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Expression of connexin 43 in breast cancer in comparison with mammary dysplasia and the normal mammary gland

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Gap junctional intercellular communication (GJIC) plays a critical role in tissue development and differentiation and probably in carcinogenesis. The purpose of the study was to evaluate the expression and localisation of Cx43 in 40 cases of mammary dysplasia and 29 cases of breast cancer (without primary chemotherapy). The tissue sections were investigated for Cx43 expression by immunohistochemistry. In the normal mammary gland there was an intercellular, punctate staining pattern, mainly between myoepithelial cells, characteristic of functional gap junctions. In dysplasias there was mainly mixed (cytoplasmic and intercellular) staining and in most cases of breast cancer we observed diffuse or granular, but cytoplasmic, staining of Cx43. Our results demonstrated that expression of Cx43 in dysplasias and breast cancer is changed and GJIC is probably impaired because of disruption of functional gap junction formation especially between breast cancer cells.

key words: gap junctions, connexin 43, benign mammary dysplasia, breast cancer

INTRODUCTION

Gap junctions are specialised cell membrane channels connecting adjacent cells that facilitate the transfer of small (M_r < 1000) molecules between cells. The mammary epithelium contains Cx43 between myoepithelial cells [4]. Loss of communication via gap junctions appears to play a role in oncogenesis [3, 4] and up-regulation of Cxs has been shown to restore normal phenotypes and retard tumour cell growth [3]. In several studies lack of connexin expression and/or function of gap junction channels was demonstrated in human tumours. However, in the breast the situation is still unclear. Different scientists have observed increased [1] as well as decreased [3] expression of connexins in breast cancer cells. There have been very few studies concerning the existence of connexins in pre-neoplastic cells and lack of evidence to evaluate the expression of Cxs in mammary dysplasia. The aim of this study was to evaluate the expression of Cx43 in breast cancer (intraductal carcinoma and invasive carcinoma of the breast) compared with the normal mammary gland and with mammary dysplasia with variable pathologial findings such as cysts, with or without apocrine metaplasia, typical or atypical ductal hyperplasia, adenosis and papillomas.

MATERIAL AND METHODS

Formalin-fixed and paraffin-embedded tissue samples were investigated as follows: 25 cases of normal human breast; 40 benign mammary dysplasias including adenosis (30 cases), cysts (30 cases),

