Immunohistochemical Expression Profiles of PRAME in Human Normal FFPE Tissue Specimens and a Review of Literature

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Introduction

PRAME (PReferentially expressed Antigen in Melanoma), is a member of the Cancer Testis Antigen (CTA) gene family, which has been detected in various tumors by Northern Blot, RT-PCR, and IHC. PRAME is commonly used as an adjunctive biomarker by IHC to differentiate benign and malignant melanocytic neoplasms. While PRAME protein expression has been extensively studied in melanoma and other types of neoplasms, its IHC expression profile in normal tissue specimens is not well documented.

Objectives

1. To describe the IHC staining profile of PRAME in normal human tissue specimens
2. To review and analyze the current literature with our data.

Materials & Methods

34 different human tissue types formalin-fixed paraffin-embedded (FFPE) human tissue specimens from multiple tissue microarrays were tested. The Genemed® anti-PRAME rabbit monoclonal antibody, clones, QR005 and EP461 were optimized by testing various pretreatment conditions, diluents, and working concentrations using Tissue-Tek Genie® Advanced Staining System with and Tissue-Tek Genie® Pro Detection Kit DAB. Evaluation of the IHC staining profile was performed by a pathologist and an external reviewer.

Results

No.	Tissue Type	Cells	Staining profile	QR005 (# Stained/Total)	EP461 (# Stained/Total)	Reference
1	Skin	Sebocytes Eccrine	C C	3/3 1/1	1/2 1/1	[1,2,5] [1,2]
2	Testis	Spermatogonia, spermatocytes, spermatids	N	6/6	6/6	[2,5]
3	Ovary	Granulosa cells	N	1/1	1/1	[2,3]
4	Uterus	Endometrial glands	N	4/6	2/6	[2,3,4]
5	Lung	Ciliated Pseudostratified columnar cells	C	6/6	6/6	[2,3]
6	Spleen	Lymphoid	-	0/6	0/6	[2,3]
7	Pancreas	Acini	-	0/6	0/6	[2]
8	Thyroid gland	Follicular	-	0/6	0/6	[2]
9	Salivary Gland	Acini	-	0/1	0/1	[3]
10	Cerebral Cortex	Neuroglial	-	0/1	0/1	[2,3]
11	Cerebellum	Neuroglial	-	0/4	0/4	[3]

Table 1. Comparable Staining profile of normal human tissue specimens amongst PRAME QR005, EP461 and literature.
A total of 46 tissue specimens from 11 different tissue types exhibited similar staining profiles from both PRAME clones when compared to literature. (-): no staining; C: cytoplasmic; N: Nuclear. *Most exhibit moderate to strong positive staining described in the table.*

No.	Tissue Type	Cells	Staining profiles			Reference
			Literature	QR005 (# Stained/Total)	EP461 (# Stained/Total)	
1	Skin	Keratinocytes*	N	N (4/8)	N (0/6)	[1,5]
2	Adrenal Glands	Adrenocortical	N/C/M -	N/C/M (4/6)	C (2/6)	[2,3,5] [4]
3	Placenta	Cytotrophoblasts	N	- (0/6)	- (0/6)	[2]
4	Testis	Leydig	M	M/C (3/6)	M/C (0/6)	[5]
5	Peripheral nerves	Neuroglial	-	N (1/1)	N (1/1)	[1,2,3]
6	Colon	Simple columnar epithelium	-	N (1/2)	N (1/2)	[2]
7	Breast	Ductal epithelium	-	C (3/5)	C (3/5)	[3]
8	Thymus	Myeloid/lymphoid	-	N (1/1)	N (1/1)	[3]
9	Stomach	Foveolar/simple columnar epithelium	-	N (4/5)	N (1/5)	[2]
10	Small intestine	Enterocytes	-	N (2/4)	- (0/4)	[2]
11	Kidney	Glomeruli Distal and proximal tubules	- -	N (6/6) N (6/6)	- (0/6) N (1/6)	[2] [2]
12	Prostate	Ductal epithelium	-	C (1/4)	C (1/4)	[3]

Table 2. Variable staining profiles observed or reported on normal human tissue specimens amongst PRAME QR005, EP461 and literature.
A total of 54 tissue specimens from 12 different tissue types exhibited variable staining profiles (including positivity, positive % cells, and staining compartment, amongst both PRAME clones and literature. (-): no staining; C: cytoplasmic; N: Nuclear; M: Membranous. *Most exhibit weak to moderate positive staining described in the table.* *Percentage of positive cells varies amongst clones and literature.

