Presentación

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Conferencia Invitada:

"INMUNHISTOCHEMICAL DETERMINATION OF TELOMERASE EXPRESSION IN TUMORAL AND NON TUMORAL BREAST TISSUE"

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SUMMARY

Telomerase is a DNA polymerase RNA dependent enzyme that adds telomere sequences at the end of the chromosomes compensating the loss of telomeres during cellular proliferation. Recent studies have reported a correlation between telomerase and clinicopathologic characteristics of certain tumors, suggesting a possible role as a future prognostic marker. Most of studies however, have detected levels of telomerase by sophisticated molecular biology techniques.

The present study evaluates the presence and distribution of telomerase in tumoral, benign and normal breast tissues by immunohistochemistry (IHC). Results are compared with established prognostic factors Estrogens, Progesterone and Her-2 neu, lymphoid nodes status and prognosis. IHC was performed on 64 cases of infiltrating breast carcinoma utilizing commercial polyclonal. In 20 cases, normal breast tissue was available and 10 had associated benign changes. Strong granular intracytoplasmic and membranous patterns of stainig were identified in malignant as well as in benign cells. Our study demonstrates that tumoral as well as non tumoral tissues can stain positive for telomerse utilizing immunohistochemistry techniques. There was no correlation between telomerase expression and other well established prognostic factors or prognosis. The role of telomerase in the prognosis of breast cancer remains to be determinated.

Telomeres are a noncoding repetition of hexameric sequencies (TTAGGG)n located at the end of the arm of each chromosome(1). They are stable structures and protected chromosome ends against fusion, recombination and degradation(1). In normal somatic cells, after each cell division, telomeres become progressively shorter and this loss of telomere lenght is considered to be one mechanisme by cellular senescence and cell mortality(2).

Male germ line cells, activated T cells, epithelial stem cells such as those in intestinal cripts, the basal layer of the epidermis and within human hair follicles, proliferative cells of renewal tissue and neoplastic cells(3) have an enzyme sistem, Telomerase, that adds telomeric repeat sequences at the end of the chromosomes compensating the loss of telomeres during the cellular replication, contributes to chromosomal stability and leads to immortalization of cells(4).

Telomerase is an RNA dependente DNA polymerase (reverse transcriptase) constitues by an RNA template (hTR) that directs the synthesis of telomeric repeat at the chromosomal ends and by a proteic catalityc subunit (hTERT) that forms a stable complex with telomerase R subunit(5). Recent studies have reported a correlation between telomerase activity and clinicopathologic characteristics of certain tumors, suggesting a possible role of telomerase as a future prognostic marker(3).

However, most of the studies realizased until now have detected levels of telomerase by sophisticated molecular biology techniques like Polimerase chain reaction (PCR)-based assay called TRAP (Telomeric repeat and amplification protocol)-assay(6) where it is necessary to use fresh-frozen tissue sections or techniques of In Situ hybridization for the RNA component(7).

To our knowledge one study using Immnohistochemistry for determinate the activity of telomerase has not been realized. The present study evaluates the presence and distribution of telomerase in tumoral, benign and normal breast tissues by IHC. Results are compared with well established prognostic factors like estrogens and progesterone, Her-2 neu, lymphoid node status and prognosis.

We examinated telomerase activity by IHC techniques in 64 cases of infiltrating breast carcinoma from 64 women undergoing elective breast surgery. The samples included normal breast tissue in 20 cases and benign breast lesions in 10 cases. Histologically, 54 of infiltrating carcinoma were ductal carcinoma and 10 were lobular carcinoma. Some of the ductal infiltrating carcinoma shown an important in situ component with necrosis. The benign breast lesions included fibroadenoma (1), ductal hyperplasia (6) and lobular hyperplasia (3). Breast cancer specimens were fixed in formalin and embedded in paraffin according to the clinical routine. The antibody used for this study was a commercial polyclonal antibody raised against a peptide mapping at the

carboxy terminus of TP1 of human origin in 1:50 dilution. Human TP1 is homologous to the Tetrahymena p80 telomerase protein and has been shown to interact with mammalian telomerase RNA. The antibodies used for the immunohistocuemical detection of estrogens, progesterone and Her-2 neu are the habitual antibodies used in our laboratory.

