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DIFFERENTIATION OF TUMOURS OF DUCTAL AND LOBULAR ORIGIN:

I. PROTEOMICS OF INVASIVE DUCTAL AND LOBULAR BREAST CARCINOMAS

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The vast majority of invasive breast tumors are ductal and lobular breast carcinomas. Despite the many similarities, some clinical follow-up data and the patterns of metastases suggest that these histological subtypes of breast cancer are biologically distinct. Few papers, however, describe immunohistochemical markers useful for differentiation of these carcinomas. Many investigations suggest that E cadherin protein expression is lost in lobular but not in ductal carcinoma. The absence of E-CD, as a partial loss of epithelial differentiation, may account for the extended spread of lobular carcinoma in situ and the peculiar diffuse invasion mode of invasive lobular carcinoma. Some investigations report the significance of E-CD associated proteins alpha-, beta-, gamma-catenin expression, as well as the usefulness of cytokeratins 5, 6, 8, 7 and thrombospondin in differentiating histological types of breast invasive carcinomas. Several reports have suggested the possibility that invasive ductal and lobular cancers differ with respect to expression of antigens involved in proliferation and cell cycle regulation. It has been shown that vascular endothelial growth factor expression, also the expression of maspin, a tumour suppressor gene product, is higher in ductal, than in lobular carcinoma. Expression of NKX3.1, a member of the NK-class of homeodomain, is highly restricted and is found primarily in lobular carcinoma. Some histological and immunohistochemical characteristics of pleomorphic lobular carcinoma are also discussed.

INTRODUCTION

Invasive ductal and lobular breast carcinomas are the most common malignancies of the breast, accounting for 80 % and 15 % of all invasive breast tumors, respectively. The two tumor subtypes are distinguished on the basis of their histology, with ductal tumors tending to form glandular structures, whereas lobular tumors are less cohesive and tend to invade in single file[1,2]. Although treatment for stage-matched ductal versus lobular tumors is similar[3,4], some studies suggest that metastatic patterns differ between lobular and ductal tumors[5,6], and lobular tumors may be less responsive to neoadjuvant therapy[7]. Such studies suggest that lobular tumor development and progression may follow a distinct pathway from ductal tumors.

At present, few papers report immunohistochemical markers useful for differentiation of lobular and ductal carcinomas of the breast or for differentiation of carcinomas derived from luminal and myoepithelial cells. Infiltrating lobular carcinoma (ILC) and infiltrating ductal carcinoma (IDC) are similar in many respects and their histologic features occasionally overlap[8–10]. Despite the many similarities, some clinical follow-up data and the patterns of metastasis suggest that ILC and IDC are biologically distinct[11,12]. Unfortunately, most breast cancer research has focused almost exclusively on the ductal subtype or has not stressed the biologic or molecular genetic distinctions between breast carcinoma subtypes.

In order to study the biological differences between histological types of breast carcinoma, Ruibal *et al* analyzed clinical and biological parameters in breast cancer patients. In negative axillary lymph node involvement (N-) patients, ILCs were more frequently associated with diploidy, the existence of multiple foci, and higher pS2 cytosolic levels than IDC. In N+ patients, ILCs were associated with multicentricity and had higher concentrations of progesterone receptors[13]. Mersin *et al* reported that although patients with ILC were older, had low grade tumor and less lymphatic vascular invasion, they had no survival advantage compared with their counterparts, and histologic type was not an independent prognostic factor for outcome[14].

E CADHERIN

Many investigations suggest that E cadherin (E-CD) protein expression is lost in ILC but not IDC of the breast[15-18]. E-CD is a calcium-dependent, epithelial-specific cell-cell adhesion molecule whose reduced or lost expression is associated with tumor dedifferentiation and increased metastatic potential in human carcinomas[19]. Lehr *et al* found that IDC express E-CD in a similar peripheral-predominant immunostaining pattern, while all ILCs are negative for E-CD, suggesting a role for E-CD in the architectural organization of the cytoskeletal scaffolding within the tumor cells[20]. Acs *et al* described E-CD as a useful diagnostic tool strongly specific for tumours of ductal origin. They found that all in situ carcinomas with mixed ductal and lobular features demonstrated complete loss of staining. Invasive carcinomas with ductal and lobular features showed 3 staining patterns: complete or almost complete lack of membrane staining, uniform membrane expression throughout the tumor, and focal loss of E-CD staining, which correlated with the histologic impression of focal lobular features[21].

