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Original Article

Immunohistochemical discrimination between intraductal adenocarcinoma of breast and its boderline malignancies with p53, Ki-67, argyrophilic (or silver) nucleolar-organizer region counts (AgNORs)

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Background

It was very difficult to discriminate histologically the intraductal adenocarcinoma (IAC) with less cellular atypism (G1) from its borderline malignancies. Southern blot analysis showed various gene mutations in breast cancer. (1) Some of frankly invasive cancerous cells were immunostained with these gene reactants but there were few reports about the difference between G1-IAC and its borderline malignancies. The present study concerned the histochemical discrimination between G1-IAC and its borderline malignancies in this study.

Methods

Examined cases consisted of nine normal controls, eight mastopathies with ductal hyperplasia, and eight early-staged invasive adenocarcinomas associated with intraductal spreading components (IC) without severe cellular atypism (G1). The ductal hyperplasias in mastopathy represented borderline malignancies and, furthermore, these G1-IC areas served as G1-IAC. All specimens were operatively extirpated ones and processed routinely. Each deparaffinized specimen was histochemically stained with cell proliferation markers (AgNORs, Ki-67) and cancer suppressor gene product (p53).

Results

AgNORs value of normal controls, hyperplasias, and G1-IC was 1.4, 2.0, and 1.9, respectively, and there was significant difference between controls and hyperplasias. Ki-67 and p53 immunostains were stronger in hyperplasias and G1-IC than that in normal controls.

Conclusions

We could not histochemically differentiate the G1-IAC of breast from its borderline malignancies with AgNORs, Ki-67, and p53.

Key words:p53, Ki-67, AgNORs, immunohistochemistry, intraductal adenocarcinoma of breast, borderline malignancy, discrimination

Introduction

In breast it was very difficult to discriminate G1-IAC from its borderline malignancies histologically because the histological atypism of G1-IAC was much less than that of borderline malignancies, that is, either the histologic monotony with cribriform pattern or the loss of myoepithelium was the only pathologic pattern for cancer diagnosis. (1) The more reliable and convenient differentiating method was required.

Many gene mutations in invasive cells of breast

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cancer were shown with Southern blot analysis, e.g. c-erbB-2, c-myc, p53, nm23, cadherin/catenin, and CD44. The number of AgNORs of invasive tumor cells was more than that of benign lesions and the significant difference was confirmed in both the size and the shape of AgNORs. (3) In this study we tried to differentiate G1-IAC histochemically from its boderline malignancies with these reactants. One of the problems in this discrimination was based on the diagnostic reliability of G1-IAC, because it lost severe cellular aypism, invasion, and metastasis, which generally helped to diagnose cancer. We solved this problem as follows: firstly the examined specimens served as G1-IAC fulfilled (i) all specimens were operatively exitirpated ones, (ii) there were definitive invasion or metastasis, (iii) G1-IC occupied in the central area of main tumor, (iv) tumor cells in G1-IC lacked marked cellular atypism, and secondly the examined specimens represented as borderline malignancies satisfied (i) there was myoepithelium confirmed with α-smooth muscle actin, i.e. preservation of two-celled layer, (ii) there was neither invasion nor metastasis.

Materials and Methods

Specimens

Examined cases consisted of nine normal controls, eight mastopathies with ductal hyperplasia, and eight early invasive adenocarcinomas associated with IC without severe cellular atypism (G1-IC). The ductal hyperplasias in mastopathy represented borderline malignancies and, furthermore, these G1-IC areas served as G1-IAC. All operatively extirpated specimens were routinely processed and deparaffinized.

Methods

Objective areas were sought in each specimens stained with Hematoxylin-eosin stain. Objective areas were sectioned and histochemically stained with cell proliferation markers (argyrophilia-detecting AgNORs, Ki-67 (Dako, Denmark)) and cancer suppressor gene product (p53 (Dako, Denmark)) in previously reported methods. (1-3) Routinely-processed paraffin-embedded routinely-immerced sections were preincubated by heat treatment with a microwave to enhance their antigenicity.

