and an up-regulation of vascular endothelial growth factor secretion, which causes the cells to switch from an antiangiogenic to an anglogenic phenotype. Oncogene activation can also influence the anglogenic properties of cancer cells, primarily by upregulating the production of angiogenesis inducers. For example, the ubiquitous *ras* oncogene is a potential stimulator of vascular endothelial growth factor secretion in many tumor types. Oncogene activation has also been associated with increased secretion of matrix degrading enzymes, which enhances tumor-associated angiogenesis (1).

Tumors of fhe prostate stimulate angiogenesis by directly secreting angiogenic substances or by activating and releasing angiogenic compounds stored within the extracellular matrix. Angiogenic substances secreted by tumor infiltrating lymphocytes, macrophages or mast cells can also enhance vascularity. The balance between angiogenesis inducers and inhibitors is tipped in favor of neovascularization as a result of an increase in the secretion of inducers and/or a decrease in the production of inhibitors. The end result is the activation of nearby endothelial cells, which respond by expressing a cell autonomous pattern of behavior that culminates in the formation of new vessels. This complex process can be broken down into several component steps. Each step is essential to the formation of tumor-associated neovascularity and represents a potential site of therapeutic manipulation (1, 2).

There are two distinct phases during prostatic carcinogenesis with regard to tumor blood vessel development (2). During the first or prevascular phase, which may persist for years, cells that have undergone some but not all of the transformation steps undergo a limited amount of net growth, producing premalignant lesions such as prostatic intraepithelial neoplasia (PIN). Most of these preneoplastic lesions do not progress to produce histologically detectable cancer. Even the PINs that do progress to cancer remain of limited virulence unless they undergo conversion to the second or angiogenic phase. Once this angiogenic phase is reached, new blood vessel development is greatly enhanced within the cancer. It is this enhanced tumor angiogenesis which allows these cancers both to grow continuously and to metastasize. There are some reports concerning neovascularity and invasive prostate cancer which, on the whole, suggest a link between increased vascular density and an aggressive tumor phenotype, but which differ in their assessment of whether vascular density offers any independent prognostic benefit (2-4).

Studies in rodents clearly demonstrate the importance of anglogenesis for prostate cancer growth and metastasis (5). For instance, treatment of animals bearing tumors derived from humans or rat prostate cancer cells lines with the angiogenesis inhibitor linomide leads to markedly decreased tumor-associated vascularity and suppression of primary and metastatic tumor growth. The antiangiogenic activity of linomide appears to be due to decreased macrophage infiltration with a resultant decrease in the secretion of the angiogenic cytokine tumor necrosis factor-ct. These preclinical data are being used for the design of clinical trials testing the safety and efficacy of antiangiogenic agents for the management of patients with prostate cancer. The use of ant angiogenic agents for the chemoprevention of prostate cancer has also been explored in experimental studies. The report has generated much interest, since such an approach would be of obvious value if it could be translated to the clinical setting (5).

In conclusion, the available data indicate that angiogenesis has an important role in the progression and metastasis of prostate cancer. Understanding the events controlling angiogenesis could allow the development of new therapeutic approaches to prevent neoplastic progression as well as to induce the regression of cancers and their precursors. The rapid pace of research in this field suggests that this aspect of tumor biology will be soon have increasingly important roles in evaluation and treatment.

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Molecular diagnosis of prostate adenocarcinoma with emphasis on screening programs

C. Lopes and R. Medeiros

Dept. of Pathology Oporto Cancer Center, Portugal.

Introduction

The use of sextant prostate biopsy associated with serum prostate-specific antigen levels has become the most common way of diagnosing prostate adenocarcinoma. However, early diagnosis in nonsymptomatic men introduced some practical problems related with the biology of the neoplasia and with the unexpected prognosis of this tumor in what concerns aggressive *versus* nonaggressive behavior. In spite of the prognostic information given by conventional histology, many questions remain to be answered. Consequently, research is also underway in the fields of molecular biology and genetics in order to better understand such matters as premalignant lesions, aggressive and nonaggressive tumors, mechanisms of metastasis, hormonal response and nonresponse tumors. In the present study we will present our results in the following fields: susceptibility to prostate carcinoma; diagnosis; immunoexpression of oncoproteins and adhesion molecules; immunoexpression of MUCi; and detection of circulating prostate cells in peripheral blood.

