strated *in vitro,* showing that prostate cancer cell lines generally require reconstituted basement membrane (Matrigel) to be tumorigenic in athymic mice. In summary, de *novo* synthesized basement membrane and adhesion via specific receptors significantly contributes to the ability of prostate cancer to penetrate the extracellular matrix during stromal invasion and metastasis (6).

Implications of PhenotyPe heterogeneity in prostate cancer progression

The most common cell type encountered in prostatic adenocarcinoma shares phenotype similarity with secretory luminal cells *(e.g.,* prostate-specific antigens, cytokeratins 8, 18). These exocrine tumor cells generally express the nuclear androgen receptor and 5os reductase 1 and 2 in primary, metastatic and recurrent lesions (1). This observation suggests that exocrine tumor cells are potentially androgen responsive, and maintain the dihydrotestosterone-forming process even in hormone refractory disease. The continuous expression of the nuclear androgen receptor in androgen insensitive carcinomas is surprising, and involves a high level of androgen receptor gene amplification, which frequently occurs in recurrent prostate cancer (7). Nevertheless, recurrent tumors also reveal androgen receptor gene mutations, leading to an abnormal androgen receptor protein, responsive to estrogens and other steroids (7). Accordingly, the presence of the nuclear androgen receptor does not imply androgen dependence. An alternative pathway by which prostate cancer cells escape hormonal control refers to their ability to acquire neuroendocrine features (6). Virtually all common adenocarcinomas of the prostate show at least focal neuroendocrine differentiation. Tumors with extensive and multifocal neuroendocrine features (accounting for approximately 10% of all prostatic malignancies) tend to be poorly differentiated, more aggressive and resistant to hormonal therapy. Devoid of the nuclear androgen receptors, the neuroendocrine phenotype constitutes an androgen insensitive cell population in prostate cancer through the various stages of the disease (6). This particular phenotype derives from exocrine tumor cells, which reflects the differentiation repertoire of prostatic stem cells. Neuroendocrine differentiation predominantly occurs in the GO phase of the cell cycle and is lost when tumor cells reenter the cell cycle (1, 6). These cell kinetic properties make the neuroendocrine phenotype more resistant to cytotoxic agents and radiation therapy than exocrine tumor cells with proliferative capacity. This concept is supported by recent clinicopathological studies, showing that neuroendocrine differentiation predicts poor survival of advanced prostatic cancer after radiation therapy. The prognostic significance of neuroendocrine tumor cells in common prostatic adenocarcinoma most likely reflects their ability to secrete a series of regulatory products with growth promoting properties, including serotonin, bombesin and parathyroid hormone-related peptides. These regulatory peptides are able to maintain cell proliferation though the paracrine, androgen independent pathway (6). Thus, neuroendocrine differentiation can affect prostate cancer progression through several pathogenetic mechanisms.

In addition to androgen related pathways, estrogens have recently been recognized to play a role in the multifactorial process of endocrine therapy failure. Androgen deprivation therapy, widely used in the medical treatment of advanced prostate cancer, is known to increase the estrogenlandrogen ratio in cancerous tissue. This therapy-induced hormonal imbalance may have potential implications. We have recently shown that a substantial number of androgen independent and metastatic lesions express the classical estrogen receptor (ERos) at high levels. Tumor cells becoming estrogen responsive in an androgen deprived milieu may survive by using estrogens for their continuous maintenance and growth. This warrants clinical trials to test the efficiency of antiestrogens in the medical treatment of androgen independent prostate cancer expressing the nuclear estrogen receptor at significant levels.

Concluding remarks

Despite its clinical magnitude, the pathogenesis of benign prostatic hyperplasia and prostate cancer remains poorly understood. Knowledge of morphogenesis can significantly contribute to this issue.

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Atypical adenomatous hyperplasia, atypical small acinar proliferation and prostatic adenocarcinoma

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In the USA over 20,000 new cases of prostate cancer are diagnosed each year, accounting for more than 35% of all cancers affecting men and resulting in 40,000 deaths annually. As a consequence of active screening, pathologists are expected to detect cancer in increasingly small tissue samples.

Atypical adenomatous hyperplasia (AAH) and atypical small adnar proliferation (ASAP) are lesions that could be confused with prostatic adenocarcinoma (PCA) and are of great importance not only as sources of diagnostic pitfalls but also for the further clinical management of the affected patients. The nature of AAH is still unclear and it is controversially discussed as a putative precancerous lesion.

