Testicular tumors are rare. It was estimated that there would be approximately 8,000 new cases reported in the United States in 2007 and only 380 cancer-related deaths. Germ cell tumors (GCT) comprise about 98% of all testicular tumors and for most of the deaths. They are the most common malignancy and one of the most common causes of cancer-related death in males between the ages of 15 and 35 years. Because of their relative rarity and diverse morphology, they present a diagnostic challenge to most practicing pathologist. However, pathologists must accept this challenge and become familiar with the cytomorphological features of those entities classified as GCT because the clinical features and treatments vary greatly among them. It is beyond the scope of this presentation to discuss in detail the pathological features of each of these tumor types and I direct your attention to many wonderful chapters and papers available in the pathology literature that do justice to this topic. Alternatively, my goal is to review the immunohistochemical features of each of these lesions and how this technology can be used in the differential diagnosis of “difficult to classify” tumors.

**Intratubular germ cell neoplasia:**
This term refers to the lesion initially described by Skakkebaek as “carcinoma in situ” as well as to other “differentiated” forms of intratubular germ cell neoplasia. Strictly speaking, the lesion originally described by Skakkebaek is now called “Intratubular germ cell neoplasia, unclassified” (IGCNU) by most, at least in the Western Hemisphere. It is characterized morphologically by the presence of enlarged, atypical germ cells located immediately above a usually irregularly thickened basement membrane. In essence, the cytologic features of classic IGCN are those of seminoma. The relationship is supported by the coexpression of a host of histochemical and immunohistochemical markers among both cell types. Tubules whose lumen is filled with these cells may be regarded as “intratubular seminoma”.

Placental-like Alkaline Phosphatase (PLAP) is one of the isoforms of alkaline phosphatase. PLAP antibodies will stain IGCNU as well as the majority of seminomas and embryonal carcinomas as well as a smaller percentage of yolk sac tumors. Immunoreactivity is seen in virtually all cases of IGCN and the staining pattern is usually membranous or cytoplasmic. No other non-neoplastic intratubular cells are immunoreactive for PLAP, but immunoreactivity may be seen in other types of non-germ cell malignancies. C-kit (CD 117) is expressed in a large percentage of IGCN as well as seminomas, but not in other germ cell tumors. Once again, the staining pattern is cytoplasmic/membranous. Despite the overexpression of this antigen, c-kit is rarely mutated in these tumors. Other antibodies which immunoreact with IGCNU but are rarely used in clinical practice include M2A and 43-F. POU5F1 (Oct3/4) is a very interesting marker which was recently described. The gene serves as a transcription factor and its product is expressed in pluripotent mouse and human embryonic stem cells and is down-regulated during...
differentiation. Since the gene is also required for self-renewal of embryonic stem cells, knocking out the gene is lethal. Early reports suggest that this antigen is expressed solely in IGCNU, seminoma and embryonal carcinoma, suggesting that these are the types of GCT cells with pluripotency, i.e. with capacity to differentiate.

**Seminoma:**
Seminomas are the most common germ cell tumors arising in the male gonad, whether they arise in a pure state or mixed with other morphologic types\(^{5, 6, 24-28}\). “Pure” seminoma account for 27%-30% of testicular GCT and another 15%-18% contain syncytiotrophoblasts. Approximately 1% to 2% are bilateral and bilaterality can occur synchronously or asynchronously. Seminomas reach a peak incidence between the 4th and 5th decade of life, which is approximately one decade later than non-seminomatous germ cell tumors. Microscopically, tumor cells are uniform and have round to vesicular nuclei with clear cytoplasm, prominent cytoplasmic membranes and a centrally-located, round nucleus with a prominent nucleolus. These cells are arranged in sheets or nests separated by thin fibrovascular bands which contain mature lymphoid cells.

Seminoma cells express Placental Alkaline Phosphatase (PLAP) and c-kit (CD-117) by immunohistochemistry\(^ {16, 17, 29-35}\) but not cytokeratins, CD-30 or inhibin. A minority of seminoma cells may express focal and weak, dot-like or linear immunoreactivity for cytokeratin; however, never diffuse and strong staining throughout the cytoplasm. Like IGCN, seminoma cells express POU5F1 (Oct 3/4) in a nuclear distribution\(^ {16, 20, 36, 37}\).

Tumors thought to be seminoma but exhibiting atypical histology should trigger consideration of a differential diagnosis of seminoma which includes a) seminoma with “early carcinomatous differentiation”, b) solid variants of embryonal carcinoma or yolk sac tumor, c) lymphoma, d) sex-cord gonadal stromal tumor, and e) metastatic disease, including poorly differentiated carcinoma and melanoma. Other causes of atypical histology in seminoma include poor fixation and faulty processing in the pathology laboratory. “Early carcinomatous differentiation” refers to areas of transition from seminoma to embryonal carcinoma. This concept suggests that seminoma cells are not terminally differentiated but rather, under certain poorly understood circumstances, may differentiate into other germ cell tumor-types\(^ {28, 38, 39}\).

**Spermatocytic Seminoma:**
Spermatocytic seminomas are rare, comprising less than 2% of testicular neoplasms\(^ {28, 40, 41}\). The peak incidence is in the sixth decade of life; however, occurrence in younger patients as early as the third decade of life is reported. This tumor occurs only in the male gonad, may be unilateral or bilateral, and is not associated with cryptorchidism. Microscopically, tumor cells are arranged in solid sheets or nests of round cells. Occasionally the tumor cells may be arranged in nests or pseudoglandular arrangements within an edematous or mucoid stroma. Cytologically, it is possible to identify three distinct cell types; small, medium and large although cells of intermediate size predominate. Immunohistochemical stains for PLAP are negative, although occasional cells may be weakly immunoreactive. Cytokeratins are negative, although occasional cells may exhibit dot-like cytoplasmic staining. CD-30 is negative, while some investigators have reported immunoreactivity for CD-117 (c-kit)\(^ {20, 28, 42, 43}\).
**Embryonal Carcinoma:**

Embryonal carcinomas comprise up to 3% of pure GCT, although it is a common component of mixed germ cell tumors. It rarely presents as pathologic stage I disease and is not associated with elevation of HCG or alfafetoprotein (AFP). Microscopically, tumor cells are large, irregular, and epithelioid. They exhibit scanty cytoplasm, large pleomorphic nuclei with coarse chromatin, and multiple irregular nucleoli. Common findings include nuclear overlap, individual cell necrosis, and apoptotic bodies. The pattern of growth is quite variable: gland-like, papillary, syncitial, and solid areas are commonly encountered. The solid variant of EC may be confused with “atypical” forms of seminoma, although the latter does not exhibit the same degree of cytologic anaplasia as embryonal carcinoma. EC may also have overlapping morphologic features with yolk sac tumor (YST) but, once again, close attention to subtle cytomorphologic differences and immunohistochemistry will resolve the majority of cases. Most ECs are immunoreactive for PLAP, low molecular weight cytokeratins, CD-30 and POU5F (Oct 3/4). They do not express CD-117, AFP or HCG.

**Yolk Sac (Endodermal Sinus) Tumor:**

Yolk sac tumors (YSTs) are characterized by multiple patterns of growth that recapitulate the yolk sac, allantois, and extra embryonic mesenchyme. It has a bimodal age distribution; infants and young children and postpubertal males. In the latter group, it rarely presents in a pure form but is present in almost half of mixed germ cell tumors. In children it commonly presents in its pure form, usually within the first two years of life. These tumors are associated with serum elevation of AFP in the overwhelming majority of cases. Microscopically these tumors are quite variable due to the multiple subtypes which are usually intermixed. Tumor cells of YST are usually immunoreactive for AFP, and low molecular weight cytokeratins. PLAP staining is variable and may be absent. CD117 (c-kit) and CD-30 (focal, weak staining may be present) are usually negative as is Oct 3/4.

**Choriocarcinoma:**

Choriocarcinoma is composed of syncytiotrophoblastic, cytotrophoblastic, and other trophoblastic cells. It comprises less than 1% of testicular GCT in its pure form; however, may be encountered as a component of a mixed GCT in up to 10% of cases. In its pure form, these tumors occur in the second and third decades of life, are commonly associated with very high levels of serum HCG, and exhibit metastatic disease at the time of initial presentation. Small foci of choriocarcinoma within a mixed germ cell tumor do not alter the prognosis. Most cases will have syncytiotrophoblasts and cytotrophoblasts with occasional intermediate trophoblastic cells present. Syncytiotrophoblasts are immunoreactive with HCG as well as inhibin, epithelial membrane antigen, and low molecular weight cytokeratins. PLAP may be positive but staining is variable.