Susceptibility to orostate carcinoma

Epidemiological studies suggest **a** correlation between prostate carcinoma and certain geographical, racial and environmental risk factors. The incidence and mortality from prostate cancer is highest in the Afro-American population (1) and lowest in the Far East, namely in Japan and China (1, 2). Japanese men have significantly reduced hormone-binding globulin and reduced 5 ɑ reductase activity and this can explain, at least in part, the recognized low incidence of prostate adenocarcinoma (3)

We studied the role of glutathione S-transferase polymorphism in several neoplasias, including prostate. The presence of a GSTM1 null genotype was analyzed by polymerase chain reaction (PCR) in DNA isolated from the blood samples of 67 patients with

clinical prostate cancer and 76 healthy male blood donors. DNA isolation was performed with the "salting-out" methodology and FOR products were analyzed by electrophoresis on an ethidium-bromide stained agarosis gem and visualized under an ultraviolet light.

Our results showed that the GSTM1 null allele did not influence susceptibility to human prostate cancer. However, concerning some differences in the biology and behavior of the tumors, namely grade, volume and response to hormonal therapy, our data suggest some positive influence of the presence of GSTM1 null allele.

Diagnosis

The diagnosis of prostate adenocarcinoma in material obtained from sextant biopsy may be difficult in rare case in which there are only a small number of isolated small glands with clear cytoplasm and without prominent nucleoli. Immunocytochemical study with high power cytokeratins may help because the immunoreactivity of basal cells is in favor of the specimen being benign. The immunostaining of inducible nitric oxide synthase (iNOS) can become a new marker of malignancy in prostate cancer (4, 5).

Nitric oxide is synthesized by iNOS and plays an important role in tumor growth and angiogenesis, as is currently being found. The immunocytochemical stain of iNOS was studied in 39 cases of prostate carcinoma and in 43 cases of benign hyperplasia and "normal" prostate. Our results confirm that prostate adenocarcinoma has a high iNOS expression while non-neoplastic tissue does not and it can be used as an important immunocytochemical marker for prostate carcinoma. In addition to its role in the process of cancerisation, nitric oxide also plays important roles in the autonomic innervation and vascularization of all compartments of prostate tissues, the consequences of which are still not completely known.

Oncooroteins

The immunoexpression of p53 in prostate cancer is variable in different series, although there is some evidence that it is infrequent and occurs late in the progression of prostate carcinogenesis. In our study, we found 12% of positive cases in benign hyperplasia; 48% in prostate adenocarcinoma and 18% in cases of high-grade prostatic intraepithelial neoplasia (FIN). In adenocarcinoma, the frequency of immunoreactivity was significantly higher in high-grade tumors (a Gleason of more than 7). A significant loss of expression was found in tumors submitted to hormonal (6, 7).

Regarding bcl-2, only a few number of cases (9%) were found to be positive in neoplastic cells. All them had a Gleason score of 7 or more and the majority presented clinical signs of hormoresistence, which confers to bcl-2 an important role in this field (8). The immunoexpression of CD44 was also uncommon (22%) and occurred more significantly in high-grade tumors, some of them developing metastasis. Our findings suggest that the immunoexpression of this adhesion molecule may be linked to the metastatic process making the neoplastic cells escape to the lymphocytes that are involved in immune surveillance.