No.	Tissue Type	Cells	Staining Pattern	QR005 (# Stained/Total)	EP461 (# Stained/Total)
1	Tonsil	Lymphoid*	N	7/7	4/7
2	Fallopian Tube	Ciliated Pseudostratified columnar cells	C	2/2	2/2
3	Omentum	Adipocytes	N/C	1/1	1/1
4	Appendix	Simple columnar epithelium*	N	2/2	1/2
5	Cervix	Stratified squamous epithelium*	N	3/3	1/3
6	Esophagus	Stratified squamous epithelium*	N	3/3	3/3
7	Bladder	Transitional epithelium*	N	2/3	1/3
8	Cardiac Muscle	Cardiomyocyte	-	0/1	0/1
9	Skeletal Muscle	Myocyte	-	0/2	0/2
10	Pituitary Gland	Acidophils, basophils and chromophobes	-	0/1	0/1
11	Parathyroid Gland	Chief and Oxyphil	-	0/1	0/1

Table 3. Staining profile observed but not well documented in literature.
A total of 26 tissue specimens from 11 different tissue types exhibited comparable staining profiles between both PRAME clones that are not well documented in literature. (-): no staining, C: cytoplasmic; N: Nuclear. *Most exhibit focally weak positive staining except Fallopian tube and Omentum described in the table.* *A subset of cells exhibit the above staining pattern.

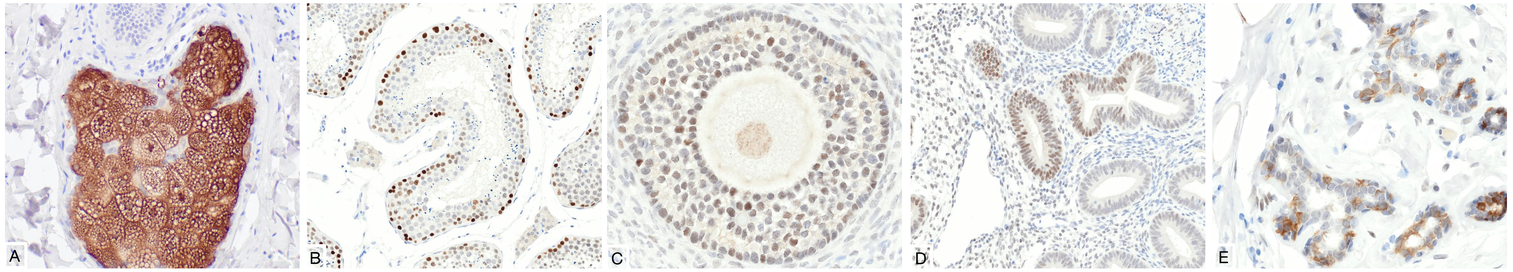

Figure 1. Comment positive staining observed for PRAME. **(A)** Strong cytoplasmic positivity in sebocytes (IHC, 200x). **(B)** Weak to strong nuclear positivity in spermatids, spermatocytes, and spermatogonia of the testis (IHC, 200x). **(C)** Moderate to strong nuclear positivity in granulosa cells of ovarian follicles (IHC, 200x). **(D)** Focally weak, moderate, and strong nuclear positivity of endometrial glands (IHC, 200x). **(E)** Scattered moderate to strong cytoplasmic positivity in breast glands (IHC, 200x).

