CONTROVERSIALS IN UROLOGICAL PATHOLOGY

Moderators:
Liang Cheng, Indiana University School of Medicine, Indianapolis, IN
Edward C. Jones, Vancouver Hospital and Health Science Center and the University of
British Columbia, Vancouver, Canada

7:00-7:10 pm
President’s Message and ISUP Gleason Grading Consensus
Jonathan I. Epstein, Johns Hopkins Hospital, Baltimore, MD

7:10-7:30 pm
Inflammatory Myofibroblastic Tumors of the Genitourinary Tract: Etiology, Diagnosis, and Management
Elizabeth Montgomery, Johns Hopkins Hospital, Baltimore, MD

7:30 -7:50 pm
Perivascular Epithelioid Cell Tumor (PEComa) in the Genitourinary Tract
Guido Martignoni, Dipartimento di Patologia, Università di Verona, Verona, Italy

7:50- 8:10 pm
Inverted Papillomas of the Bladder and Their Variants Versus Inverted Growth Patterns of Cancer
Antonio Lopez-Beltran, Cordoba University School of Medicine, Cordoba, Spain

8:10-8:30 pm
AMACR: A Help or Hindrance in the Diagnosis of Prostate Cancer?
Ximing J. Yang, Northwestern University, Chicago, Illinois

8:30-8:50 pm
Immunohistochemistry for the Diagnosis of Renal Cell Carcinoma: Does It Really Help and Make a Difference?
John C. Cheville, Mayo Clinic and Mayo Foundation, Rochester, MN
INTERNATIONAL SOCIETY OF UROLOGICAL PATHOLOGY (ISUP)

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The 2005 ISUP Consensus Conference on Gleason Grading of Prostatic Carcinoma

Epstein JI, Allsbrook WC Jr., Amin MB, Egevad LL; ISUP Grading Committee

• GENERAL APPLICATIONS OF THE GLEASON GRADING SYSTEM

• GRADING VARIANTS AND VARIATIONS OF ACINAR ADENOCARCINOMA OF THE PROSTATE

• REPORTING SECONDARY PATTERNS OF LOWER GRADE WHEN PRESENT TO A LIMITED EXTENT
  (Needle biopsy core that is entirely involved by cancer, with 98% Gleason pattern 4 and 2% Gleason pattern 3)

• REPORTING SECONDARY PATTERNS OF HIGHER GRADE WHEN PRESENT TO A LIMITED EXTENT
  (Needle biopsy which is entirely involved by cancer with 98% Gleason pattern 3 and 2% Gleason pattern 4)

• TERTIARY GLEASON PATTERNS

• RADICAL PROSTATECTOMY SPECIMENS WITH SEPARATE TUMOR NODULES

• NEEDLE BIOPSY WITH DIFFERENT CORES SHOWING DIFFERENT GRADES
Modified Gleason Grading System

**Pattern 1:**
Circumscribed nodule of closely-packed but separate, uniform, rounded to oval, medium-sized acini (larger glands than pattern 3).

**Pattern 2:**
Like Pattern 1, fairly circumscribed, yet at the edge of the tumor nodule there may be minimal infiltration. Glands are more loosely arranged and not quite as uniform as Gleason pattern 1.

**Pattern 3:**
Discrete glandular units
Typically smaller glands than seen in Gleason pattern 1 or 2.
Infiltrates in and amongst non-neoplastic prostate acini.
Marked variation in size and shape.
Smoothly circumscribed small cribriform nodules of tumor.

**Pattern 4:**
Fused microacinar glands
Ill-defined glands with poorly formed glandular lumina
Large cribriform glands
Cribriform glands with an irregular border
Hypernephromatoid

**Pattern 5:**
Essentially no glandular differentiation, composed of solid sheets, cords, or single cells
Comedocarcinoma with central necrosis surrounded by papillary, cribriform, or solid masses
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Inflammatory Myofibroblastic Tumor of the Bladder. How Does it Relate to Other Lesions With this Name?
Elizabeth Montgomery, MD
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Background:
Pulmonary lesions called “inflammatory pseudotumors” were known for many years and were regarded as part of a spectrum of lesions called “plasma cell granulomas” (1-5). Various terms were applied: inflammatory pseudotumor, plasma cell granuloma, plasma cell pseudotumor, xanthomatous pseudotumor, pseudosarcomatous myofibroblastic proliferation, and inflammatory myofibrohistiocytic proliferation (6). Subsequently, similar tumors were described in the abdomen and other soft tissue sites (6, 7). As we have learned more about a wide spectrum of lesions in this family of myofibroblastic proliferations in a host of anatomic sites (8-16), questions concerning their etiology and biologic potential remain. Advances in understanding of the molecular biology of these tumors, launched by the discovery of a “hot spot” at 2p23 flanking the ALK gene by Griffin et al (17), have provided some insights, but other questions remain unanswered. Following the report by Griffin and her colleagues (17) of these alterations in soft tissue lesions, other investigators confirmed similar alterations in other sites, including the lung, the classic site (18). Immunohistochemistry for the protein product confirmed protein expression in subsets of these lesions in a range of anatomic sites (15, 16, 19-26), although Cessna et al noted that this staining was not wholly specific (25). These tumors have been linked, on the one hand, to nodular fasciitis (27), and, on the other hand, to cells of the accessory immune system that have been variously called fibroblastic reticulum cells, myoid cells, and dictyocytes (28).

Inflammatory Myofibroblastic Tumor and Inflammatory Fibrosarcoma of Soft Tissues
Although these lesions were originally described as separate entities, they are now recognized as ends of a spectrum of tumors unified by a common molecular profile (6, 23, 29-32). They are grouped together by the WHO (33, 34). Gene fusions involving anaplastic lymphoma kinase (ALK) at chromosome 2p23 have been described (19, 32, 35, 36). By immunohistochemistry, ALK has been detected in about 60% of cases, a finding that can sometimes be exploited for diagnosis (19). In a subset of cases, ALK C-terminal kinase domain is fused with tropomyosin N-terminal coiled-coil domain and other cases have shown fusion of ALK with the clathrin heavy chain (32).

Inflammatory fibrosarcoma
This is most common in childhood with a mean age of 8 years (range 2 mos to 74 years). As described in the AFIP series (7), this tumor arises within the abdomen, involving mesentery, omentum and retroperitoneum (over 80% of cases), with occasional cases in the mediastinum, abdominal wall and liver. Sometimes there are associated systemic
symptoms. The tumor can be solitary or multinodular (30%) and up to 20 cm in diameter. The tumors are composed of myofibroblasts and fibroblasts in fascicles or whorls, and also histiocytoid cells. Pleomorphism is moderate, but mitoses are infrequently seen. There is a variable but often marked inflammatory infiltrate, predominantly plasmacytic but with some lymphocytes, and occasionally neutrophils or eosinophils as well. Fibrosis and calcification can be seen in the stroma. Immunostaining is positive for SMA and some examples express cytokeratin especially where there is submesothelial extension. The tumors invade adjacent viscera; 37% recurred and 3 cases (11%) metastasized. A quarter of the patients died of disease.

The differential diagnosis from inflammatory myofibroblastic tumor is subjective as these conditions are ends of a spectrum (one case was included in both the original papers describing these two conditions). Essentially, it depends on the presence of pleomorphism in cases designated as “fibrosarcoma”.

**Inflammatory myofibroblastic tumor**

Also known as inflammatory pseudotumor, this entity was first well-described in the lungs and later became recognized in extra-pulmonary locations(6). Recent cytogenetic and molecular evidence in both inflammatory myofibroblastic tumor and inflammatory fibrosarcoma supports a clonal origin, implying that this process is neoplastic. It is found in soft tissue, in omentum and retroperitoneum and involving viscera. IMT has been reported in patients between 3 months and 46 years, but mostly in childhood (mean age 9 years) with a slight male predominance, and some cases are associated with systemic symptoms. A small number recur, especially when multinodular. They form firm white infiltrative masses, and histologically there are three patterns: (1) fasciitis-like, with vascular, myxoid and inflamed stroma, including plasma cells; (2) fascicular MFH or leiomyosarcoma like spindle cell areas with inflammation; (3) sclerosed desmoid-like areas with calcification.

**Bladder**

A spindle cell lesion in the bladder reminiscent of nodular fasciitis was described in 1980 as “reactive pseudosarcomatous response” (37) and subsequently, this process was found elsewhere in the genitourinary tract (38-40). Identical lesions were subsequently encountered in patients who had undergone prior instrumentation, and these were called “post-operative spindle cell nodules”(41, 42). Other terms have included inflammatory pseudotumor, nodular fasciitis, pseudomalignant spindle cell proliferation, pseudosarcomatous myofibroblastic proliferation, pseudosarcomatous myofibroblastic tumor, and inflammatory myofibroblastic tumor (22). The unifying feature of these lesions is their proclivity to mimic both sarcomas (43)and spindled carcinomas(44), the latter compounded by their expression of various keratins(20, 39, 40, 43-47). It had been assumed that, since these tumors have been benign in small follow-up studies(44), that they were unrelated to lesions with similar names in other anatomic sites and, thus, more akin to nodular fasciitis. However, they differed by nodular fasciitis in their capacity to infiltrate deeply into the detrusor muscle.

