Comparison of PAX-2, RCC Antigen, and Antiphosphorylated H2AX Antibody (γ-H2AX) in Diagnosing Metastatic Renal Cell Carcinoma by Fine-Needle Aspiration

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Diagnosing metastatic renal cell carcinoma (RCC) by fine-needle aspiration (FNA) can be challenging. Existing antibodies supporting a diagnosis of RCC, including CD10 and RCC-Ma, have problems with specificity and interpretation. In this report, we evaluate the use of two newer immunostains, PAX-2 and γ-H2AX, which to our knowledge have not been studied in FNA material, in the diagnosis of metastatic RCC and in comparison with RCC-Ma. 29 cases of metastatic RCC were identified as well as a TMA of an additional 30 RCC cases. In the case cohort, RCC-Ma in a membranous pattern of staining identified 15/27 (56%) metastatic RCC, although interpretation was made difficult in many cases due to focality of staining and non-specific cytoplasmic staining. PAX-2 stained 23/29 (79%) of tumors in a nuclear stain, most strongly. Gamma-H2AX stained 19/26 (73%) of metastatic RCC strongly in a nuclear stain. In the TMA, strong, diffuse nuclear staining with γ-H2AX was present in 22/30 RCC (73%). If weak staining was also included as positive, 26/30 (87%) were positive. PAX-2 stained RCC TMA with a lower percentage at 56%, including weaker staining intensity. Both PAX-2 and γ-H2AX demonstrated patchy staining of normal renal tubules, PAX-2 to a greater extent. Both PAX-2 and γ-H2AX are sensitive markers for the diagnosis of metastatic RCC, with improved ease of interpretation when compared with RCC-Ma. A combination of all 3 markers identified 87% of cases, and failure to stain for both PAX-2 and γ-H2AX suggests against, but does not disprove, a diagnosis of RCC. Diagn. Cytopathol. 2008;36:568–573.

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Diagnosing metastatic renal cell carcinoma (RCC) by fine-needle aspiration (FNA) can be a challenge, as often very few cells are aspirated and morphologic overlap with other neoplasms and normal tissues exists, depending on the site. Although there are many features which can suggest a diagnosis of RCC in the absence of immunohistochemical stains,[1] these findings may not be entirely specific. The diagnosis often depends upon immunostains on the cell block, which is made difficult by the similar lack of specificity of some commonly used immunostains, including CD10.[2,3] The diagnosis, particularly FNA, can also be made more difficult by the small size of the sample and resultant difficulty in evaluating pathologic features as well as immunostains.[4]

The antibody known as renal cell carcinoma marker (RCC-Ma) was developed as a monoclonal antibody to normal human kidney proximal tubule,[5] and found to be specific for RCC, both primary and metastatic, in many studies.[4,6] Its use in many institutions, including our own, has been limited as the stain is often difficult to interpret and has a low sensitivity, particularly in small biopsies.[7–9] In particular, the staining pattern is frequently focal and difficult to distinguish from the frequent background, nonspecific staining which is seen in many tissues.[10] Staining by RCC-Ma is most clearly demonstrated in classic cases when immunohistochemical adjuncts to diagnosis may not even be necessary.

PAX-2 is another marker which is increasingly being studied for utility in metastatic RCC, but to our knowledge has not been studied in FNA. PAX-2 is a transcrip-

tion factor expressed in epithelial cells of fetal kidneys and enhanced in certain pathologic conditions. It is being studied as an emerging marker for diagnosing renal tumors as well as nephrogenic adenomas.[11–13] The advantages of PAX-2 over other currently used antibodies include ease of interpretation, as PAX-2 stains nuclei of cells and is less prone to false-positive interpretation than markers such as RCC-Ma and CD10, which predominantly stain cell membranes.

Gamma-H2AX is an antibody which specifically reacts with phosphorylated histone H2AX (at position Ser-139) and has been shown to be a marker of activated DNA damage response in tumor cells.[14,15] We have previously shown that γ-H2AX is also a potentially useful adjunct to the diagnosis of metastatic RCC when the differential diagnosis includes hepatocellular carcinoma and adrenocortical carcinoma, other clear cell tumors which are often in the differential diagnosis.[9] Our study aims were to see if γ-H2AX was equally sensitive, particularly in FNA, in diagnosing metastatic RCC, and in addition to compare it with PAX-2 and RCC-Ma.

