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ORIGINAL ARTICLE

Evaluation of PAX8 Expression and Its Potential Diagnostic and Prognostic Value in Renal and Extra-Renal Ewing Sarcomas/PNETs

Michael Markow[1] · Marilyn M. Bui[1,2,4] · Hui-Yi Lin[5] · Mark Lloyd[2] · Wade J. Sexton[3] · Jasreman Dhillon[1,4]

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Abstract PAX8 is a transcription factor involved in the regulation of organogenesis of the thyroid gland, kidney, and Müllerian system. It is commonly expressed in epithelial tumors of thyroid and parathyroid glands, kidney, thymus, and female genital tract. PAX8 is increasingly used in the establishment of tissue of origin in carcinomas and has recently been identified in a subset of small blue round cell tumors including Ewing sarcomas/PNETs. However, it is unclear if this association in ES/PNETs is due to renal origin or is PNET specific. In this study we investigated the PAX8 staining pattern of primary renal and extra-renal ES/PNETs to explore its potential diagnostic and prognostic role. A tissue microarray (TMA) of 22 cases of extra-renal Ewing/PNETs and two separate cases of primary renal PNET whole slide sections were immunohistochemically stained with rabbit polyclonal PAX8 antibody. PAX8 was positive in 2 of 2 primary renal PNETs and in 14 (64 %) cases of the extra renal PNETs. The association between PAX8 immunoreactivity and Ewing/PNET was identified in both primary renal and extra-renal Ewing/PNETs for the first time. Further studies are warranted to verify these findings and to shed light in the tumorigenesis of Ewing/PNET. However, PAX8 is not useful in establishing a diagnosis of Ewing/PNET due to its presence in different tumors like carcinomas, lymphomas and sarcomas. PAX8 does not seem to have prognostic value.

Keywords PAX8 · PNET · Renal PNET

✉ Jasreman Dhillon
jasreman.dhillon@moffitt.org

[1] Department of Pathology and Cell Biology, University of South Florida, 12902 Magnolia Drive, Tampa, FL 33612, USA

[2] Analytic Microscopy Core, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[3] Department of Genitourinary Oncology, Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[4] Department of Anatomic Pathology, Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[5] Department of Biostatistics and Bioinformatics, Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Introduction

PAX8 is a transcription factor involved in the regulation of organogenesis of the thyroid gland, kidney, and Müllerian-derived tissues [1]. It is commonly expressed in epithelial tumors of the thyroid gland, parathyroid glands, kidney, thymus, and female genital tract [2, 3].

Recently PAX8 staining has also been reported in neuroendocrine carcinomas of the pancreas, duodenum, and rectum [4, 5]. Increasingly, PAX8 has found usage in the determination of the tissue of origin of carcinomas [6]. Additional studies have determined PAX8 staining in non-carcinomatous tumors such as sarcomas and in lymphomas [7–9]. Recent studies published on PAX8 expression in sarcomas have shown positive staining in rhabdomyosarcomas, malignant rhabdoid tumors, and clear cell sarcomas of the kidney [10]. Chang et al. have reported PAX8 expression in one of 27 cases of PNET [7]. Recently, Zhao et al. have reported PAX8 staining in a primary renal hemangioblastoma; in contrast, central nervous system hemangioblastomas were negative for PAX8 in that study [11]. The authors hypothesized that the immunoprofile of extraneural hemangioblastoma varies with site of origin, perhaps as a result of tumor cell lineage and retention of organ-specific markers or acquisition of site-specific antigens due to local factors.

We intended to study PAX8 immunostaining pattern in renal and extra-renal Ewing sarcomas (ESs)/primitive neuroectodermal tumors (PNETs) to look for positive staining in these tumors and to determine whether there is a prognostic difference in overall survival between patients with Ewing/PNET cases that stain with PAX8 compared to cases that do not.

Materials and Methods

Tumor Sections

Following the guidelines of scientific review protocol at Moffitt Cancer Center and University of South Florida, whole slides sections of two cases of primary renal ES/PNET and a tissue microarray (TMA) section of 24 cases of extra-renal ES/PNET were obtained. Each case was represented by 2 cores of 0.6-mm diameter taken from representative areas of the tumor. Immunohistochemistry (IHC) for PAX8 was performed on ES TMA and the whole slide sections of the two renal PNETs.

Patient Data

Pertinent clinical data of the patients were compiled to include patient age, sex, tumor location and size, presentation at diagnosis and overall survival.

