

## Advances in breast pathology

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### The role of metalloproteinases in breast lesions

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During the last decades, increasing attention has been focused on the role of extracellular matrix, especially basement membrane, in the process of tumor invasion and metastasis. Although the biochemical mechanisms involved in basement membrane turnover under physiological conditions remain essentially unknown, it has been established that the basement membrane-degrading properties of tumor cells correlate with their metastatic potential. Degradation of extracellular matrices is a fundamental stage in the process of invasion and the development of metastasis.

Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes with capacity to degrade and remodel the components of the extracellular matrix. These MMPs include collagenases, gelatinases, stromelysins and others. The activity of these enzymes depends on many factors, including the presence of specific inhibitors of metalloproteinases or TIMPs.

The cellular source of extracellular matrix-degrading enzymes has important implications in our understanding of tumor biology and tissue remodeling. During invasion and the process of metastases, tumor cells must traverse epithelial and endothelial basement membrane, where type IV collagen represents a barrier as a major structural component. Type IV collagen is one of the major components of basement membrane, and it represents the structural scaffolding of these specialized sheets of extracellular matrix.

The enzymatic degradation of type IV collagen is specifically initiated by a neutral metalloproteinase, type IV collagenase. This enzyme has been found in human tumor cells, as well as in other normal cell types such as endothelium, fibroblasts, macrophages, polymorphonuclear leukocytes and keratinocytes. It is secreted in a latent form that can be activated, at least *in vitro*, by trypsin and organomercurial compounds. This activation is associated with the loss of 80 amino acid residues from the amino terminus.

Production of type IV collagenase by tumor cells have been linked to their metastatic potential in several experimental models. A possible role for this enzyme in basement membrane type IV collagen turnover has also been suggested.

Host-tumor cell interactions are known to play a role in the degradation of interstitial (I, II and III) collagens. Pepsinized type IV collagen is not degraded by classical interstitial collagenases, although it is sensitive to some extent to other nonspecific proteases such as elastase, cathepsin G, and plasmin.

The isolation and characterization of type IV collagenase introduced a new approach in basement membrane physiology and

pathology. The role of this enzyme in tumor invasion has been discussed previously. Experimental evidence of a link between the increased production of type IV collagenase by tumor cells and metastatic behavior was described in murine models, and recently it has been extended to the ras-induced metastatic phenotype.

The structure and changes of basement membrane in breast lesions have been extensively studied, and a variable loss of basement membrane is a constant feature in invasive neoplasms. Previous investigators, employing polyclonal antibodies against murine type IV collagenase, have found positive immunoreactivity in invading breast cancer cells but not in normal breast, benign lesions, or *in situ* carcinomas.

Monteagudo *et al.* evaluated the presence and distribution of type IV collagenase in normal, hyperplastic and malignant breast lesions as well as metastases of the breast, utilizing purified antibodies against different domains of the enzyme. They found intense staining in malignant breast cancers as well as metastasis. Normal myoepithelial cells also stained positive which suggested that these cells played an important role in the physiologic turnover of the basement membrane.

Recently, it has been demonstrated that immunoreactivity of MMP1 (collagenases) in stromal fibroblasts but not in tumor cells, correlated with tumor stage in patients with breast cancer. Expression of this enzyme in either epithelial or stromal cells had an inverse correlation with progesterone receptor.

Lately, correlation has been found between specific MMPs (stromelysin-1) and poor prognostic factors such as expression of p53 tumor suppressor gene products, *c-erbB-2* and progesterone receptor status.

The degradation of the extracellular matrix requires a disruption of the equilibrium maintained by the MMPs and their inhibitors (TIMPs). Overexpression of TIMP-1 has been documented in human breast cancer, and it is possible that expression of these inhibitor metalloproteinases may play a more important role in the tumor progression of breast cancer and metastasis.

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## Molecular genetic analysis of epithelial hyperplasia and normal tissues in breast

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The multistep model of carcinogenesis in the breast suggests a transition from normal epithelium to invasive Carcinoma via non-atypical and atypical hyperplasia and *in situ* carcinoma. Within the breast, these proliferations are heterogeneous in their cytological and architectural characteristics. The introduction of mammographic screening has led to the increased detection of preinvasive disease, the identification of which has highlighted deficiencies in our understanding and classification of such lesions within the breast. The morphological classification of breast disease remains controversial and difficulties are encountered in the subclassification of ductal carcinoma *in situ* (DCIS), differentiating DCIS from atypical ductal hyperplasia (ADH) and differentiating low nuclear grade (LNG) DCIS of solid type from lobular carcinoma *in situ* (LCIS).

ADH is a controversial lesion, which shares some but not all features of DCIS. It poses diagnostic difficulties in surgical histopathology and in order to address this problem, Page and Rogers (1) laid down clear criteria for the diagnosis of this entity. Rosai (2) demonstrated a high interobserver variability in the diagnosis of ADH, however, subsequently Schnitt *et al*. (3), showed an improvement with complete agreement in 58% of cases by training pathologists to use the Page criteria. Within the UK National Quality Assurance Scheme (4), agreement even among experienced breast pathologists has been low.

Lakhani *et al*. (5) showed that loss of heterozygosity (LOH) identified at loci on 16q and 17p in invasive carcinoma and DCIS is also present in ADH with a similar frequency. This indicates that ADH is a neoplastic rather than a hyperplastic proliferation and perhaps, best considered within the spectrum of *in situ* ductal neoplasia. A number of other studies have reported similar results (6-9). O'Connell *et al*. (9) studied 51 cases of ADH at 15 polymorphic loci and found LOH at at least one marker in 42% of the cases. The studies demonstrate that within the limits of available molecular investigations, ADH as currently defined is simply a small focus of DCIS.

Hyperplasia of usual type (HUT) has also been referred to as epitheliosis and papillomatosis in the past. It may range from mild to florid proliferations and retrospective studies have shown that it has a relative risk of x2 for the subsequent development of invasive cancer.

As with atypical hyperplasia, cytogenetic analysis of HUT has been limited. Some studies have reported chromosomal aberration in a proportion of HUT. O'Connell *et al*. (9) showed that LOH at many different loci can be identified in HUT with frequencies ranging from 0-15%. These figures are consistent with those of Lakhani *et al*. (10) who reported data in nonatypical hyperplasia dissected from benign breast biopsies. LOH was identified at frequencies ranging from 0% at locus on 13q (D13S267) to 13% at locus on 17q (D17S250). The frequencies of LOH in HUT are much lower than those identified in DCIS and ADH (range 25-55%). The results indicate that at least a proportion of nonatypical hyperplasias are also clonal, neoplastic proliferations. Hence, biologically, at least some HUT appears to be benign "adenomas" of the breast epithelium. The data would be consistent with HUT as a nonobligate precursor of invasive cancer. In this study, no specific morphological features were identified that predicted allelic imbalance.

Two studies over the last 2 years have also demonstrated that LOH identified in invasive carcinoma is already present in morphological normal lobules (11, 12). LOH has been demonstrated independently in luminal and myoepithelial cells suggesting the presence of a common, locally derived stem cell for the two epithelial cell types (Lakhani *et al*., unpublished observations). The extent, frequency and significance of changes in "normal" tissue remains to be evaluated, however, the data support the Concept of multistep evolution of breast cancer.

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