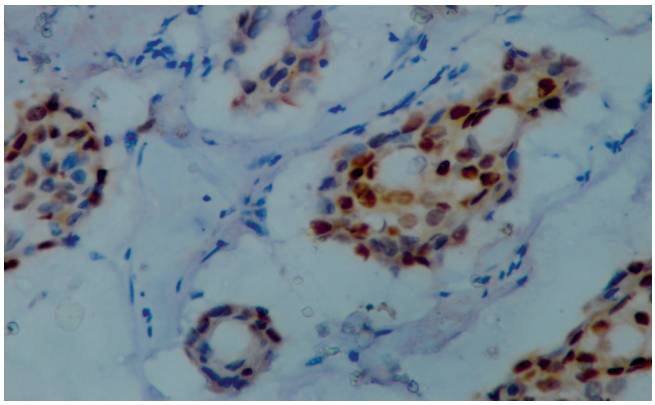

Fig. 2: IHC slide (400×) progesterone receptor positive breast cancer tissue.

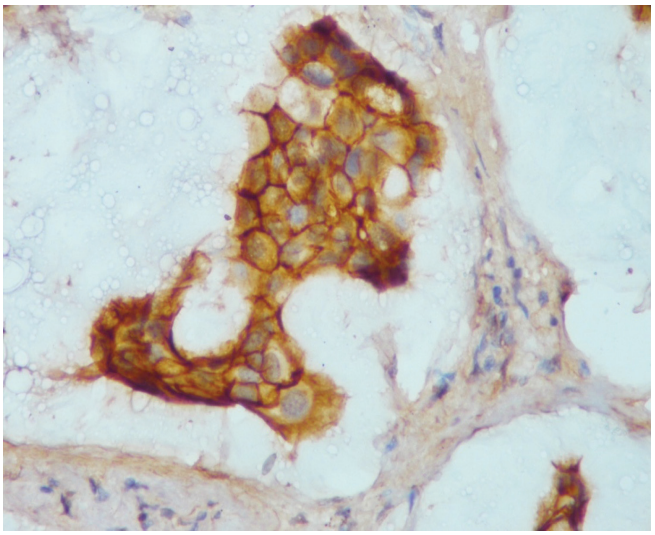

Fig. 3: ISC slide(400×) showing her 2 neu positive breast cancer tissue.

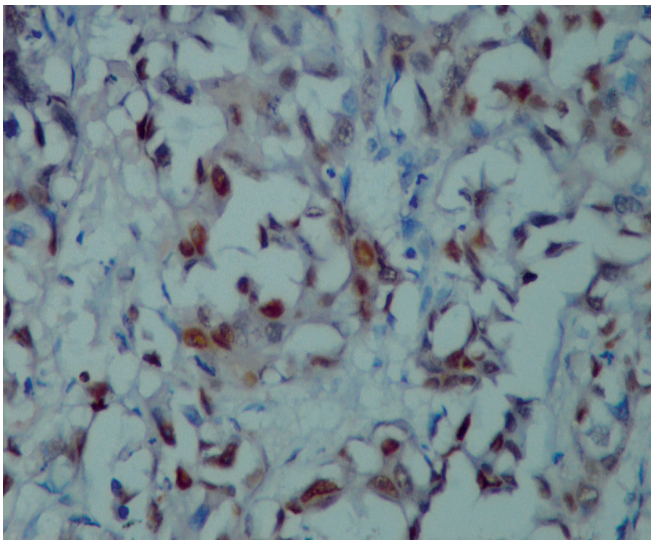

Fig. 4: IHC slides (400×) showing hTERT positive nuclear staining and breast cancer tissue.

We found that 7/20 (35%) of breast cancer patients were hTERT positive (Fig. 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).

Table 2: Correlation between hTERT positivity

	hTERT negative	hTERT positive	p-value
T-staging			0.300
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	8 (61.5)	3 (42.9)	0.423
Negative	5 (38.5)	4 (57.1)	
Positive			
Histological grading	3 (23.1)	0 (0)	0.522
Grade I	6 (46.2)	4 (57.1)	
Grade II	4 (30.8)	3 (42.9)	
Grade III			
TNBC and non TNBC	5(55%)	4(45%)	0.423
TNBC(9)	8(72%)	3(28%)	
Non TNBC (11)			

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 over-expression of hTERT and hTR together have resulted in a significant increase in telomere length[19]. Over-expression 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]. Several studies have confirmed that in many human carcinomas as well as breast cancer, the telomerase is active is very high but remains inactive in normal tissues[25].