Immunohistochemistry

PAX8 Immunohistochemical Study

The stainer, antibody source, clone, dilution, and antigen retrieval procedure for PAX8 IHC are provided in Table 3. The

Table 1 Clinicopathological features with PAX 8 staining results

Age (years)	Sex	Location of primary site	Size (cm)	Presentation at diagnosis	Molecular confirmation for Ewing sarcoma	Overall survival (months)	PAX 8 staining results	Intensity of staining
14	M	Right lower leg	15	Unknown	FISH	83	+	3+, Diffuse
15	F	Right shoulder	Not known	Regional	FISH	175	+	2+, Diffuse
15	M	Iliac bone and soft tissue	7.5	Regional	FISH	58	−	−
16	M	Pelvis	Not known	Unknown	FISH	63	+	1+, Diffuse
16	F	Left femur	Unknown	Metastatic	FISH	23	−	−
17	M	Tibia	Unknown	Regional	FISH	20	+	1+, Diffuse
24	M	Pelvis	13	Regional	FISH	88	+	2–3+, Diffuse
24	M	Chest wall	10.5	Localized	FISH	20	+	2+, Diffuse
28	M	Pelvis	14	Metastatic	RT PCR	7	+	2–3+, Diffuse
28	F	Left femur	12	Metastatic	FISH	19	+	1+, Diffuse
30	M	Thigh	17	Regional	FISH	≻ 101	+	3+, Diffuse
34	M	Chest wall	5.7	Regional	PRKCB[a]	≻ 141	−	−
35	M	Left flank	Unknown	Localized	FISH	≻ 179	+	2+, Diffuse
40	M	Pelvis	10	Unknown	FISH	70	−	−
41	F	Sacrum	Unknown	Metastatic	FISH	78	+	1+, Diffuse
48	F	Left leg	34.5	Localized	No results available	16	−	−
50	F	Chest wall	7.5	Regional	FISH	11	−	−
54	M	Chest wall	8	Localized	RT PCR	22	−	−
55	M	Thigh	4.5	Metastatic	FISH	74	+	1+, Diffuse
56	M	Left thigh	Unknown	Metastatic	FISH	74	−	−
58	M	Left thigh	8	Metastatic	FISH	23	+	2–3+, Diffuse
67	F	Buttock	7	Localized	FISH	11	+	1+, Diffuse
71	F	Uterus	20	Regional	FISH negative[b]	1	+	2+, Diffuse
72	F	Thigh	6	Localized	FISH negative[b]	9	−	−
26	M	Kidney	21	Regional	RT PCR	≻ 4	+	3+, Diffuse
32	F	Kidney	5	Localized	RT PCR	≻ 9	+	2+, Diffuse

[a] This case was positive for immunohistochemical stain PRKCB which is considered to be relatively specific for ES

[b] These two cases were originally diagnosed as ES. However both were negative for translocation via FISH study. Hence, were later reclassified as sarcoma, NOS

intensity of nuclear staining was evaluated and assigned an incremental score of 0, 1+, 2+ or 3+. The extent of staining was graded as focal (<25 %), patchy (25 to 75 %) or diffuse (>75 %).

Statistical Analysis

Patients' demographic and clinical characteristics were summarized using descriptive statistics. Mean and standard deviation were calculated for continuous factors, and frequency and percentage were generated for categorical factors. Overall survival (OS) was defined as the time from date of diagnosis to date of death due to any cause. OS data were censored by the last date on which the patient's survival status was known. The OS curve was estimated by the Kaplan-Meier method. Median survival and its 95 % confidence intervals (CIs) were estimated. The difference of OS between PAX8 positive versus PAX8 negative cases was done using the log-rank test.

Results

The PAX8 staining for the 26 cases demonstrated positive staining in 2 of 2 primary renal Ewing sarcomas/PNETs, both of which were molecularly confirmed to be Ewing sarcoma/PNET by RT-PCR. Of the 24 cases comprising the TMA slide and diagnosed as Ewing sarcoma/PNET, 22 underwent molecular testing to confirm the diagnosis either by FISH or RT-PCR. FISH was positive in 18 cases and RT-PCR was positive in two cases. One case was positive for immunohistochemical stain protein kinase PKC-ß (PRKCB) which is considered to be relatively specific for Ewing sarcoma [12]. Two cases originally diagnosed as ES were negative for translocation via FISH. Hence, were later reclassified as sarcoma, NOS. No