Goldstein studied relationships between membrane E-CD reactivity of invasive carcinoma, a dyshesive growth pattern, and lobular carcinoma-type systemic metastases. 7 % mixed, predominantly ILC, 61 % mixed carcinomas, and 67 % mixed, predominantly IDC had E-CD staining in more than 10 % of the ILC cells. Lobular carcinoma-type systemic metastases were identified in 84 % pure lobular; 11 % mixed; 4 % pure ductal cacinomas. No E-CD staining was found in 98 % of ILC in cases of lobular carcinoma-type systemic metastases and all cases of ILC systemic metastases[22]. Moll *et al* showed that loss of E-CD expression is an early event in the formation of the lobular type of breast carcinomas. The absence of E-CD, as a partial loss of epithelial differentiation, may account for the extended spread of lobular carcinoma in situ and the peculiar diffuse invasion mode of ILC. The generation of dedifferentiated IDCs can only in part be correlated with reduced expression of the intercellular adhesion molecule E-CD[23].

Gamallo *et al* showed that E-CD expression correlates with histological type and grade of breast carcinoma. None of the ILC expressed E-CD, whereas only weak immunostaining was found in areas of atypical lobular hyperplasia and lobular carcinoma in situ[24]. Siitonen *et al* showed that the loss of normal E-CD expression is an indicator of increased invasiveness and dedifferentiation in breast carcinoma and that E-CD is a potentially important prognostic factor in primary IDCs. The proportion of tumors with reduced or lost E-CD expression increased significantly from pure intraductal carcinomas (20 %) through invasive ductal (52 %) to recurrent carcinomas. None of the ILCs retained normal E-CD expression in contrast to 48 % of the IDCs. In primary IDCs, reduced E-CD expression was associated with high histologic grade, negative estrogen receptor status, and axillary node involvement. In a subset of primary IDC patients reduced E-CD expression was associated with shortened disease-free survival[25]. Brinck *et al* found that the loss of E-CD expression is related to an increase in diffuse growth pattern in both lobular and ductal types of breast cancer, and the differential proportions of growth patterns in both tumor types cause the tendency for lower E-CD expression in the lobular type. In 60 % of ILC the diffuse growth pattern and in 72 % of IDC the compact growth pattern predominated. E-CD expression was significantly lower in diffuse than in compact tumor area and not related to carcinoma type. No mutations were detected[26].

Most breast carcinomas in situ (CIS) are easily categorized as ductal (DCIS) or lobular (LCIS). However, some CIS have indeterminate histologic features (CIS-IF). Jacobs *et al* suggest that E-CD immunostaining is of value in helping to characterize breast carcinomas in situ with indeterminate features. They studied histologic features and E-CD expression by immunohistochemistry in LCIS, DCIS, and CIS-IF. CIS-IF cases were divided into three groups based on histology: Group 1 cases were typical LCISs with areas of comedo-type necrosis. Group 2 cases were CIS lesions growing in a solid or cohesive mosaic pattern. Group 3 cases had the dyshesive growth pattern characteristic of LCIS. All cases of LCIS were E-CD negative, and all DCIS cases were E-CD positive by immunohistochemistry. All cases from the CIS-IF group 1 and group 3 were negative for E-CD, suggesting a closer kinship to LCIS than to DCIS. In contrast, CIS-IF group 2 cases were heterogeneous with respect to E-CD staining. 35.3 % cases were E-CD negative (more akin to LCIS), 29.4 % cases were E-CD positive (akin to DCIS), and 35.3 % cases had both E-CD-positive and E-CD-negative tumor cells, suggesting a mixed DCIS/LCIS phenotype[27].