Mucins are a group of high molecular weight glycoproteins synthesized by glandular epithelial tissues. MUOI glycoprotein is a mammary-type apomucin and is expressed in some adenocarcinomas. We studied the immunoexpression of MUOI in 49 adenocarcinomas diagnosed by biopsy. Radical prostatectomy of all of them was also studied, 620/o of which had been treated with hormones. In the normal tissue, MUOI is expressed only in the luminal membrane of glandular cells. An increase of positive epithelial cells was found in adenocarcinoma and a strong immunoexpression occurred also in cytoplasm, suggesting that carcinogenesis induces modifications in the glycosylation which can be useful for the diagnosis of screened cases because premalignant lesions, such as high-grade FIN, also present cytoplasmic reactivity. On other hand, hormonal treatment induces a down expression that is more important in cases presenting hormonal escape.

Blood circulating prostate cells

The detection of blood circulating prostate cells has been suggested as an indicator of circulating micrometastases. The search for the presence in blood of specific prostate cell messenger RNAs (mRNA) [prostate-specific antigen (FSA) and prostate-specific membrane antigen (FSM)] can be achieved by nested FOR (9-13).

We performed nested reverse transcription-FOR (RT-FOR) for PSA mRNA and PSM mRNA in blood samples from 60 patients with prostate cancer and in 62 controls. We detected FSA mRNA in five of 33 patients (15.1%) with prostate cancer with no clinical evidence of metastatic disease and in three of six patients (50%) with prostate cancer and clinical evidence of metastatic disease. The FSM mRNA was detected in three of 11 patients (27.3%) with benign prostatic hyperplasia and in three of 10 patients (30%) who had previously undergone radical prostatectomy.

FSM mRNA was detected in 16 of 33 patients (48.5%) with prostate cancer and in two of six patients (33.4%) with prostate cancer and clinical evidence of metastatic disease. No FSA mRNA or FSM mRNA was detected in any of the controls, which included six patients who had undergone cystoprostatectomy for bladder cancer.

Our results suggest that the presence of circulating prostatic epithelial cells in the peripheral blood may be an important clue for the molecular staging of prostate cancer since it may help in the detection of patients with organ-confined prostate cancer, at risk for developing metastatic disease. Additionally, it may prove useful in the preoperative detection of potential surgical failures and in the management of metastatic disease, detecting an early hormone escape due to the clonal selection of androgen-independent cell lines.

The finding of circulating cells in nonmalignant lesions is unexpected. Additional studies on mutations in genes linked to metastasis are being performed because they could provide the clue for separating normal from malignant circulating cells.

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Benign and malignant stromal lesions of the prostate

A. L6pez Beltran

Cdrdoba University Medical School and Reina Sofia University Hospital, C6rdoba, Spain.

Hyperolastic stromal nodules and related lesions

The hyperplastic prostate may consist of purely stromal nodules with variable amounts of smooth muscle fibers. The classification proposed by Vogel and coworkers is: i) embryonal-mesenchymal; ii) fibroblastic; iii) fibromuscular; and iv) smooth muscle nodules.

All types of stromal nodules can be observed simultaneously in the prostate and are located predominantly in the transition zone. Transitional forms with a changing mixture of either embryonal-mesenchymal and fibroblastic or fibroblastic and smooth muscle cells exist. The majority occurs in the central periurethral regions, although they are also encountered in the intermediate and subcapsular regions (1). The stromal nodules contain thin-walled capillary-like vascular channels and thick-walled vessels in proportions that change with each morphological type of nodule. No elastic fibers are found in any type of nodule, in contrast to the diffuse hyperplastic stroma. Hyperplastic stromal nodules are infiltrated by T lymphocytes. Stromal nodules of predominantly fibroblastic and fibromuscular types can be infiltrated by glands such as fibroadenomas in the breast. In contrast, stromal hyperplasia with atypia is a rare lesion with very few well-documented reports (2). In this situation, there is hyperplasia of the stroma with no associated epithelial hyperplasia. The spindle cell proliferation is atypical and shows hyperchromasia and pleomorphism with bizarre nuclei. Bizarre hyperchromatic nuclei have occasionally been reported in the stroma between normal prostatic glands. Mitotic figures are usually absent or scanty. These changes are currently considered degenerative. Some of these lesions resemble phylloides tumors of the breast and the term phyllbides-type atypical prostatic hyperplasia has been applied (3). More recently, the term prostatic stromal proliferation of uncertain malignant potential has been applied to this group of lesions (2, 4). Rare prostatic stromal lesions of a regressive nature are stromal edema and myxoid changes, scars following infarction and postinflammatory fibrosis.

