行政院國家科學委員會專題研究計畫成果報告

計畫名稱：台灣乳癌病理成因之研究－
TELOMERASE之活化與乳惡化過程之研究探討
計畫編號：NSC86-2314-B002-099-M37
執行期限：86年8月1至87年7月31日
主 持 人：黃俊升
執行機構：台大醫學院 外科

1.中文摘要：

染色體尾端（Telomere）由特定的重覆基因序列組成，此尾端重覆基因序列被認為與染色體的穩定度有關。尾端脢(Telomerase)是一種反轉錄脢（Reverse transcriptase），它可藉由本身所含有的RNA 成份，在染色體DNA的尾端做為合成(TTAGGG)n重覆基因序列的模板，進而可能維持細胞的不死。檢測乳癌組織的尾端（telomerase）活性可了解其活性是否與乳癌細胞的不死有關，並可做為辨視組織是否具有癌細胞的存在，更可能做為判定乳癌病人預後之指標。另外，在乳癌組織的變化與乳癌細胞的不死，其尾端脢活性是否與抑癌基因、腫瘤基因及荷爾蒙接受體等因子相關，亦值得進一步探討。故吾人檢測乳房腫瘤的尾端脢活性，結果發現：四個良性乳房疾病標本並未檢測出尾端脢活性，相對地，腋下淋巴結無癌轉移的乳癌檢體中有52.9%顯示尾端脢活性，而腋下淋巴結有癌轉移的乳癌檢體中，則有88.6%顯示有尾端脢活性；顯示尾端脢活性與病理分期亦有相關。

2. ABSTRACT

Breast cancer is a major affliction of women in many countries; it is the second most frequent female malignancy in Taiwan. With continuing westernization and urbanization, the incidence of breast cancer has almost tripled in the past two decades. Recently, the expression of telomerase and stabilization of telomeres have been thought to be concomitant with the attainment of immortality in tumor cells. The sustaining ability in chromosome integrity will be strongly associated with the immortality of cells, and thus, the measurement of telomerase activity in breast cancer may provide information useful both as a diagnostic marker to detect immortalized cancer cells in clinical materials and as a prognostic indicator of patient outcome. In the present study, we try to (1) determine the role of telomerase activity in breast lesions for differentiation of cancer vs atypical hyperplasia or dysplasia. (2) Correlate the status of telomerase activation with different pathologic stages and specific histopathologic grades/or subtypes of breast tumors. (3) Evaluate the telomerase expression in axillary lymph nodes micrometastases. (4) Determine the prognostic significance of telomerase expression in breast cancer and their role in treatment decision.

Among 96 samples we tested, telomerase activities were not detected in 4 benign lesions. For breast cancers, 52.9% (27 of 51) of cancers without lymph node metastasis and 88.6% (31 of 35) of cancers with lymph node metastasis showed telomerase activities. There was 72.6% (45 of 62) telomerase positive in high stage breast cancers. It indicated that there was correlation between telomerase reactivation and pathological stage.

Keyword: Breast cancer, telomerase, telomere

3.BACKGROUND

Cancer is a disease with a feature of out of controlled proliferating in a certain cell. The etiology of breast cancer until now is still

not well understood. The pathogenesis of human cancer was predicted that multiple independent genetic instability could alter the structures and functions of oncogenes/or tumor suppressor genes that leads cell immortality [1-3]. Many studies had suggested that breast cancer be due to a constellation of multiple factors including genetic predisposition, adverse hormonal milieu, immunological incompetence, exposure to carcinogens, and personal and demographic factors [4-10].

The changes of specific oncogenes or tumor suppressor genes per se, however, cannot explain the immortalization of tumor cells. Instead, recent studies in telomerase have provided the information for a better understanding of the development of the immortal phenotype (unlimited cell division potential) in tumor cells [11]. The "reactivation of telomerase" that results in immortalized cancer cells up-regulate or reactivate telomerase overcoming the two stages of M1 and M2 in human cellular senescence [12] and the activation of telomerase will closely connect to cell proliferation with a malignant progression and differentiation stage of carcinogenesis [13].