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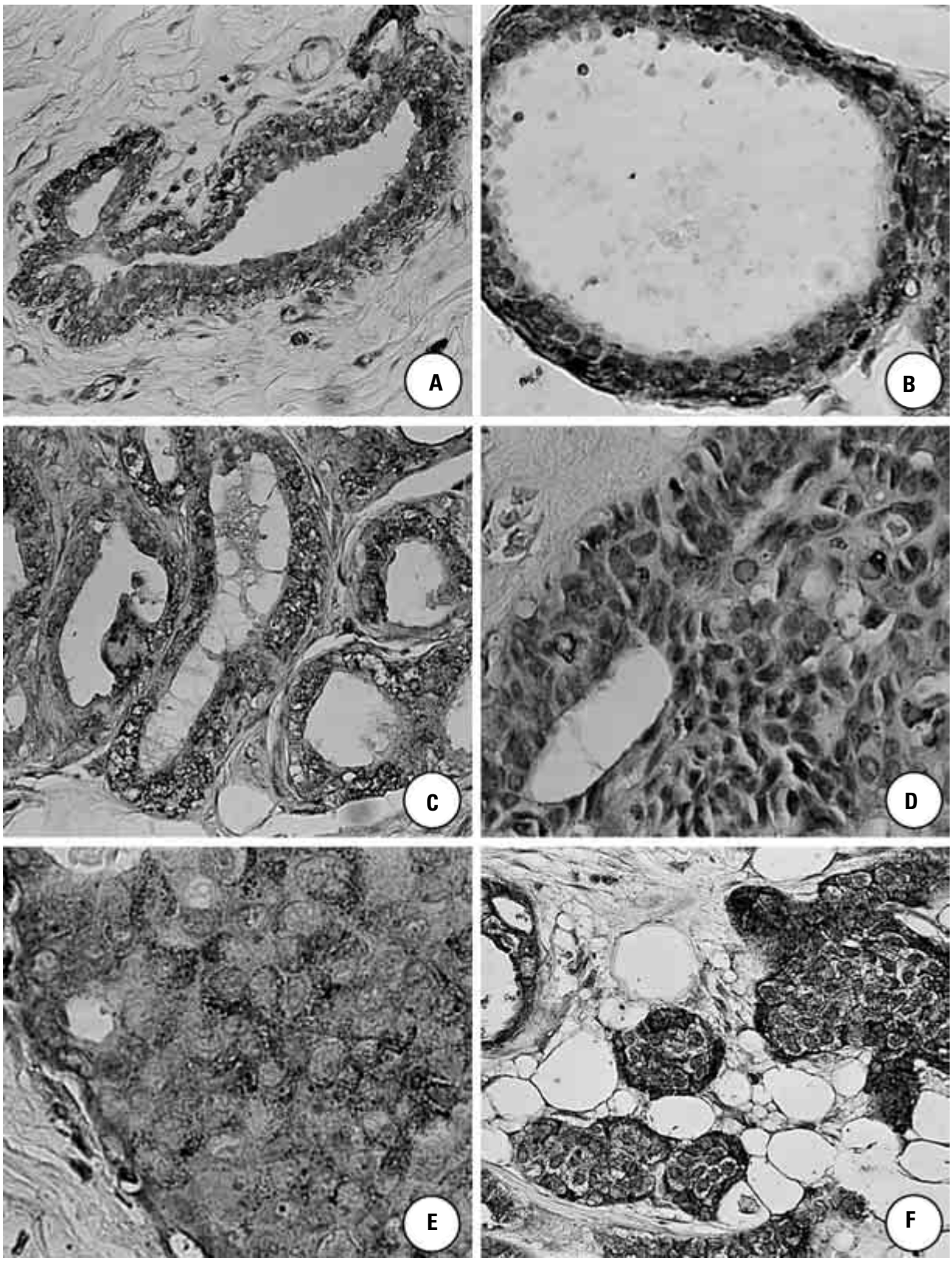

Figures 1. Immunohistochemical detection of Cx43 expression in the human breast. **A, B.** Granular, intercellular expression of Cx43 between myoepithelial cells in the normal breast duct and in the small cyst. Magn. 200 ×, 400 ×; **C.** Mixed (intercellular and cytoplasmic) staining pattern in the adenosis. Magn. 200 ×; **D.** Weak cytoplasmic staining in the atypical intraductal hyperplasia. Magn. 400 ×; **E.** Heterogenous cytoplasmic staining pattern in the intraductal carcinoma. Magn. 400 ×; **F.** Strong cytoplasmic staining in breast cancer cells. Magn. 200 ×.

intraductal hyperplasia (20 cases), intraductal atypical hyperplasia (10 cases), papilloma (15 cases), as well as 11 intraductal carcinomas and 29 cases of ductal carcinoma (18 cases in grade G2 and 11 cases in grade G3). Immunostaining was performed using goat polyclonal anti-Cx43 antibody (Santa Cruz Biotechnology, USA) in a dilution rate of 1:200. A streptavidin-biotin-peroxidase complex technique was used to reveal antibody-antigen reactions (LSAB + Kit, Dako, Denmark) according to the protocol provided by the manufacturer. Staining was routinely developed using 3,3'-diaminobenzidine as a chromogen (Dako, Denmark). Negative controls were carried out with omission of the primary antibody. Sections were counterstained with haematoxylin. The results of immunostaining were evaluated by light microscopy using a 200× magnification by two independent pathologists.

