

RENAL EPITHELIAL TUMORS 2009: THE ROLE OF ELECTRON MICROSCOPY IN UNDERSTANDING PATHOGENESIS, DIAGNOSIS, AND CLASSIFICATION.

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Epithelial renal cell tumors comprise a heterogeneous group of neoplasms with diverse biologic potential, different response to therapies, and variable clinical outcomes. In recent years the discovery of new molecular and cytogenetic markers has resulted in the recognition of new tumor entities or subtypes of renal epithelial tumors. Electron microscopy has contributed to the morphological characterization of these new important categories of neoplasms which have become specific clinicopathologic entities that must be recognized by surgical pathologists in order to manage patients appropriately. Surgical pathologists must intelligently select the ancillary diagnostic techniques that will provide the information needed to address the differential diagnosis under consideration in these tumors.

For many years renal cell carcinomas were considered a group of relatively “boring” tumors from the surgical pathologists’ point of view. Only clear and granular cell renal cell carcinomas were recognized and in a significant number of cases both cell types coexisted. This was a very simplistic approach to the categorization of what now we recognize as a rather complex group of neoplasms. More recent classifications have recognized several additional categories of renal carcinomas. Molecular biology and cytogenetics information is being actively incorporated into our understanding of renal neoplasia providing new insights into recognition of new types of neoplasms with specific clinical correlates. Despite all these advances, the clear / granular cell carcinoma category still encompasses the majority (approximately 70%) of the renal epithelial tumors. The role that ancillary diagnostic techniques play in the evaluation of renal neoplasms in the daily practice of pathology remains unclear to most practicing surgical pathologists. Recommendations addressing the proper utilization of adjunct diagnostic techniques are still not well defined. As a result, the temptation is to make the best possible diagnosis using light microscopy and immunohistochemistry. This is rather fast and delivers a diagnosis with “some apparent degree of sophistication”. Because the immunohistochemical profiles of the great majority of renal epithelial tumors are not specific, this approach opens the way for arriving to diagnoses that are not correct which may ultimately adversely affect patients’ management. This approach may also create fertile grounds for legal litigation.

The role of electron microscopy will be highlighted and it should be clearly understood that it still plays an important role in the diagnostic algorithm of these epithelial renal neoplasms in diagnostic laboratories where electron microscopy remains as a viable diagnostic technique.

Electron microscopy is also very helpful in situations where separating primary epithelial renal tumors from metastases is required. Sending difficult cases to regional electron microscopy laboratories should be seriously considered when this technique is not available locally. The use of more than one ancillary diagnostic technique to resolve a difficult case is encouraged.

DIAGNOSIS AND CLASSIFICATION OF RENAL EPITHELIAL TUMORS- ROLE OF ELECTRON MICROSCOPY

Renal cell carcinomas are encountered in approximately 10 individuals per 100 000 population. Approximately 15 000 new cases are diagnosed each year and 5 to 7000 deaths occur per year as a result of renal carcinomas. There has not been much improvement in the treatment or management of these patients in the last 10 years. However, our understanding of the biology, morphology, genetics and clinical behavior (prognosis) of these neoplasms has increased markedly.

Approximately 30% of patients with epithelial renal malignancies present with metastatic disease at the time of diagnosis. The clinical and pathological manifestations of renal cell carcinomas can be quite diverse and these tumors are often referred to as the “great mimickers”. This is an area where electron microscopy can be very helpful i.e. determining primary vs. metastatic renal epithelial tumors. There is not an immunocytochemical marker that indicates renal cell origin for a given epithelial neoplasm.

In the AFIP fascicle published in 1975 only clear and granular renal cell carcinomas were recognized. Although oncocytoma was first reported in 1942, the publication by Klein and Valensi in 1976 popularized the concept. The same year papillary renal cell carcinoma became a recognized entity. Fleming and Lewi published the first series of collecting duct carcinomas in 1977. Electron microscopy was an integral part of these seminal publications.

The classification of renal epithelial tumors in 1981 expanded the accepted categories to include conventional (clear cell), papillary, granular cell and sarcomatoid carcinomas but did not include oncocytomas or collecting duct carcinomas. The next attempt at classification of renal epithelial neoplasms was in 1994, and at that time oncocytomas were added. Thoenes and associates in 1985 described and characterized chromophobe renal cell carcinomas and began a revolutionary attempt to redefine the classification of renal epithelial neoplasms, based not only on morphology but also on cytogenetics and molecular information. Merely 14 years ago the first series of metanephric adenomas was published. Electron microscopy played a pivotal role in the recognition of each one of the epithelial tumor categories. In fact their specific definition virtually always carried with it an ultrastructural seal authenticating their separation from other types well recognized at the time a new entity was coined.

Thus the Heidelberg classification of 1997 amalgamated all the information available at the time to generate the latest classification of epithelial renal tumors. However, significant new information has been obtained since and a new classification scheme awaits us all in the near future.