**Teratoma:**

The term teratoma refers to neoplasms composed of tissues that have differentiated along any of the three somatic pathways: ectoderm, mesoderm, or endoderm. Teratomas may be composed of mature tissues, embryonal-type tissues, or a mixture of both. Historically they were subclassified as immature and mature forms based on their degree of differentiation. The World Health Organization now recommends that these morphologies be considered as a single entity.
based on their overlapping genetic features. The immunohistochemical profile will be consistent with the degree of differentiation. The epithelial component will express cytokeratins while the mesenchymal components will express vimentin. Few epithelial cells lining cysts may express oncofetal proteins such as CEA, AFP and the levels of these proteins in cyst fluid may markedly elevated. However, these findings rarely are associated with significant serum elevation of the corresponding oncofetal protein.

Recently novel markers have been developed that may be very useful in classifying GCT. As with Oct 3/4, markers such as Sox 2, GDF-3, Lefty, NANOG (and others), have the added value of helping us better understand the stem cell origin of this disease and how “somatic” differentiation translates into predictable patterns of antigenic expression. Whether these makers will take hold in clinical practice remains to be seen.
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Recent Developments in the Diagnosis of Adrenal Neoplasia

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Recent Developments in the Diagnosis of Adrenal Neoplasia

- Distinguishing adrenal cortical adenomas from carcinoma relies on the Weiss criteria which are a very useful guideline.
- Criteria for malignancy of adrenal cortical carcinomas in pediatric patients are different from those in adults.
- In addition to typical adrenal cortical tumors, oncocytic and myxoid variants may also be present.
- There are several new systems to evaluate malignancy in pheochromocytomas. However, the presence of metastatic disease remains the most reliable predictor of malignancy.
Adrenal Cortical Tumors

One of the major problems in diagnostic endocrine pathology is distinguishing adrenal cortical adenomas from carcinomas. This is especially true with borderline lesions compared to small adenomas less than 20 grams or very large tumors more than 500 grams that are usually obvious carcinomas.

Adrenal cortical adenomas may be functioning or non-functioning (1). Many small adenomas less than 3 cm in diameter are discovered accidentally during working up for various other conditions. These tumors are referred to as “incidentalomas”.

Adrenal cortical carcinoma may be functioning malignancies in some cases, while in other cases, they are non-functioning (1-6). The gross appearance of adrenal cortical carcinoma can be very helpful in making the diagnosis. Most carcinomas in adults are greater than 100 grams, while adenomas generally weigh 50 grams or less. Adrenal cortical tumors weighing less than 50 grams have, on occasion, metastasized, but this is extremely uncommon. In pediatric patients, however, adrenal cortical adenomas may weigh up to 500 grams. In addition to tumor weight, the presence of necrosis usually indicates an adrenal cortical carcinoma unless the necrosis resulted from a traumatic insult such as FNA. A variegated appearance with nodularity and intersecting fibrous bands should also suggest the possibility of a carcinoma.

Various studies have outlined specific criteria used to diagnose adrenal cortical carcinomas (7-10). The criteria of Weiss (8) are most useful because of their reliance on histologic features. These include high nuclear grade, mitotic rate greater than 5 per 50 high power fields, atypical mitotic figures, eosinophilic tumor cells (≥ 75%), diffuse architecture (> 33% of tumor), necrosis, venous invasion, sinusoidal invasion and capsular invasion (8).
Three of more of the nine criteria are indicative of an adrenal cortical carcinoma, while two or less would be more in keeping with an adenoma. Other systems such as that of van Slooten et al, (11) attach numeric values to the various criteria, and an index of eight or higher was consistent with a carcinoma.

One of the older systems is that of Hough et al (12) who used histologic criteria and clinical parameters in their assessment of adrenal cortical neoplasms. A numeric value of 2.91 was indicative of malignancy, while a value of 0.17 or less was consistent with a benign lesion. The disadvantage of this system is the reliance on clinical parameters as well as histologic features, and some of these clinical parameters may not be available when examining the specimen.

From a practical perspective, the most useful criteria to separate adenomas from carcinomas include tumor size, presence of necrosis, mitotic activity including atypical mitoses, invasive growth, and high nuclear grade. Capsular invasion may be difficult to recognize because the expanding capsule may be a pre-existing adrenal capsule. Invasion of adjacent soft tissue, kidney or liver, are definitive signs of malignancy. Special studies may be useful in confirming the nature of the malignant tissue (12-19). Ultrastructural studies may show the distinct features of ACC tissues including abundant smooth endoplasmic reticulum and mitochondria with prominent tubular or vesicular cristae. Immunohistochemical studies that are most useful in adrenal cortical carcinoma include melan A, inhibin alpha and calretinin. Stains for cytokeratin are usually weakly positive, while vimentin is strongly positive. Synaptophysin is usually weakly positive in these tumors. Chromogranin is consistently negative. A marker for adrenal cortical cells, Ad4BP/SP-1, is relatively restricted in its distribution (20,21) and may be
useful in the diagnosis of adrenal cortical tissues. This protein is a transcription factor that is needed for embryonic development of adrenal cortical cells.

Adrenal cortical neoplasm in pediatric patients is more difficult to diagnose and to separate adenomas from carcinomas (22). In a study of 83 adrenal cortical neoplasms, only 31% of histologically malignant tumors behaved in a clinically malignant fashion. Features of malignancy included tumor weight >400 g, tumor size >10.5 cm, vena cava invasion, confluent necrosis, periaxial soft tissue invasion greater than 15 mitoses per hpf and atypical mitoses.

There are several variants of adrenal cortical tumors. The most common include the oncocytic tumors (23,24), and the myxoid variant (25). Criteria for the diagnosis of oncocytic carcinomas were recently proposed by Weiss’ group (24). Major criteria for oncocytic tumors included high mitotic rate, atypical mitoses and vena cava invasion. Minor criteria included large size and weight, necrosis, capsular invasion and sinusoidal invasion. One major criteria indicated malignancy, while 1 to 4 minor criteria indicated borderline tumors. Absence of all major and minor criteria indicated benign oncocytic tumors. The myxoid variant of adrenal cortical tumors looks different morphologically, but the criteria for malignancy should be similar to conventional adrenal cortical tumors.

The differential diagnosis of adrenal cortical carcinoma includes renal cell carcinoma, hepatocellular carcinoma, pheochromocytomas, and metastatic carcinomas and melanomas. Insulin-like growth factor-2 has been useful in the classification of adrenal cortical tumors (26).

Recent studies of these various markers and techniques separate adrenal cortical carcinomas from adenomas. Some of these include DNA flow cytometric analysis and nucleolar organizer regions have not been very effective. However, some markers of proliferation have
been shown to be useful in the distinction. Ki-67 labeling index with MIB-1 antibody are somewhat promising (19,20).

**Molecular Studies**

Molecular studies have characterized various genes that are differentially expressed in normal and benign compared to malignant adrenal cortical tumors (27). The phenotypes of Ki-67 negative, p53 negative, mdm-2 positive, cyclin D1 negative, Bcl-2 negative, p21 negative and p27 positive cells was found in 83% of normal adrenal tissues, but only in 3% of malignant tumors (27). Giordano and colleagues performed microarray analysis of adrenal cortical tumors and reported up-regulation of IGF2 in 10% of adrenal cortical carcinomas (90.9%). Proliferation in related genes such as TOP2A and Ki-67 were also up-regulated in carcinomas (28,29). Velazquez-Fernandez, et al (30) performed expression profiling of 7 patients with adrenal cortical carcinomas and 13 with adenomas and reported up-regulation of ubiquitin-related genes (USP4 and UFD1L) and insulin-like growth factor related genes (IGF2, IGF2R, IGF\(\beta\)P3 and IGF\(\beta\)P6). A cytokine gene (CXCL10) and cadherin 2 gene (CDH2) were down-regulated in carcinomas compared to adenomas (30).

**Pheochromocytomas**

Pheochromocytomas (“dusky colored tumor”) are chromaffin derived tumors that develop in the adrenal gland (31). When the tumor is immersed in chromaffin salts or other weak oxidizing agents, it develops the dusky color. Most tumors are sporadic and benign. The reported incidence is about 0.4 to 9.5 per 10^6 people. The tumors occur most frequently in the 4th
and 5th decades. Familial tumors develop at a younger age. Most familial tumors are bilateral, while sporadic tumors are unilateral.