Methods

Through a search of the University of Michigan pathology database, cases of metastatic RCC with cell block material available were identified. Standard cytology smears, cell blocks, and available immunostains were reviewed to confirm the diagnosis in each case. Cell block preparation included initial fixation in CytoLyt (Cytyc Corporation, Boxborough, MA), which is a methanol-based fixative. They were then processed by routine cell block processing. Paraffin embedded cell block sections were then stained for γ-H2AX (Cell Signaling Technologies, Danvers, MA), PAX-2 (Zymed laboratories), and RCC-Ma (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) according to the manufacturer's recommended protocols with some modifications for optimal conditions (described in our previous paper[9]). Gamma-H2AX was used at a dilution of 1:50, RCC-Ma at a dilution of 1:100, and PAX-2 at 1:100. In addition, a tissue microarray (TMA) of primary RCC was stained for PAX-2 and γ-H2AX.

Stains were reviewed by both pathologists (MJW and RTP) and the patterns (membranous, cytoplasmic, etc) of staining as well as the amount (in percentage) of tumor cells staining were recorded for each tumor. These characteristics were identified by visual estimation. Any background staining was also recorded. A case was considered positive when more than 25% of tumor cells stained and the tumor could easily be distinguished from the background. Staining was considered negative or equivocal if background staining and tumor were difficult to distinguish, or if fewer than 25% of tumor cells stained. Data were analyzed using simple statistics to determine the sensitivity of each marker.

Table I. Distribution of Cases in the Cohort

Site	*Number of cases*
Adrenal	1
Femoral head	2
Inguinal lymph node	2
Kidney	1
Liver	2
Lung	9
Neck lymph node	3
Pancreas	1
Parotid gland	1
Retroperitoneal lymph node	4
Supraclavicular lymph node	1
Thyroid	1
Vertebral bone	1

Results

A total of 29 cases of metastatic RCC were identified. Cases from a wide spectrum of site were available, with the most common site being lung (Table I). Seventeen of the cases were clear cell RCC, 3 were papillary, and 8 were of unknown or unclassified subtype. For two of the cases, lesional tissue was no longer present on the slide stained for RCC-Ma. This was also true for three of the 29 cases for the γ-H2AX-stained slide. Most cases had more than 100 cells available for immunostaining on the cell block, the minimum was 25 cells.

Case Cohort Results

A summary of staining of the metastatic RCC cohort with all three markers is as follows. RCC-Ma in a membranous pattern of staining identified 15/27 (56%) cases, although interpretation was made difficult in many cases due to focality of staining and some non-specific cytoplasmic staining (Fig. C-1). PAX-2 stained 23/29 (79%) of tumors in a nuclear stain. The majority were strong staining. Gamma-H2AX stained 19/26 (73%) of metastatic RCC strongly in a nuclear stain (Figs. C-1–C-3). For all antibodies, the average percent of cells staining in positive cases was over 50% (range 30–90%). Both γ-H2AX and PAX-2 stained some background lymphocytes and macrophages in a minority of cases. There were no significant differences in staining patterns or intensity among the different subtypes of RCC, although all three examples of papillary RCC were positive for all three antibodies. Both PAX-2 and γ-H2AX were easy to interpret when positive, as positive cases generally displayed a strong nuclear stain in the majority of tumor cells, with minimal background staining.

For PAX-2 positive cases, the average percentage of cells staining positive was 68%. For γ-H2AX, the average was 56% of cells. Of metastatic RCC that were negative for RCC marker, 4/12 were positive for γ-H2AX (in two

