

Expression of E2F-1, Rb and ER in Peripheral Papilloma and Ductal Carcinoma in Situ of the Breast and its Significance

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OBJECTIVE To investigate the correlation of E2F-1, Rb and ER expression with peripheral papilloma (Peri-PM) and ductal carcinoma in situ of the breast (DCIS), and further explore some molecular mechanisms of the canceration of Peri-PM.

METHODS Immunohistochemistry was used to examine the expression of E2F-1, Rb and ER in 60 Peri-PM, 60 Peri-PM with atypical ductal hyperplasia (Peri-PM with ADH) and 60 DCIS. Normal breast tissues were selected as a control group.

RESULTS Based on immunohistochemical staining, the positive rate of E2F-1 expression in Peri-PM, Peri-PM with ADH and DCIS was 21.7%, 46.7% and 78.3% respectively. The positive rate of Rb expression was 83.3%, 53.9% and 21.7% and the ER expression was 86.7%, 61.7% and 55.0%. Significant differences were found among the 3 groups (Peri-PM, Peri-PM with ADH and DCIS) ($P < 0.05$). Significant differences existed between any 2 groups ($P < 0.05$) except for the rate of ER positive expression comparing Peri-PM with ADH versus DCIS ($P > 0.05$). The expression of E2F-1 was negatively correlated with ER and Rb, and at the same time the expression of ER was positively correlated with Rb. Following the degree of breast epithelial hyperplasia involved and its development into carcinoma, the positive rate of E2F-1 expression displayed an elevating tendency, but that of Rb and ER expression showed a tendency to decline.

CONCLUSION The interaction of the 3 indexes studied may play an important role in the conversion of precancerous lesions to early in situ breast carcinoma, and the evaluation of these indexes might provide a valuable basis for screening high-risk cases of Peri-PM.

KEYWORDS: E2F-1, Rb, ER, breast peripheral papilloma, ductal carcinoma in situ.

INTRODUCTION

The E2F-1 gene is an active factor in cell transcription which has a significant role in regulating the transition of cells from the G1 to the S-phase in the cell cycle^[1]. Retinoblastoma (Rb) acts as a suppressor gene which has a considerable negative regulatory influence in restraining the growth and proliferation of cells^[2]. In addition, the estrogen receptor (ER) is known to be associated closely with the development of breast carcinoma. Since reports have not been published in English relating the significance of the expression of E2F-1, Rb and ER in Peri-PM and DCIS to breast cancer, the purpose of our study was to explore these relationships and present this report.

MATERIALS AND METHODS

Samples and groups

A total of 180 cases were randomly selected and pathological sections from their paraffin blocks were obtained from the Breast Cancer Pathological Department and Research Laboratory of Tianjin Medical University Cancer Institute and Hospital. The patients were treated during the period from January, 1998 to December, 2003. The pathologic diagnoses were rechecked in reference to the WHO Histopathology Classification^[3]. The 180 cases were divided into 3 tissue types, each of 60 cases as follows: Peri-PM, Peri-PM with ADH and DCIS. Normal tissues served as a control group. All of the patients were followed-up for 37~96 months.

Reagents

Anti-E2F-1 monoclonal antibodies were obtained from the NewMarker Co.(USA). The mouse monoclonal antibodies against human ER and a S-P kit were the products of the American ZYMED Co, purchased from the Zhongshan Biotechnology Co. Anti-Rb monoclonal antibody and a super-sensitivity S-P immunohistochemistry (IHC) kit were purchased from the Fujian Maixin Biotechnology Ltd Co.

Staining methods

Immunohistochemical (IHC) staining was performed by the labeled streptavidin-biotin method (S-P) with the E2F-1 primary antibody (1:20) and with the Rb primary antibody (working solution). Antigens were restored by high-pressure and boiling water for 5 min. The dilution of the ER primary antibody was at 1:50, and antigens were restored by microwave treatment for 10~20 min before the blocking with blood serum. The intensity of the reaction was developed with 3,3'-diaminobenzidine (DAB), and hematoxylin was used to counter-stain the sections. Positive and negative controls were included with every batch.

Standardization assessment of the results

For the results of the immunohistochemical (IHC) staining, positive signals of E2F-1, ER and Rb expression were located in the nucleus as indicated by a yellow-brown staining. If the percentage of the positive cells of E2F-1 or Rb was $\geq 20\%$ ($\times 100$ HP) and that of ER was $\geq 15\%$ ($\times 100$ HP), the case was considered to be positive case.

Statistical analysis

Analysis of the data was performed with a SPSS 10.0

software package. The differences among the groups were compared by the Chi-square Tests, partition of Chi-square, Fisher's Exact Test and Spearman Rank Correlation. The level of significance was set to be 0.05.