Patients usually present with throbbing headaches, sweating, palpitations, chest and abdominal pains. The “spells” may last from 10 to 60 minutes and may be triggered by positional changes.

Malignant pheochromocytomas comprise only about 10% of all pheochromocytomas. Signs and symptoms are similar to those in patients with benign disease; however, catecholamine production and the degree of hypertension may be more marked with metastatic disease.

Imaging studies cannot distinguish benign from malignant pheochromocytomas unless there is metastatic disease. CT studies and 123I-meta-iodobenzyl-guanidine (MIBG) is very useful in imaging especially for locally recurrent or metastatic disease.

Malignant pheochromocytomas tend to be larger tumors than benign ones. They may be more nodular, lobular and show areas of necrosis. They may infiltrate periadrenal adipose tissue. Metastatic disease is the most reliable evidence of malignancy (32-45).

Histological features suggesting malignancy include: capsular invasion, vascular invasion, extension into periadrenal adipose tissue, diffuse growth, necrosis, tumor cell spindling, increased cellularity, marked nuclear pleomorphism. Macronucleoli, increased mitoses including atypical mitoses, absence or decreased hyaline globules.

Sustentacular cells were reported to be decreased or absent in malignant pheochromocytomas (31). MIB 1 labeling index may be helpful in separating benign and malignant pheochromocytomas. However, in some larger studies using 2.5% or 3.0% of cut off points had a sensitivity of only 50% in identifying proven malignant tumors.
The Pheochromocytomas of the Adrenal Gland Scaled Score (PASS) was developed by Thompson to distinguish benign from malignant pheochromocytomas (46). It uses features such as growth pattern, necrosis, cellularity, cellular monotony, tumor cell spindling, mitotic count, atypical mitosis, invasion, nuclear pleomorphism and hyperchromasia to try to separate tumors. A PASS sure of $\geq 4$ is associated with a higher probability for malignancy. Other studies of malignant pheochromocytomas have been recently reported (47-50). The proposed system of Kimura et al used an assigned score that adds up to a maximum of 10 (50) Ki-67 immunoreactivity along with catecholamine and phenotype are included. With a score of 7 to 10, 100% of patients were found to have malignant tumors (50).

Paragangliomas are tumors arising from the paraganglia which are distributed along the parasympathetic nerves in the head, neck and mediastinum and along the sympathetic chain such as the cervical, intrathoracic, supraneural inferior paraaortic and urinary bladder. Although morphologic distinction between pheochromocytomas and paragangliomas is difficult, molecular differences between tumors arising in the adrenal medulla and other sites are more evident. With respect to malignancy, the general impression is that tumors arising in the organs of Zuckerkandl close to the bifurcation of the aorta have the highest incidence of malignancy.

Histopathologic features of pheochromocytomas and paragangliomas include chief cells with basophilic to amphophilic cells with abundant cytoplasm and large vesicular nuclei. A prominent Zellballen or cell nesting pattern may be present. Some tumors may have scant cytoplasm. Cellular and nuclear pleomorphism may be prominent. Cytoplasmic hyaline globules are frequently present. Melanin-like pigment may be present. Mitotic figures are uncommon. Tumors may have scattered ganglion cells which does not indicate a composite tumor.
Immunohistochemical studies show that the chief cells of the tumors are positive for chromogranin and synaptophysin. The sustentacular cells are positive for S100 acidic protein. The absence of positivity for EMA helps to distinguish pheochromocytomas from renal cell carcinomas. Adrenal cortical tumors are positive for melan A, inhibin alpha and calretinin and weakly positive for keratin; but negative for chromogranin A. Pheochromocytomas and paragangliomas are positive for chromogranin A and negative for melan A and keratins.

**Molecular Genetics**

Pheochromocytomas associated with a variety of inherited conditions including MEN2, Von Hippel-Lindau (VHL) disease, neurofibromatous type 1 (NF1), heredity paraganglioma (PGL) syndromes and Sturge-Weber disease (Table 1). The genetics of these disorders are summarized in a recent report(51).

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Chromosome Location</th>
<th>Pheo</th>
<th>PGL</th>
<th>Genetics</th>
</tr>
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<tbody>
<tr>
<td>MEN 2A &amp; 2B</td>
<td>10q11.2</td>
<td>+</td>
<td></td>
<td>RET mutation</td>
</tr>
<tr>
<td>Von Hippel Lindau</td>
<td>3p26-29</td>
<td>+</td>
<td></td>
<td>VHL mutation</td>
</tr>
<tr>
<td>Neurofibromatosis I</td>
<td>17q11.2</td>
<td>+</td>
<td></td>
<td>NF1 mutation</td>
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<tr>
<td>Familial PGL1</td>
<td>11q23</td>
<td></td>
<td>+</td>
<td>SDHD mutation</td>
</tr>
<tr>
<td>Familial PGL3</td>
<td>1q2</td>
<td></td>
<td>+</td>
<td>SDHC mutation</td>
</tr>
<tr>
<td>Familial PGL4</td>
<td>1p36</td>
<td>+</td>
<td>+</td>
<td>SDHB mutation</td>
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</table>
Multiple Endocrine Neoplasia Type 2 (MEN2):
Approximately 50% of patients with MEN2 develop pheochromocytomas. De novo germ line mutations occur in about 6% of MEN2A and familial medullary thyroid carcinoma (FMTC) cases and in around 50% of MEN2B cases.

Von Hippel-Lindau (VHL):
The frequency of pheochromocytomas in VHL patients ranges from 10-30% and is restricted to the type 2 kindreds. Type 1 VHL patients with renal cell carcinomas, hemangioblastomas and retinal angiomas do not usually develop pheochromocytomas.

Neurofibromatosis Type 1 (NF):
Pheochromocytomas are associated with 1-4% of NF1 patients.

Hereditary Paraganglioma Syndromes (PGL):
The frequency of SDHB and SDHD mutations in pheochromocytomas is about 3 to 5%. These mutations are much more common in paragangliomas or extra-adrenal pheochromocytomas. SDHB mutations have been associated with malignant paragangliomas. The various PGL1 mutations are shown in Tables 1 and 2.
Table 2 Features of paragangliomas/pheochromocytomas with SDH mutations.

<table>
<thead>
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<th>Mutations</th>
<th>PGL</th>
<th>Comment</th>
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</thead>
<tbody>
<tr>
<td>SDHD</td>
<td>PGL1</td>
<td>Multifocal, extra-adrenal disease &lt; age 35. If mutation will develop paragangliomas or pheochromocytomas.</td>
</tr>
<tr>
<td>SDHB</td>
<td>PGL4</td>
<td>More common in sympathetic paragangliomas associated with malignancy.</td>
</tr>
<tr>
<td>SDHC</td>
<td>PGL3</td>
<td>More common in head and neck paragangliomas. Usually benign. Rare in adrenal.</td>
</tr>
</tbody>
</table>
References


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Recent Developments in the Pathology of Renal Cell Neoplasia

John N. Eble, Indiana University School of Medicine, Indianapolis, IN

Mucinous Tubular and Spindle Cell Carcinoma

Histologically distinctive renal neoplasms composed of cuboidal and spindle cells with mucinous extracellular matrix have been described in reports of single cases and in small series since 1998. Authors have offered a variety of names for these tumors, often referring to their resemblance to tubules of the lower nephron or loop of Henle and to the remarkably low nuclear grade for a carcinoma with areas of spindle cell morphology.

Clinical Features

Clinical information is presently available from 22 cases described individually and 7 cases for which the data were combined. All have been adults (age range 20 to 82 years, mean 55 yr, median 58 yr). There is a 3:1 predominance of women over men (22 women and 7 men). Three of the 11 patients reported by Hes et al. also had nephrolithiasis. Approximately 50% of the tumors have been large (stage pT2); in one patient the tumor invaded perirenal fat and in 2 others there was metastasis to a lymph node. All of the tumors have been solitary except in one case in which three tumors arose and were resected over a span of 22 years. The patients have all been treated solely with surgery. With limited periods of clinical follow up, no recurrence has been reported.

Gross Pathology

Mucinous tubular and spindle cell carcinomas have ranged in size from 22 mm to 130 mm in diameter. The cut surfaces are solid and tan brown to pinkish. Some authors have variously described the cut surfaces as gray to white, tan, and yellow to pinkish. Foci of hemorrhage or necrosis have been found in a few of the tumors. One small tumor appeared to be centered on the renal medulla. All but one of the tumors has been well-circumscribed and contained within the renal capsule.

