Published in final edited form as:

Exp Mol Pathol. 2013 February ; 94(1): 29–32. doi:10.1016/j.yexmp.2012.05.008.

LMP2, a novel immunohistochemical marker to distinguish renal oncocytoma from the eosinophilic variant of chromophobe renal cell carcinoma

Gang Zheng, M.D., Ph.D., Alcides Chaux, M.D., Rajni Sharma, Ph.D., George Netto, M.D., and Patrizio Caturegli, M.D.

Department of Pathology, the Johns Hopkins Hospital, Baltimore, Maryland

Abstract

LMP2 is a subunit of the immunoproteasome that is overexpressed in oncocytic lesions of the thyroid gland. This study was designed to assess the expression profile and diagnostic utility of LMP2 in two renal oncocytic tumors that share similar morphologic features but have different clinical outcomes: renal oncocytoma (RO) and the eosinophilic variant of chromophobe renal cell carcinoma (CHRC-EO). A total of 56 RO, 38 classic CHRC, and 7 CHRC-EO cases, as well as 84 normal kidney controls, were selected from the Johns Hopkins surgical pathology archive and stained for LMP2 using a standard immunohistochemical protocol. Sections were scored for cellular location (nuclear versus cytosolic), intensity (from 0 to 3), and percent of area involved (from 0 to 100%), and an H score was calculated multiplying the intensity by the extent of the staining signal. The cytoplasmic expression of LMP2 was similar among the renal lesions, being present in 44 of 56 (79%) ROs, 27 of 38 (71%) CHRCs, and 7 of 7 (100%) CHRC-EO cases. The nuclear expression of LMP2, however, was more informative. All CHRC-EO cases (7 of 7, 100%) strongly showed nuclear LMP2 staining, as opposed to only 2 of 56 (4%, $P < 0.0001$) ROs and 9 of 38 (24%, $P = 0.0001$) classic CHRCs. These results suggest that the nuclear LMP2 expression can be used in clinical scenarios where histological distinction between RO and CHRC-EO remains challenging.

Keywords

LMP2; renal oncocytomas; chromophobe renal cell carcinoma

INTRODUCTION

Renal oncocytomas (RO) and the eosinophilic variant of chromophobe renal cell carcinoma (CHRC-EO) are sometimes difficult to differentiate histologically because of overlapping features. RO cells display dense granular eosinophilic cytoplasm and relatively bland nuclei with occasional degenerative atypia (Kuroda et al., 2003). CHRC cells typically show prominent cell membranes, pale cytoplasm, perinuclear halo, and occasional binucleation (Stec et al., 2009), but have an eosinophilic variant that has an abundant and granular

© 2012 Elsevier Inc. All rights reserved

Correspondence: Patrizio Caturegli, MD, MPH Johns Hopkins Pathology – Ross 656. 720 Rutland Avenue Baltimore, MD 21205 pcat@jhmi.edu Phone: 443-287-8911; Fax: 410-614-3548.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

cytoplasm similar to that of RO. The distinction between the two histologically similar entities is critical due to their different behavior and prognosis. RO is a benign tumor, although it can extend into the perinephric fat and the renal vein (Hes et al., 2008; Perez-Ordóñez et al., 1997). CHRCC, in contrast, is malignant, giving rise to metastasis (Renshaw et al., 1996) and undergoing necrosis or sarcomatoid differentiation (Abrahams et al., 2003).

Immunohistochemistry has been used in recent years to characterize protein markers that could aid in distinguishing RO from CHRCC (Liu et al., 2007). Examples include cadherin (Adley et al., 2006; Mazal et al., 2005), caveolin-1 (Garcia and Li, 2006), cytokeratin-7 (Carvalho et al., 2011; Memeo et al., 2007), c-kit (Carvalho et al., 2011; Memeo et al., 2007), PAX-2 (Memeo et al., 2007), claudin-7 and 8 (Osunkoya et al., 2009), MAGE-A3/4 and NYESO-1 (Demirovic et al., 2010). Although collectively these markers have improved the diagnostic sensitivity and specificity, a reliable marker that distinguishes RO from CHRCC is still missing. Goal of the study was to investigate the potential diagnostic utility of a novel proteasome marker: LMP2.

The constitutive proteasome degrades ubiquitin-tagged self and foreign proteins to generate peptides that are then presented on the cell surface in the context of MHC class I molecules (Navon and Ciechanover, 2009). It has a highly conserved barrel-shaped structure made of a 20S core and a 19S cap at either end. The 20S core is composed of 28 subunits arranged into four axially stacked rings. The two outer rings contain seven alpha subunits ($\alpha 1 - \alpha 7$) that participate in the assembly and regulation of the proteasome. The two inner rings contain seven beta subunits ($\beta 1 - \beta 7$) endowed with the proteolytic activity. In particular, $\beta 1$ has caspase-like activity, $\beta 2$ trypsin-like activity, and $\beta 5$ chymotrypsin-like activity. When a cell is exposed to pro-inflammatory stimuli like interferon-gamma ($\text{IFN}\gamma$) and tumor necrosis factor-alpha, the nascent proteasome replaces four of its elements: the 19S cap is replaced by a 11S cap (or PA28) and the three proteolytic beta subunits are replaced by $\text{i}\beta 1$ (LMP2), $\text{i}\beta 2$ (LMP10 or PSMB10), and $\text{i}\beta 5$ (LMP7 or PSMB8) (Angeles et al., 2012). This new structure, called immunoproteasome, is more proteolytically efficient and restricted in its cleavage specificity since it preferentially hydrolyzes proteins after non-polar amino acids (Gaczynska et al., 1994). The peptides produced by the immunoproteasome stimulate lymphocytes potently since their hydrophobic C-terminus fits perfectly in the groove of MHC class I molecules (Romero et al., 1991). The crystal structure of the immunoproteasome has recently been solved (Huber et al., 2012), and compounds that selectively inhibit its activity, like epoxyketone PR-957 (Muchamuel et al., 2009), are being developed for the treatment of auto-inflammatory conditions.