Total AgNORs numbers of 200 cells were micr-oscopically counted in the magnification of X1000 and the mean number of AgNORs by the nucleus was calculated. (1) Positive immunoreaction was judged in the magnification of X100, and both the positive cells and negative ones for p53 and Ki-67 were counted in the magnification of X400. The percentage of immunoreactive cells was calculated. For further statistic analysis the positive concentration more than 5% was classified as true positive (semiquantitatively valued as 1.0) and that less than 5% was classified as weakly positive (valued as 0.5) (Fig. 1). The

Fig. 1. Histochemical analysis between borderline malignancy and intraductal components of well-differentiated invasive adenocarcioma of breast (G1-IC)

characteristic	normal control	ductal hyperplasia	G1-IC	p-value
AgNORs &	1.39±0.29*	2.01±0.22*	1.85±0.43	0.05*
Ki-67 #	0.36±0.05	0.88±0.13	0.66±0.09	no significance
p53 #	0.25±0.06	0.31±0.05	0.34±0.03	no significance

& mean silver-positive number in each nucleus

\# judging of positivity with a microscope of 100 magnification and classified as following semiquantitative scoring:

rate of immunoreactive cells	semiquantitative score
0%	0
<5%	0．5
5%≦	1
diffuse	2

negative staining was valued as 0 and, conversely, the diffuse and strong positivity confirmed in the magnification of x40 was valued as 2.0. Mann-Whitney testing was performed for the statistic significance with computer soft of Lotus 1-2-3/W. (1)

Results

AgNORs value of normal controls, hyperplasias, and G1-IC was 1.4, 2.0, 1.9, respectively, and there was the significant difference between controls and hyperplasias (Fig. 1, $p=0.05$). The shape of AgNORs was round and uniform in normal controls, but oval and various in G1-IC. The borderline malignancies showed a configurational anomaly of moderate degree.

Ki-67 value of normal controls, hyperplasias, and G1-IC was 0.36, 0.88, and 0.66, respectively (Fig. 1). The positivity of borderline malignancies was slightly stronger than that of G1-IC.

p53 value of normal controls, borderline malignancies, and G1-IC was 0.25, 0.31, and 0.34, respectively (Fig. 1). All the positivities of p53 were weak and no difference could not be confirmed.

Discussion

AgNORs corresponded to the cell proliferation. Ductal hyperplasias had significantly more AgNORs than that of normal controls. AgNORs of ductal hyperplasias were more than that of G1-IAC. In a previous paper, the value of AgNORs in benign lesions was higer than that in invasive carcinoma of breast by 9 to 14, which was higher than our values just because of sample pretreatment. (3) In previous report about atypical bronchioloalveolar hyperplasia of borderline malignancy, the AgNORs in aneuploidy was more than that in diploidy and, furtheremore, similar to well-differentiated pulmonary adenocarcinoma of Goblet cell type or Clara/II-type. This indicated that borderline malignancy of aneuploidy was more similar to adenocarcinoma than benign lesions. (1) From this, we suppose the following possibilities: (i) our ductal hyperplasias would contain carcinoma of G1-IAC, (ii) carcinoma of G1-IAC would be misdiagnosed as ductal hyperplasias, (iii) these two proliferative lesions were almost similar in the degree of cell proliferation, whether benign or malignant.

Ki-67 was the marker of cell proliferation or tumor growth, and was found in divisional phases of G1-S-G2-M. Ki-67 reaction was stronger in both ductal hyperplasias and G1-IAC than in normal controls. The significant difference in this cell proliferation marker could not be confirmed between ductal hyperplasias and G1-IAC. This implied the same possibilities as described in AgNORs.

The p53-gene mutation of breast cancer occurred by as much as 20 or 40%. (1) But this gene anomaly could not induce the marked accumulation of abnormal p53 products and the immunostaining was too weak to detect in this study.

We failed to differentiate histochemically the G1-IAC of breast from its borderline malignancies with AgNORs, Ki-67, and p53.

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原　著

p53, Ki-67, argyrophilic (or silver) nucleolar-organizer region counts (AgNORs)による乳腺境界病変と分化型乳癌の病理組織化学的鑑別

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乳腺組織における、分化型腺癌と境界病変の鑑別について組織化学的に検討した。細胞増殖マーカーとしてのG1・S・G2・M細胞周期関連マーカー(Ki－67)と核小体形成関連部位認識マーカー(argyrophilic or silver nucleolar-organizer region counts (AgNORs))、並びに、異常癌抑制遺伝子としての癌抑制遺伝子(p53)産物蓄積に関する組織化学的検討がなされた。細胞増殖マーカーは、境界病変と癌で強い反応が認められ、境界病変に最も強い反応が認められた(p＝0.05)。p53は、正常部には認められず境界病変と癌に陽性が認められたが、境界病変と癌の間には有意差は認められなかった。現時点における、分化型腺癌と境界病変の免疫組織学的鑑別は困難と思われた。

キーワード：p53, Ki-67, AgNORs, 乳腺境界病変, 組織学的鑑別

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