RESULTS AND DISCUSSION

The anti-Cx43 antibody gave punctate intercellular immunostaining mainly for the myoepithelial cells of the normal human breast (Fig. 1A). In the lesions of papilloma, adenosis and intraductal hyperplasia (Fig. 1C) was observed mixed, intercellular and cytoplasmic immunoreactivity (28/30), and in some cases membrane-associated staining for myoepithelial cells was also seen. The majority of cysts were lined with apocrine epithelium, in this case cytoplasmic, but it seemed that no specific immunoreactivity was shown. In the case of cysts without apocrine metaplasia we observed a very weak, intercellular staining pattern (Fig. 1B), but only in a few cells. In the atypical ductal hyperplasia heterogenous (intercellular and cytoplasmic) staining was seen (Fig. 1D), but this was sparse compared to the typical intraductal hyperplasia. In the majority of cases (7/11) of intraductal carcinoma no immunoreactivity was seen in the cancer cells. In one case, the carcinoma cells had intercellular, granular staining for Cx43 while in other cases (3/11) there was a weak, cytoplasmic staining pattern (Fig. 1E). The majority (26/29) of invasive carcinomas exhibited cytoplasmic, mainly diffuse immunostaining in carcinoma cells (Fig. 1F) and there was a predominantly strong reaction (15/26). In two cases we observed punctate staining aggregations between cancer cells. In only three cases of the completely evaluated samples was no reaction found. Furthermore, in the breast arterioles the endothelial and smooth muscle cells stained positively for Cx43.

It has been shown that in normal human breast epithelium Cx26 and Cx43 are present [4]. Our finding of punctate aggregations of Cx43 between myoepithelial cells is in agreement with the results of a recent study made by Jamieson et al. [1]. Furthermore, the present study demonstrated for the first time that in various pathological components of benign mammary dysplasia expression of Cx43 is changed, ranging from the characteristic intercellular pattern present in cysts, the mixed (intercellular and cytoplasmic) pattern in the case of papilloma, adenosis and intraductal hyperplasia, and finally down-regulation of Cx43 expression in the atypical ductal hyperplasia. In the letter case, our observations are compatible with those of King et al. [2], in which a loss of Cx43 expression is described in premalignant lesions of the cervix (cervical dysplasia). Parallel to our results, Jamieson et al. [1] and Laird et al. [3] failed to identify Cx43 in the intraductal carcinoma cells. It is, therefore, probable that loss of Cx43 expression may be an early event in the carcinogenic process.

In several studies the absence of connexin expression and/or function of gap junction channels has been demonstrated in human tumours [3, 5]. It has been suggested that genes-encoding connexins could play a tumour suppressor role. On the other hand, Jamieson et al. [1] investigated the immunohistochemical expression of Cx26 and Cx43 in breast cancer cells and found mainly cytoplasmic heterogenous Cx26 and Cx43 immunostaining in breast cancer cells. Our findings are consistent with the results of Jamieson et al. [1]. This could suggest that persistent expression of Cx43 might be inconsistent with a tumour suppressor role of Cxs, but it is possible that breast cancer cells express Cx43 in cytoplasm, which fails to traffic and assemble Cx43 into functional gap junction channels at the cell surface. Further investigation of Cxs expression, regulation and gap junction traffic may shed light on the relationship between gap junctions and mammary carcinogenesis.

REFERENCES

1. Jamieson S, Going JJ, D'arcy R, George D (1998) Expression of gap junction proteins connexin 26 and connexin 43 in normal human breast and in breast tumours. J Pathol, 184: 37–43.
2. King TJ, Fukushima LH, Hieber AD, Shimabukuro KA, Sakr WA, Bertram JS (2000) Reduced levels of connexin 43 in cervical dysplasia: inducible expression in a cervical carcinoma cell line decreases neoplastic potential with implications for tumour progression. Carcinogenesis, 21: 1097–1109.
3. Laird DW, Fistouris P, Batist G, Alpert L, Huynh HT, Carystinos GD, Alaoui-Jamali MA (1999) Deficiency

of connexin 43 gap junctions is an independent marker for breast tumors. Cancer Res, 59: 4104–4110.
4. Lee SW, Tomasetto C, Paul D, Keyomarsi K, Sager R (1992) Transcriptional down-regulation of gap-junction proteins blocks junctional communication in human mammary tumor cell lines. J Cell Biol, 118: 1213–1221.
5. Wilgenbus KK, Kirkpatrick CJ, Knuechel R, Willecke K, Traub O (1992) Expression of Cx26, Cx32 and Cx43 gap junction proteins in normal and neoplastic human tissues. Int J Cancer, 51: 522–529.