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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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Multistep model of the genetic alterations leading to breast cancer

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Breast cancer development is the result of the accumulation of genetic alterations in the epithelial cells of the breast. The main genetic alterations occurring in breast cancer are amplification of a number of oncogenes (approximately 10) and the inactivation of tumor suppressor genes. There are approximately five known tumor suppressor genes in breast cancer, but in view of the high frequency of loss of heterozygosity found for various chromosomal locations, many more tumor suppressor genes must play a role in breast cancer development. Translocations and activating mutations in oncogenes do not seem to play an important role in breast cancer development.

There are many reports in the literature on genetic alterations in invasive breast carcinomas; a limited number of reports describe the genetic alterations in carcinoma *in situ*, which is the only known precursor to invasive breast cancer. More recently, genetic abnormalities have also been described in normal epithelial cells adjacent to invasive breast cancer, as well as in benign epithelial proliferations.

From these data we can begin to construct a model of the development of breast cancer. It is not known in what cell in the normal breast carcinomas develop. The candidates for these precursors include ductal epithelial cells, myoepithelial cells and ductal "stem cells"; it has been hypothesized that the latter exist, but they have not been identified based on morphological or other characteristics. It is not known with certainty in what part of the ductal tree most carcinomas develop, but the most likely site is the terminal ductolobular unit.

It has been discovered that most genetic alterations found in invasive breast carcinoma are already present in carcinoma *in situ*; the genetic alterations involved in the progression from carcinoma *in situ* to invasive carcinoma remain to be elucidated. Very little is also known concerning the genetic alterations involved in the development of lymph node metastases and distant metastases.

It has become clear that, unlike the situation for colorectal cancer, there is not one linear route from a normal epithelial cell to invasive carcinoma, but that there are several distinct pathways leading to distinct histological types of carcinoma.

Based on the study of genetic alterations in relation to histological type, the following routes of invasive cancer development can be hypothesized to exist:

- i) Normal epithelial cell → lobular carcinoma *in situ* (LOIS) → invasive lobular carcinoma (ILC). Inactivating mutations in the E-cadherin gene are almost always found in ILC and never in invasive ductal carcinoma (IDC). Inactivation of E-cadherin is already present in LCIS, indicating that this is an early step in the development of lobular cancer. Amplification and overexpression of cyclin D1 are also frequently found in ILC.
- ii) Normal epithelial cell → well-differentiated ductal carcinoma *in situ* (DCIS) → IDC grade I. Loss of heterozygosity (LOH) on chromosome 16q is frequently found in well-differentiated DCIS; inactivation of a tumor suppressor gene located on 16q has not yet been identified in these tumors. LOH on 16q is less frequently found in poorly differentiated DCIS, indicating that inactivation of a tumor suppressor gene in this region is specific for the development of well-differentiated DCIS. Genetic alterations involved in the progression to invasion have not yet been identified.
- iii) Normal epithelial cell → poorly differentiated DCIS → IDC grade III. Amplification of the *c-erbB-2/neu* gene and inactivating point mutations in the p53 gene are frequently found in poorly differentiated DCIS and these genetic alterations are absent in well-differentiated DCIS. There are no genetic alterations which have been shown to be involved in the progression to invasion. Amplification of the *c-myc*, fibroblast growth factor receptor I and II genes and of the cyclin D1 gene may be involved in the progression to invasion, not only in poorly differentiated breast cancer, but also in well-differentiated breast cancer.
- iv) Based on morphological characteristics, an intermediately differentiated DCIS and IDC grade II are recognized. It is not known whether this represents a specific subgroup of breast carcinomas, characterized by specific genetic alterations.
- v) A number of histological breast cancer types, which are all relatively rare, can be recognized. These types include mucinous carcinoma, metaplastic carcinoma and others. At present, only very little is known with regard to the genetic alterations in these tumor types.

In the coming years, more genetic alterations will be described and fully characterized in breast cancer. This will enable us to obtain a full genetic understanding of breast cancer development. Many of the genetic alterations identified are already present in DCIS; it will be particularly challenging to find the genetic alterations involved in the development of invasive and metastatic breast cancer.

Myoepithelial differentiation in breast carcinoma

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Myoepithelial cells have long been considered as a sort of residual component in breast parenchyma, devoid of specific function (be-