Studies dealing with differentiation of epithelial renal tumors have arrived to the conclusions that conventional and papillary renal cell carcinomas differentiate along proximal tubular cell lines while oncocytomas and chromophobe carcinomas express distal tubular markers and appear to be closely associated in their genesis to intercalated cells of distal tubules. Finally, medullary and collecting duct carcinomas reflect collecting duct cell differentiating features. Immunohistochemical markers include CD10 for those neoplasms with proximal tubule differentiation while kidney specific cadherin labels tumors differentiating

along distal nephron lines. There is not a good marker for those neoplasms with collecting duct lineage. The combination of several immunohistochemical stains may be used to address the differential diagnosis of these epithelial renal tumors but significant overlap is often present among the reactions observed in different types of neoplasms. Combinations of CK7 and 20 staining have also been used to try to speciate renal epithelial tumor with similar overlap occurring among different groups. Numerous manuscripts have been written (some listed in the references) trying to use immunohistochemical markers to classify renal epithelial tumors and, invariably, the results reported are less than ideal for a diagnostic setting where specificity is crucial. Interestingly, electron microscopy has provided rather specific morphologic markers for neoplasms with proximal and distal nephron differentiation, and even medullary carcinomas, and ultrastructural evaluation is in most instances better than immunohistochemistry when the objective is typing these tumors.

The role that cytogenetics/molecular and gene profiling techniques play in the current assessment of renal epithelial tumors is still not completely defined. This topic will be discussed by one of the speakers in the session and placed in proper perspective.

PRACTICAL APPLICATIONS

The various subtypes of renal epithelial neoplasms exhibit rather specific ultrastructural features that permit their accurate diagnosis. The virtual replacement of the cytoplasm of neoplastic cells by mitochondria remains the criterion for a diagnosis of oncocytoma. The finding of coalescent cytoplasmic vesicles in the cytoplasm of an epithelial renal neoplasm is indicative of chromophobe differentiation. The diagnosis of the eosinophilic variant of chromophobe carcinoma can be a source of difficulty to surgical pathologists; this diagnosis can be made with precision ultrastructurally. The presence of certain specific cytoplasmic granules in the cytoplasm of neoplastic cells in a renal neoplasm can define a given renal tumor (i.e. juxtaglomerular cell tumor). These neoplasms can easily be confused with papillary carcinomas. The features and complexity of the microvillous surface in the neoplastic cells can provide indications of either proximal or distal nephron differentiation in a given renal epithelial neoplasm. Diagnosis of a sarcomatoid component in a renal cell carcinoma is generally much easier and accurate using electron microscopy than immunohistochemistry.

The area where electron microscopy findings still remain very broad is in the large category of conventional renal cell carcinomas (granular/clear cell carcinomas). Specific subtypes of these tumors (i.e. Xp11.2- TFEB translocation carcinomas) have been recognized and these share similar ultrastructural features, suggesting that this large category of tumors will be segregated into different clinically significant subtypes in the future. It is likely that in this process ultrastructural evaluation will play a significant role, as it has before in the speciation of other renal epithelial tumors.

As non-surgical therapies (i.e. tumor ablation techniques) and surgical nephron sparing techniques become more sophisticated, it becomes even crucial to properly speciate renal epithelial tumors with small amounts of tissue. Furthermore, the advent of new therapies targeting well defined molecular markers further emphasizes the need for a clinically relevant classification and accurate speciation of these renal epithelial tumors. These newer approaches to treatment are tailored to specific tumor entities. Prior to institution of therapy, confirmation of the diagnosis is needed and a small sample of the tumor is usually obtained, either by fine needle aspiration or surgical biopsy. The pattern of tumor growth that is so important for surgical pathologists to recognize the different varieties of renal epithelial neoplasms may not

be readily recognizable in these less than ideal samples. Furthermore, the material available for evaluation may not be sufficient for performing extended panels of immunohistochemical stains. These specimens become prime candidates for ultrastructural diagnosis. In most instances, a small sample is more than enough for thoroughly characterizing an epithelial renal tumor. The value of electron microscopy is enhanced by the fact that the great majority of renal epithelial neoplasms lack specific reliable immuno histochemical profiles to diagnose subtypes.

It is disappointing that a large percentage of renal tumors remain unclassified. In some series unclassifiable renal neoplasms may account for as many as 5-7% of all tumors. This fact by itself clearly indicates that there is plenty of work ahead to better understand and segregate these tumors into clinically meaningful groups. Incorporating molecular, cytogenetics and morphologic criteria into a unified data bank to be used for diagnostic purposes resulting in sound clinico-pathologic correlates will be the challenge of the future. Electron microscopy will play an important role in the characterization of these currently “unclassifiable” neoplasms.

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Fine Needle Aspiration of Renal Masses

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Fine needle aspiration (FNA) biopsy of the kidney is a valuable diagnostic tool primarily reserved for radiographically indeterminate lesions, work-up for metastatic disease to the kidney, and for the diagnosis of inoperable patients. The use of ancillary techniques such as electron microscopy, molecular genetics, and immunohistochemistry enhances the accuracy of the FNA diagnosis.

Renal FNA diagnosis, though accurate, suffers from a high false negative rate. The sensitivity of FNA biopsy of the kidney for malignancy averages about 85% and the specificity averages about 98%. False positive diagnoses are exceedingly rare and are related to sampling adjacent organs, such as the adrenal or the liver. Chronic inflammation, infarction, cysts, hematomas, angiomyolipomas, and other benign lesions may also result in false positive diagnoses.