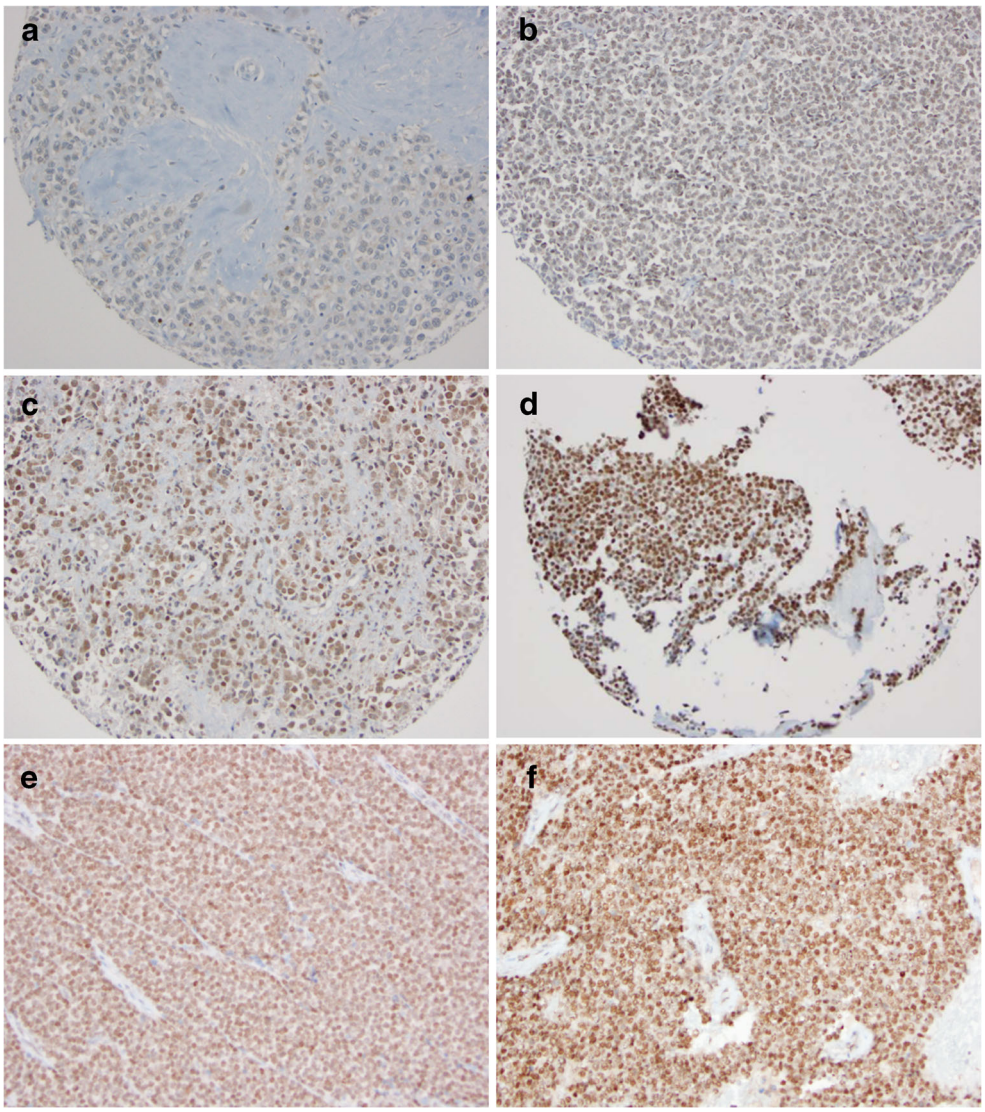

Fig. 1 **a**–**d** Staining results obtained with PAX8 immunostain in non-renal PNETs: **a** negative; **b** 1+; **c** 2+; **d** 3+. 20×. **e** and **f** Two separate cases of primary renal PNETs with positive PAX8 immunostaining 20×

information on molecular testing was available on one of the cases (Table 1).

The intensity of staining in the renal PNETs was graded as 2+ in one tumor and as 3+ in the second one. Fourteen of 22 (64 %) cases of the extra-renal Ewing sarcomas/PNETs stained positive for PAX 8. The intensity of staining ranged from 1+ up to 3+ (Fig. 1a–f). Overall, 65 % (17/26) of sarcomas stained positive for PAX8 (Table 1). Interestingly, there was a single case of Medulloblastoma on the TMA slide which stained weakly positive (1+) for PAX 8. Nearly 60 % (16/26) of the patients in our cohort were 40 years or younger in age, Ewing sarcoma also occurs in older patients. Since our hospital is not a children's hospital, some of our patients were older. However, there were two patients (71 and 72 years old) in our cohort where the diagnosis of Ewing sarcoma/PNET could not be confirmed by molecular testing; FISH was negative in both the cases. Both of these cases were reclassified as sarcoma, NOS. The difference of OS between PAX8 positive versus PAX8 negative cases done using the log-rank test is shown in Fig. 2.