Microscopic Pathology

Mucinous tubular and spindle cell carcinomas have a distinctive histologic appearance, consisting of cuboidal cells arranged in long cords and tubules and making abrupt transitions to spindle cell morphology. These epithelial structures are arrayed against a background of lightly basophilic mucinous or myxoid material. The nuclei usually are spherical or oval, have a few small chromatin clumps and small nucleoli Mitotic figures are uncommon. The spindle cell component may form sheets. The mucinous background material may focally dominate and the epithelial elements form small cords in lakes of mucinous material. The mucinous background material has little affinity for mucicarmine but reacts strongly with alcian blue. Plasma cells, mast cells, and clusters of foamy histiocytes are sometimes present. The degree of morphologic
variability which occurs in mucinous tubular and spindle cell carcinoma is not clear. One of the tumors reported by Hes et al contained areas of typical clear cell renal cell carcinoma.

Immunohistochemical Profile

The immunohistochemical results obtained in different series have been variable. Positive reactions with antibodies to vimentin and epithelial membrane antigen have been the most frequent findings. Reaction with the cytokeratin cocktail AE1/3 was positive in 10 of 14 tumors in two studies. The lectin *Ulex europaeus*, which binds to collecting duct epithelium, has consistently failed to bind to the cells of mucinous tubular and spindle cell carcinoma.

The immunohistochemical case for lower nephron differentiation is mixed and inconclusive. The positive reactions with antibodies to high molecular weight cytokeratins reported by some authors appear to favor this and the negative reactions for CD15 (which marks proximal tubules) also support the idea. Rakozy et al. found that the lectins soybean agglutinin and peanut agglutinin (which bind to the epithelium of the distal tubule and collecting duct) bound to mucinous tubular and spindle cell carcinoma but antibody to THP (a marker of the ascending loop of Henle and distal convoluted tubules) and *Ulex europaeus* failed to bind, weighing against the idea of collecting duct origin.

Tubulocystic Renal Cell Carcinoma

Clinical Features

In the published series, men have predominated (21:3). All patients have been adults (age range 30 years to 97 years, median 58 years). All but one (an autopsy case) have been treated with total or partial nephrectomy. Follow up data are limited but only one patient has been reported to have had metastasis to lymph nodes. This patient is alive without evidence of recurrent carcinoma 90 months after nephrectomy. In the Yang series, 5 of 13 patients also had papillary renal cell carcinoma or adenoma in the same kidney.

Gross Pathology

The tumors have ranged from 2 mm to 85 mm in diameter. Twenty-one of 24 patients have had pT1 tumors. In 3 patients from the Yang series, tubulocystic carcinoma was multifocal. Tubulocystic carcinoma has a distinctive gross appearance: well-circumscribed with an off-white cut spongy surface which shows innumerable cysts filled with clear fluid. The cyst lining is smooth and the cysts are fairly uniform in size, compared to the highly variable sizes of the cysts of clear cell renal cell carcinoma.

Microscopic Pathology
Tubulocystic carcinoma is seen microscopically to be composed of cysts of variable size ranging down to ones of the diameter of a cross section of a renal tubule. The cysts are lined by a single layer of carcinoma cells with eosinophilic cytoplasm. The contours of these cells varies from cuboidal to hobnail or flattened. The nuclei are spherical and nucleoli are usually prominent in many of the nuclei. Necrosis and mitotic figures are rare. The septa between the cysts are thin and composed of fibrous tissue.

Immunohistochemical Profile

In the series of Azoulay and Yang, all tubulocystic carcinomas reacted strongly with antibody to α-methyl-CoA racemase (AMACR). Azoulay et al. found 11 of 11 tumors to react with antibody to CD10. Yang et al. found 7 of 8 to react focally or weakly with antibody to cytokeratin 7.

Acquired Cystic Disease-associated Renal Cell Carcinoma

Long-term renal dialysis has long been known to predispose to the development of acquired cystic disease and renal cell neoplasms; 3 to 7% of patients with acquired cystic disease develop renal cell carcinoma, indicating a risk 2 orders of magnitude greater than that of the general population. Many of these appear to be typical clear cell, papillary, and chromophobe renal cell carcinomas. Recently, a morphologically distinctive carcinoma has been discovered in patients with acquired cystic disease. Some appear to be unique to end-stage renal disease and acquired cystic disease.

Clinical Features

Males predominate in a ratio of 2:1. The patients have had end-stage renal disease of varied etiology and have been treated with dialysis for a median duration of 8 years. In the study by Tickoo et al 1 patient died of widespread metastases and 2 other patients had lymph node metastases at the time of nephrectomy. The metastases showed the morphology of acquired cystic disease-associated carcinoma. Sarcomatoid change was present in both the primary and the metastases of of the patient who died of disease. Sarcomatoid change was present in a second acquired cystic disease-associated carcinoma who was alive without disease at 29 months.

Gross Pathology

The kidneys are atrophic and have numerous cysts, typical of acquired cystic disease. The carcinomas are usually well-circumscribed and the larger ones often have a thick fibrous pseudocapsule which often contains foci of calcification. More than half
the tumors appear to arise in a cyst. The larger tumors also often have foci of hemorrhage and necrosis.

**Microscopic Pathology**

The architecture is complex with acinar, compact sheets, microscopic and macroscopic cysts in various combinations. Papillary structure ranging from very focal to more than 50% of the tumor, are present in roughly half the tumors. A nearly ubiquitous (present in > 94% of carcinoma) and diagnostically helpful feature is a pattern of sharply defined lumens giving a cribriform appearance. Oxalate crystals are readily visible in routine sections in about 80% of the tumors. The carcinoma cells are large and have eosinophilic cytoplasm. The nuclei often have prominent nucleoli.

**Immunohistochemical Profile**

Acquired cystic disease-associated carcinomas gave consistently positive reactions with antibodies to AMACR and vinculin and mostly gave negative reactions with antibodies to cytokeratin 7 and parvalbumin in Tickoo’s series. Cossu-Rocca et al. found in three tumors positive reactions for CD10 and cytokeratin AE1/3 and negative reactions with antibodies to cytokeratin 7 and epithelial membrane antigen.

**References**

**Mucinous Tubular and Spindle Cell Carcinoma**


Tubulocystic Renal Cell Carcinoma


Acquired Cystic Disease-associated Renal Cell Carcinoma


Molecular Alterations in Prostate Cancer as Diagnostic, Prognostic and Therapeutic Targets

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Introduction

Prostatic adenocarcinoma is extremely common in Western cultures, representing the second leading cause of cancer death in American men. The recent application of increasingly sophisticated molecular approaches to the study of prostate cancer, in this “post-genomic” era, have resulted in a rapid increase in the identification of somatic genome alterations and the role of heritable factors in this disease. These findings are leading to a new understanding of the pathogenesis of prostate cancer and to the generation of new targets for diagnosis, prognosis, and prediction of therapeutic response. Although we are still in the very early phase of clinical development, some of the molecular alterations identified in prostate cancer are being translated into clinical practice.

The purpose of this presentation is to update the practicing surgical pathologist, and residents-in-training in pathology, regarding recent findings in the molecular pathobiology of prostate cancer. This presentation will highlight some of the somatic molecular alterations associated with prostate cancer development and progression, with a focus on newer discoveries. In addition, recent studies in which new molecular diagnostic approaches have been applied in the clinic will be discussed. Finally, the presentation will conclude with some predicted future directions.

Epidemiology of Prostate Cancer

The major risk factors for the development of prostate cancer are advanced age, race (African Americans and African Caribbean’s have the world’s highest rates), inherited susceptibility and environmental factors such as diet. In terms of diet, vitamin E, lycopene (or other carotenoids found in tomato based products) and selenium may exert a protective effect, while diets rich in fat and red meat, and well-done meats may exert a promotional effect. Since each of the dietary factors that appear to protect against prostate cancer are potent antioxidants, it is widely held that oxidative stress (which can directly damage DNA) may contribute to prostate carcinogenesis. Potential sources of oxidant stress are endogenous metabolism, inflammation, and dietary factors. Circulating levels of insulin like growth factor 1 (IGF-1), which can be influenced by diet or genetics, have been implicated in the development of aggressive prostate cancer.
Many, albeit not all, prostate adenocarcinomas are believed to be derived from high grade prostatic intraepithelial neoplasia (PIN)\(^6\). Over the last several years, our group has been working on a model whereby focal atrophy lesions, which are extremely common in the prostate and occur as a number of morphological variants, result from cellular injury imparted by dietary and inflammatory insults\(^7\). These atrophy lesions show morphological transitions to high grade PIN lesions, and at times directly to “microcarcinoma”\(^8\) lesions. They also show clear evidence of a stress response, and some also contain molecular alterations that are generally found at much higher frequencies in high grade PIN and carcinoma lesions. Thus, some atrophy lesions, which are often found in association with chronic inflammation, may be “risk factor lesions” for the development of PIN and adenocarcinoma of the prostate. One of the potential important aspects of this research is that since inflammation and diet are known to play a prominent role in the development of cancer in many other organ systems\(^9,10\), they may become future targets for the deployment of novel prevention strategies for prostate cancer.