AAH (adenosis

AAH is a common lesion and is considered to be a benign glandular lesion with architectural features that could be confused with

low-grade carcinoma (1-3). The architectural atypia can consist of benign looking glands or overcrowded glands that are difficult to distinguish from carcinoma. The distinction of AAH from low-grade adenocarcinoma is mainly based on architectural growth pattern and cytologic features (3). In the presence of a predominantly atypical growth pattern, the diagnosis of adenocarcinoma should be considered (4, 5).

Clinical significance of AAH

AAH is considered to be a putative premalignant lesion of the prostate because of its increased incidence in association with carcinoma. Efforts are being made to establish its biological significance. The considerable difficulty in distinguishing AAH from carcinoma is with lesions containing nucleoli larger in size between benign and malignant. In case of diagnostic difficulties, one should consider the following histoarchitectural criteria for the diagnosis of AAH.

The features characteristic of AAH include: i) preserved lobular growth pattern composed of small crowded glands admixed with larger glands, with nucleoli larger than 3 pm being usually absent; ii) other important features that help in the diagnosis of AAH is a direct comparison of the cytological and nuclear features of AAH with that of the surrounding benign acini; and iii) the cytoplasm of AAH is usually clear, blue mucinous secretion is very rare and in most cases of AAH corpora amylacea are common and in some acini basal cells could be demonstrated. There are difficult cases where features common to AAH and PCA exist: these are the presence of overcrowded back to back glands, frequent intraluminal crystalloids, the presence of few larger nucleoli and signs of infiltrative and irregular growth pattern, features commonly found in PCA. Infiltrative growth pattern can be seen in about 19% of all cases (3) and huge nucleoli can occasionally be found in AAH. The presence of nucleoli alone should not be given more diagnostic weight than the many other criteria. Intraluminal crystalloids are other features considered by some authors to be helpful in distinguishing AAH from PCA (6-8). Crystalloids can commonly be found in up to 39% of AAH (1, 3, 9). Basal cells are important features that are not seen in PCA but are found in AAH. Basal cells are not always easy to identify and the use of high molecular weight cytokeratin could be helpful in this respect.

Due to the fact that AAH shares certain morphological features with PCA, it has been suggested that AAH could be a precursor lesion for PCA. To date, there is no convincing molecular biological or cell kinetic data to support this (4, 10-14).

Based on the information we have to date, the practical consequence of AAH for the pathologist would be to embed all tissues with subsequent additional serial sectioning of the suspected area. Since the clinical importance of these lesions is not understood, there is no immediate consequence for patients, unlike the case of high-grade prostatic intraepithelial neoplasia. It is generally advised to perform dose surveillance and follow-up.

Atypical small acinar proliferation

In about 2.5-5.5% of prostate needle biopsies, one can find so-called atypical small acinar proliferation (ASAP) which is suspicious for PCA but not diagnostic (15, 16). ASAP as a diagnostic category was introduced in 1993 by a study group. This category was created to describe specific lesions (foci) that are small in size (1), which disappear on step levels and which lack significant cytologic abnormalities, raising the possibility of PCA. To accommodate this borderline group, the further separation of small acinar proliferations into AAH (benign) and ASAP (possibly benign but having some features of carcinoma) has been suggested (1).

One important feature in the diagnosis of ASAP is the maintained fragmented basal cell layer which is similar to AAH and prostatic intraepithelial neoplasia. The most important question arising in the diagnosis of ASAP (suspicious for malignancy) is if it represents undiagnosed PCA. The lack of concordance for this lesion indicates that there is uncertainty among pathologists (1). The criteria for distinguishing ASAP from AAH and cancer can be very difficult to apply in practice. Causes of difficulty include the presence of distorted acini with a lack of convincing cytologic features, prominent inflammation in which the adjacent benign acini show distortion and inflammatory reactive atypia with nuclear and nucleolar enlargement. In such cases, it is safe to call the lesion ASAP (suspicious for but not diagnostic for malignancy).

PCA *versus* ASAP

In cases where few acini reveal clear-cut nuclear anaplasia in significant contrast with the adjacent benign epithelium without inflammation around the lesion and after excluding seminal vesicle or ejaculatory duct epithelium without inflammation around the lesion, the diagnosis of PCA can be made. Since the diagnosis of PCA has serious consequences, it should be made when there is absolute confidence. Supporting clinical (PSA) and immunohistochemical (high molecular weight cytokeratin) evidence can be useful. Keeping this in mind, the range of uncertainty can be categorized in three groups as follows: i) ASAP favor benign, ii) ASAP suspicious and iii) ASAP highly suspicious. The latter two groups show a proliferative activity (MIB 1), similar to low grade PCA (16).