Telomeres, specific structures with TTAGGG tandem repeats located at the ends of chromosome, will shorten after each cell division because lacking of continuous replication of the lagging strand [14,15]. Telomeres cap the ends of eukaryotic chromosomes and are essential structures for chromosome stability and function [15]. Telomeric DNA acts as a buffer against the loss of terminal sequences resulting from replication of linear DNA molecules by unidirectional RNA-primed DNA polymerase [16] and provides sites for the de novo elongation of these molecules by the ribonucleoprotein enzyme telomerase [17,18]. Progressive telomere shortening has been proposed to be the major mechanism of mitotic clock [19]. In immortalized cells, reactivation of telomerase can elongate the telomeres *de novo* and stabilize the integrity of telomeric length.

Recently, Telomeric repeat amplification protocol (TRAP) assay, based on the method of polymerase chain reaction (PCR), had been developed in 1994 [12]. In humans, telomerase expressed in most cancers and immortal cell lines, but is inactivated in normal somatic cells except for germ cells, activated lymphocytes, and proliferating cells of renewal tissues. [20,21]. To date, more than 85% of primary tumor biopsies from different tumor types had been detected telomerase activity [12,22-24]. These data suggested that expression of telomerase could be treated as a suitable marker for the diagnosis of human tumors and furthermore, telomerase may be an important therapeutic target for the treatment of many different forms of cancers. Expression of telomerase was first reported by Kim et al. in 19 of 24 cases of Caucasian patients with breast cancers (histologic subtypes, unspecified) and in two long-term culture cell lines of breast tumor [12]. The same group published a larger scale of screening of telomerase activity in breast cancer, including 140 patients, in Jan., 1996

[25], but telomerase activity was not detected in 32% of stage I breast cancers in contrast to less than 4% negative results in more advanced breast cancers. Whether telomerase activity has a prognostic implication, however, is not shown in their study.

4.RESULTS AND DISCUSSION

We have investigated telomerase activity in 96 breast tumors, 4 benign lesions and 92 cancers. There was not any telomerase activity detected in 4 benign lesions. Telomerase activities in the 92 primary breast cancers were shown in the following table. The earlier the stages were, the less frequent the telomerase activities were detected. The 86 primary breast cancers were classified into two groups according to the status of axillary lymph node metastasis. For the node negative group, which is supposed to have favorable outcome. We found that 31 of 35 (88.6%) cancers within the node positive group revealed telomerase activity, the telomerase positive rate was higher than that of cancer group with node negative (52.9%; 27 of 51). As regards to the three recurrent tumors, two were positive of telomerase assay. Our results showed that telomerase reactivation could be used for the evaluation in axillary lymph nodes micrometastases. In the present study, both of 60% telomerase activities were found in stage I (12 of 20) and stage II (15 of 25) cancer patients. The frequencies were lower than that published by Hiyama et. al. [25]. In their study, 15 of 22 (68%) stage I patients and 51 of 52 (98%) stage II patients showed telomerase activity. Based on our findings, we might have given a short comment that the same stage of our patients will have better prognosis. To make a conclusion, further investigation of more samples and survival analysis will be performed. Besides, we had also collected and tested 8 specimens of ductal carcinoma in situ (stage 0) breast cancers. There was only 12.5% telomerase activity in this group, the telomerase expression and/or the PCR reaction probably inhibited by some other factors in the cell extracts. The real reason is unknown and worthy to further investigated. To summarize, our data indicate that telomerase activity is associated with aggressiveness of breast tumors and could served as an indicator of prognosis.

