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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.
ii) 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-

sides contraction during lactation) and of proliferative activity. In non-neoplastic lesions, features of myoepithelial cells hyperplasia have been described, but in carcinomas of the breast their role has been restricted to rare spindle cell tumors, supposedly of myoepithelial cell origin (subcutaneous myoepitheliomas). It was only thanks to the availability of myoepithelial cells markers that the role of these cells in breast lesions could fully be appreciated.

Tumors showing a predominant or a significant component of spindle cells (subcutaneous myoepitheliomas and adenomyoepitheliomas) are a rare event in the human breast, while relatively frequent in experimental animals and in dogs. Unlike that observed in rodents, in the human breast the presence of a dual cell component is regarded to be diagnostic of a benign lesion. The whole spectrum of myoepithelial cell differentiation in breast carcinomas cannot be appreciated on the basis of morphology alone, but needs to be defined following combined criteria of morphology (light and electron microscopic), immunophenotypic markers and hormone receptors. The spindle cell tumors of myoepithelial cell origin and differentiation which are described in the literature are relatively indolent neoplasms, most being invasive, although single cases of *in situ* myoepithelial cell lobular carcinomas have been described. Rare instances of aggressive (mainly recurrent) myoepithelioma have also been described.

Following immunocytochemical criteria, myoepithelial differentiation in carcinomas of the human breast is not a frequent event, but novel information is provided by expression of hormone receptors. In the normal breast, myoepithelial cells are lacking estrogen and progesterone receptors, while sensitive to stimulation by the hypothalamic hormone oxytocin, Expression of receptors for oxytocin is indeed a marker of (normal) myoepithelial cells, as revealed by affinity binding and by immunocytochemical procedures employing specific antibodies. Surprisingly, oxytocin receptor expression is relatively frequent in human breast cancers, both *in situ* and invasive, and is often associated with presence of progesterone receptors. These data might suggest that the origin of breast cancer cannot properly be traced back to the epithelial (columnar) cells, while a stem cell capable of a dual (epithelial and myoepithelial cell) differentiation seems a better candidate. Whatever the histogenetic meaning, oxytocin receptor expression opens up interesting prospects in tumors of hormone dependency since experimental evidence indicates that oxytocin alone or in association with antiestrogenic drugs, inhibits proliferation of breast cancer cells.

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The role of molecular prognostic factors in breast cancer

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Introduction

In the past, the importance of the pathology laboratory in the field of tumor pathology has been underestimated and underutilized, the clinician wanting (and receiving) only a one-line report stating the diagnosis. However, it has now become widely accepted that more information can be gleaned from the histopathological appearance of a tumor and that this may be used to predict the biological behavior and clinical outcome in many malignancies, including breast cancer. In addition, the use of other techniques, such as immunohistochemistry and molecular examination, has been expanded in the search for features of the tumor which can predict reliably not only the prognosis of the patient but also the potential for response to a given treatment.

Prognostic factors, although not specific predictors of response to a therapy, can be used for appropiate treatment selection of patients with malignancies: those patients who have an extremely good prognosis after tumor excision may not warrant noxious adjuvant therapies which themselves carry a significant morbidity, but those with a poor prognosis may benefit from an aggressive adjuvant approach. Identifying the prognostic features of an invasive breast carcinoma for these reasons is particularly important (1) as the disease has a markedly variable course; a group of women with "curable" carcinomas who do not receive significant benefit from adjuvant therapy can be identified, while others will succumb relatively rapidly to the disease. Because of this widely differing clinical outcome and because breast carcinoma is common, prognostic factors, and more recently predictive factors, in this malignant disease are probably the most widely studied.

Many of the recent studies of possible prognostic factors in primary breast carcinoma have examined novel variables, either morphologically, immunohistochemically or biochemically, which experimentally at least, are associated with invasion, metastasis, differentiation or growth rate of the tumor. Thus, cell adhesion molecules such as integrins and cadherins, proteases, metalloproteinases, growth factors and their hormone receptors, tumor suppressor gene such as p53 and oncogene expression including *c-erbB-2* status have been assessed. Others have investigated the tumor cell DNA content and the proliferation index in breast carcinomas. The latter has been examined using immunohistochemical techniques with determination of the proportion of nuclei demonstrating the MIB1/Ki67 antigen either subjectively or by image analysis and alternatives such as thymidine and brornodeoxyuridine labeling index can be assessed. Several of these studies have found that in univariate analysis, these molecular markers show a significant association with prognosis of patients with primary breast cancer. However, as novel treatment strategies directed at specific cellular mechanisms *(e.g.,* growth control or regulation) are being developed, tests recognizing the activation or amplification of such pathways in a specific tumor will be required.

In breast cancer estrogen receptor is the best, and at present the only widely applied example of a specific test used to predict response to a specific therapy in breast cancer management. It is not in our hands of independent significance in predicting prognosis in breast cancer patients due to its close relationship with histological grade. We nevertheless assess estrogen receptor status routinely on all patients with invasive breast carcinoma as a predictive factor to determine the probability of a response to hormone treatment (2).