The recent identification of ALK alterations in bladder lesions (20, 22, 48) suggests that, despite the lesions’ frequent similarity to nodular fasciitis, they are probably neoplastic. It has also led to re-evaluation of their relationship to similar proliferation in the soft tissues. Since those in the bladder often appear fasciitis-like with
a loose myxoinflammatory appearance, whereas those in other sites can be fascicular, sclerosed, or laden with plasma cells and foam cells, bladder lesions had been regarded simply as counterparts of nodular fasciitis.

In our own material, bladder lesions are highly likely (about 70%) to be ALK reactive on immunohistochemistry and to harbor ALK alterations on FISH studies (about 75%), certainly supporting that most are not simply reactive processes. Most cases display nuclear p53 on immunohistochemistry as well as keratin reactivity.

Most patients with bladder lesions are adults (mean age in 40s with a range from childhood to elderly patients) males (about 3:1) who present with hematuria. There is a history of instrumentation in about 20% of patients. Some lesions are quite cellular with mitoses and necrosis, and bladder wall invasion is not uncommon.

The vast majority of patients have an indolent course (although 10-25% experience recurrences), but we have recently encountered 2 cases in which biopsies showing bladder IMT preceded (1 and 2m, respectively) biopsies showing sarcomatoid carcinoma associated with high grade invasive urothelial carcinoma accompanied with separate fragments of bladder IMT; even on re-review the bladder IMT in these 2 cases were morphologically indistinguishable from other cases of bladder IMT, with FISH demonstrating ALK alterations in the bladder IMT areas in 1 of the 2 cases. These 2 patients both died of their carcinomas. A further case displayed overtly sarcomatous features and displayed ALK alterations by FISH and the patient subsequently died of this malignant neoplasm. As such, currently, when we encounter atypical features in these lesions, we now advise caution in our reports and do not render an unequivocally benign interpretation. When lesions appear typical and fasciitis-like, we note that most are benign but mention recurrences and even association with malignant neoplasms as remote possibilities.

The question remains as to whether bladder IMT is the same lesion as lesions called IMT in the rest of the body. Bladder lesions are far more likely to express keratin than those in other sites and are probably less likely to recur and certainly less likely to metastasize [although metastases are rare in soft tissue examples and remain the subject of debate]. They do share molecular alterations and should for the present at least be regarded as a subtype of the general family of IMT. Conservative management and follow-up is advised for most cases.

The most important components of the differential diagnosis are sarcomatoid urothelial carcinoma, leiomyosarcoma, and rhabdomyosarcoma. It is well known that some sarcomatoid urothelial carcinomas exhibit myxoid features mimicking IMT. IMT often expresses cytokeratin, and sarcomatoid urothelial carcinoma sometimes shows weak or focal immunoreactivity for cytokeratin, making the differential diagnosis even more difficult. Finding marked cytologic atypia, atypical mitotic figures, and nonmyxoid areas with marked increased cellularity usually allows for a diagnosis of sarcomatoid carcinoma, but the most useful feature is the identification of an in situ or invasive “typical” epithelial component.

Some leiomyosarcomas of the bladder display myxoid zones and can also express cytokeratin. The lack of a delicate vascular network and interspersed inflammatory cells and red blood cells, which are usually observed in IMT, and the presence of marked cytologic atypia and atypical mitoses in leiomyosarcomas may be helpful in the differential diagnosis. Leiomyosarcomas and leiomyomas lack ALK-1. In the pediatric setting, embryonal rhabdomyosarcoma is the key contender in the differential diagnosis,
an entity readily separated by application of an immunohistochemical panel that includes MyoD1 or myogenin.

References:


Perivascular Epithelioid Cell Tumor (PEComa) in the Genitourinary Tract
Guido Martignoni, Maurizio Pea, Giuseppe Zamboni, Franco Bonetti.
Anatomia Patologica, Università di Verona, Verona, Italy.

Perivascular Epithelioid Cell

The Perivascular Epithelioid Cell (PEC) is a “novel” cell type showing morphological, immunoistochemical and ultrastructural distinctive features. It is characterized by an epithelioid appearance, a clear to granular cytoplasm, a central located round to oval nucleus with inconspicuous nucleoli, slight, if any, atypia and a perivascular distribution (1). PEC coexpresses myogenic and melanocytic markers. Immunoreactivity with HMB45, HMSA-1, Melan A/Mart1 and Microophthalmia Transcription Factor has been demonstrated in this cell, together with immunoreactivity for actin and, less consistently, desmin (1, 2, 3). Ultrastructurally, it exhibits microfilament bundles with electron-dense condensation, numerous mitochondria and membrane-bound dense granules (4, 5).

PEC is thought to be capable of dramatically modulating its morphology and immunophenotype. It can become spindle, with elongated nucleus and cytoplasm showing obvious muscular features or it can become vacuolized acquiring the characters of an adipocyte. The morphologic modulation of PEC is mirrored by its immunophenotypic modulation. Thus, a PEC with prevalent spindle morphology expresses muscle markers like actin more strongly than HMB45 whereas when it is purely epithelioid displays HMB45 immunoreactivity and focal, if any, actin positivity. The presence of progesterone receptors in the PEC with spindle morphology suggests a role of this hormone in its morpho-phenotypical modulation (1, 6). As today, the normal counterpart of PEC has not been identified.

In 1991, Pea et al. (7) first noted this unusual cell in both renal angiomyolipoma and clear cell sugar tumor of the lung. One year later Bonetti et al. (8) advanced the concept of a family of neoplasms composed by this distinctive cell and its association with Tuberous Sclerosis in a letter published in the American Journal Surgical Pathology. In 1996, Zamboni et al. (9) reported the first case of pancreatic clear cell sugar tumor and suggested to name PEComa those neoplasms composed by a pure proliferation of PECs.
Perivascular Epithelioid Cell Tumor (PEComas) of the genitourinary tract.

The World Health Organization defines PEComas as “mesenchymal tumors composed of histologically and immunohistochemically distinctive perivascular epithelioid cells” (10). In the genitourinary tract they can occur in the kidney, in the bladder, in the prostate, in the uterus, in the ovary and in the vulva.

KIDNEY

PEComas of the kidney include classic angiomyolipoma, microscopic angiomyolipoma (so called microhamartoma), intraglomerular lesions, epithelioid angiomyolipoma, oncocyto ma-like-angiomyolipoma and lymphangiomyomatosis of the renal sinus.

Classic angiomyolipoma is the most common mesenchymal tumor of the kidney. The increasing diagnosis of asymptomatic angiomyolipoma seems to be due to the widespread use of ultrasonography performed to evaluate other conditions. Classic angiomyolipoma is characterized by the presence of a variable mixture of adipose tissue, spindle and epithelioid smooth muscle cells and abnormal thick-walled blood vessels (11, 12). It has been considered for a long time to be a hamartoma rather than a true neoplasm, but there is currently strong evidence arguing for its clonal nature (13, 14, 15). Angiomyolipoma can occur sporadically or in patients with Tuberous Sclerosis, a syndrome due to losses of TSC1 (9q34) or TSC2(16p13.3). Tuberous Sclerosis is a complex disease characterized by mental retardation, seizures, and cellular proliferations, including angiomyolipomas, subependymal giant cell tumors, cutaneous angiofibromas, cardiac rhabdomyomas, lymphangioleiomyomatosis, and pulmonary multifocal micronodular hyperplasia. In patients with Tuberous Sclerosis, renal angiomyolipomas are found in both sexes, in the third and fourth decades of life, with a female predominance; they are usually asymptomatic, bilateral, small and multifocal. Sporadic angiomyolipomas occur in older patients, in the fourth to sixth decade of life, with a female predominance; they are single, unilateral and larger than those associated with Tuberous Sclerosis (12). Classic angiomyolipoma contains more than one cell type, but occasionally adipocytes (lipoma-like angiomyolipoma) or spindle smooth muscle cells (leiomyoma-like
angiomyolipoma) predominate in a particular lesion. Classic angiomyolipoma have a benign outcome. Multifocality and regional lymph node involvement can occur and this is considered to represent a multifocal growth pattern rather than metastasis (16, 17). Three cases of sarcoma developing in sporadic angiomyolipoma have been reported, although a similar event has not been described in Tuberous Sclerosis patients (18, 19, 20). Angiomyolipoma frequently shows loss of heterozigosity of variable portions of the TSC2 gene locus in both sporadic and Tuberous Sclerosis-associated tumours. The TSC1 gene occasionally shows loss of heterozigosity (21, 22).

Microscopic angiomyolipomas (so called microhamartomas) are small nodules often present in the kidney bearing angiomyolipomas. They are not homogeneous in appearance and display all the varied morphologic features of angiomyolipoma less the thick-wall blood vessels (1, 23).