RESULTS

Expression of E2F-1, Rb and ER in Peri-PM, Peri-PM with ADH and DCIS

The positive signals of E2F-1, Rb and ER were located in the nucleus of the epithelial cells which showed a brown-yellow color that could be seen in the 3 tissue types (Figs.1~5). The positive rates of E2F-1, Rb and ER expression are shown in Table 1.

The positive expression of the E2F-1 and Rb in the nucleus of Peri-PM, Peri-PM with ADH and DCIS varied in degrees (Table 1). Particularly in most cases of DCIS, E2F-1 showed positive expression, and in most cases of Peri-PM, ER and Rb displayed positive expression. The expression of Rb and ER was observed in the normal breast ductal tissue and lobular epithelium in almost all cases, but E2F-1 was not detected in normal breast tissue.

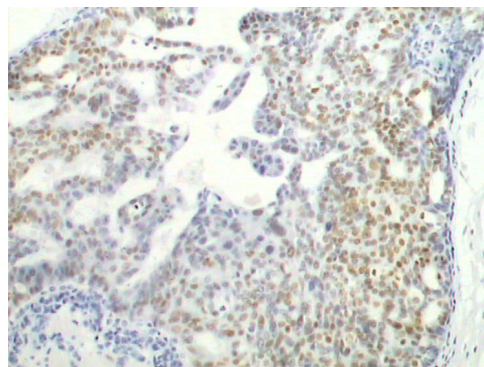


Fig.1. Positive expression of the E2F-1 protein in Peri-PM with ADH (IHC S-P method $\times 100$).

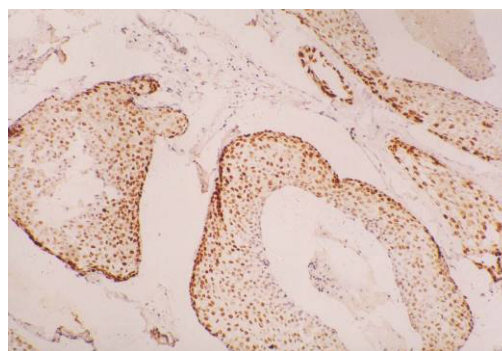


Fig.2. Positive expression of the E2F-1 protein in DCIS (IHC S-P method $\times 100$).

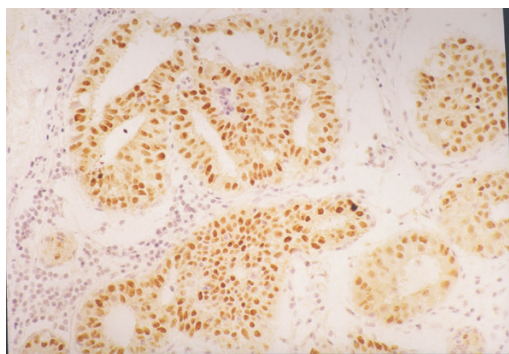


Fig.3. Positive expression of the Rb protein in Peri-PM with ADH (IHC S-P method x100).

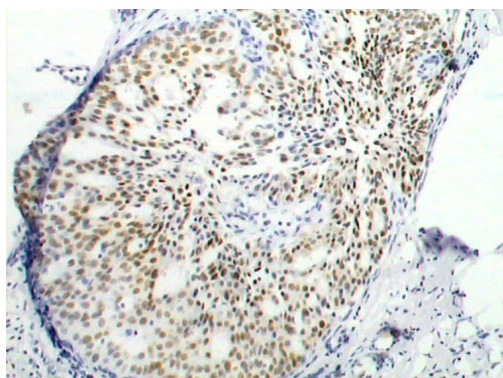


Fig.4. Positive expression of the Rb protein in Peri-PM (IHC S-P method x100).

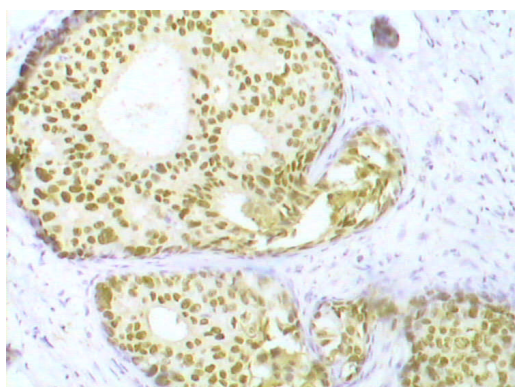


Fig.5. Positive expression of the ER protein in Peri-PM with ADH (IHC S-P method x100).