Bratthauer et al found that E-CD and high molecular weight (HMW) cytokeratins in combination are extremely useful in distinguishing lobular (LIN) and ductal (DIN) intraepithelial lesions and clarifying the nature of morphologically intermediate cases. Antibodies to E-CD and HMW cytokeratins 1, 5, 10 and 14 were used in classic LIN, DIN1c to DIN3. All samples of LIN showed complete negativity for E-CD, whereas the LIN lesions displayed cytoplasmic positivity, often in a distinct peinuclear pattern. Morphologically indeterminate cases could be classified as either ductal or lobular based on the immunoprofile but some cases differed from either typical DIN or classic LIN which can be separated as MIN (mammary intraepithelial neoplasia, not otherwise specified)[28].

CATENINS

Some investigations suggest the role of E-CD associated proteins alpha-, beta-, gamma-catenin expression and their significance in ILC and IDC[29-31].Beta-Catenin plays a central role in the E-CD/catenin cell-cell adhesion complex and is possibly involved in cellular signalling pathways. De Leeuw *et al* report that simultaneous loss of E-cadherin and alpha-, beta- and gamma-catenin is an

important step in the formation of lobular carcinoma in situ, as a precursor of invasive lobular breast cancer[32]. Karayiannakis *et al* found that quantitative and qualitative changes in beta-catenin expression occur in in situ and IDC and are more prominent in ILC. Altered beta-catenin expression was found in 68 % of tumours including 77 % of ILC and 64 % of IDC with 46 % of lobular cases showing complete absence of beta-catenin immunoreactivity. Cytoplasmic beta-catenin localization was seen only in IDC[33]. However, Han *et al* showed that alpha-, beta-, gamma-catenin expression was almost identical in ILC and IDC of breast, indicating significant loss and reduction of protein expression in tumor cells[34].

CYTOKERATINS

Some investigations suggest the usefulness of cytokeratins in differentiating histological types of breast invasive carcinomas. Immunohistochemistry using antibodies to cytokeratin 8 can serve as a valuable diagnostic tool for the differentiation of ILC from IDC. IDC exhibits a peripheral-predominant immunostaining pattern, adjacent tumor cells "molding" to each other, while ILC shows a ring-like perinuclear immunostaining pattern. This immunostaining pattern is stable even in tumors that otherwise do not exhibit characteristic histomorphologic features[20]. Heatley *et al* showed that cytokeratin profiles in the luminal epithelium of benign breast lesions and most carcinomas are similar in most cases, but in a few IDCs none of the tumour cells reacted for cytokeratins 7, 8, or 18. Three IDCs expressed cytokeratin 14. Only occasional cases expressed cytokeratins 3,4,10,13[35]. Tot analysed the expression of cytokeratins 20 and 7 in different histological types of invasive breast carcinomas. Typical IDC as well as ILC carcinomas expressed CK7 but not CK20. 92 % were CK20 negative. 4 % of ILC stained diffusely with CK 20.98 % of the tumors were CK7 positive. Like their ductal counterparts, invasive breast carcinomas of special type were CK20(–)/CK7(+)[36].

Otterbach *et al* found that basal-type cytokeratins (CKs) 5 and 6 can distinguish typical ductal hyperplasias (UDH) from the spectrum of atypical ductal hyperplasias (ADH), DCIS and LCIS. In normal breast tissues, myoepithelial immunoreactivity for CK5/6 was most pronounced in the duct system, while luminal epithelial immunoreactivity was strongest in the terminal duct lobular units. In ductal hyperplasias, luminal epithelial cells revealed predominantly CK 5/6 immunoreaction. In contrast, neoplastic epithelial cells in atypical ductal and lobular hyperplasias (ADH and ALH) lacked such an expression, whereas 3.7 % of DCIS and 7.7 % of IDCs showed positive immunostaining[37]. Lacroix-Triki *et al* confirmed that D5/16B4 antibody directed against CK5/6 is useful in distinguishing UDH from the spectrum of ADH/DCIS/LCIS and that D5/16B4 is a far more specific marker than 34betaE12 antibody. Abundant immunostaining was observed in all UDH using both antibodies[38].