Cystic Lesions

Most cystic lesions are benign and are either acquired or familial. Solitary cysts are usually acquired and sporadically occur in elderly men, whereas acquired multiple cysts occur in patients who have end stage kidney disease. Foci of renal cell carcinoma can develop in association with a small percentage of these cysts and the majority are either of the clear cell or papillary subtypes. Tumors can have cystic components (eg. cystic nephroma) and tumors can undergo cystic degeneration. Aspirates from benign cysts are usually clear and may contain macrophages. In approximately 20% of the cases, the samples are bloody raising suspicion for malignancy. The smears may exhibit pleomorphic spindle cells admixed with other epithelioid cells and contain vacuoles resembling fat. In such cases, differentiating cystic nephroma from renal cell carcinoma or angiomyolipoma can be increasingly difficult based on cytological grounds alone.

Other benign cystic lesions of the kidney include polycystic disease which are generally not aspirated. Aspirates from benign cysts are usually clear and may contain macrophages. In approximately 20% of the cases, the samples are bloody raising suspicion for malignancy. Other cystic lesions include polycystic kidney disease which are generally not aspirated.

Solid Lesions

In contrast to cystic lesions, the majority of solid kidney lesions are malignant. In childhood, Wilm's tumor is the most common renal malignancy and is usually not biopsied. Renal cell carcinoma (RCC), a solid tumor that may undergo cystic degeneration is the most common renal malignancy, accounting for more than 90% of adult cases. Renal tumors are generally solitary, although bilateral tumors are encountered in approximately 2% of patients. The most common type of renal cell carcinoma is the clear cell type (also referred to as conventional).

Clear Cell Carcinoma Subtype

Most common subtype of renal cell carcinoma and is associated with deletion of chromosome 3p, the site of the tumor suppressor gene involved in Von Hippel-Lindau disease. At the time of diagnosis, 25% of the patients have metastasis. Smears are very cellular comprised of large sheets of discohesive vacuolated cells with capillaries traversing through sheets of tumor cells. The tumor cells have abundant vacuolated cytoplasm with indistinct borders, a low N:C ratio and a centrally located round to slightly irregular nucleus with delicate and well-defined nuclear

membranes on both Papanicolaou and Diff Quik stains. The cytoplasm is generally translucent but may be also be granular and eosinophilic in some cases. The grading of the tumor is based on Fuhrman grading of the nuclei. Generally, low-grade tumors have bland nuclear features with delicate chromatin. Higher grade tumors are more likely to have prominent nucleoli, anisonucleosis and nuclear pleomorphism. The sample may be paucicellular if the lesion is sclerotic, or bloody when the lesion is highly vascular.

Differential diagnosis: normal tubular cells, macrophages, inadvertently aspirated hepatocytes, and adrenal tissue. The tumor cells are positive for glycogen and fat and negative for mucin.

IHC: positive for cytokeratin, vimentin, EMA and the RCC antigen. These are A103/Melan A and inhibin positive and clear cell RCCA is the opposite with Inhibin and A103/Melan A being negative. In addition, RCC antigen is positive in most RCCA and negative in adrenal neoplasms.

Ultrastructural evaluation becomes the arbiter when IHC is equivocal. By EM, clear cell RCCA has the classic combination of intimately admixed lipid and glycogen within the cytoplasm, a rather classic appearance. The adrenal and adrenocortical neoplasms will demonstrate abundant SER, in addition to mitochondria with tubulovesicular cristae and stacks of RER.

Papillary Renal Cell Carcinoma Subtype

Accounts for 7-15% of all renal cell carcinomas and is defined by the presence of true papillae in at least 50% of the tumor. Chromosomal abnormalities, such as trisomy of chromosomes 7, 16, and 17, or the loss of the Y chromosome are associated with this tumor. Papillary RCCs are frequently multiple and may be associated with cortical adenomas. Since papillary renal cell carcinoma is usually hypovascular on angiography and may have cystic degeneration, therefore, the tumor is frequently diagnosed by FNA. The tumor has an arborizing arrangement with a fibrovascular core that is better appreciated in cell block sections. The nuclei are bland, sometimes with a groove, and there may be foamy macrophages and psammoma bodies.

IHC: positive for low molecular weight cytokeratin and EMA, RCC antigen and CD10, while negative for CEA and mucin. Differential diagnosis: other papillary neoplasms, such as metastatic disease. The diagnostic pitfalls include differentiating this tumor from high-grade clear cell RCC.

Ultrastructural evaluation shows papillary features with proximal tubule differentiation such as tall microvilli lining tubular structures and abundant mitochondria.