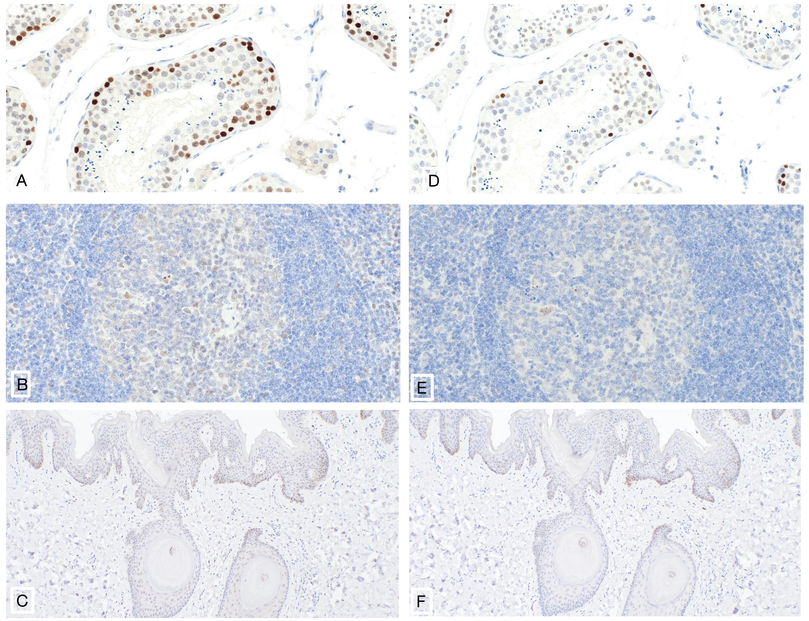

Figure 2. Variable IHC expression profile and intensity amongst PRAME QR005 (left), EP461 (right), and reported literature.
PRAME QR005 **(A-C)** exhibited an overall stronger staining intensity than EP461 **(D-F)**. Moderate and weak membranous and cytoplasmic positivity in Leydig cells of testis were observed in PRAME QR005 (**A**, IHC 200x) and EP461 (**D**, IHC 200x), respectively, when these cells exhibited only membranous positivity in the reported literature. In the reported literature, no to rare nuclear positivity was observed in keratinocytes or not documented for tonsil. A weak to moderate nuclear staining was present in tonsillar lymphoid cells using QR005 (**B**, IHC 200x) whereas scattered nuclear positivity was observed using EP461 (**E**, IHC 200x). Weak to moderate nuclear positivity was observed in keratinocytes of orthokeratinized stratified squamous epithelium of the skin and hair follicles for QR005 (**C**, IHC 100x), while EP461 exhibited no to weak nuclear positivity (**F**, IHC 100x).

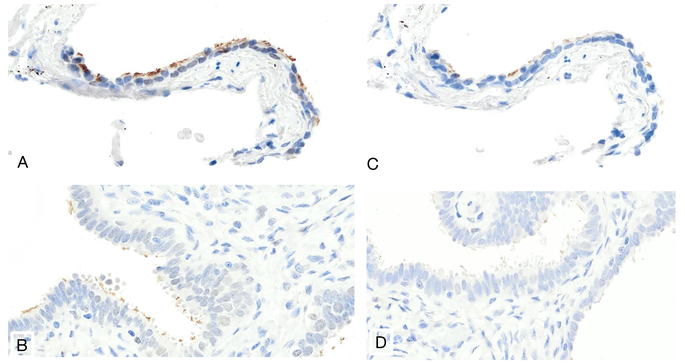

Figure 3. PRAME expression profile in lung and fallopian tube
PRAME QR005 exhibited strong, and weak to moderate cytoplasmic positivity in the ciliated pseudostratified columnar epithelium of the lung (A, IHC 200x) and fallopian tube (B, IHC 200x), respectively. Similar findings were observed using PRAME EP461 in the lung (C, IHC 200x) and fallopian tube (D, IHC 200x), with EP461 exhibiting an overall weaker staining pattern.

Discussion

- PRAME staining profile observed in this study and reported in the literature may be influenced by the number of specimens tested as well as antibody clone and assay used.
- QR005 and EP461 exhibit predominantly the same staining profile, and the differences observed are mainly due to the staining intensities detected and the analytical sensitivity of the clone and assay.
- New observations of staining profile in tonsil, fallopian tube, omentum and etc. (Table 3) need to be confirmed with more studies using larger sample sizes.

Conclusion:

- The staining profile of PRAME clones, QR005 and EP461, is predominantly comparable to literature for human normal tissue specimens.
- To the best of our knowledge, new staining profiles for normal tissue specimens are described in this study.
- A larger sample size of normal tissue specimens needs to be analyzed by IHC to confirm and define the staining profile of PRAME.

References

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MPUB0047 Rev A

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