Intraglomerular lesions with features overlapping with those of angiomyolipoma have been reported in patients with and without Tuberous Sclerosis and in the TSC2/PKD1 contiguous gene syndrome, a disease with a deletion disrupting both TSC2 and PKD1 (autosomal dominant polycystic disease gene) (24).

Epithelioid angiomyolipoma is a recently described variant of angiomyolipoma. This tumor is composed of purely epithelioid cells with melanogenesis markers immunoreactivity arranged in sheets and characterized by the absence of both adipocytes and abnormal blood vessels. The cytoplasm of the neoplastic cells in these tumors varies from faintly eosinophilic to clear. These cells can display considerable nuclear atypia and can resemble ganglion cells. Epithelioid angiomyolipoma closely resembling high-grade or sarcomatoid renal cell carcinomas is responsible for the occasionally misdiagnosed angiomyolipoma. This tumor can recur locally, metastasise and cause death. On the basis of histology alone it is not possible to predict malignant behaviour in these neoplasms and further data are needed to better define it. However, at the present time, all epithelioid angiomyolipomas should be closely followed clinically. Epithelioid angiomyolipoma has been described in patient with or without evidence of Tuberous Sclerosis and in the TSC2/PKD1 contiguous gene syndrome.

Loss of heterozigosity of TSC2 have been reported in one case of sporadic epithelioid angiomyolipoma (24, 25, 26, 27).
Tumors composed of a homogeneous population of HMB45 positive polygonal cells with deeply eosinophilic cytoplasm have been identified in patients with and without Tuberous Sclerosis and are called oncocytoma-like angiomyolipoma. Recognition of this variant is significant because oncocytomas in the same kidney with angiomyolipomas have been reported repeatedly, and in patients with Tuberous Sclerosis, oncocytomas seem to occur more frequently than in general population (28).

Lymphangiomyomatosis is a rare and progressive disease that affects the lungs of women usually during their reproductive years. In the lung, it consists of an interstitial proliferation of HMB45 positive smooth muscle cells which can vary from small spindle-shaped cells to large epithelioid cells (5). This lesion has also been reported in extrapulmonary sites including mediastinal and retroperitoneal lymph-nodes and soft tissue of the mesentery and the renal sinus. Lymphangiomyomatosis of the renal sinus is a plaque-like mass in the wall of the renal pelvis. All three cases reported to date also had renal angiomyolipomas, but in only two out of the three cases careful post-mortem examination of the lungs revealed pulmonary lymphangiomyomatosis (24, 29).

**BLADDER AND PROSTATE**

In 2003, Pan et al have reported two PEComas of the genitourinary tract occurring in patients without Tuberous Sclerosis (30, 31). One of them measured 8 cm in diameter and involved the prostate and the left seminal vesicle of a 46-year-old male whereas the other of 4 cm arose from the muscularis propria of the urinary bladder in a 33-year-old-woman. Both tumors were composed of a variable percentage of epithelioid and spindle cells with clear to granular cytoplasm arranged in nests separated by a vascular stroma. The neoplastic cells were positive for HMB45, but not for epithelial markers, vimentin and S100 protein. The prostatic tumor showed a low mitotic activity, coagulative necrosis and a malignant behaviour whereas the neoplasm of the bladder, lacking these histologic findings, behaved in a benign fashion. The major differential diagnosis of PEComa, especially around the anatomic site of prostate and urinary bladder, should include smooth muscle tumors (leiomyoma and leiomyosarcoma), malignant melanoma, clear cell sarcoma of the soft part, paraganglioma, postoperative spindle cell nodule/inflammatory myofibroblastic proliferation and clear cell or sarcomatoid carcinomas.
UTERUS, OVARY AND VULVA

PEC neoplasms can rarely involve the female genital tract. The first case reported by Pea et al (32) was a polypoid neoplasm involving the endometrium, which showed morphological features closely related to the clear cell “sugar” tumor of the lung. Vang and Kempson (33) described eight cases of uterine perivascular epithelioid cell tumor (“PEComa”). Patients ranged in age from 40 to 75 years (mean 54 years). They distinguished a morphologic spectrum of neoplasms varying from tumors with a tongue-like growth pattern composed of sheets of HMB45-positive clear epithelioid cells, which they called group A, to circumscribed tumors composed of hyalinized stroma and neoplastic cells focally positive for HMB45 and extensively immunoreactive for actin and desmin, which they referred to as group B. A tumor with a strong and diffuse HMB45 expression morphologically corresponding to an epithelioid angiomyolipoma has been reported in the ovary (34). Finally, one case described as primary extrapulmonary sugar tumor of the vulva has been reported by Tazelaar et al. (35). Lesions considered to be uterine involvement of lymphangiomyomatosis are usually asymptomatic and some of them correspond to an incidental finding in patients bearing stigmata of Tuberous Sclerosis. The PEComas of the uterus have usually shown benign behaviour, but thirteen tumors, two of them associated with Tuberous Sclerosis, were aggressive (36). Recently Folpe et. al reported 26 cases of PEComas of soft tissue and gynecologic origin (vagina, cervix and uterus) proposing criteria for the classification of these tumors as “benign”, “of uncertain malignant potential”, and “malignant” (37). In this study they observed a significant association between tumor size >5 cm., infiltrative growth pattern, high nuclear grade, necrosis and mitotic activity > 1/50 HPF and subsequent aggressive clinical behaviour.

REFERENCES


INVERTED PAPILLOMA OF THE BLADDER AND THEIR VARIANTS
VERSUS
INVERTED GROWTH PATTERNS OF CANCER

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Inverted papilloma of the bladder is thought to be benign, but some urothelial carcinomas show a prominent inverted growth pattern which may pose a diagnostic dilemma. A recent meta-analysis of 322 cases reported with respect to inverted papilloma of the lower urinary tract identified a recurrence rate of 3.85%. Moreover, 1.55%, 5.90% and 1.54% were associated with previous, simultaneous and subsequent urothelial cell carcinoma, respectively (1). These findings together with the known potential for misinterpretation of urothelial carcinoma with endophytic growth as inverted papilloma emphasizes the need of an appropriated approach to differential diagnosis of endophytic lesions of the bladder.

INVERTED PAPILLOMA (UROTHELIAL ADENOMA; BRUNNIAN ADENOMA)

Inverted papilloma comprises less than 1% of urothelial neoplasms and occurs at all ages with a few examples in children (2, 3). The mean age at diagnosis was 64 years (range, 37-87 years) and a peak frequency in the 6th and 7th decades (3, 4). It is more common in men than women (4:1 ratio) and usually presents with hematuria and irritative symptoms. The etiology is uncertain. Some consider this to be a neoplasms with malignant potential (5), whereas others consider it a reactive process similar to proliferative urothelial lesions such as cystitis glandularis and cystitis cystica. Ultrastructural, DNA-ploidy, and immunohistochemical studies indicate a similarity to normal urothelium and low-grade urothelial carcinoma (3, 6). Recent molecular data supports its benign nature based in the low amount of genetic anomalies found in most cases (7).

Most cases of inverted papilloma are located in the bladder trigone, but inverted papilloma can also be found in the ureter, renal pelvis, and urethra (3). Macroscopically, it is characteristically sessile or pedunculated, smooth surfaced, small 0.2-to-4.3 cm (mean, 0.9 cm in diameter), and single, but large multifocal lesions may occur (8, 9, 10). Microscopically, inverted papilloma consists of intramucosal and submucosal anastomosing islands and trabeculae of urothelium. The surface urothelium may be normal, attenuated, or elevated. There are two main patterns of inverted papilloma, including trabecular and glandular patterns (11). The trabecular pattern is composed of anastomosing cords and sheets of urothelium that are arranged at various angles to the mucosal surface. In some cases, cystic spaces lined by attenuated urothelium are present within the epithelial islands. These spaces may contain eosinophilic secretions which stain with PAS but not mucicarmine. The glandular pattern is composed of nests of urothelium with pseudoglandular or true glandular differentiation with goblet cells. Pseudoglandular spaces are lined by urothelium, whereas true glandular spaces contain mucous-secreting cells with mucicarmine-positive secretions, sometimes with intestinal metaplasia with goblet cells (11). In both patterns of inverted papilloma, the epithelial
elements are surrounded by an intact basement membrane and delicate fibrovascular stroma. Unusual growth patterns of inverted papilloma include basaloid, hyperplastic, spindle cell (also called as medullary), and neuroendocrine patterns, often with mixed forms. Neuroendocrine differentiation in inverted papilloma is characterized by numerous granular eosinophilic cells that are immunoreactive for chromogranin. Such cells are present in 40% of cases of typical inverted papilloma. Non-keratinizing squamous metaplasia is also common.