Renal Cell Carcinoma with sarcomatoid features

On FNA, sarcomatoid features may be seen in association with other RCC types. Sarcomatoid areas are described in chromophobe (most commonly), clear cell, collecting duct or papillary RCC and this feature is associated with a bad prognosis. FNA of RCC with sarcomatoid features produce cellular aspirates composed primarily of high-grade spindle cells with anisonucleosis, marked nuclear membrane irregularities, and prominent nucleoli; occasional epithelioid cells are also seen. Differential diagnosis: high-grade soft tissue sarcoma and clear cell RCC if the epithelioid component predominates. IHC: positive for cytokeratin and EMA, focally at least, while negative in sarcomas. Vimentin is positive in both sarcomas and in RCC with sarcomatous features and therefore is of no utility. Specific immunomarkers useful for speciating sarcomas such as smooth muscle actin and muscle specific actin for smooth muscle tumors and CD34 for vascular neoplasms such as angiosarcoma and others. Pitfall: poor sampling, since the sarcomatoid spindle cell component may be focal.

EM: RCC with sarcomatous features may have focal epithelial elements such as junctions while sarcomas will have features that pertain to the type of sarcoma having either smooth muscle, striated muscle, fibroblastic differentiation or others.

Collecting Duct Carcinoma

Arising from the collecting duct (distal nephron), this is an aggressive tumor located in the medulla of the kidney, occurring in young individuals and occasionally associated with transitional cell carcinoma of the renal pelvis. Cytogenetic studies may reveal loss of one or more of chromosomes 1, 6, 14, 15, and 21. Medullary carcinoma (a variant of collecting duct carcinoma), occurs in young adults with sickle cell trait or disease and has a worse prognosis. On FNA, collecting duct carcinoma cells have a varied morphology, sometimes resembling a high-grade papillary renal cell carcinoma. The cells are arranged in cohesive groups with the cells having a high nuclear grade with irregular nuclear membranes and scant cytoplasm, which may be vacuolated, suggesting the possibility of metastatic disease. This tumor can have papillae, psammoma bodies and sarcomatoid features on the smears. Differential diagnosis: papillary RCC, high grade urothelial carcinoma and metastatic disease. IHC: positive for high molecular weight cytokeratin, 34BE12, this is in contrast to RCC. Vinculin has recently been proposed as a possible marker for tumors with collecting duct differentiation.

Ultrastructurally, these tumors show evidence of distal nephron differentiation with lumina lined by a poorly developed microvillus border.

Chromophobe Renal cell Carcinoma Subtype

Comprises 3-5% of all renal cell carcinomas. Chromosomal abnormalities: loss of chromosomes 1, 2, 6, 10, 13, 17, and 21. Chromophobe RCC can be confused morphologically with oncocytoma especially on cytologic grounds. The smears are cellular, with discohesive cells with well-defined cytoplasmic cell borders and a granular eosinophilic cytoplasm. The cells are large and may be pleomorphic with centrally located, round, regular, and sometimes hyperchromatic nuclei. The presence of a perinuclear halo is a clue to the diagnosis. Binucleation, intranuclear inclusions and flocculent cytoplasmic features are some distinguishing characteristics of this tumor. Pitfalls may arise if hepatic tissue or oncocytoma is aspirated. It may be impossible to distinguish oncocytoma from chromophobe carcinoma on cytologic grounds alone. Special stains may aid in differentiating these two tumors; Hale's colloidal iron is positive in chromophobe RCC while negative in other RCC tumor subtypes. IHC: chromophobe RCC is positive for cytokeratin and negative for vimentin. Ultrastructurally, chromophobe carcinoma has distinguishing characteristics, with numerous mitochondria and aggregates of coalescent round to elongated cytoplasmic vesicles.

Benign Lesions

Oncocytoma

Benign tumors and account for 5% of all renal tumors. Grossly, the tumors are well circumscribed, mahogany-brown on the cut surface, and reveal a central, stellate white scar in the center. Cytogenetic studies: translocation of chromosome 11 and loss of chromosomes 1 and Y. On FNA, uniform, discohesive cells with abundant granular cytoplasm and well-demarcated cell borders can be observed. Sometimes, hyaline globules are present within the cytoplasm. The cells have round to oval nuclei with regular contours, with or without inconspicuous nucleoli (low nuclear grade, mostly Fuhrman grade I). The low nuclear grade, the oncocytic nature of the cells and the pushing margin of the tumor grossly are characteristic features that distinguish this tumor from low-grade chromophobe renal cell carcinoma. Differential diagnosis: clear cell RCC and benign hepatocytes that may sometimes be aspirated inadvertently. Pitfalls include differentiating this tumor from other types of RCC. It is important to note that for a tumor with oncocytic cells to be classified as an oncocytoma, the tumor cells must have Fuhrman grade I

nuclei. IHC: negative for vimentin, usually positive for cytokeratin in contrast to RCC where the cells are positive for both cytokeratin and vimentin. Hale's colloidal iron stain on the cell block is negative in oncocytoma and strongly positive in chromophobe RCC which helps distinguishing oncocytoma from the eosinophilic variety of chromophobe. EM: easiest way to diagnose oncocytoma since the cells have abundant mitochondria explaining the eosinophilic (oncocytic) appearance of the tumor. Besides, oncocytoma lacks the round vesicles that can be seen in chromophobe carcinomas.