Table1. Positive rate of E2F-1, Rb and ER expression in the 3 tissue types (cases, %).

| Type | n | E2F-1 (%) | Rb (%) | ER (%) |
|------------------|-----|-----------|-----------|------------|
| Peri-PM | 60 | 13 (21.7) | 50 (83.3) | 52 (86.7) |
| Peri-PM with ADH | 60 | 28 (46.7) | 32 (53.9) | 37 (61.7) |
| DCIS | 60 | 47(78.3) | 13(21.7) | 33(55.0) |
| Total | 180 | 88 (48.9) | 95 (52.8) | 122 (67.8) |

Comparison of the positive rate of E2F-1 and Rb expression in the 3 tissue types

The positive rate of E2F-1 expression is shown in Table 1. There were statistically significant differences among the 3 tissue types ($\chi^2=38.730, P<0.05$). For example, the difference in E2F-1 positive expression between Peri-PM and Peri-PM with ADH was significant ($\chi^2=8.336, P<0.05$) and E2F-1 expression was higher in DCIS compared to Peri-PM with ADH ($\chi^2= 12.836, P<0.05$) .

Similarly, significant differences of Rb positive expression were observed among the 3 tissue types ($\chi^2=45.786, P<0.05$). There was a statistically significant difference between Peri-PM and Peri-PM with ADH ($\chi^2=12.478, P<0.05$), and Rb was expressed at a higher level in Peri-PM with ADH compared to DCIS ($\chi^2= 12.836, P<0.05$)

As shown in Table 1, with the development of the breast epithelial hyperplasia (from Peri-PM to Peri-PM with ADH to DCIS), the positive rate of E2F-1 expression increased. Simultaneously, that of Rb expression showed a decreasing tendency.

A correlation analysis of the positive rates of E2F-1 and Rb expression showed that the positive rates of E2F-1 expression was negatively correlated to that of Rb in Peri-PM ($r=-0.4257, P<0.05$), Peri-PM with ADH ($r=-0.4211, P<0.05$) and DCIS ($r=-0.3401, P<0.05, Fig.6$).

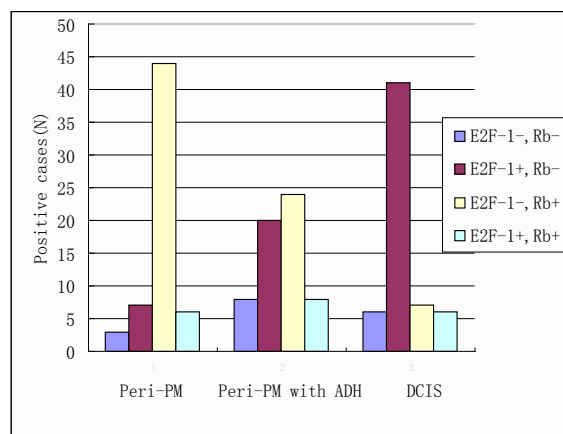


Fig.6. The correlation of E2F-1 and Rb expression in the 3 tissue types (Peri-PM: $r=-0.4257, P<0.05$; Peri-PM with ADH: $r=-0.4211, P<0.05$; DCIS: $r=-0.3401, P<0.05$)

Comparison of the positive rates of ER expression with E2F-1 and Rb

The positive rates of ER expression in the 3 tissue types are shown in Table 1. There was a statistically significant difference among the 3 tissue types ($\chi^2=15.314, P<0.005$). ER expression in Peri-PM differed from Peri-PM with ADH ($\chi^2=9.786, P<0.05$)

and was expressed at a higher level in Peri-PM compared to DCIS ($\chi^2=14.561, P<0.05$). But the difference in ER expression between Peri-PM with ADH and DCIS was not significant ($\chi^2= 0.549, P>0.05$). Fig.7 shows that the expression of ER was negatively correlated to E2F-1 in Peri-PM ($r=-0.4527, P<0.05$), Peri-PM with ADH ($r=-0.5068, P<0.05$) and DCIS ($r=-0.2988, P<0.05$).

As shown in Table 1, following the descending tendency of the Rb positive expression rate, the positive rate of ER expression was similar. The results also showed that the positive rate of ER expression was positively correlated to that of Rb in Peri-PM ($r=0.4345, P<0.05$), Peri-PM with ADH ($r=0.4939, P<0.05$) and DCIS ($r=0.2988, P<0.05$), respectively (Fig.8).