Mild cytologic atypia is often encountered in inverted papilloma, and the precise demarcation with carcinoma is unresolved; fortunately, this is an uncommon problem, but there are cases that are difficult to resolve. In rare cases nuclear atypia may be prominent but these atypical nuclear features are most probably best considered degenerative in nature at present. Mitotic figures are rare or absent in inverted papilloma, unlike carcinoma. It is possible that inverted papilloma and papillary urothelial carcinoma are related, but this possibility is controversial and recent molecular data argues against (12, 13, 14, 15). The number of cases with coexistent urothelial carcinoma in situ or carcinoma has increased recently (16). In some cases, foci of papillary urothelial carcinoma appear to arise from an otherwise typical inverted papilloma (15, 16). Some cases diagnosed as inverted papilloma probably represent urothelial carcinoma with an inverted growth pattern (17, 18, 19, 20, 21, 22). A unique case associated with leiomyoma has been reported (5). Inverted papilloma are usually diploid (4, 5), although one of three cases with associated urothelial carcinoma was aneuploid (19). Recurrent lesions have been observed in less than 1% of the reported cases. An initial diagnosis of inverted papilloma should be challenged if progression is observed (3).

UROTHELIAL CARCINOMA, INVERTED GROWTH

In 1976, Cameron and Lupton (18) described 2 cases of urothelial carcinoma which mimicked inverted papilloma architecturally, but possessed high grade cytologic abnormalities. The potential for misinterpretation of such cases as inverted papilloma has been confirmed by other authors (17, 19, 20). In some cases the tumor has an identical architecture to inverted papilloma while others grow with more of a broad pushing front analogous to carcinoma (17).

By definition, this variant of urothelial carcinoma has significant nuclear pleomorphism, mitotic figures, and architectural abnormalities consistent with low- or high-grade urothelial carcinoma (WHO, 2004) (23). In most cases, the overlying epithelium has similar abnormalities and often contains typical urothelial carcinoma. Inverted papilloma-type carcinoma with minimal cytologic and architectural abnormalities has high mitotic activity and high ki67 labeling index. An exophytic papillary or invasive component is often associated with the inverted element. However, in cases of inverted papilloma fragmented during transurethral resection, a pseudoexophytic pattern may result. The stromal “cores” in this instance are wider and more variable than the fibrovascular cores of true papillary neoplasms (17). In some instances, both inverted papilloma and inverted papilloma-type carcinoma are intimately admixed. Large papillary tumors with prominent endophytic growth “invade” the lamina propria with a pushing border (17). Unless this pattern is accompanied by true destructive stromal invasion the likelihood of
metastasis is minimal, because the basement membrane is not truly breached (17, 18). The main problem associated with this type of growth is assessing the presence of invasion, especially when the tumor is seen intermingling with slender muscle bundles of lamina propria or in juxtaposition with well-defined fascicles of muscularis propria. The diagnosis of invasion should be made when there are irregularities of the contours of the neoplastic nests, if they have jagged edges, and if desmoplastic or inflammatory stroma is noted surrounding these nests (23).

Main differential diagnostic consideration is inverted papilloma with atypia (22). Available data confirms that these cases have rare mitotic figures and very low proliferation index as seen by ki67 labeling index. Some authors have proposed a cut off of <5% to favor a diagnosis of inverted papilloma (7). In addition, to date there has been no association with urothelial carcinoma in the follow up of individuals diagnosed with inverted papilloma with atypia. These atypical nuclear features are most probably best considered degenerative in nature.

There is insufficient data on the literature to indicate whether carcinoma with endophytic growth behave in a manner different to than what would be expected on a stage for stage basis. Main distinguishing features are summarized in Table 1.

REFERENCES


24
23. Eble JN, Sauter G, Epstein JI, Sesterhenn IA. Pathology and genetics. Tumors of the urinary system and male genital organs. IARC Press, Lyon 2004
TABLE. Differences between urothelial carcinoma with inverted growth and inverted papilloma.

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>UROTHELIAL CARCINOMA, INVERTED GROWTH</th>
<th>*INVERTED PAPILLOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>Variable, usually exophytic papillary lesion present</td>
<td>Smooth, dome shaped, usually intact surface cytologically unremarkable</td>
</tr>
<tr>
<td>Growth pattern</td>
<td>Endophytic, lesional circumscription variable</td>
<td>Endophytic, expansive, sharply delineated, anastomosing cords and trabeculae</td>
</tr>
<tr>
<td>Cytologic features</td>
<td>Maturation, spindling or palisading minimal to absent</td>
<td>Orderly polarized cells, some spindling and palisading at periphery, diffuse severe atypia absent. None-to-rare mitosis. No tumor necrosis</td>
</tr>
<tr>
<td>Biologic potential</td>
<td>Recurrences and progression may occur</td>
<td>Benign, no recurrences**</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>Variable, usually high p53 accumulation or Ki67-MIB1 counting (varies according to grade of differentiation)</td>
<td>Low p53 accumulation and Ki-67-MIB1 counting</td>
</tr>
<tr>
<td>Molecular analysis</td>
<td>Frequent FGFR3 mutation, chromosome 9 and 17 deletions</td>
<td>Rare deletions at chromosome 9 or 17, and rare FGFR mutations</td>
</tr>
</tbody>
</table>

*May be associated with concomitant urothelial carcinoma
**Rare recurrences related to incomplete surgical excision
APPLICATION OF ALPHA-METHYLACYL COENZYME A RACEMASE IMMUNOHISTOCHEMISTRY: A HELP OR HINDRANCE IN THE DIAGNOSIS OF PROSTATE CANCER?

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ABSTRACT
Since the discovery of alpha-methylacyl CoA racemase (AMACR) in prostate cancer in 2000, there have been a number of publications studying the diagnostic value of this enzyme. Although the role of this enzyme is still unknown, the application of AMACR immunohistochemistry in pathology practice has been increased sharply in the last few years. We are going to review the recent studies of the AMACR expression in prostate cancer and several benign and malignant entities by us and other investigators. Then we will discuss clinical application, limitation and pitfall of using this marker in details. This discussion is by no means a guidance for pathology practice, but it may provide some reference when interpreting AMACR immunostaining.

INTRODUCTION
Widespread use of PSA has resulted not only in increased number of prostate needle core biopsies performed each year, but also an increasing number of small foci of uncertain diagnoses of limited biopsy material (1-3). Although histological features of prostatic adenocarcinoma such as growth pattern, nuclear atypia, absence of basal cells, and presence of characteristic extra cellular material are important (4-5), when used alone, they are not entirely sensitive or specific to establish a definitive diagnosis of prostate cancer. Immunohistochemical stains for high-molecular weight keratins such as 34beta12 and more recently p63 have been used to identify basal cells which are typically present in benign glands but absent in prostatic adenocarcinoma (6-). Unfortunately, negative staining for basal cells in a few suspicious glands is not a definitive proof of malignancy, as benign conditions can have a patchy or discontinuous distribution of basal cells (8). Rarely, prostatic adenocarcinoma may also contain cells positive for basal cell markers. Therefore, a sensitive yet specific “positive” marker of prostatic adenocarcinoma would be very useful in raising the confidence level in diagnosis on limited specimens for prostate needle cores.

DISCOVERY OF AMACR
Alpha-methylacyl coenzyme A racemase (AMACR) is an enzyme first purified and characterized by investigators studying lipid metabolism. It was characterized in human tissues in 1995 (9). Later, Ferdinandusse et al demonstrated that AMACR, a protein with 382 amino acid residues, played a role in the beta-oxidation of branched chain fatty acids and fatty acid derivatives (10). In 2000, Xu et al (11), using cDNA subtraction in conjunction with high-throughput cDNA microarray screening, identified 3 three genes: P503S, P504S and P510S that showed differential expression in malignant and benign prostate glands. P504S, one of the gene product named with the cDNA clone number, was clearly identified as human alpha-methylacyl coenzyme a racemase (AMACR) (11). AMACR mRNA was overexpressed in the majority of prostate adenocarcinomas compared to low to undetectable levels in benign prostatic glands by Northern blots and real-time PCR analysis. Furthermore, they generated rabbit monoclonal antibodies specific for P503S and P504S (AMACR), respectively. It was demonstrated that AMACR (P504S) immunoreactivity in prostatic adenocarcinoma but not in benign prostatic glands, while P503S immunoreactivity was present in both malignant and benign glands in 5 prostate cases on paraffin-embedded human prostate tissues (11).

AMACR IN PROSTATE CANCER

In 2001, Jiang et al (12) examined at AMACR expression in 137 prostate cancer cases and 70 benign cases by immunohistochemistry using the same rabbit monoclonal antibody to AMACR (P504S). All 137 cases of carcinoma reportedly showed strong cytoplasmic expression regardless of Gleason grades. In addition, 88% of benign tissue samples were completely negative for AMACR, with the other 12% were only weakly and focally positive. In addition, benign prostatic lesions, such as, atrophy, and basal cell hyperplasia were completely negative. The authors therefore concluded that AMACR may be a useful adjunct in the diagnosis of prostate cancer on paraffin tissue (12).