Angiomyolipoma

Triphasic benign tumor composed of smooth muscle, mature adipose tissue and variably sized blood vessels. It occurs sporadically in young to middle age women or as familial in young adults with tuberous sclerosis. The majority of the lesions are not biopsied because angiomyolipoma contains fat that can be visualized radiologically. On the contrary, when the tumor fat content is low, aspiration may be warranted. Aspiration poses particular difficulty in interpretation of the smears because of the presence of spindle cells. The presence of predominantly spindle cells may be misidentified as malignant sarcoma. In addition, the presence of epithelioid spindle cells with nuclear pleomorphism and prominent mitotic activity may make it more difficult. The presence of occasional eosinophilic crystals in the spindle cells of angiomyolipoma may aid in differentiating this tumor from others. Another helpful fact is that the spindle cells within the tumor are positive for HMB-45 immunostain.

EM: smooth muscle differentiation in a tumor with fat may be a helpful hint. But since this tumor has a characteristic radiologic appearance; it rarely goes for ultrastructural evaluation, except when epithelioid features are prominent and the differential diagnosis is renal cell carcinoma.

Metanephric Adenoma

Metanephric adenoma first described in 1995 is a benign tumor that occurs most commonly in women in the fifth decade. It may be associated with polycythemia and the tumor cells are invariably diploid cytogenetically. On FNA, the cells are uniform with round to oval nuclei, inconspicuous nucleoli, and scant cytoplasm. They are arranged in tubules and papillae lined by bland looking cells, forming "glomeruloid bodies". Psammoma bodies may be observed in the smears. Differential diagnosis: papillary RCC, metastatic lung carcinoma and metastatic papillary thyroid carcinoma. IHC: papillary renal cell carcinomas are positive for EMA while metanephric adenomas are negative. Other useful differentiating markers include TTF-1, CEA and thyroglobulin, which are all negative in metanephric adenoma while CD57 is usually expressed by these neoplasms. Ultrastructurally, the cells of metanephric adenoma have basal lamina and microvilli.

Metastatic Disease

Metastasis to the kidney is not uncommon and most of these will have history of a known primary diagnosis. The lung is a common primary tumor site for metastatic disease to the kidney. A diagnosis can be made by histological correlation with the previous biopsy together with the clinical history and the use of ancillary diagnostic techniques. IHC for TTF-1 is positive in many metastases from the lung. Other useful markers are prostatic specific antigen, (PSA) for prostate; leukocyte common antigen, (LCA) for lymphoid and other leukocytic processes, alpha fetoprotein for liver, RCC antigen for kidney and a combination of cytokeratins, CK7/CK20 to differentiate other organs. The ancillary technique of choice for solving the problem of metastatic disease with an unknown primary is ultrastructural evaluation. Ultrastructurally, there are features, by which tumors can be speciated and the primary site determined.

Other Tumors

Urothelial carcinoma(UC)

UC involves the kidney primarily in the renal pelvis and may be confused radiographically with a RCC large. It accounts for 5-10% of all renal tumors. High-grade tumors usually harbor an aneuploid population of cells. The cytologic appearance depends upon the grade of the tumor. On FNA, low-grade UC have cells typically arranged in sheets and papillae in a clean background. The tumor cells have large hyperchromatic nuclei and moderate amounts of opaque, granular cytoplasm. In high grade UC, the cells are dispersed as isolated cells and occasionally appear in small clusters with a high N:C ratio, scant cytoplasm and hyperchromatic nuclei which may be irregular and have indented nuclear membranes. "Cercariform cells" having long cytoplasmic tails if present in the smears, are very characteristic of high grade UC with squamous differentiation. Pitfall: high grade UC may be difficult to differentiate from a metastatic disease. Immunohistochemistry and molecular studies may be helpful whenever applicable to further characterize the tumor. Typically, UC is positive for keratins 34BE12 and CK20, CEA and mucin.

EM: cells lined with sparse microvilli, joined by junctions, the nature of which depends upon the degree of differentiation of the tumor and have large vesicular nuclei known as telolysosomes.

Lymphoma

Primary lymphoma of the kidney is a controversial and rare disease; however, a handful of cases have been reported in the literature and the majority are diffuse large B cell type. On-site rapid smear interpretation will help in triaging the specimen for flow cytometry and molecular studies. If necessary, an IHC panel may be ordered on the cell block specimen.

Ultrastructurally, lymphomas have very few characteristic features, the lack of which may be helpful especially differentiating them from other tumors that have similar cytologic features such as small cell carcinoma. No intercellular junctions, few organelles which are usually polarized within the cytoplasm of the neoplastic cells.

Conclusion

FNA continues to be the biopsy of choice in the evaluation of renal lesions. FNA biopsy is convenient, has less morbidity, is less expensive, and it is best suited for renal lesions since they are deep seated and difficult to biopsy otherwise. The diagnostic results are comparable to those of a surgical biopsy, especially when combined with ancillary techniques. On-site evaluation of the smear greatly assists in reducing insufficient diagnostic samples. In addition, FNA biopsy of the kidney has proved useful in evaluating incidental renal lesions found during imaging studies for problems unrelated to the kidneys. Overtime, FNA biopsy is a valuable tool, one that has allowed physicians the opportunity to make bold therapeutic decisions while avoiding unnecessary and expensive investigative procedures. The FNA biopsy is also excellent for obtaining material for IHC, molecular studies and EM. Because immunohistochemistry may show increased background, an intrinsic problem with FNA samples, EM can be very useful in addressing diagnostic challenges.