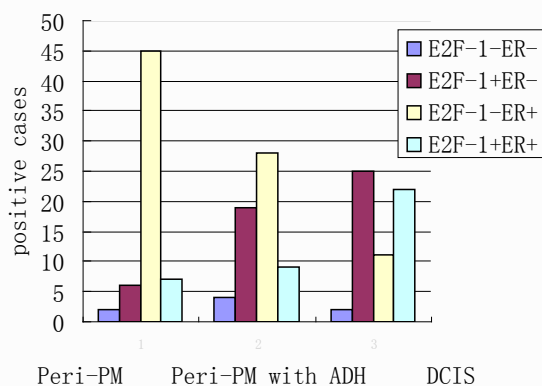


Fig.7. The correlation of ER expression with E2F-1 in the 3 tissue types (Peri-PM: $r=-0.4527, P<0.05$; Peri-PM with ADH: $r=-0.5068, P<0.05$; DCIS: $r=-0.2988, P<0.05$).

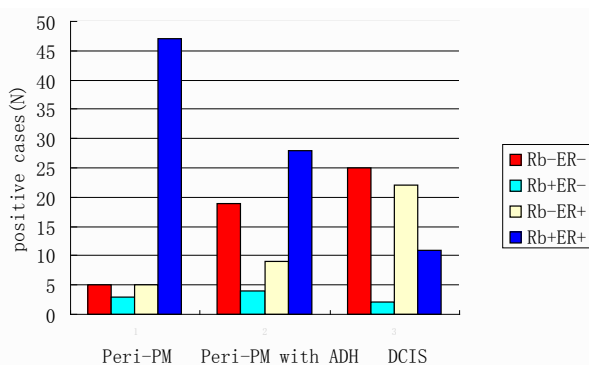


Fig.8. The correlation of ER expression with Rb in the 3 tissue types (Peri-PM: $r=0.4345, P<0.05$; Peri-PM with ADH: $r=0.4939, P<0.05$; DCIS: $r=0.2988, P<0.05$).

DISCUSSION

DCIS is considered to be the most common type of early in situ carcinoma of the breast, and most scholars believe that ADH is a precursor lesion of breast cancer^[4]. As a proliferative lesion, intraductal papillomatosis is usually multiple, originating within the terminal duct lobular units (TDLUs) form where they may extend into the larger ducts.

Papillomatosis was renamed as Peri-PM in the 2003 WHO histological classification, and it was more frequently observed in association with concomitant ADH which was confirmed to correlate closely with breast carcinoma in our previous studies^[5,6]. Some cases of ADH or Peri-PM with ADH might have already changed at the molecular level before the histological lesion develops, and these changes are regulated by many factors. The purpose of our study was to investigate the correlation of E2F-1, Rb and ER expression with Peri-PM and DCIS of the breast in order to elucidate the molecular mechanism of Peri-PM canceration.

It is well known that the G1/S transition is the most essential^[7] among many check points in different phases of the cell cycle, as it determines whether or not the cells continuously progress through the cell cycle. The E2F-1 protein is an active factor in cell transcription which plays a significant role in regulating the G1/S transition. E2F-1 controls cellular proliferation through an interaction with Rb, and many proteins that are involved in the cell cycle. E2F-1 protein can activate C-myc, N-myc and DNA polymerases directly, and thus start the synthesis of DNA, initiating the S-phase^[8]. Recent findings have shown that Rb can bind to the functional domain of E2F-1 to suppress DNA synthesis, thereby keeping the cells in G1 with inhibited mitosis^[9]. Therefore it has an important role in restraining breast epithelial proliferation and the development of breast cancer. Additional studies^[10] have confirmed that other oncogenes can contribute to E2F-1 function including the important estrogen-RB-E2F-1 cell cycle regulating pathway.

The finding that the degree of differentiation of the breast epithelium associates with positive ER expression has gained wide-spread interest. Estrogen can regulate the transcription of target genes directly or indirectly through allosteric activation of the ER, which also plays a central role in regulating the growth and differentiation of breast epithelium as well as in the expression of other genes^[11, 12]. Gewirtz et al^[13] investigated the contribution of estrogen in the MCF-7 human breast carcinoma cell line related to the RB-E2F-1 cell cycle regulatory pathway. Continuous exposure of the MCF-7 cells to estrogen de-

creased the number of the cells in the G1-phase and S-phase cells markedly, but in contrast, the cells at the G2/M transition increased. This study also found that: because of the dephosphorylation of Rb, the transcriptional activity of E2F-1 was inhibited, the cells could not pass into the S-phase and were thus retained in G1^[13,14]. Several studies have also confirmed that the level of E2F-1 in cells with high ER expression is remarkably lowered. Perhaps the effect of estrogen on cyclin inhibited the function of E2F-1 indirectly^[15].

