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).

Estrogen receptor

These steroid receptors belong to a "superfamily" of proteins whose function is to control the transcription of a repertoire of other cellular genes (3). Steroid receptors such as estrogen and progesterone receptors are located in the cell nucleus. Hormone is believe to diffuse or be transported to the nucleus where a steroid-receptor complex is formed with receptor dimerization. Some of the genes regulated by steroid receptors are involved in controlling cell growth and it is currently believed that these effects are the most relevant to estrogen receptor influences on the behavior and treatment of breast cancer.

Approximately 30% of unselected patients with breast cancer will respond to hormone therapy such as oophorectomy (or chemical castration) or tamoxifen treatment. However, by assay of estrogen receptor status, using the standard radioligand binding assay on tissue cytosol samples, a response is seen in between 50% and 60% of patients with estrogen receptor positive tumors, in contrast to a <10% response observed in patients with estrogen receptor negative tumors (4).

Immunocytochemical methods, despite being less readily quantifiable, have superseded both the ligand binding and enzyme immunobiochemical assays as they require less tissue, allow fornial histological assessment, thereby reducing sampling error (5), and have a closer association with response to hormone therapy (6), probably because of avoidance of sampling error and lack of influence of tumor cellularity and type.

c-erbB-2 HER-2/neu

Studies of the humanized anti-HER-2 neu (c-erbB-2) monoclonal antibody trastuzumab (Herceptin) have demonstrated a potent inhibitory activity of the antibody against tumor cell lines overexpressing the c-erbB-2 protein and also increase the sensitivity of experimental tumors to chemotherapy, possibly by lowering the threshold for cells to undergo apoptosis following drug exposure. Recently, two large multicenter trials have reported objective responses in 16% of patients (8% complete) in a group of patients with c-erbB-2 overexpressing tumors and who had received prior combination chemotherapy (7), and improved response rates when combined with other chemotherapeutic agents (8). Patients suitable for this new therapy will require selection by demonstration of overexpression of the c-erbB-2 protein by immunocytochemistry or amplification of the gene by fluorescent in *situ* hybridization.

Future predictive markers

There is evidence that response to chemotherapy can be predicted in patients with breast cancer through measurement of the proliferative activity or growth factor expression of the tumor. It is widely accepted that tumors with a very high proliferative rate, such as acute leukemias, high grade lymphomas and germ cell tumors, can respond dramatically to chemotherapy schedules. Similar although less dramatic behavior has been reported in breast cancer. A relationship has been shown between S phase fraction and tumor response in patients with stage II-lila disease (9). Our own results using MIBI (Ki61) growth fraction assessment are less encouraging (10).

Despite this evidence there is currently no biological feature in routine clinical usage for prediction of response of breast cancer to chemotherapy. Recently it has been suggested that *c-erbB-2 (Her2 neu)* gene amplification may influence response to some chemotherapy regimen. Some studies using cyclophosphamide/methotrexate/flourouracil (11, 12) and MMM (10) chemotherapy have show an inverse relationship between c-erbB-2 membrane immunocytochemical reactivity and response. Although with other regimens, including doxorubicin, c-erbB-2 protein membrane expression was associated with a favorable response (13, 14). These conflicting results with differing forms of chemotherapy raise the possibility of differential drug sensitivity related to growth factor regulation.

Other growth factors or molecular markers such as p53 and bcl-2 may be of importance in predicting response to chemotherapy and radiotherapy, respectively, but require further evaluation in prospective series (15).

Conclusion

The histopathology laboratory has a major role to play in both the diagnosis and the prediction of prognosis of breast cancer patients. At the present time the most valuable prognostic factors appear to be those which can be assessed on routinely fixed, processed and stained material: histological grade, lymph node stage, tumor size, vascular invasion and tumor type. The accurate determination of these features requires well prepared hematoxylin and eosin sections. Using a combination of histological features; it is possible at this time to predict reliably an individual patient's likelihood of survival. Additional information predicting response to specific therapies can be obtained from immunohistochemically stained sections; in particular estrogen receptor status can be used to identify those patients who are likely to receive benefit from hormone therapies. In the future, it is to be hoped that other markers of response to specific treatments can be identified so that patients can be started on the most suitable therapy for their individual case without delay.

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Newly described entities

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In recent years, more attention has been paid to breast tumors displaying granular cytoplasm. This granular aspect is variable depending on the different organelles of which it is constituted, such as mitochondria, secretory products, or lysosomes.

Oncocytomas (oncocytic carcinomas) were recently described by Damiani *et al.* (1). Most of these lesions have low-grade nuclei and show eosinophilic granular cytoplasm. Antimitochondrium antibody is strongly positive, while the apocrine markers are frequently negative. At ultrastructure these lesions show numerous enlarged mitochondria scattered throughout the cytoplasm. Secretory granules, when present, are located at one pole of the cell. In recent years we have seen grade III DCI cases loaded with mitochondria as well as myoepithelial cell carcinomas with oncocytic changes. This latter type of tumor is easily overlooked in the breast.

An acinic cell carcinoma of the breast have been described by Roncaroli *et al.* (2) as a single case report. Since then we have seen a number of tumors with a distinct morphology (similar to that seen in acinic cell carcinoma of salivary glands) and in which salivary gland amylase and lysozyme showed immunocytochemical positivity.

Lysosome rich cells have been seen in leiomyoma of the breast which is a rare lesion, newly described by Roncaroli *et al.* (3). Adenohibernoma (4) is a benign lesion that has to be recognized in order to avoid erroneous diagnoses of malignancy such as in the cases of solitary fibrous tumors (5). In contrast low-grade adenosquamous carcinomas which look like benign lesions and otten interpreted as scierosing adenosis, show a locally aggressive attitude and frequent lymph node metastases (6).

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