Somatic Molecular Alterations in Prostate Cancer

Prostate cancer cells, like other cancer cells, usually contain a large number of somatic genome alterations\(^11-15\) that contribute to the cancer phenotype. Some of the somatic alterations are genetic (changes in DNA sequence), such as point mutations, deletions, amplifications, and translocations. Other changes are epigenetic\(^1\), including modifications in deoxycytidine methylation patterns and chromatin structure. A major challenge for researchers is to decipher which changes are causal in the disease process and which occur as bystanders unrelated to disease pathogenesis.

Except for telomere shortening\(^16,17\) (a genetic change), somatic hypermethylation of deoxycytidine residues within CpG dinucleotides in the upstream regulatory regions of a number of genes occurs earlier and more consistently in prostate cancer than genetic changes do. Several of the genes silenced by epigenetic alterations have been identified, providing new potentially useful molecular biomarkers of prostate cancer and insights into prostate cancer etiology, and some of these will be discussed briefly below.

Genes Silenced by Hypermethylation in Prostate Cancer

**GSTP1**

*GSTP1* encodes the \(\pi\)-class Glutathione S-transferase (GST-\(\pi\)). GSTs are an enzyme family that can detoxify reactive chemical species by catalyzing their conjugation to reduced glutathione. Thus, *GSTP1* likely serves as a “caretaker” gene, defending prostate cells against genomic damage mediated by carcinogens or various oxidants. Loss of *GSTP1* function may render prostatic cells sensitive to carcinogenesis driven by inflammation and diet.

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\(1\) The term epigenetic refers to changes in a cell’s phenotype that are stably inherited through cell division but have not resulted from a change in DNA sequence. Epigenetic mechanisms include methylation of deoxycytidine residues within CpG dinucleotides, histone modifications such as methylation and acetylation, RNA interference and others.
Since the first study of CpG island hypermethylation within the \textit{GSTP1} promoter region\textsuperscript{18}, a large number of studies have verified this finding\textsuperscript{19} which occurs in over 90% of prostate cancers\textsuperscript{19-21}.

**Other Genes Methylated in Prostate Cancer**

A number of other genes have also been found to be hypermethylated in prostate cancer\textsuperscript{21-25}. Using quantitative real-time methylation specific PCR (Real Time-MSP), Yegansubramanian et al. assessed the extent of hypermethylation in 16 different genes in prostate cancer and found strikingly common hypermethylation in the CpG islands associated with \textit{GSTP1}, \textit{APC}, \textit{RASSF1a}, \textit{PTGS2} and \textit{MDR1}, but virtually no methylation in normal prostate tissues\textsuperscript{22}.

While hypermethylation of specific genes is likely to be useful diagnostically and perhaps prognostically (see below), whether these methylated genes (other than \textit{GSTP1}) are etiologically involved in prostatic carcinogenesis has not been studied.

**Somatic Genetic Alterations and Prostate Cancer**

Like other cancer types, prostate cancers often contain genetic changes at the chromosomal or sub-chromosomal level\textsuperscript{11-15, 26-29}. The most common chromosomal abnormalities are losses at 8p, 10q, 13q, and 16q and gains at 7p, 7q, 8q, and Xq.

**Telomere Shortening**

Telomeres are composed of repeat DNA sequences bound to specific binding proteins at the termini of chromosomes. Telomeres serve to protect against loss of chromosome sequences and illegitimate recombination between chromosome arms or at DNA double strand breaks. Telomerase is a multi-component enzyme that acts to extend telomere sequences in order to maintain chromosomal length despite loss of telomeric sequences due to the “end replication problem”. Telomeres become critically shortened during the development of most cancers, to the point where chromosomal instability ensues\textsuperscript{30}. Mice carrying disrupted genes encoding telomerase subunits show increased numbers of cancers, especially when crossed to mice with deleted \textit{p53} genes\textsuperscript{31}. In the human prostate, somatic telomere shortening occurs in the luminal cells of the vast majority of cases of high grade PIN and carcinomas\textsuperscript{16, 17}. At the same time, prostate cancers\textsuperscript{32} and some PIN lesions\textsuperscript{33} paradoxically show telomerase activity, whereas normal prostate tissue and BPH do not. Thus, telomere shortening may be a nearly universal feature of early prostate cancer and may promote chromosomal instability leading to disease progression. While the telomere FISH assay is generally used to demonstrate that telomeres are short in tissue sections, recently Meeker et al., have developed a chromogenic in situ hybridization approach that may prove useful in applications of prostate biopsies or other specimens in the clinic\textsuperscript{34}.

**Selected Tumor Suppressor Genes and Loss of Heterozygosity (LOH)**

Deletions of genomic sequences from sites on chromosome 8 occur frequently in prostate cancer\textsuperscript{35}. Loss of 8p appears to be an early event since high grade PIN may show LOH at this location\textsuperscript{35}, albeit the fraction of high grade PIN lesions with this change may be less than previously thought\textsuperscript{36}. Several
genes located on chromosome 8p have been examined as candidate tumor suppressors, with one of the most promising being *NKK3.1*.

The product of the *NKK3.1* gene, which is a prostate restricted homeobox protein that is involved in the regulation of prostate development, is expressed in normal prostate epithelium and is often decreased in PIN lesions and in prostate tumor cells. Further, mice lacking either one or both *Nkx3.1* alleles develop abnormal prostate ductal branching, prostatic hyperplasia and lesions similar to human PIN. *NKK3.1*, however, may not be the only target for deletion in this region, since chromosome 8p is also deleted frequently in other tumors, such as those of the colon/rectum, and these other tissues do not express *NKK3.1*.

In a very recent study Chang et al., used high-resolution Affymetrix SNP arrays to define detailed deletion patterns at chromosome 8p and reported that two small deleted regions at 8p21.3 and 8p23.1 were commonly found, and, that these same regions showed evidence for linkage to hereditary prostate cancer patients. These relatively small “consensus” regions will likely facilitate more effective searches for prostate cancer genes at 8p, perhaps by large scale DNA sequencing analyses in this region.

The *PTEN* gene on 10q23 is mutated in up to 1/3 of hormone refractory prostate cancers and homozygous deletions and mutations have been identified in a subset of primary prostate cancers. Loss of PTEN protein in primary prostate cancer, as determined by immunohistochemistry, correlates with high Gleason score and advanced stage. PTEN is a dual protein and lipid phosphatase that is responsible for dephosphorylation and inactivation of phosphatidylinositol 3,4,5-trisphosphate (PIP3), a second messenger that is produced after activation of PIP3 kinase in response to ligation of several growth factor receptors, including IGF-1. PIP3 activates the protein kinase AKT. AKT signaling results in inhibition of apoptosis in response to a variety of signals and to increased cell proliferation.

Another potential role for AKT related to prostate cancer is the finding that AKT can phosphorylate p27 protein, resulting in cytoplasmic retention of p27 and lack of p27 mediated cell cycle arrest. Levels of p27, encoded by the *CDKN1b* gene, are often downregulated within the nucleus of prostate cancer and high grade PIN cells. Inactivation of p27 cannot, however, be the only function of the PTEN pathway during prostate carcinogenesis; in the mouse, *Pten* can cooperate with either *Nkx3.1* or *Cdk1b* (encoding p27Kip1) in increasing the frequency and extent of high grade PIN lesions and perhaps early cancers. Since this pathway is commonly altered in prostate cancer, inhibition of signaling through PI3K and AKT is a promising therapeutic strategy in this disease.