Strong granular intracytoplasmic and membranous patterns of staining were identified in malignant as well as in benign cells. We divided the positive stain in two groups: 1) + stain in which the stain is focal or generalized but always weakly positive; and 2) ++ stain in which the pattern of stain is strongly positive.

Telomerase activity was detected in 34 (53%) of infiltrating ductal carcinoma and in 7 (10'9%) of infiltrating lobular carcinoma. 23 (31'9%) of all infiltrating carcinoma vere negative.

Between the benign breast lesions, 7 shown positive stain or telomerase and 3 were negative. 9 of 20 normal breast tissues stained positively with telomerase and 11 were negative.

TELOMERASE IHC	IDC	ILC	BENIGN LESIONS	NORMAL BREAST
- STAIN	20 (31'2%)	3 (4'7%)	3	11
+ STAIN	17 (26'5%)	4 (6'2%)	3	9
++ STAIN	17 (26,5%)	3 (4'7%)	0	0

IDC: Infiltrating ductal carcinoma; ILC: Infiltrating Lobular carcinoma

In the other hand we made a study of correlation between the telomerase and other well established prognostic factors like estrogens and progesterone, Her-2 neu and lymphoid node status and we did not observe correlation

In these last years it had had a great proliferation of articles about the telomerase and its relation with the tumorogenesis in the medical bibliography. They report the increasing activity of telomerase in tumor cells and attach importance to the role of telomerase as a tumor marker well as in the monitoring tumor borden in patients undergoing therapeutics regimes. In all of them, the techniques reported for determining the levels of telomerase activity were based on biology molecular techniques, all above the TRAP-assay.

We think, own to the importance of telomerase as a possible tumor marker, it will be useful to have a technique for the determination of telomerase activity that can be applied in any Surgical Pathology Laboratory. Probably the most simple technique will be the Immunohistochemistry due to we can use the paraffin embebbed tissues and because we don't need sophisticated apparatus

which are not accessible for a modest Surgical Pathology Laboratory.

In this study the presence and distribution of telomerase determined by immunohistochemical techniques have been evaluated in benign and tumoral breast tissue. The results, just as they appear in their correspondent section, suggest several reflections. First of all, we must determinate the pattern of stain of telomerase; according to our experience, the pattern of stain observed is granular intracytoplasmic and membranous. Secondly we observed that the benign as much as the tumoral breast tissue were stained with the polyclonal antibody used against the telomerase in a similar way, and so we couldn't use the telomerase as a tumor marker.

Finally we observed that there were not correlation between the expression of telomerase and the expression of the other well established prognostic factors like estrogens and progesterone, Her-2 neu, lymphoid node status and prognosis.

This study stresses on the one hand the importance of defined and controlled criteria for evaluation of telomerase in immnohistochemistry, and on the other hand the role of telomerase in the prognosis of breast cancer which remains still to be determinated.

BIBLIOGRAPHY

Morin GB. The human telomere terminal tranferase enzyme is a Ribonucleoprotein that syntheses TTAGGG repeats. Cell 1989; 59:521-529.

Rhyu MS. Telemeres, telomerase and inmortality. J Natl Cancer Inst. 1995; 87:884-894.

V. Urquidi, D. Tarin, S. Goodison. Telomerase in cancer: clinical applications. Ann Med 1998; 30:419-430.

K. Mokbell, C.N. Parris, M. Ghilchick, F. Newbold. Telomerase activity in Breast Cancer. The breast (1999) 8: 208-211.

K. Yashima, S. Milchgrub, L.S. Gollahon, A. Maitra, M.H. Saboorian, J.W. Shay, A.F. Gazdar. Telomerase Enzyme Activity and RNA expression during the Multistage Pathogenesis of Breast Carcinoma. Clinical Cancer Research 1998, Vol 4, 229-234.

M.A. Piatyszek, M.W. Kim, S.L. Weintich et al. Detection of Telomerase activity in human cells and tumors vy a telomeric repeat amplication protocol (TRAP). Methdos in Cell Science, 1995; 17: 1-15.

C. Morales, E.L. Lee, J.W. Shay. In situ Hybridization for the Detection of Telomerase RNA in the Progression from Barrett's Esophagus to Esophageal Adenocarcinoma. Cancer 1998: 83/4: 629-824.