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The Current Role of Electron Microscopy in the Diagnosis of Pediatric Renal Tumors

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Key Words: Ultrastructure, neoplasia, renal, pediatric

Key Points:

- Nearly all of the pediatric renal tumors exhibit distinctive ultrastructural features, the demonstration of which can reliably enable their positive identification.
- Electron microscopy is, in this setting, a generally more useful ancillary diagnostic technique than either immunohistochemistry or molecular genetics.

Wilms' Tumor (Nephroblastoma)

Wilms' tumor is the most common of the pediatric renal tumors. Typical examples, which exhibit a triphasic mixture of blastemal, epithelial and stromal components, should present little diagnostic difficulty. Examples exhibiting only one of these components can, however, prove quite challenging. The blastemal predominant tumors can be confused with other small round blue cell tumors. A particular problem here results from the propensity of these tumors to display rosette formations similar to those associated with PNET, which happens to be one of the more common "non-renal tumors" occurring in the kidney. Monophasic epithelial tumors can be confused with renal cell carcinoma, metanephric adenofibroma or others. Pure stromal tumors can be mistaken for embryonal sarcoma of kidney, metanephric stromal tumor, rhabdomyosarcoma, mesoblastic nephroma or others. Immunohistochemistry is not very useful in resolving such diagnostic dilemmas because Wilms' tumor cells exhibit no specific markers and may be reactive with CD99, cytokeratin, desmin, and other antibodies. Wilms' tumors (of any sort) are easily identified by the electron microscopic demonstration of a uniquely characteristic thick flocculent glycocalyx surrounding the cells and a virtual absence of banded collagen within the extracellular matrix. The demonstrated presence/absence of this feature also enables discrimination of multicystic Wilms' tumor (malignant) from cystic partially differentiated nephroblastoma (benign).

Mesoblastic Nephroma

The (generally benign) cellular variant of mesoblastic nephroma histologically mimics a malignant tumor and can be confused, among others, with clear cell sarcoma, rhabdoid tumor or Wilms' tumor. They bear a characteristic t(12:15)(p13;q25) translocation and can be positively identified using FISH or RT-PCR technology; or, alternatively, by the ultrastructural demonstration of an unusual electron dense granulofibrillar material occupying the extracellular matrix.

Clear Cell Sarcoma of Kidney

In instances where the 'chicken wire' vascular pattern characteristic of this aggressively malignant tumor cannot be demonstrated, diagnostic confusion with a number of entities may result. Immunohistochemistry is of little value in establishing a positive diagnosis, as these tumors are reactive only for vimentin. No molecular diagnostic markers are available. No specific ultrastructural features are displayed but the electron microscopic demonstration of 1) nuclei devoid of heterochromatin and containing small peripherally located nucleoli, 2) sparse cytoplasmic organelles, and 3) small wisps of banded collagen within the extracellular matrix can contribute substantially to the establishment of this diagnosis.

Rhabdoid Tumor of Kidney

Rhabdoid tumor of kidney is a highly aggressive chemotherapy resistant malignancy of early childhood. It can display a variety of histologic appearances and, especially when the characteristic 'owl's eye' nuclei and/or eosinophilic hyaline cytoplasmic inclusions are not apparent, can be confused with a number of other entities. Immunohistochemical staining results can prove confusing and even misleading, as its immunophenotype is highly variable. The cytoplasmic inclusions are usually reactive for vimentin, making this stain useful in drawing attention to inclusion bearing cells when these are few in number. Although perhaps more a marker of its biological aggressiveness than of its specific identity, inability to detect the INI gene product by immunohistochemical means is another useful technique. Electron microscopic demonstration of the dense whirls of cytoplasmic filaments responsible for the eosinophilic inclusions provides strong morphologic evidence to support this diagnosis.

Renal Cell Carcinoma

Pediatric patients display variants of renal cell carcinoma different usually from those affecting the adult population. Most common among these are tumors displaying translocations involving the TFE3 gene at Xp11.2. These can most reliably be identified by immunohistochemical means. Electron microscopy is of more limited usefulness in this setting but can prove helpful in distinguishing renal medullary carcinoma (which similarly may not exhibit INI immunoreactivity) from rhabdoid tumor of kidney. Also, the ultrastructural finding of unusually large accumulations of mitochondria can draw attention to the diagnostic possibility of RCC resembling oncocytoma, or that of RCC following neuroblastoma.

Juxtaglomerular Cell Tumor

Electron microscopic demonstration of numerous rhomboid granules within the cytoplasm of the tumor cells enables a positive diagnosis of juxtaglomerular cell tumor. It is noteworthy, however, that an occasional morphologically similar granule may sometimes be seen in angiomyolipoma and in Xp11.2 associated RCC.