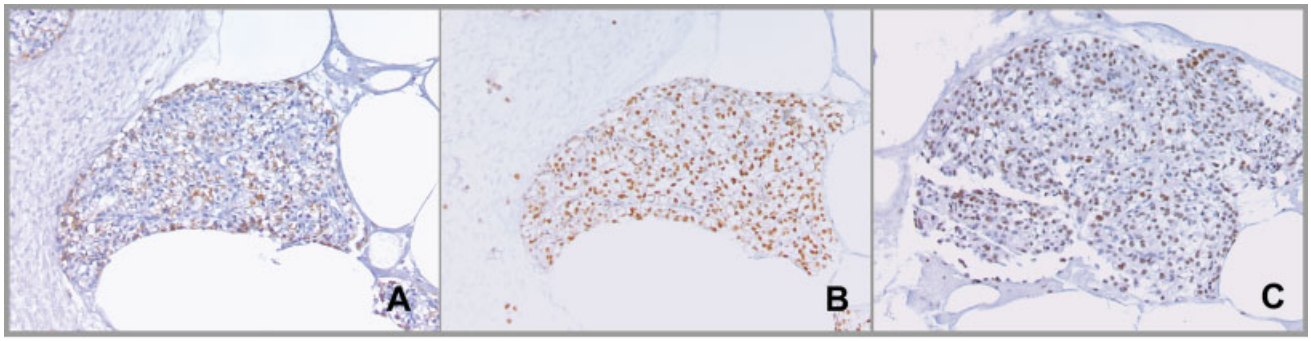

Fig. C-1

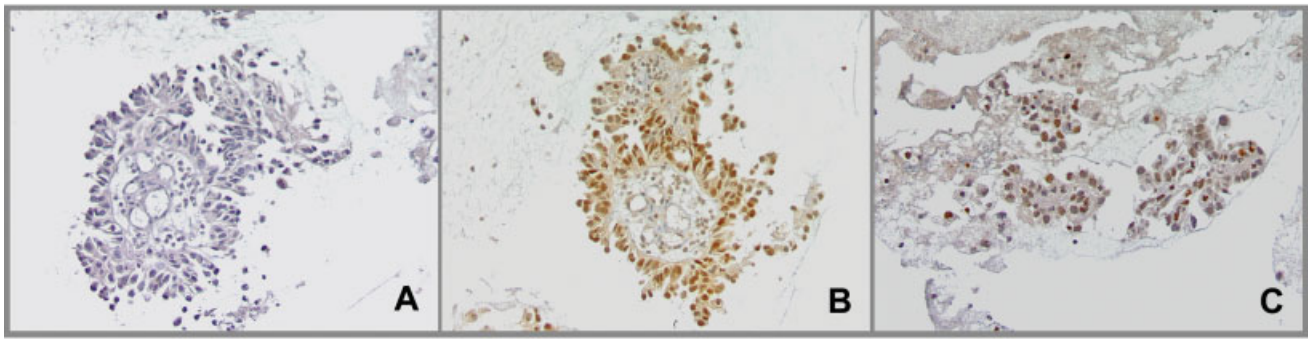

Fig. C-2

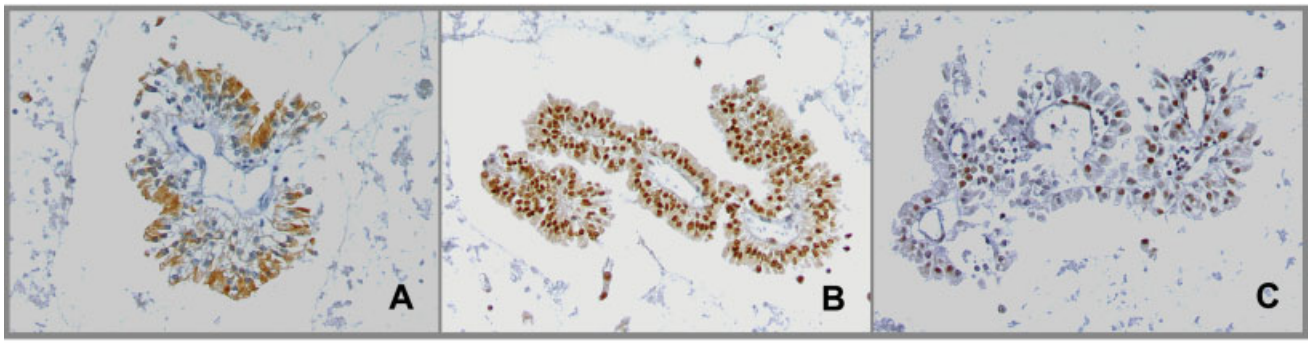

Fig. C-3

Figs. C-1–C-3. Fig. C-1. A case of metastatic RCC to the neck with weak, non-specific or equivocal staining for RCC-Ma (**A**), positive for PAX-2 (**B**), and weakly positive for γ-H2AX (**C**). **Fig. C-2**. A case of metastatic RCC to the neck negative for RCC-Ma (**A**), and positive for both PAX-2 (**B**) and γ-H2AX (**C**). **Fig. C-3**. A case of metastatic RCC to the parotid positive for RCC-Ma (**A**), strongly positive for PAX-2 (**B**) and focally positive for γ-H2AX (**C**).

of the 12 cases, tumor was no longer present on the γ-H2AX stained slide) and 6/12 were positive for PAX-2. Two of the RCC-Ma negative tumors were positive for PAX-2 but negative for γ-H2AX. Four cases were negative for all three antibodies. There were no cases that were positive for RCC-Ma but negative for the other two markers.