Other sites of loss/deletion in prostate cancer mainly occur in the late stages of cancer progression. Genetic inactivation of the classic tumor suppressor genes *p53, RB1, p16*, are seen rarely in primary cancers, but occur at higher frequencies in metastatic and/or hormone refractory lesions, suggesting that these genes may be involved in prostate cancer progression.
Selected Gene Targets in Regions of Chromosomal Gain

High-level amplification of the ERBB2 gene (often referred to as HER2 or HER2/NEU) does not occur in prostate cancer to any great extent. However, amplification of regions on chromosome 8q correlate with aggressiveness of tumors. One candidate for amplification on 8q is the C-MYC oncogene (see more on C-MYC below). Another gene on chromosome 8q that is often amplified in prostate cancer is PSCA, encoding prostate stem cell antigen, which is also accompanied by demonstrable corresponding protein overexpression. PSCA is a cell surface marker, and humanized antibodies or fragments thereof are currently being investigated in clinical trials in patients with metastatic prostate cancer. Other genes on chromosome 8q have also been implicated recently as potential targets of amplification, including the Elongin C gene and the EIF3S3 gene. Other regions of gain include the AR gene itself (located on Xq12), where amplification occurs almost exclusively in the hormone refractory state.

Selected Oncogenes/Growth Promoting Genes in Prostate Cancer

Androgen receptor

The prostate requires androgenic hormones and an intact androgen receptor (AR) for normal growth and development. In the normal prostate, AR is expressed highly in the luminal epithelial cells where it is present largely within nuclei. Much lower levels of AR are expressed in prostatic basal epithelial cells, and, many prostatic stromal cells also contain nuclear AR. High grade PIN luminal cells and the vast majority of prostatic adenocarcinoma cells express AR at relatively high levels. Metastatic prostate cancer is almost always treated with androgen deprivation, anti-androgens, or a combination of the two. However, despite such treatment, androgen-independent prostate cancer cells eventually emerge. Despite their apparent androgen independence, however, in most hormone refractory prostate cancers androgen-receptor (AR) expression and AR signaling remain intact, and AR is critical for androgen-refractory prostate tumor cell proliferation. In fact, AR expression itself is often increased in hormone refractory prostate cancer.

Somatic alterations of AR have been reported for many prostate cancers, especially for androgen-independent prostate cancers, and these mutations are often “activating” mutations. AR mutations can also result in altered ligand specificity in which even anti-androgens can act as agonists. In addition, AR gene amplification, accompanied by high-level expression of AR mRNA and protein, may promote the growth of androgen-independent prostate cancer cells by increasing the sensitivity of the cells to low androgen levels.

In addition to somatic AR gene changes, androgen-independent prostate cancer cells with wild-type AR may activate AR signaling even in the absence of androgens, through post-translational modifications of the AR and/or AR coactivators in response to other growth factor-signaling pathways.
While there is abundant data indicating AR can adapt to function in the setting of very low androgen levels, recent studies have suggested that prostate cancer cells may manufacture androgens themselves\(^61,63\). Thus, AR signaling may be intact as a result of relatively high local androgen levels in the tumor microenvironment, despite castrate levels of androgens in circulation.

**Oncogene Addiction and Lineage Survival in Prostate Cancer**

Another emerging concept that may be related to AR in prostate cancer is the notion of oncogene addiction—i.e., the dependence of a cancer cell on one overactive gene or pathway for the cell's survival and growth\(^64,65\). Evidence for this concept stems from a number of mouse models, and, in human cancers such as: (i) chronic myeloid leukemia or gastrointestinal stromal tumors treated with imatinib (Gleevec\(^\text{®}\)); (ii) lung cancers containing epidermal growth factor receptor mutations treated with gefitinib (Iressa\(^\text{®}\)) and erlotinib (Tarceva\(^\text{®}\)); and (iii) ERBB2 amplified breast cancers treated with Herceptin (Trastuzumab).

While AR is not a classical oncogene, evidence certainly indicates that prostate cancer cells that express AR are indeed “addicted” to AR signaling. Along these lines is the concept of “lineage dependency”\(^66\) which suggests that “master regulator” genes, such as AR in the prostate, when deregulated in certain contexts can become an oncogene. This implies that as a function of their prior lineage development in the prostate, prostate cancer cells may be “hardwired” to use AR signaling for growth and survival in a manner that generally cannot be later bypassed. Another way to look at this is that all of the genome changes that drive prostate cancer growth, prevent apoptosis, induce angiogenesis, etc, have occurred in a setting of a cell that has been epigenetically “programmed” by androgen receptor signaling. Therefore, without AR signaling, these oncogenic changes are not tolerated by the cells. If correct, this hypothesis implies that the cell of origin (tumor stem cell or tumor progenitor cells) in prostate cancer is an AR positive cell and not likely an AR negative “stem cell”. Clearly, the androgen receptor is still a major therapeutic target in prostate cancer and new ways to inhibit its function are continually under development\(^67\).

**C-MYC**

The C-MYC protein is a nuclear transcription factor that regulates a number of cellular processes including cell cycle progression, metabolism, ribosome biogenesis, protein synthesis and mitochondrial function\(^68\). C-MYC is over-expressed in a large variety of tumor types, often associated with somatic genetic alterations such as translocations and gene amplification\(^69\). In prostate cancer, there is evidence that C-MYC is involved in disease progression since a region encompassing the MYC locus (8q24) is somatically amplified at low levels in a subset of patients\(^69,69-71\), and the presence of amplification in this region correlates with both high histological grade and a worse prognosis\(^49-51,70\). Whether there is amplification of MYC in high grade PIN is controversial since MYC amplification has been reported in up to 50% of HGPIN lesions\(^71\), but more recent experiments revealed a lack of MYC amplification in such lesions\(^36\).

It has been long known that a subset of prostate cancer lesions express elevated levels of MYC mRNA, often in parallel with increased expression of PIM-1, a gene known to cooperate with MYC in
other malignancies\textsuperscript{15}, and that is often overexpressed in prostate cancer\textsuperscript{72,73}. Further, targeted overexpression of the human \textit{MYC} gene in the mouse prostate results in PIN\textsuperscript{74,75}, early invasive prostate adenocarcinoma\textsuperscript{75} and rare metastatic adenocarcinoma\textsuperscript{75}. These findings provide definitive evidence that C-MYC overexpression can drive neoplastic transformation in the mouse prostate, and support a model whereby C-MYC may play a role in initiation of human prostate cancer.

Nevertheless, due to a lack of suitable antibodies that can be readily applied for cellular and subcellular localization in archival tissues, the phase of prostate cancer development in which C-MYC protein is expressed in humans has been unclear.

In very recent work from our laboratory\textsuperscript{76} (Poster # 117, Monday Morning), using genetically-defined control experiments, we found strong nuclear staining for C-MYC in the majority of human clinical prostate cancer and high grade PIN samples, and much less staining in benign tissue. Although the levels were somewhat lower than Gleason score 6 tumors, high grade lesions (7-9) and hormone naïve metastatic lesions also showed marked overexpression in most cases for C-MYC protein. These new results suggest that the view of this key oncogenic transcription factor in prostate carcinogenesis should be revised to include activation in the majority of cases at a very early time point in the neoplastic process.

\section*{Gene Fusions in Prostate Cancer}

Arul Chinnaiyan and colleagues have made a series of discoveries recently that have begun to transform prostate cancer research and how we think of this disease. Tomlins et al.\textsuperscript{77} used a novel analysis method, cancer outlier profile analysis (COPA), to discover genes with marked overexpression in a subset of prostate cancer cases. Starting with clinical prostate cancer specimens, COPA identified outlier profiles for v-ets erythroblastosis virus E26 oncogene like (\textit{ERG}) and ets variant gene 1 (\textit{ETV1}), two ETS family transcription factors. Strikingly, the group showed the presence of aberrant mRNA transcripts containing sequences from the 5 prime region of the androgen regulated gene, \textit{TMPRSS2}, that were fused to 3 prime exons from the \textit{ERG} or \textit{ETV1} genes. By using multiple approaches, including FISH looking for rearrangements of \textit{TMPRSS2:ERG} loci, the Chinnaiyan group and others have concluded that rearrangements in \textit{ERG} or other ETS family members occur in the majority of all PCA cases\textsuperscript{15,78}(current estimates vary between \textasciitilde40-70\%). The same group has since shown that other ETS family members and other androgen regulated genes, or even housekeeping genes\textsuperscript{79-81}, may also be involved in gene fusions in prostate cancer. A number of other groups have verified these overall findings\textsuperscript{29,82-86}, and at least one study indicates that \textit{TMPRSS2:ERG} gene fusions can be identified in a subset of high grade PIN lesions\textsuperscript{86}. These gene fusions, therefore, have become a prime target for the development of novel diagnostic, prognostic, predictive and therapeutic approaches in prostate cancer. Of special interest for surgical pathologists, Mark Rubin and colleagues have recently shown that some of the morphological features of prostate cancer correlate with the presence of the \textit{TMPRSS2:ERG} rearrangement\textsuperscript{87}. 