Other Entities

Because of their rarity, entities such as PNET, lymphoma, rhabdomyosarcoma and synovial sarcoma are apt not to be given due consideration when occurring as primary renal tumors. Immunohistochemical and molecular techniques require a preconceived hypothesis to test but electron microscopy, which does not, can provide answers even when the diagnostic questions have not been correctly formulated.

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**CYTOGENETICS IN THE DIAGNOSIS AND UNDERSTANDING OF RENAL
NEOPLASIAS**

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There are 150,000 new cases of renal cell carcinoma (RCC) diagnosed every year worldwide, representing 1.9% of all malignant tumors. It has been estimated that there will be 35,710 new cases and 12,480 deaths due to RCC in the United States this year, which is one of the highest rates in the world, along with that of Europe. Although the incidence of RCC is modest compared to that of other tumors, renal cancer is a disease that it is frequently detected in advanced stage and it is difficult to treat. Oberling in 1960 demonstrated that these neoplasms originate from renal tubular epithelium, a finding that was the first step in developing a valid classification system of renal tumors.

Recently, cytogenetic and molecular biology techniques have been used to study these tumors and have demonstrated a clear relationship between RCC and the presence of chromosomal alterations. These findings have led to a better understanding of the pathogenesis of RCC and to the development of new classification systems, including that of Heidelberg (1997) which integrated morphologic, clinical and cytogenetic elements. The last classification system from the World Health Organization (WHO) in 2004 includes recently described entities and incorporates morphologic, clinical, and genetic correlations.

These molecular biology techniques include in situ hybridization (ISH), particularly fluorescent in situ hybridization (FISH), which has been used in the study of various tumors for the detection of numeric and structural chromosomal alterations. Enumerating chromosome copies with centromeric probes has been performed using chromogenic detection methods since the 1990s, the chromogenic detection of unique sequences such as the HER-2 oncogene in breast cancer has shown some advantages over FISH, including lower cost and ease of application in conventional pathology laboratories, because it does not require use of a fluorescent microscope and histologic evaluation of the tissue under study can be performed concomitantly.

We evaluated the use of CISH, chromosome in situ hybridization in the diagnosis of renal tumors.

Tissue Samples

The histology slides and paraffin blocks from 91 cases of various types of renal tumors were obtained for the study. Each case was reviewed and classified according to the 2004 WHO classification. The cases under study included 37 cases of PRCC, 28 of which were type 1 (9 sporadic cases, 11 hereditary, and 8 of unknown history) and 9 of which were type 2. The 54 remaining cases represented a substantial number of different diagnoses within the spectrum of renal tumors, including 18 clear cell RCC, 10 oncocytomas, 8 chromophobe renal cell carcinoma, 4 medullary carcinomas, 6 collecting duct (of Bellini) carcinomas (CDC), 4 hybrid tumors, 2 metanephric adenomas, and 2 carcinomas which could not be classified.

CISH

CISH was done on 4 µm thick archival formalin-fixed paraffin-embedded (FFPE) tissue sections following the technique of Tanner et al.

In order to obtain satisfactory visualization of the chromosome alterations, a count of 100 to 500 nuclei was performed in randomly selected fields from each of the slides based on the size of the sections and the degree of nuclear overlapping, in accordance with Hopman's recommendations. The final result was an average expressed as a percentage of nuclei with 2, 3, or 4 signals (spots), classifying them as disomic, trisomic, and tetrasomic, respectively.

We scored the intensity of the stain with 1, 2, or 3 crosses: +/+++, when the signals could only be seen with the X40 and X60 objectives; ++/+++, when they could be seen with the X20 objective; and +++/+++, when they were visible with the X1