CYCLINS

Several reports have suggested the possibility that ILCs and IDCs differ with respect to expression of antigens involved in proliferation and cell cycle regulation. Soslow *et al* studied the expression of estrogen receptor (ER), progesterone receptor (PR), HER-2/neu, Ki-67, cyclin D1, p27, p53, mdm-2 and bcl-2. They found that ILCs and well-differentiated IDCs show similar proliferation and cell cycle control antigen profiles. Despite their unusual histologic features, most ILC variants appear to maintain a characteristic ILC immunophenotype[39]. Sasano *et al* found different patterns of cyclin D1, cyclin E, cdk2 and cdk4 expression in human breast IDC and ILC. In IDC a significant correlation was found between Ki-67 and cyclin D1, Ki-67 and cdk2 and cyclin D1 and cdk4. Only cyclin D1 correlated with the pathologic stages of the disease and histological grades of invasive IDC. Cyclin D1 and cdk2 expression correlated with cell proliferation and cyclin D1 expression with expression of cdk4 in IDC but not ILC[40]. Naidu *et al* found that cyclin D1 expression differs according to histological type of breast neoplasm. Cyclin D1 was strongly positive in 61 % of the comedo subtype, 61 % of the non-comedo subtype, 59 % of the comedo DCIS and 63 % of the adjacent IDC, 53 % of the non-comedo DCIS and 58 % of the adjacent invasive lesions, 58 % of the IDC, 73 % of the ILC[41].

THROMBOSPONDIN

The biological differences between ductal and lobular breast carcinomas is reflected in the different expression patterns of TSP (thrombospondin). TSP is present in normal breast secretions, and high levels of TSP are observed in malignant breast secretions and cytosols. Three genes encoding for three distinct TSPs (TSP1, TSP2, TSP3) have recently been described. TSP1 has two known cell surface receptors for TSP1: CD36 and CD51. Clezardin *et al* showed that TSP1 codistributed with CD51 in most ILC cells (40 to 80 %) and with CD36 in a subpopulation (30 to 40 %) of these invasive tumor cells[42]. TSP could account for the peculiarly diffuse invasive behaviour of breast ILC cells. Serre *et al* studied the ultrastructural distribution of TSP and its cell surface receptor, integrin alpha V in DCIS with an invasive component and ILC. In the invasive part of IDC, most of the malignant cells were negative for TSP, while integrin alpha V was moderately expressed. In contrast, typical strands of ILC cells in „Indian file" showed moderate TSP immunostaining in the rough endoplasmic reticulum and strong immunoreactivity for TSP at the plasma membrane and in the extracellular matrix. Moderate to strong immunoreactivity for integrin alpha V was also observed in ILC cells[43].

p63

p63 is a member of the p53 gene family, and its germline mutations are associated with severe mammary developmental defects in both rodents and humans. Different p63 isoforms have been identified, some of which ([DELTA]Np63) are preferentially expressed in the epithelial basal cells of different organs and have been considered as possible markers of stem cells/reserve cells. Barbareschi *et al* demonstrated that in normal and pathologic breast tissue myoepithelial cells (MCs) consistently express the [DELTA]Np63 isoforms. They suggest that p63 is a reliable, highly specific, and sensitive MC marker in both histologic and cytologic preparations. Since p63 immunoreactivity in adult epithelia is normally restricted to progenitor cells, it can be speculated that it might be a clue for the identification of the still elusive breast progenitor cells[44].

VEGF

Angiogenesis is essential for tumour growth and important in tumour metastasis and prognosis. Vascular endothelial growth factor (VEGF) stimulates endothelial proliferation in vitro and angiogenesis in vivo[45,46]. It has been shown that VEGF expression is correlated with high tumour vascularity, including carcinoma of the breast[47]. Lee *et al* found a difference in VEGF expression between IDC and ILC. They investigated VEGF expression by immunohistochemistry and in situ hybridization in ILC, IDC and pure DICS of the breast. Vascular density was assessed by staining for von Willebrand factor. There was more expression of both VEGF protein and mRNA in IDC than in ILC. VEGF protein and mRNA correlated with vascular density in IDC. In ILC, vascular density did not correlate with VEGF mRNA and was inversely related to VEGF protein. There were no significant differences in vascular density between the two types of invasive carcinoma, suggesting that VEGF is important in angiogenesis in IDC, but that other angiogenic factors are important in ILC[48].