Median overall survival for PAX8-positive patients was 4.4 years (95 % CI = 0.9–6.2), and for PAX8-negative patients was 1.9 years (95 % CI = 0.8–6.2). There is no statistical difference in overall survival (Fig. 2) between PAX8 positive and negative cases (log-rank test $P = 0.69$).

Discussion

PAX8 is a transcription factor involved in the regulation of organogenesis of the thyroid gland, kidney, and Müllerian-derived tissues [1, 2]. As a diagnostic marker, PAX8 has been rigorously studied since 2008. It is commonly expressed in epithelial tumors of the thyroid gland, parathyroid glands, kidney, thymus, female genital tract and neuroendocrine carcinomas of the pancreas, duodenum, and rectum [2–5].

PAX8 immunostaining is utilized in determining the site of origin of carcinomas. However, besides carcinomas other non-epithelial tumors such as sarcomas and certain lymphomas also stain for PAX8. Table 2 contains a review of the immunohistochemical expression of PAX8 in various sarcomas reported in the literature. It is not clear yet if the PAX8 staining pattern in sarcomas is site dependent and follows the pattern seen in carcinomas or is dependent on the sarcoma subtype. Recently, Zhao et al. have reported PAX8 staining in a primary renal hemangioblastoma [11]. Since, central nervous system hemagioblastomas are always negative for PAX8, it was hypothesized by the authors that the immunoprofile of extraneural hemangioblastoma varies with the site of origin, perhaps as a result of tumor cell lineage and retention of organ-specific markers or acquisition of site-specific antigens due to local factors.

We studied the immunoreactivity for PAX8 in primary renal and extra renal PNETs/Ewing sarcomas in order to determine whether reactivity was dependent on the site of origin. We had two cases of primary renal PNET, both of which stained for PAX8. Interestingly, 14 of 22 (64 %) extra renal PNETs also stained with PAX8. The intensity of staining varied from 1+ up to 3+ in the positive cases. Although, there are only two cases of renal PNETs in our current study, we propose that the staining of PNETs with PAX8 is not dependent on the site of origin. More studies with more number of cases are needed to confirm this impression.