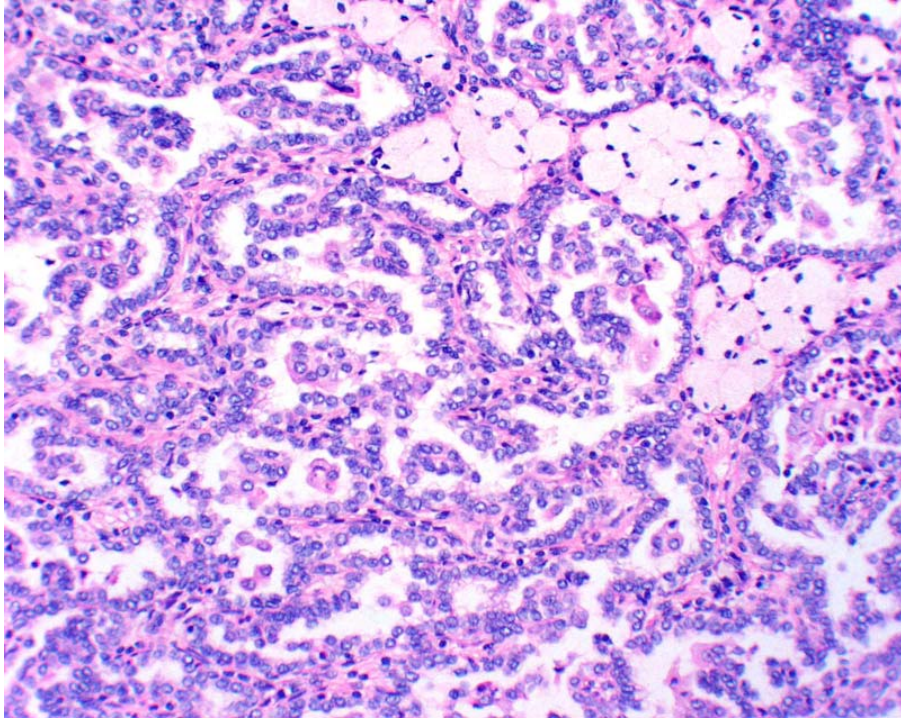
Results

Our study demonstrated that gains in chromosomes 7 and 17 are more frequent in PRCC than in nonpapillary RCC. Specifically, polysomy for chromosomes 7 and 17 was observed in 100% and 54.5% of cases of familial type 1 PRCC, respectively, higher figures than those obtained in cases of type 2 PRCC or nonpapillary RCC. These findings confirmed the differences in cytogenetic profile between type 1 and type 2 PRCC.

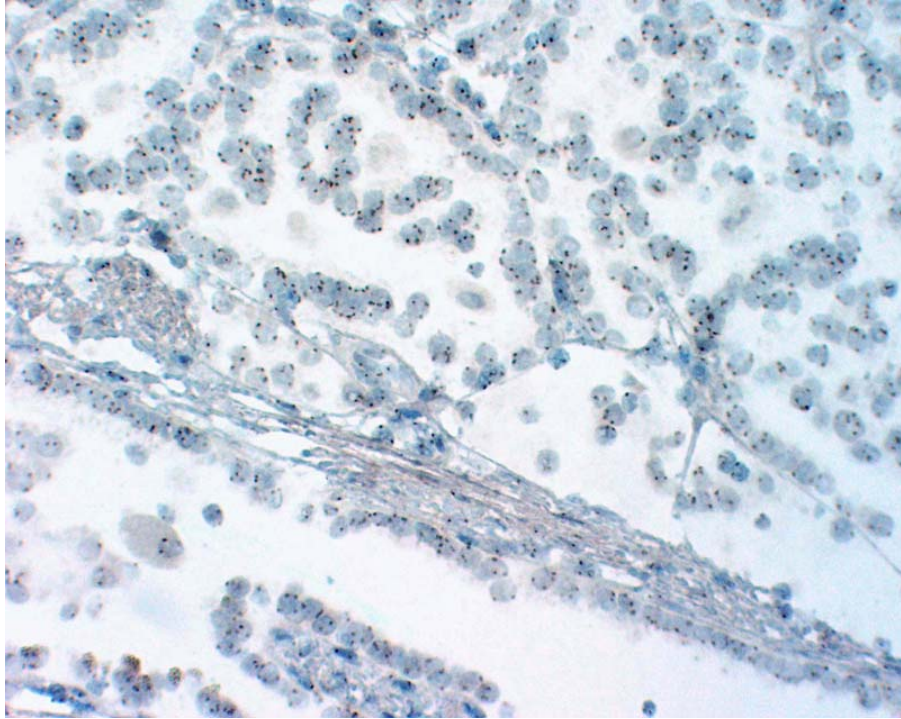
This was the first study in which chromogenic in situ hybridization was used to evaluate numeric alterations of specific chromosomes in RCC, and our results correspond to those reported in prior studies in which different techniques were used. In sporadic type 1 PRCC, we found a lack of numeric alterations of chromosome 17, data that has not been previously reported and that may prove helpful in the differentiation of these tumors. We found no alterations in the 2 metanephric adenomas studied, a finding that may prove useful in the differential diagnosis between these tumors and PRCC.

We found chromosome 7 trisomy in a case of ChRCC, which is one of the alterations that has been described in this type of tumor, in addition to hypodiploidy of various chromosomes, which is its most characteristic feature.

We conclude that CISH can be a very useful tool in the identification of numeric abnormalities in specific chromosomes and can improve the diagnosis of renal tumors. There is a clear correlation between chromosome 7 aneuploidy identified by CISH and familial type 1 PRCC. The absence of chromosome 17 aneuploidy can help identify cases of sporadic type 1 PRCC. The identification of chromosome 7 aneuploidy in clear cell RCC with sarcomatous features as well as in one unclassified tumor suggests a high grade and the possibility of new genetic alterations as the tumor progresses.



Papillary Renal Cell carcinoma type I



PRCC Chromogenic In Situ Hybridization showing trisomy for chromosome 7

RESULTS

RESULTS									
Histology	Cases	Chromosome 7 Aneuploidy	%	Chromosome 7 Diploidy	%	Chromosome 17 Aneuploidy	%	Chromosome 17 Diploidy	%
TYPE 1 PRCC	28	15	53.6	13	46.4	7	25	21	75
Hereditary	11	11	100	0	0	6	54.5	5	45.5
Sporadic	9	3	33.3	6	66.7	0	0	9	100
Undetermined	8	1	12.5	7	87.5	1	12.5	7	87.5
TYPE 2 PRCC	9	3	33.3	6	66.7	2	22.2	7	77.8
OTHERS	54	6	11.1	48	88.9	3	5.6	51	94.4

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