MASPIN

Maspin is a recently described member of the serpin family or protease inhibitors known to be a tumour suppressor gene product. Loss of maspin expression has been found in most breast cancer cases and is correlated with cell motility and tumour invasiveness[49,50]. However, its precise role in human breast cancer remains unknown. Some results indicate that different biological mechanisms may be responsible for maspin expression in different histological types of breast cancer. Kim *et al* found that maspin expression was more frequently detected in IDC (36.4 %) than in ILC (7.1 %). They analysed the expression of maspin in stages I and II of primary breast cancers by immunohistochemistry. 34.4 % of these cases showed maspin expression, which was not associated with overall and disease-free survival rate of breast cancer[51].

NKX3.1

NKX3.1 is a member of the NK-class of homeodomain. It can be expressed in a broad spectrum of human cancers and normal tissues[52–54]. Gelmann *et al* showed that expression of NKX3.1 is highly restricted and is found primarily in ILC. NKX3.1 expressed in 9 % of primary and 5 % of metastatic IDC, and 27 % of primary and 26 % of metastatic ILC. In a cohort of primary breast cancers with median follow-up over 62.5 month survival, NKX3.1 expression had no effect on prognosis[55] (Table 1).

Table 1. Putative immunohistochemical markers for invasive ductal and lobular breast carcinomas

Immunohistochemical markers	ILC	**IDC**
E cadherin	No expression	Expression
Alpha-, beta-, gamma-catenin	No expression	Expression
Cytokeratin 8	Ring-like perinuclear immunostaining	Peripheral-predominant immunostaining
Cytokeratins 18	Expression	No expression
Thrombospondin	Expression	No expression
VEGF	No expression	Expression
Maspin	No expression	Expression
NKX3.1	Expression	No expression

DIFFERENTIATION OF PLEOMORPHIC LOBULAR AND DUCTAL CARCINOMA

Pleomorphic lobular carcinoma (PLC) is a recently described entity separate from classical ILC by cytologic pleomorphism[56,57]. It can have an aggressive clinical course with a higher frequency of recurrence. Histologic differentiation from IDC may be difficult but it is important for this differentiation to be made[58,59]. Complete loss of E-CD expression has been observed in ILC and LCIS. IDC retains at least some expression of E-CD. Wahed *et al* examined the pattern of E-CD expression in a series of cases of PLC by immunohistochemistry. 86 % of cases showed no staining, and the remaining cases exhibited 10 % to 25 % positive cells. In cases with histologically equivocal features, immunohistochemical detection of

E-CD expression can be a useful diagnostic aid in the differentiation of PLC and IDC[60]. Frolik *et al* studied PLC, poorly differentiated IDC and well-differentiated ILC as controls. They detected more frequent over-expression of Her2 among PLCs (G3) as well as the generally low apoptosis which can contribute to their aggressive behaviour[61]. Sneige *et al* found that the cytologic features and biomarker expression profile of of PLC (ductal-lobular) CIS (PL/DLCIS) are similar to those of PLC in situ (PLCIS) with invasion but somewhat different from those of classic LCIS and DCIS[62].

CONCLUSION

The data discussed above suggest that the proteomics of infiltrating ductal and lobular carcinomas are different. The biological differences between these two types of breast cancer are reflected in the different expression of E cadherin, E cadherin associated proteins alpha-, beta-, gamma-catenin, basal type cytokeratins, cyclins, thrombospondin, p63, VEGF, maspin, and NKX3.1. The distinct expression patterns of these proteins in atypical epithelial hyperplasias, ductal or lobular carcinoma in situ lesions and infiltrative ductal and lobular tumours suggest that the molecular basis of breast cancer is not uniform. Different histological types of breast tumours show different phenotypes which seem to be associated with different gene expression profiling. More investigations focused on correlations between tumour phenotype and gene expression profile are needed for translation of molecular results in clinical practice.

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