The clinical follow-up of all these findings is similar: repeated biopsy.

AAH *versus* ASAP

Usually, the diagnosis of AAH is more unlikely in needle biopsies and it is more likely that needle-biopsies contain ASAP. In general, there is a tendency to diagnose all small acinar proliferations as AAH in needle biopsies. A diagnosis of AAH should be reserved for small acinar proliferations that are in intimate association with nodular hyperplasia (5, 17).

The histological features of ASAP can be divided into two groups:

i) Architectural features: acini that vary in size, shape and spacing without cellular atypia. In a few cases, acini with luminal crystalloids and inflammatory infiltrations accompany the lesion.

ii) Cytologic features: in this second group the acini display most of the cytologic features necessary for the diagnosis of cancer, in particular nuclear and nucleolar enlargement. The diagnosis of cancer cannot be made with certainty because of the limited number of acini, cell kinetic features: proliferative activity of ASAP favors benign (group 1) corresponds to AAH (16).

The predictive value of ASAP for cancer biopsy is estimated to be 73% (18) with special emphasis on ASAP 3 as indicated by cell kinetic data (16).

Small acinar orostatic carcinoma

The diagnosis of PCA relies on a combination of architectural and cytologic findings, which in some cases are difficult to distinguish from those of AAH and ASAP. In most cases, light microscopy is

sufficient for a diagnosis of PCA whereas in some cases the application of immunohistochemistry is necessary.

Architectural features are assessed at low to medium power magnification. Malignant acini usually have an irregular, haphazard arrangement. The suspicious acini are usually small. In the experience of many pathologists, low grade PCA and Gleason grade 3 PCA are particularly difficult to separate from benign acini in needle biopsies due to the relatively uniform size and spacing of acini. The basal cell layer is completely absent in PCA, an important feature which sometimes needs immunohistochemical verification.

Concerning cytologic features, the most important in the majority of cases are nuclear and nucleolar enlargement. Since practically every single cell reveals a nucleolus, one looks for larger ones (1.25-1.50 microns). The presence of two or more nucleoli in a single cell is virtually diagnostic for PCA (19). Nucleoli are found in virtually all cells. Nuceolar size is a good indicator of cancer when compared with benign lesions (20). Nuclear size is an important finding used to differentiate AAH from PCA. Crystalloids and luminal mucin are often seen in larger quantities in PCA. Crystalloids are needle-like eosinophilic structures found in well and moderately differentiated PCA and they are not specific for PCA (21, 22). The pathogenesis of crystalloids is not clear. They are considered to be the result of abnormal protein and mineral metabolism within benign and malignant acini. Mucin (acidic sulfated and non sulfated) mucin is often seen in the acini of PCA. This mucin can stain with alcian blue, whereas the normal prostatic epithelium contains periodic acid-Schiff reactive neutral mucin. Again, acidic mucin is not specific for PCA (8, 23). Collagenous micronodules are specific but rare findings in PCA. They are nodular masses of eosinophilic fibrillar stroma that impinge on acinar lumens (15, 24). They are present in mucin-producing adenocarcinoma and result from extravasation of acid mucin into the stroma. They are not present in benign lesions or in prostatic intraepithelial neoplasia. Their frequency in PCA is estimated to be 13%.

Perineural invasion is a strong indicator of malignancy but is not pathognomonic: it can rarely occur in benign prostate (25).

Microvascular invasion is strong evidence for PCA and its presence correlates with histological grade. It can sometimes be difficult to distinguish from retraction artifact of acini due to fixation (26).

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Angiogenesis in prostate cancer and prostatic intraepithelial neoplasia

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Tumors evolve from normal cells by accumulating a series of genetic alterations that either activate oncogenes or inactivate tumor suppressor genes. In addition to increasing mitogenesis and preventing apoptosis, the accumulation of activated oncogenes and br loss or mutation of tumor suppressor genes also leads to the development of an angiogenic phenotype of prostate neoplasia. For example, mutation of the p53 suppressor gene in human fibroblasts is associated with a sharp decrease in the expression of thrombospondin-1