Currently the prognostic significance of these gene fusions is uncertain. While some studies suggest that the presence of fusion transcripts, or altered types or numbers of copies of the fusion, portend a worse prognosis, other data using ERG mRNA overexpression do not support this\textsuperscript{91}.

Given the excitement regarding these gene fusions, it is certain that a great deal of new information will be generated over the next few years, and the challenge will be to discover ways to implement these findings into clinical practice (see below).

**Approaches to Using Molecular Alterations as Early Diagnostic Markers**

There are several major challenges in prostate cancer care in which molecular markers are expected to become highly useful in the clinic. These include: (i) early detection, including the determination of who may or may not require an initial prostate biopsy, and, who may require rebiopsy after an initial negative biopsy; (ii) monitoring of low risk prostate cancer patients who do not elect immediate treatment and are undergoing “active surveillance”; (iii) prediction of recurrence after initial treatment to stratify patients into risk groups for emerging adjuvant therapies; (iv) detection of recurrence after treatment; and (v) as surrogate markers for assessing the efficacy of treatments in advanced disease. Most efforts to apply non-prostate specific antigen (PSA) related molecular markers have so far have been in the area of early detection, so this will be the focus of this part of this presentation.

**Applications to Urine or Post-Prostate Massage Urine**

Most prostate cancers are now discovered by transrectal prostate needle biopsy in men who are found to have elevated serum PSA levels (usually ≥4 ng/mL, but often as low as 2.5 ng/mL). Several groups have been attempting to examine urine specimens for molecular alterations associated with prostate cancer in order to improve upon the ability of serum PSA to predict the presence of prostate cancer. This is an important area of research since elevated serum PSA levels can be reasonably sensitive for prediction of prostate cancer in a needle biopsy (~80%), but at a cost of poor specificity (~25-40%). In addition, the negative predictive value of a low serum PSA is also not very robust. As reported in the Prostate Cancer Prevention Trial (PCPT) when men without an elevated PSA or abnormal digital rectal exam underwent random prostate needle biopsy, the prevalence of prostate cancer was 6.6 percent among men with a PSA level of up to 0.5 ng/mL, 10.1 percent among those with values of 0.6 to 1.0 ng/mL, 17.0 percent among those with values of 1.1 to 2.0 ng/mL, 23.9 percent among those with values of 2.1 to 3.0 ng/mL, and 26.9 percent among those with values of 3.1 to 4.0 ng/mL\textsuperscript{92}.

It should be also be noted that a major limitation of all studies designed to improve upon PSA testing is that the gold standard (prostate biopsy) is still approximately only 80-85% sensitive. This reflects the reality that prostate needle biopsies are typically carried out in a blinded fashion and may miss cancer in up to 15-20% of patients. Therefore the test performance parameter measurements...
(sensitivity, specificity, and positive and negative predictive values) of tests designed to predict the presence of prostate cancer by testing for molecular markers in bodily fluids or serum are necessarily only relevant to the prediction of a positive prostate needle biopsy, and not specifically to the presence or absence of cancer.

Nevertheless, one major application has been in attempts to determine which patients with elevated serum PSA actually need a biopsy. Given the false negative rate of prostate needle biopsies, another way to state this is to ask: can the molecular marker help to determine which patients have a high risk for the finding of cancer on a prostate needle biopsy? The goal of such a test is to prevent “unnecessary biopsies”.

**DNA Markers**

As mentioned above, methylation of deoxycytidine residues within CpG within upstream regulatory regions of a number of genes occurs in a very high percentage of prostate cancers and is not found to any significant extent in normal prostate tissues in most studies. Therefore a number of groups have attempted to improve on the ability of serum PSA to predict a positive biopsy using methylation of *GSTP1* and other genes in the urine (as well as other bodily fluids), and a number of these studies have been reviewed.

One of the first studies using DNA based tests was by Goessl et al., who used MSP to detect *GSTP1* hypermethylation in bodily fluids. Whereas *GSTP1* promoter hypermethylation was not detectable in prostate tissue and bodily fluids from patients with BPH, these authors reported that methylation was detected in 94% of tumors (16 of 17), 72% of plasma or serum samples (23 of 32), 50% of ejaculate (4 of 8) and 36% of urine (4 of 11) from patients with prostate cancer. Additionally, MSP identified circulating tumor cells in 30% (10 of 33) of prostate cancer patients.

Goessl et al., also used MSP to detect *GSTP1* hypermethylation in urine sediments from patients after prostate massage and found an overall sensitivity of 73% and a specificity of 98%, although some of these patients had advanced prostate cancer.

Rouprêt et al., recently used a 10 gene MSP approach in which urine samples were obtained from 95 consecutive radical prostatectomy patients and from 38 age-matched males (controls) with no history of genitourinary malignancy, negative prostate biopsies, and with or without BPH. Radical prostatectomy patients underwent prostate massage and the first urine stream was then collected. The authors reported a sensitivity of 86% and a specificity of 89% for the 10 gene panel.

Results of another very recent study have been reported by Woodson et al., in which 100 men were referred for prostate needle biopsy due to increased PSA, abnormal DRE or related symptoms. In this study methylation of *GSTP1* in post-massage urine had a 75% sensitivity and a 98% specificity for cancer. It is not clear why this latter study showed such high performance, but the results imply that perhaps the use of *GSTP1* alone will be valuable as a molecular marker in prostate cancer in urine specimens.
Until now all of the studies that have detected methylation of genomic DNA in bodily fluids (and serum—see below) for the detection of prostate cancer have relied on some form of methylation-specific PCR. One major limitation of this approach is that the DNA must be first treated with sodium bisulfite, which is a very harsh treatment and results in damage to what are often already low quantities of DNA. As a result, Yeungasubramanian et al., have developed a first generation assay, referred to as COMPARE-MS (combination methylated-DNA precipitation and methylation sensitive restriction enzymes) that does not rely on bisulfite treatment of DNA and this approach promises to increase the sensitivity of these types of assays. The approach, which results in very high sensitivity and specificity, is based upon capture of methylated DNA using a methyl domain specific binding protein, followed by PCR for the gene of interest. The assay was found to be highly sensitive and specific. It is anticipated that this type of approach may indeed improve upon existing approaches for both specific genes and for the ability to multiplex a number of genes.

RNA Markers

Another series of studies that have been employing molecular tests using urine to help predict prostate cancer has used RNA-based approaches. Most studies have employed the RNA product of a gene originally named $DD3$ and now commonly referred to as $PCA3$. $PCA3$ is expressed nearly exclusively in the prostate, with much higher levels in prostate cancer, and it encodes an RNA product of unknown function that does not contain a protein coding open reading frame.

Hessels et al., studied post-prostate massage urine samples in men with elevated serum PSA (>3) and found, using a quantitative RT-PCR based approach to detect $PCA3$, that the sensitivity for prediction of a positive biopsy was 67%, with a negative predictive value of 90%.

These results ultimately led to the development of a clinical test by Gen-Probe Inc. referred to as the APTIMA® PCA3 assay. This test uses whole urine specimens and includes target capture, transcription-mediated amplification, and a hybridization protection assay. In the initial study using this method, post prostate massage urines were obtained from 3 groups: men scheduled for prostate biopsy (n = 70), healthy men (<45 years of age with no known prostate cancer risk factors; n = 52), and men who had undergone radical prostatectomy (n = 21). ROC curve analysis showed an area under the curve of 0.746 and a sensitivity of 69% and specificity of 79%. In this study, the negative predictive value was 90%.

Fradet et al., used what was referred to as the uPM3 test in post-massage urines in a multicenter study in Canada enrolling 517 patients (of which 86% were informative) with elevated serum PSA and reported essentially similar findings and added the fact that the test added value at all serum PSA levels. The overall accuracy was 81% compared with 43% and 47% for total PSA at a cutoff of 2.5 and 4.0 ng/mL, respectively.

Schalken and colleagues used a second generation of this test based on RT-PCR, in a large multicenter Dutch trial consisting of 534 men, and, this study too showed very promising results.
A different commercial version of this urine test referred to as PCA3Plus® is offered currently by Bostwick Laboratories®. It should be noted that none of these urine based tests have been FDA approved for the diagnosis of prostate cancer, but that they are being offered so far as an aid to decision making regarding who should undergo a prostate needle biopsy or a repeat biopsy.