Pseudosarcomatous lesions

Postoperative spindle cell nodule is a well-described but rare condition that occurs within a few months of prostate surgery, generally in a periurethral site. This condition is characterized by a proliferation of plump spindle cells arranged as intersecting fascicles each of which are surrounded by a delicate network of blood vessels. When small, these appearances may simulate Kaposi's sarcoma. Mitotic figures may range from 1-25 per 10 high power field but atypical mitotic figures are exceptional. Pleomorphism and focal necrosis are occasionally observed. Spindle cell proliferation without previous surgery (inflammatory pseudotumor and inflammatory myofibroblastic tumor) are rare in the prostate. Recent studies suggest a clonal myofibroblastic proliferation. Patient age and clinical history are essential in this setting.

Benign soft tissue tumors

Benign soft tissue tumors of the prostate are rare. Those more frequently encountered are lebomyomas, fibromas and hemangiomas. These tumors show features similar to those at any other site with no pleomorphism and no significant mitotic rate. The variant atypical leiomyoma has also been described in the prostate and shows atypical, sometimes bizarre nuclei, some of which may be multinucleated but with no increase in mitosis. Criteria used to separate benign from malignant soft tissue tumors of the prostate are the criteria of malignancy applied to other soft tissue sites. A single case of leiomyoblastoma has also been described. Most fibromas described in the old literature are currently considered examples of hyperplastic stromal nodules.

Malignant soft tissue tumors

Sarcoma of the prostate accounts for less than 0.1% of prostatic neoplasms. Prostatic malignant soft tissue tumors occur in a younger age group than do carcinomas. Fifty percent of prostatic sarcomas are rhabdomyosarcomas, 25% are lelomyosarcomas, 15% are unclassifiable and the remainder includes examples malignant peripheral nerve sheath tumors, fibrosarcomas, malignant fibrous histiocytoma, chondrosarcoma and osteosarcomas. Leiomyosarcomas are the dominant in type in adults (5). The histopathological features of prostatic sarcomas are similar to those in other sites (3). Immunohistochemistry, which is essential to distinguish these tumors from other pleomorphic sarcomas, undifferentiated carcinoma and undifferentiated lymphoma can be supplemented with electron microscopy.

Mixed malignant stromal-eDithelial tumors

Most prostatic mixed malignant stromal-epithelial tumors are malignant phylloides tumors (6) and carcinosarcomas (7-9).

Malignant phylloides tumor of the prostate exhibits a spectrum of histological features similar to its counterpart in the breast, It may be subdivided into low-grade, intermediate-grade and high-grade groups but even low-grade tumors may recur. High-grade prostatic phylloides tumor has a high stromal-epithelial ratio, prominent stromal cellularity and overgrowth, marked cytological atypia and increased mitotic activity. Tumors with overtly malignant stroma rarely give rise to distant metastases of sarcoma. Some of these tumors are designated as prostatic stromal sarcoma (4).

Carcinosarcoma of the prostate is defined as a malignant neoplasm composed of histologically differentiated carcinoma plus malignant mesenchymal elements (7-9). The most common mesenchymal elements are poorly differentiated spindle cell sarcoma, chondrosarcoma and osteosarcoma but myosarcoma, liposarcoma and angiosarcoma may occur (9). Most reported cases have developed after treatment for apparently pure prostatic adenocarcinoma but some cases present at time of diagnosis with both adenocarcinoma and sarcoma (synchronous carcinosarcoma). Some authors