In 2002, four additional studies supported the notion that AMACR may be a useful marker for prostate cancer. Rubin et al (13) found significant over-expression of AMACR in prostate cancer in three of four independent cDNA microarray analyses. By immunohistochemical analysis using a polyclonal AMACR antibody on tissue microarrays and needle core biopsy specimens, they showed that AMACR had 97% sensitivity and 100% specificity in the detection of prostate cancer (13). Similarly, Luo et al (14) showed 95% of prostate cancer cases were positive for AMACR compared to less than 4% of normal tissue and supported the findings that AMACR as a new positive marker that complements basal cell markers to enhance prostate cancer diagnosis. Jiang et al (15) investigated 73 small foci (<1mm) of prostate cancer on the prostate needle core biopsies and found 69 of 73 (95%) foci to be positive for AMACR while all 69 benign biopsies were all negative. This is of particular importance because the diagnosis of limited prostate cancer on needle biopsy is a major challenge for pathologists. Furthermore Beach et al (16) reported 153 of 186 (82%) prostate cancer specimens were positive for AMACR, while 21% of benign foci showed focal, faint, and noncircumferential staining, and concluded circumferential and diffuse luminal positivity was specific for prostate cancer.

Furthermore, in a multi-institutional study, a total of 807 prostatic specimens from 6 US
medical centers were analyzed using conventional and quantitative immunostaining analyses and found that of the 454 cases of prostatic adenocarcinoma, 441 (97%) were positive for AMACR, while 254 of 277 cases of benign prostate were negative for AMACR. Moreover, by using quantitative automated imaging analyses, AMACR immunostaining intensity and percentage in prostate cancer were also significantly higher than those in benign prostatic tissues (17).

Kumar-Sinha et al (18) found evidence that AMACR enzymatic activity is consistently elevated in prostate cancer tissue specimens indicating AMACR in prostate cancer is enzymatically active. The first study examining the predictive capacity of AMACR in patient outcome came in 2005, when Rubin et al (19) reported that lower AMACR expression in cancer cells was associated with worse patient outcome, independent of Gleason score, PSA, and margin status.

In 2004, in an effort to evaluate diagnostic utility of AMACR immunostaining in establishing definitive diagnosis from suspicious prostate biopsies, three urological pathologists analyzed 93 cases of atypical small acinar proliferations (ASAP) with combination of histology, immunostains for AMACR and HMWCK. It was found that AMACR immunoreactivity contributed a resolution of 12% to 24% cases unanimously or by consensus (20). Therefore, AMACR can increase the level of confidence in establishing a definitive malignant or benign diagnosis in atypical cases. In another study, AMACR immunoreactivity contributed the conversion from atypical diagnosis to cancer in 50% (34/76) of atypical cases by a genitourinary pathology expert and 10% (34/307) of atypical cases diagnosed by contributing pathologists (21). Even for experts in prostate pathology, AMACR is helpful for definitive diagnoses in difficult cases.

AMACR IN VARIANTS OF PROSTATE CANCER

In addition to typical prostatic adenocarcinoma, there are several morphologic variants of prostate cancer such as transition zone cancer, foamy gland cancer, hyperplastic cancer. Most of the earliest studies of AMACR expression in prostate cancer dealt mainly with conventional acinar prostatic adenocarcinoma, making little note of expression in various morphologic subtypes of prostate cancer. In 2003, Leav et al (22) demonstrated AMACR expression in 25 of 25 cases of prostatic carcinoma of the transition zone, but noticed staining was less intense in tumors of lower Gleason grade.

The previously mentioned study by Beach et al (16) stated that 5 of 6 carcinomas in prostatectomy specimens with pseudohyperplastic patterns did express AMACR. In 2003, Zhou et al (23) looked at thirty needle core biopsies containing the so-called foamy gland carcinoma, a deceptively benign-appearing variant of prostatic adenocarcinoma. They reported that 68% of these tumors were positive for AMACR using the monoclonal antibody P504S and 62% were positive using the polyclonal antibody specific for AMACR. They also examined 17 needle biopsies with pseudohyperplastic carcinomas and reported 77% and 70% positive in AMACR immunoreactivity, respectively (23). Similar findings have recently reported 72% of malignant foamy glands positive for AMACR in 23 prostatectomy specimens (24).
The atrophic variant of prostatic adenocarcinoma is a mimicker of benign atrophy, often difficult to diagnose on needle core biopsy. Farinola et al (25) looked at AMACR expression in 19 cases of atrophic carcinoma and 16 cases of benign atrophy on needle core biopsy and found expression in nearly 70% of atrophic cancers but none of the cases of benign atrophy. In summary, expression of AMACR may be helpful in diagnosing deceptively bland variants of prostate cancer (i.e. pseudohyperplastic, foamy gland, and atrophic variants) when positive. One must interpret a negative stain in these variants with caution, because approximately 30% could be negative.

AMACR IN PROSTATE AFTER RADIATION OR HORMONAL THERAPY

Radiation therapy, a common treatment modality for prostate cancer, can induce marked histological changes in both malignant and benign prostatic tissues such as nuclear enlargement and hyperchromasia. Benign prostatic glands after radiation may be difficult to distinguish from malignant ones based on histological features alone. An additional marker such as AMACR might be useful as a tool to aid in the diagnosis of malignancy in prostatic glands with post radiation atypia. Yang et al (26) examined AMACR expression in 40 irradiated prostate specimens (28 with carcinoma) and 40 nonirradiated specimens (20 with carcinoma) and found that all 48 malignant cases showed strong expression of AMACR while all 32 benign cases were negative. Amin et al (27) looked at 26 patients with post radiation prostate cancer and reported AMACR expression in 94% of cases. The authors also observed that decreased AMACR expression in tumors correlated with treatment effects (27).

Hormonal treatment modalities such as androgen antagonists are also commonly used against prostate cancer, especially for advanced stage disease. Rubin et al reported a significant decrease in AMACR expression in metastatic hormone-refractory prostate cancers compared with hormone naïve cancers (13). On the other hand, Luo et al (14) reported 13 of 14 cases of hormone refractory metastatic cancers were positive including 71% showing strong expression of AMACR. In the study by Beach et al (16), all eight hormonally treated cases were positive. The largest study examining AMACR expression in hormone treated tumors was done by Suzue et al in 2005 (28). They looked at 64 patients with residual or recurrent prostate cancer following hormonal therapy. They found that AMACR expression was reduced significantly in the majority of post hormonal residual carcinomas, whereas in hormone-refractory metastatic tumors, AMACR expression was retained (28). This finding also indicates that AMACR may be functionally related to the development and progression of prostate cancer rather than a by-stander.

AMACR IN PUTATIVE PRECURSOR LESIONS OF PROSTATE CANCER

Two prostatic lesions have been considered as potential premalignant lesions of the prostate. High grade prostatic intraepithelial neoplasia (HGPIN) is most likely a precursor of peripheral zone prostatic adenocarcinomas (29-30). Studies have shown that the risk of carcinoma on re-biopsy is increased when HGPIN is present (31-32). Several of the early studies of AMACR in prostate found increased expression in HGPIN, but rates varied from 13 to 72% (12-17). In 2004, Wu et al (33) analyzed AMACR
expression by immunohistochemistry in 3954 prostatic ducts and acini with HGPIN from 140 prostatectomy specimens. They found AMACR expression in 126 of 140 cases, but only 41% of prostatic ducts or acini involved by PIN showed AMACR immunoreactivity. Significantly, 56% of HGPIN glands close to adenocarcinoma were positive compared to only 14% of HGPIN glands away from adenocarcinoma (33) which suggests a higher risk of finding cancer or developing cancer in areas adjacent to AMACR positive HGPIN.

Using gene expression profiles, Ashida et al showed that AMACR was one of 21 up-regulated genes seen in PIN lesions and considered it to be involved in the early stages of prostate carcinogenesis (34). Recently, Ananthranarayanan et al (35) looked at AMACR expression in 45 patients with isolated HGPIN in needle core biopsy, 12 radical prostatectomy specimens with prostatic carcinoma, and 6 cystoprostatectomies without prostatic carcinoma. They found that AMACR expression was increased in HGPIN lesions, but that proximity to carcinoma did not affect expression levels. This study was limited by the choice of small specimens from biopsy. Significantly, they also observed that AMACR expression was significantly increased in benign glands adjacent to adenocarcinoma and postulated a possible field effect in prostatic carcinogenesis (35).

Atypical adenomatous hyperplasia (AAH), also known as adenosis, is a lesion characterized by a well-circumscribed lobule of closely packed, crowded small glands without significant cytologic atypia, occurring mostly in the transition zone (30, 36). Seen in approximately 5-20% of transurethral and radical prostatectomy specimens, AAH may be difficult to distinguish from low-grade carcinoma because of their architectural similarities (30, 36, 37). Yang et al (38) examined AMACR in 40 cases of AAH and found focal expression in 10% of cases and diffuse expression in 7.5% of cases. Similarly, Gupta et al (39) observed AMACR expression in 31% of cases of AAH. These findings support the notion that a small subset of AAH may be a precursor of prostate cancer. They also indicate that AMACR expression in a lesion, in which AAH is a diagnostic consideration, must be interpreted with caution. Other features such as the presence of basal cells by high molecular weight keratin stains might also be useful in these circumstances.