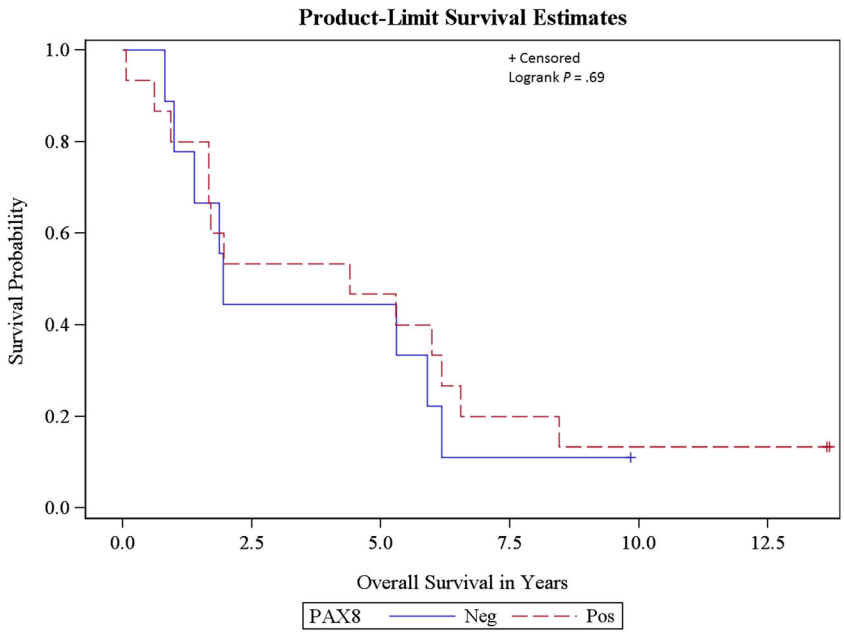

Fig. 2 Comparison of overall survival between PAX8 positivity status

Table 2 PAX8 positivity reported in different sarcomas

Organ/site	Neoplasm	Positive cases N (%)	Author
Uterus	Sarcomatous component of Malignant Mesodermal Mixed Tumor (MMMT)	10 in 37 (27 %)	Holmes et al. [13]
Pediatric – any site	Embryonal Rhabdomyosarcoma	3 in 20 (15 %)	Fan R [10]
Pediatric – any site	Alveolar Rhabdomyosarcoma	5 in 14 (35.7 %)	Fan R [10]
Pediatric – any site	Malignant Rhabdoid Tumor	7 in 25 (28 %)	Fan R [10]
Pediatric – any site	Ewing Sarcoma/PNET	0 (0 %)	Fan R [10]
Kidney	Clear cell sarcoma	0 (0 %)	Fan R [10]
Uterus	Endometrial Stromal Sarcoma	1 in 6 (16 %)	Chang et al. [7]
Not known	Ewing Sarcoma	1 in 27 (3.7 %)	Chang et al. [7]

PAX8 expression in sarcomas has been studied by Fan et al. [10] and Chang et al. [7]. The method of staining, including the clone utilized, source of PAX8 antibody and the antigen retrieval step, for these studies is compared our study in Table 3. All of the three studies used polyclonal PAX8 antibody but the method of antigen retrieval was different for all three studies.

Fan R studied expression PAX8, PAX5 and PAX2 antibodies in 123 cases of poorly differentiated small round cell tumors of childhood [10]. In that study all the 37 cases of ES/PNET were negative for PAX8 stain. Whereas 5 of 14 (35.7 %) of alveolar rhabdomyosarcomas, 3 of 20 (15 %) of embryonal rhabdomyosarcomas and 7 of 25 (28 %) of malignant rhabdoid tumors stained positive for PAX8. Chang et al. evaluated 161 sarcomas in TMAs and found a single case (1 of 27; 3.7 %) of ES/PNET which stained for PAX8. All other sarcomas including seven cases of rhabdomyosarcoma and five cases clear cell sarcoma besides others were reported to be negative for PAX8 [7]. Our results interestingly show 15 of 24 (62.5 %) cases of the extra-renal Ewing sarcomas/PNETs and 2 of 2 cases of primary renal Ewing sarcoma/PNET to be positive for PAX 8.

PAX8 positivity in tumors especially in small biopsy samples should be interpreted with caution. PAX8 reactivity can be seen in epithelial and non-epithelial tumors such as lymphoma, hemangioblastoma and some sarcomas. Detailed study of histopathological features, clinical and radiographic correlation and staining profile with pertinent immunostains is required to render a correct diagnosis. PAX8 staining is not lineage specific. Differential diagnoses of PNET, rhabdomyosarcoma [10], Merkel cell carcinoma [14] hematopoietic neoplasms [15] and pancreatic and rectal neuroendocrine carcinomas [4, 5] should be considered in a tumor with small, round cell features and positive staining for PAX8.

Our study leads us to hypothesize that the association between PAX8 staining and ES/PNETs is due to ES/PNET-specific PAX8 antigen expression that is unrelated to the site of origin in these tumors. However, more studies are needed to confirm this impression. ES and PNET are entities with continued unclear histogenesis, and are now widely considered as two entities of a continuum [16].

PAX8 immunoreactivity had no prognostic significance on survival in our study. This conclusion is however, limited by the fact that evaluations of survival have been based solely on PAX8 immunostaining and multiple clinical and pathological variables have not been accounted for due to a small case number in our study. A previous study using the same cases, except for the two renal cases, identified that metastasis had a significant effect on overall survival ($p = 0.003$), while age, sex, tumor size and tumor location did not [17]. More studies with larger number of cases are indicated to confirm these results.

Conclusion

In conclusion, the association between PAX 8 immunoreactivity and ES/PNET was identified in both the renal and extra-renal ES/PNETs for the first time. PAX8 is not useful in establishment of tissue origin for Ewing/PNET due to its presence in both renal and extra-renal ES/PNETs. Further studies are warranted to verify these findings and to shed light in the

Table 3 Comparison of staining methods for PAX8 in different studies

Authors	Stainer	Source	Clone	Dilution	Antigen retrieval method
Fan [10]	NA	Cell Marque, NA	Polyclonal	Ready to use	EDTA in pressure cooker
Chang et al. [7]	Bond Max	BD Pharmingen, San Jose, CA, USA	Polyclonal	1:100	Bond enzyme
Markow et al. (current study)	Bench Mark	Cell Marque, Rocklin, CA, USA	Polyclonal	1:5	Heat activation

tumorigenesis of ES/PNET. PAX8 immunoreactivity did not show prognostic value in Ewing sarcoma.

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Conflict of Interest The authors report no conflicts of interest.

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