Laxman et al. showed the ability to detect \textit{TMPRSS2:ERG} gene fusion transcripts in urine from prostate cancer patients\textsuperscript{108}, and, more recently added the detection of such transcripts to a multiplex RNA based assay that included \textit{PCA3}\textsuperscript{109}. In this study, which consisted of patients with known prostate cancer (\(n = 86\) positive needle biopsies and 52 radical prostatectomy patients) and patients with negative needle biopsies (\(n = 96\)), the authors reported the area under the ROC curve was 0.758 for the multiplexed assay versus 0.66 for PCA3 alone.

Another application of this type of test is to apply it to men that have already undergone a biopsy that was negative for cancer, but there is still clinical suspicion of prostate cancer. Marks et al., used the APTIMA®PCA3 test on men with a prior negative prostate biopsy but with a persistently elevated serum PSA of >2.5(ng/mL)\textsuperscript{110}. Receiver operating characteristic curve analysis yielded an area under the curve of 0.68 for the PCA3 score and 0.52 for PSA. The assay sensitivity was 58\% and specificity 72\%, with an odds ratio of 3.6.

An important potential limitation of most of the studies mentioned is that the authors generally have not compared the predictive ability of their molecular markers to that of measurements of the relative levels of free to total serum PSA, which have been shown in a number of studies to improve the predictive ability of PSA in terms of classifying patients into those that will have a positive biopsy\textsuperscript{111, 112}.

### Assessment of Molecular Markers in Tissues Remaining in Paraffin Blocks from Negative Prostate Biopsies

A number of groups have been attempting to determine whether assessment of methylation of \textit{GSTP1}, and other genes, in DNA isolated from tissue remaining in paraffin blocks in samples that were considered benign by pathologists\textsuperscript{113}. As with all of the other approaches that do not examine the cells directly under the microscope, it is not clear whether the assay is detecting cancer cells, high grade PIN cells, or rare methylated atrophic cells\textsuperscript{114} that were not originally sampled by microtome sections, or, whether it is detecting a “field effect” whereby normal appearing prostate tissues harbor molecular alterations that are predictive of cancer on subsequent biopsies. It should be noted that this approach, while promising, results in destruction of the remaining tissues in the biopsies, precluding their use for subsequent studies such as prediction of response to therapies in the future.

### Other Bodily Fluids and Serum

A number of studies have been performed that have also attempted to examine methylated DNA isolated from ejaculate fluid or from serum. Most of these studies have been used for prediction of prognosis and will be described below.
How Will The Adoption of These Types of Tests Affect The Practice Of Surgical Pathology?

At present it is not clear how a future potential widespread deployment of these types of molecular assays will affect the number of men undergoing prostate needle biopsy. For example, what if these tests become the gold standard for screening populations, replacing PSA? On one hand the number of overall men that are biopsied may go down as the tests are more specific than PSA. However, it is not clear just how sensitive these tests will be in the population at large. At present it would appear that these tests are not likely to identify more small “insignificant” cancers than currently employed screening approaches do based on PSA (which is estimated to be approximately 20% of all cases in the United States). However, if these tests improve further in sensitivity, it is possible that they may indeed begin to pick up more cancers since it is estimated that more than 50% of men who are 50-75 years of age have microscopic prostate cancer lesions (as determined by autopsy studies). In the short term it would appear that the use of these tests will, at the very least, increase the fraction of needle biopsies sets that contain cancer, since they are specifically designed to predict positive biopsies.

Molecular Alterations as Prognostic Markers

A number of biomarkers have been studied (both in needle biopsy specimens and in radical prostatectomy specimens) in order to enhance the prediction of outcome in prostate cancer. Older studies have shown that ploidy status, immunohistochemical staining for markers such as Ki67, bcl-2, p53, and p27

\textsuperscript{kip}, FISH analysis for chromosome 8q24 amplification, and nuclear morphometry measurements can add value to the prediction of outcome in prostate cancer patients. However, as of yet none are currently employed routinely in clinical practice. There may be a number of reasons for this. First, there is often a lack of inter-study reproducibility. This can either result from lack of standardization of measurements, variable study designs often employing small numbers of select patients, or simply a lack of a market (i.e., the lack of adjuvant therapy for high risk prostate cancer patients results in a lack of need to stratify patients beyond the available clinic-pathological parameters) for the development of such tests.

In order to address whether a number of different markers can add value to the prediction of biochemical recurrence in patients undergoing prostate needle biopsy, a group of investigators from 11 National Cancer Institute funded prostate SPORE (Specialized Projects of Research Excellence Awards) programs have begun to accrue moderate to high risk patients with prostate cancer to a prospective study (n = 700). This study will hopefully determine whether selected markers applied to prostate needle biopsies may be clinically useful to predict outcome beyond typical clinic-pathological measurements such as Gleason score, serum PSA, number of cores positive, etc.

In terms of current use, the most promising marker right now is actually based on serum PSA itself. That is, serum PSA velocity or the PSA doubling time appears to be a very powerful predictor of disease progression, both after biochemical recurrence following primary treatment, and even when measured within 18 months prior to the initial diagnosis\textsuperscript{115,116}. In fact, the latter may indeed become a useful biomarker to better stratify patients with positive biopsies into risk groups such that more men
may safely elect active surveillance as opposed to immediate treatment. Certainly a number of other molecular markers are under development that may be applied to serum for similar uses.

Role of Methylation Markers in Predicting Prognosis

A number of studies have begun to examine the ability of quantitative changes in DNA methylation, as measured either in prostate cancer tissues or in serum, to augment prediction of outcome for prostate cancer patients. Although large trials are needed before clinical implementation, several of these studies suggest that methylation markers may add value to existing models in predicting outcome in prostate cancer.

Molecular Alterations as Predictive Markers

Molecular markers that are expected to be widely used in the future are the so called “predictive markers” that help to stratify patients into groups that will likely respond to specific targeted therapeutic interventions. While this type of approach has been commonly used in breast cancer and more recently in lung cancer, it is also expected to become widely employed in prostate cancer care as well. The most promising pathway in which this is likely to be employed in the near future is the PTEN/PI3K pathway as a number of clinical trials using inhibitors of this pathway are in development or underway in prostate cancer. Thus, the measurement of PTEN protein levels and downstream targets of AKT in prostate needle biopsies may have value in the future if these trials show promise.

What is on the Horizon for Prostate Cancer?

One the most promising areas that has been accelerated greatly by the human genome project is the development of methods to perform “Genome Wide Association Studies” (GWAS) in which disease risk is related to germ line polymorphic variants. These studies, which currently employ roughly 500,000 genetic makers referred to as SNPs (single nucleotide polymorphisms), are beginning to revolutionize medicine. Within the last year alone, a number of different research groups have identified regions on chromosome 8q24 (not within the C-MYC gene), and other novel loci, that harbor SNP variants that affect the risk of prostate cancer. What is striking about many of these new studies is that the reproducibility across different patient populations appears to be remarkably high. At least one of these recent studies has resulted in the formation of a company that is developing tests that will attempt to provide patients with information regarding their genetic risk of prostate cancer. While this may not be ready for “prime time”, it does appear that these types of tests will become highly popular in the future. In the short term, these findings point to new areas of research to attempt to decipher how these variants, which are often in non-protein coding areas and even in areas devoid of any known genes, influence prostate cancer risk.

Another area of research that has exploded onto the scene of cancer research is the study of microRNAs. These non-protein coding small RNAs that are encoded by specific genes, are double stranded and range in size from 20-25 nt in their mature form, were discovered in worms in the 1990s but have already revolutionized basic science research and are poised to change medicine.
soon. In fact, altered expression patterns of microRNAs are found commonly in cancer. It is expected that these molecules will become useful as diagnostic targets, therapeutic targets, and as the therapeutic agents themselves.

**Concluding Remarks**

Although we are still at the very beginning phase of understanding prostate cancer at the molecular level, there is great promise for employing recent findings to some of the ongoing vexing problems in clinical practice. Nevertheless, translation of this new knowledge into new clinical tests that can ultimately better serve patients, at all phases of the disease process, is a daunting task. To accomplish this, there is a great need to conduct sufficiently designed and powered studies. These studies also need to be performed in conjunction with acquisition of well annotated biospecimens and detailed clinical follow-up information. Such studies require an integrated approach that includes investigators from multiple disciplines such as bench scientists, epidemiologists, biostatisticians/bioinformatics specialists, pathologists, urologists, medical oncologists and radiologists. What would appear to be “good news” for pathologists is that the widespread implementation of such tests will require highly experienced diagnostic pathologists/laboratory medicine experts for both proper selection and interpretation of such tests.
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