**AMACR IN BENIGN CONDITIONS**

Many benign conditions such as small, crowded glands, atrophy, inflammatory atypia, and basal cell hyperplasia can mimic prostatic adenocarcinoma on needle core biopsies (40). Moreover, small foci of such conditions may be negative for high-molecular weight keratins (HMWK) (8). The initial studies examining AMACR expression in prostate noted an occasional small amount of expression in benign prostatic epithelium (12 to 21% of benign glands) using both polyclonal and monoclonal antibodies (12-17). The staining was reported as almost always fine granular, focal, weak, and noncircumferential (12-17). Benign prostatic hyperplasia (BPH) was often negative for AMACR (12, 16). In the study by Leav et al (22), the authors noted AMACR expression in eight BPH samples adjacent to adenocarcinoma but none of the other BPH cases. This could be a “field effect” phenomenon similar to that described above by Ananthranarayanan et al (35) in which benign glands adjacent to cancer seemed to show increased expression of
Recently, Herawi et al. (42) examined AMACR expression in benign mimickers of prostate cancer seen on needle biopsies in consultation. They reported 15 of 19 (79%) cases of partial atrophy and 7 of 11 (64%) cases of crowded glands were positive for AMACR, although they did not mention extent or intensity of staining (42). We have also noticed increased AMACR expression in cases of partial atrophy (unpublished data), although the staining tends to be non-circumferential and less intense compared to carcinoma.

Nephrogenic adenoma (NA) is a benign lesion composed of small glandular structures that develops along the urothelium which is felt to be derived from shedding renal tubules (43). Although not commonly seen on prostate needle core biopsies, it may be mistaken for carcinoma when present. Skinnider et al. (44) reported moderate to strong circumferential AMACR in 3 of 4 NAs of the prostatic urethra. Gupta et al. (45) found AMACR expression in 28 of 38 (58%) NAs, ranging from patchy, focal staining to diffuse positivity. Some of these lesions were also negative for HMWK. Thus, nephrogenic adenomas are both morphologic as well as immunohistochemical mimickers of prostate carcinoma and must be kept in mind when examining small foci of suspicious glands on needle biopsy.

There seems to be some differences in AMACR staining levels in benign prostatic conditions when comparing the monoclonal (P504S) or polyclonal antibodies specific for AMACR. Typically polyclonal AMACR antibodies demonstrated slightly higher background than monoclonal ones. Using the monoclonal antibody, Beach et al. (16) reported that small benign glands including atrophy, basal cell hyperplasia, urothelial metaplasia, and most cases of adenosis were completely negative for AMACR. On the other hand, Rubin et al. (13), using the polyclonal antibody, found increased expression in benign conditions such as post-atrophic hyperplasia. In 2003, Kunju et al. (41) directly compared the two antibodies and found that 68% of benign glands showed weak expression of AMACR with the polyclonal antibody compared to only 7% using the monoclonal antibody. Sensitivity for prostatic adenocarcinoma was 100% using the polyclonal antibody compared to 94% for P504S. We have been using the rabbit monoclonal antibody, as it seems to have good specificity while not sacrificing much sensitivity for prostatic adenocarcinoma. Because of the wider application of AMACR antibodies, the staining conditions for AMACR immunohistochemistry are essential for interpretation. Overstaining can be a major problem since both benign and malignant glands would be all positive. Optimal staining condition for each AMACR antibody has to be tested out. When interpreting AMACR stains in prostates, one should always use the benign prostatic glands as a negative control, which should have very low AMACR staining, and compare to the lesions of interest.

**AMACR IN OTHER NEOPLASMS**

Since the original prostate cancer studies, several investigators have looked at AMACR expression in other tumors. Zhou et al. (46), using the polyclonal antibody, found AMACR overexpression in colorectal, ovarian, breast, bladder, lung, and renal cell
carcinomas, as well as lymphomas and melanomas. Greatest overexpression was seen in colorectal carcinomas (92%) (46). Using the monoclonal antibody, Jiang et al (47) reported that 81% of hepatocellular carcinomas, 75% of renal cell carcinomas, 31% of urothelial carcinomas, and 27% of gastric carcinomas were positive for AMACR. They also reported that lung, breast, pancreas, bile duct, adrenal gland, salivary gland, ovary, thyroid, and endometrial cancers were negative or rarely positive, while AMACR expression was found in normal liver, kidney, and salivary gland tissue (47). Using a high-density tissue microarray, Witkiewicz et al (48) determined that AMACR was overexpressed in 42 of 160 invasive breast carcinomas, and was associated with a decrease in tumor differentiation.

In 2003, Jiang et al (49) examined AMACR expression in 242 cases of colonic tumors including 176 carcinomas, 38 adenomas, and 28 hyperplastic polyps. Using immunohistochemistry, they determined AMACR was highly expressed in 75% of carcinomas and 79% of adenomas but only 4% of hyperplastic polyps. It was postulated that AMACR overexpression might be an early event in the adenoma-carcinoma sequence in colorectal tumor genesis (49). Recently, Chen et al (50) examined AMACR expression in 59 small intestinal adenocarcinomas and 66 colorectal adenocarcinomas and reported that 62% of colorectal tumors were positive compared to only 5% of small intestinal tumors. Therefore, AMACR expression level is important for differentiation of small bowel adenocarcinoma from colonic adenocarcinoma.

There are several studies in the literature regarding AMACR expression in renal tumors. Tretiakova et al reported expression in 41 of 41 papillary renal cell carcinomas (RCC) compared with 13 of 52 clear cell RCCs, 3 of 20 oncocytomas, 0 of 18 chromophobe RCCs and 0 of 15 sarcomatoid RCCs. In addition, they reported a 5.2-fold increase of mRNA levels in 7 of 8 papillary RCCs but no increase in 60 of 62 non-papillary renal tumors (51). Other papillary carcinomas including thyroid, breast, endometrium, ovary, and pancreas carcinomas were rarely positive (51). Lin et al (52), using AMACR immunohistochemistry, also observed 100% expression in 15 papillary RCCs but also reported expression in 69% of clear cell RCCs, 29% of chromophobe RCCs, and 25% of oncocytomas.

Suh et al (53) stained 17 cases of enteric-type primary adenocarcinomas of the bladder and observed 65% positivity, similar to the 70% expression rate they observed in colorectal carcinomas but much higher than the 14% expression rate they observed in conventional transitional cell carcinomas of the bladder. This data suggests that AMACR expression might be related to an “enteric phenotype” in certain tumors. Logani et al (54) reported P504S overexpression in 32% of metastatic colorectal carcinomas to the ovary compared with none of the 23 primary ovarian mucinous and endometrioid carcinomas. Perhaps AMACR expression in a mucinous ovarian neoplasm may suggest a metastasis rather than a primary ovarian neoplasm.

DOUBLE OR TRIPLE STAINS

Based on the above data, one can reasonably assume that positive staining for AMACR in small atypical glands with absence of basal cells can help establish a definitive
diagnosis of prostatic adenocarcinoma when HGPIN, AAH, and nephrogenic adenoma have been excluded. Therefore, the development of double or triple staining distinguishing prostate cancer from benign glands became attractive. In 2004, Jiang et al reported the double immuno florurcense with AMACR in red and HMWCK in green as well as double immunohistochemistry with AMACR in brown and HMWCK in blue. In 2004, Browne et al (55) further showed that AMACR, when used in combination with a basal cell stain such as HMWKR or p63, can render a definitive diagnosis in up to 70% of cases that would otherwise have been called atypical and recommended both stains for such lesions. The authors also noted that a limitation of this approach at the time was the loss of tissue in these small lesions, suggesting that combining the two or three stains on a single slide would be more optimal (55).

The same year, Sanderson et al (56) examined 40 cases containing small foci of adenocarcinoma, HGPIN, atypical small acinar proliferations (ASAP) with and without PIN, and atypical favor benign glands using a p63/P504S cocktail. After the combined stain, one third of cases were re-classified to carcinoma (56). Molinie et al (57) observed an increase in both sensitivity and specificity for prostate adenocarcinoma when using a p63/P504S cocktail compared to using a basal cell marker alone and that the combined stain supports a diagnosis of cancer in 40% of cases previously considered as ASAPs. Another study in 2004 examined 101 small foci of prostate cancer, 104 foci of ASAP, 19 small foci of PIN, and 36 benign mimics of cancer and found that with the P504S/p63 cocktail, 89% of ambiguous lesions were reclassified more definitively versus 53% when using CK 5/6 alone (58).

In 2005, Hameed et al (59) reviewed 31 consecutive radical prostatectomy specimens and 150 prostate needle biopsies and selected sections showing foci of minimal prostatic adenocarcinoma, HGPIN, and common benign mimickers of prostate cancer. They reported that a cocktail containing p63 and AMACR was very useful in highlighting adenocarcinoma associated with HGPIN, flat and cribriform HGPIN, and distorted foci of minimal prostatic carcinoma and suggested the cocktail is essentially equivalent to using each antibody separately for immunohistochemical confirmation of cancer (59).