The results of our study showed that the difference in E2F-1, Rb and ER expression among the 3 tissue types was significant. With the atypical development of breast epithelial hyperplasia progressing to DCIS, the over-expression of E2F-1 and lower-expression of ER or Rb acted as an important promoting stimulus. The positive expression rate of E2F-1 and Rb were compared between Peri-PM with ADH and DCIS showing that there was statistically significant difference, so both indicators may possibly aid diagnosis. However the comparison of ER expression between Peri-PM with ADH and DCIS did not show a significant difference. This may relate to the fact that cytologically ADH corresponds to low grade DCIS and therefore they share many similarities. So using the ER marker alone to distinguish between Peri-PM with ADH and DCIS is not reliable.

In addition, the expression of ER was negatively correlated to that of E2F-1 and positively correlated to that of Rb which suggested that simultaneous abnormalities in these proteins could stimulate the cells to pass into the S-phase with unlimited DNA synthesis. Similarly, the progression from normal epithelium through hyperplasia, ADH and DCIS to invasive cancer would be speeded up. In routine clinical work the cases which were diagnosed as Peri-PM in pathological diagnosis could be detected with the 3 indicators at the same time. We suggest that if all 3 indicators are abnormal, the case should be considered to be of high risk, indicating the need for immediate therapy.

REFERENCES

- 1 Ho GH, Calvano JE, Bisogna M, et al. Expression of E2F-1 and E2F-4 is reduced in primary and metastatic breast carcinomas. *Breast Cancer Res Treat* 2001; 69:115-122.
- 2 Carlson CA, Ethier SP. Lack of RB protein correlates with increased sensitivity to UV-radiation-induced apoptosis in human breast cancer cells. *Radiat Res* 2000;154:590-599.
- 3 Tavassoli FA, Devilee P. Editors IARC Press, Lyon. The WHO classification of tumors of the breast and female genital organs. 2003;10-112.
- 4 Page DL, Jensen RA, Simpson JF. Premalignant and malignant disease of the breast: the roles of the pathologist. *Mod Pathol* 1998; 2:120-128.
- 5 Fu XL. Histopathologic typing of breast tumors. See: Liu FS Chief Editor, *Histopathologic Classification of Tumors of Chinese*. Beijing: Scientific and Technical Documents Publishing House. 2001;3:409-411 (Chinese).
- 6 Niu Y, Li Y, Niu RF, et al. Correlation of E2F-1 and Rb expression with papillomatosis and ductal carcinoma in situ of the breast. *Chin J Oncol* 2004;26:290-293 (Chinese).
- 7 Nielsen-NH, Loden-M, Cajander-J, et al. G1-S transition defects occur in most breast cancer and predict outcome. *Breast Cancer Res Treat* 1999;56:105-112.
- 8 Zacharatos P, Kotsinas A, Evangelou K, et al. Distinct expression patterns of the transcription factor E2F-1 in relation to tumour growth parameters in common human carcinomas. *J Pathol* 2004;203:744-753.
- 9 Garcia-Tumon I, Ricote M, Ruiz A, et al. Cell cycle control related proteins (p53, p21, and Rb) and transforming growth factor beta (TGFbeta) in benign and carcinomatous (in situ and infiltrating) human breast: implications in malignant transformations. *Cancer Invest* 2006;24:119-125.
- 10 Bindels EM, Lallemand F, Balkenende A, et al. Involvement of G1/S cyclins in estrogen-independent proliferation of estrogen receptor-positive breast cancer cells. *Oncogene* 2002;21:8158-8165.
- 11 Nobert GS, Kraak MM, Crawford S. Estrogen dependent growth inhibitory effects of tamoxifen but not genistein in solid tumors derived from estrogen receptor positive (ER+) primary breast carcinoma MCF7: single agent and novel combined treatment approaches. *Bull Cancer* 2006;93:E59-66.
- 12 Zheng W, Zheng J, Ma L, et al. Comparison of HER-2/neu, ER and PCNA expression in premenopausal and postmenopausal patients with breast carcinoma. *APMIS* 2005;113:175-181.
- 13 Gewirtz DA, Di YM, Ramdolph JK, et al. Rb dephosphorylation and suppression of E2F activity in human breast tumor cells exposed to a pharmacological concentration of estradiol. *Arch Biochem Biophys* 2001; 388:243-252.
- 14 Jensen SS, Madsen MW, Lukas J, et al. Inhibitory effects of 1alpha, 25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. *Mol Endocrinol* 2001;15:1370-1380.
- 15 Schuur ER, Loktev AV, Sharma M, et al. Ligand-dependent interaction of estrogen receptor-alpha with members of the forkhead transcription factor family. *J Biol Chem* 2001;276:33554-33560.