Tissue Microarray

For the tissue microarray, strong, diffuse nuclear staining with γ-H2AX was present in 22/30 RCC (73%). If weak staining was also included as positive, 26/30 (87%) were positive. PAX-2 stained RCC TMA with a lower percentage at 56%, including weaker staining intensity (Fig. C-4). Both PAX-2 and γ-H2AX demonstrated patchy staining of normal renal tubules, PAX-2 to a greater extent.

Discussion

Diagnosing metastatic renal cell carcinoma can be difficult, in part due to the propensity of the tumor to spread to a wide variety of sites and tendency to mimic other types of neoplasm.[16] Current strategies include appraisal

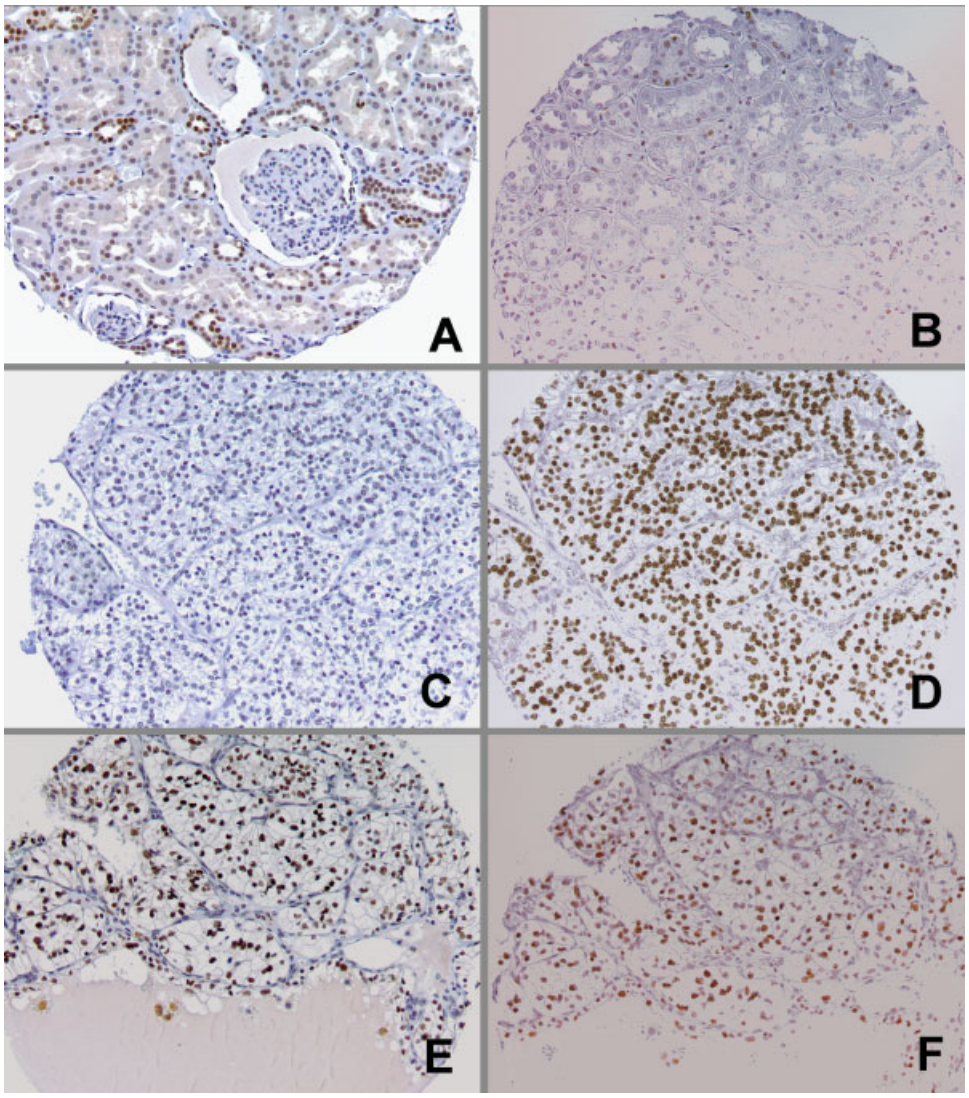

Fig. C-4. A TMA was stained with γ-H2AX and PAX-2. Patchy staining of normal renal tubules was seen with both PAX-2 (**A**) and γ-H2AX (**B**). Pictured is a case of RCC that was negative for PAX-2 (**C**) and positive for γ-H2AX (**D**), and also, a case which was positive for both PAX-2 (**E**) and γ-H2AX (**F**).