Jiang et al (60) recently assessed the usefulness of a 3-antibody cocktail combining AMACR, 34betaE12, and p63 (triple stain) with a double chromogen reaction. Examining 138 needle biopsies including 82 with small foci of cancer, they found that 95% of the malignant cases expressed AMACR and none expressed basal cell markers, while a positive AMACR and negative basal cell phenotype was 100% specific for cancer (60). Performing the triple stain seems to be a very sensitive and specific way to detect small foci of prostate cancer on needle core biopsies while utilizing as little tissue as possible, and we recommend its routine use on all cases of small atypical foci suspicious for cancer.

The major reason for PIN-4 triple stains (AMACR, P63 and HMWCK) becoming more popular is using only one slide and revealing the prostate cancer marker and basal cell markers on the same focus. However, it is important to know that condition of double or triple stain is more complicated than single stain and subject to tissue distortion understaining or overstaining.
LIMITATION AND PITFALLS

Although AMACR immunohistochemistry has shown promise to surgical pathologists when diagnosing small foci of prostate cancer, there are some diagnostic pitfalls to keep in mind. First, like many other immunohistochemical stains, AMACR staining has shown variability from laboratory to laboratory. In 2003, Magi-Galluzzi (61) et al found that although all 34 in-house cases of prostate cancer performed at an immunohistochemistry laboratory at a major teaching hospital were positive for AMACR, while only 80% of prostate cancer cases seen in consultation but performed from various immunohistochemistry laboratories were positive. Zhou et al (23) reported that close to 20% of their prostate cancer cases seen in consultation were negative for AMACR. These rates are significantly less than most initial reports on AMACR expression in prostate cancer (12-17). This finding may represent the selection of difficult cases for consultation but nevertheless indicated the increasing utility and difficulty in performing and interpretation of AMACR staining in different institutions.

As stated earlier, some histologic variants of prostate cancer show decreased expression of AMACR compared to conventional prostatic acinar adenocarcinoma. Approximately 30% of atrophic, foamy gland, and pseudohyperplastic variants of adenocarcinoma are reportedly negative. In addition, many hormonally treated residual prostatic adenocarcinomas show decreased AMACR expression (28). Therefore, a lesion with negative expression of both AMACR and basal cells should be interpreted with caution when suspecting such entities.

Conversely, some benign conditions have been shown to express AMACR on occasion, although the staining pattern is usually weaker and non-circumferential. These include adenosis, atrophy, and benign glands adjacent to cancer. One must also be aware that HGPIN and nephrogenic adenoma often strongly express AMCAR. Many tumors from other organ systems have been shown to express AMACR as well. Therefore AMACR may not be as useful as markers such as PSA to define a metastatic carcinoma from other organ.

Although AMACR immunochemistry has been used in clinical practice, the utility of AMACR in serum tests are only at experimental stages. A screening test in the clinical setting based on urinary AMACR may develop as a useful adjunct to serum PSA and digital rectal exam in the early detection of prostate cancer. Using Western blot analysis, Zielie (62) et al detected AMACR in the urine in all twelve patients with biopsy-proven prostate cancer, and showed AMACR detection was associated with cancer status by biopsy in 21 of 26 patients. Rogers et al (63), using quantitative reverse transcriptase-PCR to detect AMACR-to-PSA transcript ratios, also were able to predict prostate cancer status in patients. These initial studies hint at perhaps not only a promising future for AMACR immunohistochemistry, but also in other clinical applications.

In summary, multiple studies have shown that AMACR immunohistochemistry of is only a sensitive and specific marker for prostate cancer, but also practical for pathologic utility. However, we need to be aware that occasionally benign lesions may show positive AMACR staining and prostatic adenocarcinoma can be negative for AMACR.
Therefore, it is a good practice to use a combination of AMACR and basal cell markers such as 34bE12 and p63. Neoplasms from other organs may also express AMACR when a specimen outside the prostate is evaluated. Like any other good markers, application of AMACR should be used as an adjunct test. The diagnosis of prostate cancer should be primarily established on the morphological basis.

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REFERENCES


32. Kronz JD, Allan CH, Shaikh AA, et al. Predicting cancer following a diagnosis


The majority of renal epithelial neoplasms are diagnosed on morphologic grounds. In renal cell carcinoma (RCC), there are unique histologic features that help to distinguish subtypes, and separate them from benign tumors such as oncocytoma and metanephric adenoma. These unique morphologic features are associated with specific genetic and chromosomal abnormalities as well as different tumor behaviors. However, renal epithelial tumors can exhibit overlapping features. In this setting, immunostains may play a role in the differential diagnosis (Table 1).

Table 1. Differential Diagnosis in Select Renal Epithelial Neoplasms

1. Renal epithelial neoplasms with eosinophilic granular cytoplasm
   - Clear cell renal cell carcinoma
   - Papillary renal cell carcinoma (type II)
   - Chromophobe renal cell carcinoma (eosinophilic variant)
   - Oncocytoma

2. Renal epithelial neoplasms with “small blue cells”
   - Papillary renal cell carcinoma (type I)
   - Metanephric adenoma
   - Wilms tumor

3. Renal epithelial neoplasms with tubular, tubulopapillary or papillary architecture
   - Papillary renal cell carcinoma
   - Metanephric adenoma
   - Translocation-associated renal cell carcinomas (TFE3 gene fusions)
   - Oncocytoma
   - Urothelial carcinoma of the collecting system
   - Collecting duct carcinoma

4. Renal epithelial neoplasms with spindle cells
Renal cell carcinoma (all subtypes) with sarcomatoid differentiation
Primary renal sarcomas
Retroperitoneal sarcomas with secondary involvement of the kidney
Solitary fibrous tumor
Angiomyolipoma

5. Metastatic renal epithelial neoplasms

Adrenal gland
Primary adrenocortical adenoma/carcinoma
Metastatic clear cell renal cell carcinoma
Identification of primary site for metastases
Clear cell carcinomas from other sites

1. Renal Epithelial Neoplasms with Eosinophilic Granular Cytoplasm

This differential diagnosis includes clear cell, chromophobe, and type II papillary RCC and oncocytoma, and number of published studies have shown the utility of immunostains in separating these various renal tumors (Table 2). The immunostains of note are CD10, parvalbumin, RCC, KIT, alpha-methylacyl-CoA racemase or AMACR (P504S), PAX-2, antimitochondrial antibody (112-1), vimentin, and cytokeratins (CK) including CK7.

CD10

CD10, the common acute lymphoblastic leukemia antigen, is a cell surface metalloproteinase, and expressed by lymphoid precursor cells, germinal center B cells, some myeloid cells, and a number of non-hematolymphoid tissues including the normal proximal nephron of the kidney. A number of studies have shown that CD10 is expressed in the majority of clear cell and papillary RCC, and a minority of chromophobe RCC and oncocytoma, and therefore may be useful in the diagnostic distinction between these tumor types. It is of note that in clear cell RCC, increasing tumor grade is associated with decreased expression of CD10. In one study, low-grade clear cell RCC (grade 1 and 2) was positive in 89% of cases compared to 69% of higher-grade RCC. Also, CD10 appears to be more frequently expressed in type II papillary RCC (88% of tumors positive) compared to type I papillary RCC (45%). CD10 is expressed in a number of other tumors including urothelial carcinoma, prostatic adenocarcinoma, melanoma, pancreatic adenocarcinoma, and others. Therefore, in the immunostains workup of a tumor of unknown primary, CD10 may have limited utility.

Parvalbumin
Parvalbumin is a cytosolic calcium-binding protein that regulates intracellular calcium and this protein is expressed in the collecting ducts of the adult kidney. A limited number of studies have shown that the immunostain to this protein is positive in chromophobe RCC and oncocytoma, and negative in papillary and clear cell RCC. This finding has potential diagnostic utility and supports that concept that chromophobe RCC is derived from the intercalated cell of the collecting duct and has a close relationship with oncocytoma.

**RCC**

RCC is a glycoprotein found in the brush border of proximal tubule of the nephron. The immunostain for RCC is found in 47 to 85% of clear cell RCC and 63 to 91% of papillary RCC in contrast to a minority (0 to 4%) of chromophobe RCC and (0 to 14%) of oncocytoma. The staining pattern is either surface membrane or a combination of surface membrane and cytoplasmic.

**KIT**

KIT is a transmembrane tyrosine kinase receptor protein encoded by the proto-oncogene c-kit. Mutations in certain exons of the gene are found in a number of tumors, most notably gastrointestinal stromal tumors. Malignant GISTs that exhibit these mutations are responsive to imatinib mesylate therapy. Recently, gene expression studies have identified that c-kit is upregulated in chromophobe RCC, and immunostains have confirmed the presence of KIT protein in both chromophobe RCC and oncocytoma and its absence in clear cell RCC and papillary RCC. However, a recent study reported that a significant percentage of high-grade clear cell RCC including those with sarcomatoid differentiation expressed KIT, and these patients could potentially benefit from imatinib mesylate therapy. We recently reported that KIT protein staining is very infrequent in high-grade RCC and its presence is not associated with the specific mutations indicative of response to imatinib mesylate. KIT protein immunostaining is not specific to GIST, oncocytoma or chromophobe RCC, and it is seen in a number of different tumor types.