Stage	Telomerase activity no.positive / no. tested
0	1/8(12.5%)
I	12/20(60%)
IIa	15/25(60%)
IIb	21/23(95.6%)
III	6/7 (85.7%)

5.自評

這是針對台灣乳癌與尾端脢關係較為完整性的研究。相較於歐美的研究文獻，吾人發現台灣乳癌有尾端脢活性的比例較低，其是否對乳癌預後較佳仍須長期追蹤了解。另者吾人發現早期乳癌或預後較好的乳癌（如淋巴結無癌轉移者）其有尾端脢活性的比例亦較低。有關早期乳癌病變(DCIS) 其尾端脢活性比例甚低更值得吾人做更深入的探討。

REFERENCE

1. Vogelstein B, Kinzler KW: The multistep nature of cancer. Trends Genet 9:138-141, 1993.
2. Land H, Parada LF, Weinberg RA: Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperation oncogenes. Nature (Lond.) 304:596-602, 1983.
3. Shay JW, Wright WE, Werbin H: Defining the molecular mechanisms of human cell immortalization. Biochem Biophys Acta 1072:1-7, 1991.
4. Fisher ER, Fischer RSB, Wickerham L, Paik SM: Pathologic findings from the national surgical adjuvant breast project (protocol 6). I. Intraductal carcinoma (DCIS). Cancer 57:197-208, 1980.
5. Hutter RVP: The influence of pathologic factors on breast cancer management. Cancer 46:961-76, 1982.
6. Lagious MD, Westdahl PR, Margolin FR, Roses MR: Duct carcinoma in situ. Cancer 50:1309-14, 1982.
7. McDivitt RW: Breast carcinoma. Hum Path 9:3-21, 1982.
8. Page DL, Dupont WD, Rogers LW, Landenberger M: Intraductal carcinoma of the breast, Follow-up after biopsy only. Cancer 49:751-9, 1982.
9. Rosen PP, Senie R, Schottenfeld D, Ashikari R: Non-invasive breast carcinoma: frequency of unsuspected invasion. Implication for treatment. Ann Surg 189:377-82, 1979.
10. Rosen PP, Braun DW, Kin DE: The clinical significance of preincasive breast carcinoma. Cancer 46:919-25, 1980.
11. Shay JW, Werbin H, Wright WE: Telomere shortening may contribute to aging and cancer: A perspective. Mol Cell Differ 2:1-21, 1994.
12. Kim NW, Piatyszek KR, Proese KR, Harely CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific associations of human telomerase activity with immortal cells and cancer. Science 266:2011-2015, 1994.
13. **Shay JW, Wright WE. Thee reactivation of telomerase activity in cancer progression. Trends Genet 12:129-131,1996.**
14. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer 33:787-791, 1997.
15. Blackburn EH: Structure and function of telomeres. Nature 350:569-573, 1991.
16. Watson J: Origin of concatemeric T7 DNA. Nature New Biol 239:197-201, 1972.
17. Greider CW, Blackburn EH: Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 43:405-413, 1985.
18. Blackburn EH: Telomerases. Ann Rev Biochem 61:113-129, 1992.
19. Harley CB: Telomere loss: mitotic clock or genetic time bomb? Mut Res 256:271-282, 1991.
20. Shay JW , Gazdar AF. Telomerase in the early detection of cancer. J Clin Pathol. 50:106-109, 1997.
21. Hiyama E, Tatsumoto N, Kodama T,

Hiyama K, Shay JW, Yokohama T. Telomerase activity in human intestine. Int J Oncol 9:453-458; 1996.

22. Klingelhutz AJ, Barber SA, Smith PP, Dyer K, McDougall JK: Restoration of telomeres in human papillomavirus-immortalized human anogenital epithelial cells. Mol Cell Biol 14:961-969, 1994.
23. Morin GB: The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell 59:521-529, 1989.
24. Counter CM, Hirte HW, Bacchetti S, Harley CB: Telomerase activity in human ovarian carcinoma. Proc Natl Acad Sci USA 91:2900-2904, 1994.
25. **Hiyama E, Gollahon L, Kataoka T, Kurio K, Yokoyama T, Gazdar AF, Hiyama K, Piatyszek MA, Shay JW. Telomerase activity in human breast tumors. J Natl Cancer Inst 88:116-22, 1996.**