of various morphologic features as well as immunostains such as RCC-Ma,[8,16] CD10,[3] and increasingly, PAX-2.[11]

In this article, we report the comparison of RCC-Ma, PAX-2, and a new antibody, γ-H2AX, in the diagnosis of metastatic RCC in fine-needle aspiration material. Previously, we have reported the utility of γ-H2AX, primarily in the differentiation of metastatic RCC from two of its common mimickers, adrenocortical carcinoma and hepatocellular carcinoma.[9] Gamma-H2AX, similarly to PAX-2, has a nuclear pattern of staining (generally strongly) and has less of the background staining which makes RCC-Ma a problematic marker for usage in routine clinical practice in many laboratories.[10] These difficulties with RCC-Ma interpretation often come into play in FNA material, when only limited amounts of tumor tissue are available for study.[8]

Thus far, there have been limited reports of PAX-2 expression in non-renal tumors,[18] and it is to date considered to be a fairly specific antibody for tumors of renal origin. But use of this antibody has not yet been widely accepted, and clinical utility needs to be confirmed by additional studies. An interesting finding in early studies is that PAX-2, in some reports, may be less useful in higher grade renal tumors than in conventional, more clearly differentiated RCC.[19] In our previous study, one

of the useful features of γ-H2AX was that it stained a high percentage of high-grade (equivalent of Fuhrman nuclear grades 3–4) tumors when compared with RCC-Ma.[9] As these are the tumors that often defy diagnosis, it may have clinical relevance particularly in these cases. Tumors from some other sites have been reported to be positive for γ-H2AX, including breast, melanoma, prostate, and urinary bladder,[14,15] although these studies are limited and preliminary and have not been supported by additional studies of clinical relevance.

The results of our study show that all three antibodies are relatively sensitive for metastatic renal cell carcinoma, in particular clear cell and papillary subtypes. Our study did not include any cases of chromophobe RCC, although several cases were of indeterminate subtype. RCC-Ma (in a membranous pattern) identified 56% of metastatic RCC, with several cases equivocal due to difficulty of interpreting the stain. PAX-2 and γ-H2AX (nuclear stain) each were more sensitive markers, identifying 79% and 73%, respectively, of metastatic RCC in our cohort. Both were similarly easy to interpret, staining approximately half of tumor cells and occasionally staining background lymphocytes and histiocytes in a minority of cases. Both γ-H2AX and PAX-2 were helpful in over 50% of the cases in which RCC-Ma was negative or equivocal, suggesting the utility of these stains. Additionally, RCC-Ma was never the sole positive antibody. In total, 87% of cases were positive for at least one of the markers.

Of note, the data from the TMA of renal cell carcinomas cases provided only a 56% sensitivity for PAX-2 (including weak staining cases), compared with 87% for γ-H2AX. This may suggest that the sensitivity of PAX-2 is lower than that reported previously, although in the actual case cohort the sensitivity was higher.

Although the relationship of the PAX-2 antibody as a relatively specific marker for renal epithelium has been studied,[12,13] the reasons for relative specificity of γ-H2AX for renal tumors are less clear. Gamma-H2AX is involved in the cellular repair process, particularly in regards to double stranded DNA breaks. The exact role of γ-H2AX in the repair process is unknown, but it is known that foci of H2AX phosphorylation are created within the damaged DNA areas as part of the multifactorial repair process.[20] H2AX and other linker histones have been shown to be crucial for nucleosome formation and consequently gene expression controls.[14,15] We have shown previously that most RCC mark with γ-H2AX while another type of tumor often in the differential, hepatocellular carcinoma, does not. The different staining pattern by γ-H2AX suggests that the double-stranded DNA damage control pathway might be involved differently during the tumorigenesis of these two tumors. Similarly, epigenetic change such as DNA methylation also involves different tumor suppressor genes in RCC and HCC.[21,22]

In conclusion, γ-H2AX and PAX-2 are antibodies with fairly high sensitivity for metastatic RCC. They both have improved sensitivity and ease of interpretation on FNA material when compared with RCC-Ma. The combination of all three markers identifies 87% of metastatic RCC. Failure to stain for all three markers suggests against a diagnosis of RCC, although occasional cases can be negative for all three. PAX-2 may be less sensitive than previously reported, although still clinically useful, for the diagnosis of metastatic RCC. More clinical studies may be warranted to fully clarify the utility of both PAX-2 and γ-H2AX in the spectrum of diseases and differential diagnosis.

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