**Alpha-methylacyl-CoA Racemase (AMACR or P504S)**

Alpha-methylacyl-CoA racemase was identified in gene expression analyses of prostate cancer where the mRNA (followed by protein studies) was found to be increased in prostate cancer and absent in benign prostatic tissue. Gene expression analyses followed by immunohistochemical staining indicated that AMACR was also increased in papillary RCC but not the other RCC subtypes or oncocytoma. In addition, AMACR protein expression was identified in metastatic papillary RCC. Like other tumor markers, AMACR is not specific to papillary RCC and may be seen in other tumor types so its utility in determining a specific tumor lineage is limited.
Pax-2

Pax-2 is a homeogene that is expressed in the metanephric mesenchyme of the developing human kidney, and it functions in the mesenchymal-epithelial transformation that occurs in fetal kidney development. Although the number of studies and cases examined are low, the preliminary findings suggest that Pax-2 protein is more frequently expressed in clear cell RCC than papillary and chromophobe RCC and oncocytoma. In addition, Pax-2 expression appears to be higher in higher grade tumors. Additional studies are needed to determine the utility of this diagnostic marker in the evaluation of primary and metastatic renal epithelial tumors.

Vimentin

Immunohistochemical studies examining vimentin expression in renal tumors indicate that vimentin is more frequently expressed in clear cell and papillary RCC compared to oncocytoma and chromophobe RCC. However, as in most of the immunostains discussed here, there is overlap in expression of this protein between tumor types. In addition it is thought by some pathologists that cytokeratin and vimentin co-expression is supportive of a diagnosis of metastatic RCC. However, this co-expression profile is not specific to either papillary or clear cell RCC, and can be seen in other tumor types particularly spindle cell carcinomas from a number of sites.

Cytokeratins

The cytokeratins compromise a family of 20 distinct intermediate filaments, and their expression is indicative of epithelial differentiation. Differential CK protein expression has become common in diagnostic pathology with the use of CK7, CK20, CK5/6, and HMWCK. Recently, differential cytokeratin expression has been identified in the human nephron with the CKs of simple epithelia (CK7, CK8, CK18, and CK19) predominating particularly CK8 and CK18. Using this differential CK expression, Skinnider et al examined a panel of various CK subtypes and vimentin to differentiate between various renal cortical neoplasms consisting of clear cell, papillary, chromophobe, collecting duct, renal medullary, tubulocystic, mucinous tubular and spindle cell RCC, oncocytoma and metanephric adenoma and they identified the following profiles:

- Clear cell RCC (15 cases): CK7/-;CK8/-;CK18+;vimentin+
- Papillary RCC (15 cases): CK7+;CK8+;CK19+;vimentin+
- Chromophobe RCC (15 cases): CK7+/--;CK8+/-;CK18+;vimentin-
- Oncocytoma (10 cases): CK7--;CK8+;CK18+;vimentin--;CK14-
- Urothelial Ca (12 cases): CK5/6+/-;CK17++;vimentin+/--;HMWCK+/-
- Collecting duct RCC (6 cases): CK5/6--;CK17-/+;vimentin+;HMWCK+
Renal medullary RCC (3 cases):
CK7+;CK8+;CK18+;CK19+;vimentin+

Tubulocystic RCC (3 cases): 7-/+;CK8+CK18+;CK19+;vimentin+

Skinnider et al concluded that immunohistochemical determination of CK subtypes can be helpful in the diagnostic workup of some renal tumors. Cytokeratin 20 has also been examined in RCCs and oncocytoma, and is negative in clear cell RCC, positive in papillary RCC (type II), negative in chromophobe RCC and variably positive in oncocytoma (53-80% of cases positive).

Other Markers

Other potentially useful diagnostic markers of renal cell carcinoma include Ron protein. The protein is derived from a RON proto-oncogene that encodes for a tyrosine kinase receptor. In immunohistochemical analyses, Patton et al identified Ron protein expression in 99% (69 of 70 cases) of oncocytomas, 96% (55 of 57 cases) of chromophobe RCC, and in only 17% of other renal cell carcinoma subtypes. Antimitochondrial antibody (113-1) is a monoclonal antibody that recognizes a protein portion of the human mitochondria. In the assessment of granular renal cell tumors, distinctive staining profiles for this antibody were detected in oncocytoma (diffuse and fine granularity), chromophobe RCC (peripheral accentuation with coarse granularity), and granular variant of clear cell RCC (irregular distribution of coarse cytoplasmic granules). These distinctive staining patterns were reported as useful in the diagnostic workup of renal tumors with granular eosinophilic cytoplasm. E-cadherin is positive in chromophobe RCC and oncocytoma, and negative in clear cell RCC. In papillary RCC, e-cadherin is positive in approximately 40% of type II tumors, and 0% of type I tumors.
<table>
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2. Renal Epithelial Neoplasms with “Small Blue Cells”

The differential diagnosis in this setting includes type I (basophilic) papillary renal cell carcinoma (immunophenotype already discussed), metanephric adenoma and Wilms tumor. The following immunostains may be helpful with the following typical immunophenotype:

- Metanephric adenoma: EMA--; CK7-/+; WT-1+
- Papillary RCC, type I: EMA++; CK7+; WT-1-
- PNET: EMA--; CD99+
- Wilms: EMA--; CK7-/+; WT-1++; CD99-/+ 

In the differential diagnosis of metanephric adenoma and Wilms tumor, immunostains play a minimal role as the tumors exhibit identical immunoreactivities including positivity for WT-1. In addition, it must be remembered that WT-1 is not specific to Wilms tumor and can be seen in a number of other tumors including carcinomas of the ovary, breast, lung, pancreas, endometrium, and colon as well as mesotheliomas.

3. Renal Epithelial Neoplasms with Tubular, Tubulopapillary or Papillary Architecture

The immunophenotype of the tumors in this diagnostic category have been covered previously (Table 2). One very important exception is renal cell carcinomas that arise from TFE3 gene fusions, the so-called “translocation carcinomas of the kidney”. These tumors most commonly affect young individuals and have a characteristic chromosomal translocation that involves chromosome Xp11.2 that results in gene fusions that involve the TFE3 transcription factor gene. Although renal cell carcinomas comprise less than 5% of renal tumors in young patients, it is likely that these translocation carcinomas of the kidney compromise a significant proportion of these childhood RCCs. The gene fusion combinations include ASPL-TFE3, PRCC-TFE3, PSF-TFE3, CLTC-TFE3 and nonO-TFE3. A polyclonal antibody to the C-terminal portion of TFE3 has been developed, and is a sensitive and specific marker for these tumors. Finally, another translocation carcinoma involves t(6;11)9p21;q12) that results in a Alpha-TFEB gene fusion whose product can be recognized by an antibody to TFEB. These translocation carcinomas of the kidney have malignant potential and will require further study to determine their frequency in the childhood and adult populations as well as their biologic behavior. Utilization of immunostains to identify these tumors will help greatly in this regard. In rare instances, urothelial carcinoma can have an unusual architecture. In addition to some stains listed in Table 2, p63 may be helpful as p63 is reported as positive in urothelial carcinoma and negative in RCC.
4. Renal Epithelial Neoplasms with Spindle Cells

The primary differential diagnosis in this setting rests between RCC with sarcomatoid differentiation, spindle cell sarcoma, and some variants of angiomyolipoma. The presence of an epithelial component (of any RCC subtype) associated with a malignant spindle cells is diagnostic of sarcomatoid RCC, and no immunostains are warranted. In most instances, adequate sampling will reveal an underlying RCC. In cases where the tumor is composed entirely of spindle cells, cytokeratin positivity is indicative of epithelial differentiation and sarcomatoid RCC. Immunostains are helpful for the other spindle cells tumors as well including smooth muscle markers for leiomyosarcoma and leiomyoma, CD34 for solitary fibrous tumor, and smooth muscle actin, muscle-specific actin, melan A and HMB45 for angiomyolipoma (particularly useful for the epithelioid variant). In cases where the kidney is involved secondarily from a retroperitoneal sarcoma (which can be difficult to determine), dedifferentiated liposarcoma must always be kept in mind, and the tumor sampled well to evaluate for a diagnostic low grade component.

5. Metastatic Renal Epithelial Neoplasms

The identification of the kidney as a primary site in the immunostains workup of metastatic tumors of unknown primary is problematic. When the adrenal gland is involved by a tumor with clear cells and the differential includes adrenocortical tumors and metastatic clear cell RCC in a patient with known RCC, immunostains are potentially helpful (Table 3). At other sites, however, the lack of specificity of the immunostains (except in the unusual cases ) presented here prevents an unequivocal identification of kidney as the primary site. In addition, a thorough clinical examination including radiologic studies will determine if the kidney contains the likely primary tumor . In patients with known RCC who develop tumors at other sites, histologic comparisons of the metastasis with the renal tumor can be extremely helpful particularly knowledge of the primary tumor subtype, nuclear grade and stage. Hopefully, in the future additional specific immunostains will become available through gene expression profiles of RCC subtypes that will help in this regard.

Table 3. Renal Versus Adrenocortical Neoplasia.

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