Although, a study conducted by Shay *et al.* reported 88% of all stages of breast carcinoma having positive TRAP[26]. Another study conducted by Carey *et al.* studied careful histological confirmation and micro-dissection reveal telomerase activity in otherwise telomerase negative breast cancers revealed the value may be closer to 95%[27]. In a study conducted by Shay and Bacchetti reported 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.* studied telomerase enzyme activity and RNA expression during the multistage pathogenesis of breast carcinoma and found to detect a mean telomerase levels is increase in severity of histo-pathological change: 14%, 92% and 94% in benign breast diseases, carcinoma in situ lesions, and invasive breast cancers respectively[28]. In a study conducted by Bieche *et al.* evaluate the quantitation of hTERT gene expression in sporadic tumors with real time reverse transcription PCR assay revealed a statistical link between hTERT mRNA levels and the aggressiveness of breast tumors[29].

Hoos *et al.* conducted a study to correlates with tumor aggressiveness and reflects therapy effects in breast cancer. They found a significant correlation between telomerase activity with tumor size, lymph node status, and stage[30].

Mokbel *et al.* found a significant association between expression of telomerase-positivity and lymphovascular invasion, they also observed telomerase a useful prognostic marker of breast cancer metastasis and survival[31]. Another study conducted by Clark *et al.* examined the telomerase activity and survival of patients with 398 patients with node positive breast cancer. They concluded 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 Immuno-histochemistry. Results are compared with well established prognostic factor like estrogen and progesterone and Her-2 neu and Lymph node status. Our study predict the possibility in patient will respond to particular treatment modality is becoming important with increasing range of cancer therapies; the healthcare provider should receive treatment guideline as to which patient should be treated with which therapy. It is

Important that the biological markers are available in future will predict whether a breast neoplasm will be sensitive to therapy.

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 occasions 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 occasions.

Lu *et al.*[33], conducted a study to determine the telomerase expression and telomerase length in breast cancer and their associations with adjuvant treatment and disease outcome. They found telomerase expression is slightly increased in tumors with longer telomeres and also in large tumors or advanced disease. They also found the telomerase expression was not associated with disease outcome but this finding may be marked by adjuvant 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 concluded that telomerase activity is a useful marker in determining the choice of adjuvant chemo 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, ascetic 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 effects 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.

ACKNOWLEDGEMENTS

Authors would like to thanks all patients who participated in the study.

REFERENCES

1. DeSantis C, Siegel R, Bandi P, Jemal A. Breast cancer statistics, 2011. CA Cancer J Clin. 2011;61:409–18.
2. Hiyama E, Hiyama K, Yokoyama T, Shay JW. Immuno-

histochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. Neoplasia. 2001;3:17–26.

3. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994; 266 (5193):2011-5.
4. Carey LA, Kim NW, Goodman S, Marks J, Henderson G, Umbricht CB, *et al.*. Telomerase activity and prognosis in primary breast cancers. J Clin Oncol. 1999; 17:3075–81.
5. Allsopp, R. C., E. Chang, *et al.*. (1995). "Telomere shortening is associated with cell division in vitro and in vivo." Exp. Cell Res. **220**: 194-200.
6. Bryan, T. M., A. Englezou, *et al.*. (1997). "Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines." Nat Med **3**(11): 1271-4.
7. Bryan, T. M., A. Englezou, *et al.*. (1995). "Telomere elongation in immortal human cells withough detectable telomerase activity." EMBO J. **14**: 4240-4248.
8. McElligott, R. and R. J. Wellinger (1997). "The terminal DNA structure of mammalian chromosomes." EMBO J **16**(12): 3705-14.
9. Liu, D., A. Safari, *et al.*. (2004). "PTOP interacts with POT1 and regulates its localization to telomeres." Nat. Cell Biol. **6**: 673.
10. Palm, W. and T. de Lange (2008). "How Shelterin Protects Mammalian Telomeres." Annual Review of Genetics **42**(1): 301-334.
11. de Lange, T. (2009). "How telomeres solve the end-protection problem." Science **326**(5955): 948-52.
12. Shore, D. and A. Bianchi (2009). "Telomere length regulation: coupling DNA end processing to feedback regulation of telomerase." EMBO J **28**(16): 2309-22.
13. d'Adda di Fagagna, F., P. M. Reaper, *et al.*. (2003). "A DNA damage checkpoint response in telomere-initiated senescence." Nature **426**(6963): 194-8.
14. Herbig, U., W. A. Jobling, *et al.*. (2004). "Telomere Shortening Triggers Senescence of Human Cells through a Pathway Involving ATM, p53, and p21CIP1, but Not p16INK4a." Molecular Cell **14**(4): 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. USA **25**: 405-416.
17. Reddel, R. R. (2010). "Senescence: an antiviral defense that is tumor suppressive?" Carcinogenesis **31**(1): 19-26.
18. Klingelhutz, A. J., S. A. Barber, *et al.*. (1994). "Restoration of telomeres in human papillomavirus-immortalized human anogenital epithelial cells." Mol Cell Biol **14**(2): 961-9.
19. Cristofari, G. and J. Lingner (2006). "Telomere length homeostasis requires that telomerase levels are limiting." Embo J **25**(3): 565-574.
20. Pickett, H. A., A. J. Cesare, *et al.*. (2009). "Control of telomere length by a trimming mechanism that involves generation of t-circles." EMBO J **28**(7): 799-809.
21. Harley, C. B., A. B. Futcher, *et al.*. (1990). "Telomeres shorten during ageing of human fibroblasts." Nature **345**: 458-460.
22. Hemann, M. T., M. A. Strong, *et al.*. (2001). "The Shortest Telomere, Not Average Telomere Length, Is Critical for Cell Viability and Chromosome Stability." Cell **107**(1): 67-77.
23. Britt-Compton, B., R. Capper, *et al.*. (2009). "Short telomeres are preferentially elongated by telomerase in human cells." FEBS Letters **583**(18): 3076-3080.
24. Xu, L. and E. H. Blackburn (2007). "Human Cancer Cells Harbor T-Stumps, a Distinct Class of Extremely Short Telomeres." Molecular Cell **28**(2): 315.
25. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997;33:787–91.
26. Shay JW, Bacchetti S: A survey of telomerase activity in human cancer. Eur J Cancer 1997, 33:787–791.
27. Carey LA, Hedican CA, Henderson GS, Umbricht CB, Dome JS, Varon D, Sukumar S: Careful histological confirmation and microdissection reveal telomerase activity in otherwise telomerase-negative breast cancers. Clin Cancer Res 1998, 4:435–440.
28. Yashima K, Milchgrub S, Gollahon LS, Maitra A, Saboorian MH, Shay JW, Gazdar AF: Telomerase enzyme activity and RNA expression during the multistage pathogenesis of breast carcinoma. Clin Cancer Res 1998, 4:229–234.
29. Bieche I, Nogues C, Paradis V, Olivi R, Bedossa P, Lidereau R, Vidaud M: Quantitation of hTERT gene expression in sporadic breast tumors with a real-time reverse transcription-polymerase chain reaction assay. Clin Cancer Res 2000, 6: 452–459.
30. Hoos A, Hepp HH, Kaul S, Ahlert T, Bastert G, Wallweiner D: Telomerase activity correlates with tumor aggressiveness and reflects therapy effect in breast cancer. Int J Cancer 1998, 79:8–12.
31. Mokbel KM, Parris CN, Ghilchik M, Amerasinghe CN, Newbold RF: Telomerase activity and lymphovascular invasion in breast cancer. Eur J Surg Oncol 2000, 26:30–33.
32. Clark GM, Osborne CK, Levitt D, Wu F, Kim NW: Telomerase activity and survival of patients with node-positive breast cancer. J Natl Cancer Inst 1997, 89:1874–1881.

33. Lu Lingeng, Zhang Chong, Zhu Gongjian, Irwin Melinda, Risch Harvey, Menato Guido, Mitidieri Marco, Katsaros Dionyssios and Yu Herbert. Telomerase expression and telomere length in breast cancer and their associations with adjuvant treatment and disease outcome. Breast Cancer Research 2011, 13:1-8.

34. Hess JL, Highsmith WE Jr. Telomerase detection in body fluids Clinical chemistry 2002;48:1;18-24.

35. Lanzelli e, Fuggetta MP, Tricarno M *et al*.. Resveratrol down regulates the growth and telomerase activity of breast cancers cells in vitro. International Journal of Oncology 2006; 28:641-648.