We have previously characterized a mouse model of Hashimoto thyroiditis induced by the transgenic expression of $\text{IFN}\gamma$ in the thyroid gland (Caturegli et al., 2000). In this model, the thyroid cells undergo an oncocytic metaplasia that resembles the Hürthle cells typically found in Hashimoto thyroiditis (Kimura et al., 2005; Kimura et al., 2009a; Kimura et al., 2009b). Using genetic and pharmacologic tools we have shown that this thyroid oncocytic transformation depends upon the over-expression of the immunoproteasome subunit LMP2 (Kimura et al., 2009a). Considering that oncocytes are found not only in the thyroid gland but also in other tissues such as the kidney (Guaraldi et al., 2011), we hypothesized that the overexpression of LMP2, rather than being unique to the thyroid oncocytes, is found in other oncocytic lesions. We therefore designed the presented study to assess by immunohistochemistry the expression profile of LMP2 in renal oncocytic lesions and evaluate its diagnostic potential in distinguishing RO from CHRCC.

MATERIALS & METHODS

Renal tissues

The study included 101 renal tumors, 84 derived from tissue microarrays and 17 from individual surgical pathology specimens, and 84 normal kidney controls for the microarray cases. The renal tumors included 56 ROs, 38 CHRCC, and 7 CHRCC-EO. They were identified through a search of the Johns Hopkins Surgical Pathology database spanning the years 1985–2011, and classified histologically according to the 2004 WHO classification (Eble et al., 2004). The study was approved from the Johns Hopkins Hospital Institutional Review Board for human subject research.

Immunohistochemical staining for LMP2

Immunohistochemical staining for LMP2 was developed on a Bond-Leica autostainer using a biotin free polymer detection systems, as per manufacturer's instructions (Leica Microsystems Inc., Bannockburn, IL). Briefly, formalin-fixed and paraffin-embedded tissue sections of the tumor with adjacent non-neoplastic kidney were first deparaffinized and rehydrated and then treated for 20 minutes in citrate buffer pH 6.0 for heat-induced antigen retrieval. Sections were then incubated for 30 minutes at room temperature with a rabbit polyclonal antibody directed against LMP2 (from Biomol International, L.P., Plymouth Meeting, PA), diluted 1:200 in PBS supplemented with 1% bovine serum albumin. After washing, sections were incubated for 8 minutes with the provided post-primary antibody, then washed and incubated for 8 minutes with polymer detection. Endogenous peroxidase was blocked by incubation with 3% hydrogen peroxide and color development obtained by addition of DAB substrate and hematoxylin counter-stain. Each immunohistochemistry experiment included two controls: a non-neoplastic kidney area stained with the LMP2 antibody, and a known LMP2 positive RO stained without addition of the LMP2 antibody.

Scoring and statistical analysis of the LMP2 staining

The LMP2 staining was analyzed based on cellular location (cytoplasmic or nuclear), intensity (from 0, negative, to 3, strongly positive), and percent of the area involved. A cytosolic and nuclear H score was derived multiplying the intensity of the staining by the percent involvement. H scores >0.1 were considered positive. Differences in the frequency distribution of positive staining were assessed by chi-squared test, considering significant p values smaller than 0.05. Analyses were performed using Stata statistical software, release 12 (Stata Corp., College Station, TX).

RESULTS

Cytoplasmic expression of LMP2

ROs expressed LMP2 in the cytoplasm in the majority of the cases (44 of 56, 79%), a frequency significantly higher than observed in normal kidney (8 of 84, 10%, Table 1). This staining ranged from low (18 of 56, 32%, Figure 1A), to moderate (16 of 56, 29%, Figure 1B), to high intensity (10 of 56, 18%, Figure 1C), and was easily distinguishable from the scattered and weak staining found in a minority of normal controls (Figure 1D, the inset shows the negative control where no LMP2 antibody was added during the primary incubation, indicating lack of endogenous biotin staining). Cytoplasmic LMP2 positivity was greatest in the CHRCC-EO, where all 7 cases (100%) stained positive, most of them at high intensity, and lowest in CHRCC (27 of 38, 71%, Table 1). Overall these results indicate that oncocytic lesions of the kidney, similarly to those of the thyroid (Kimura et al., 2009a), express LMP2 in the cytoplasm. The expression pattern, however, is not adequate to distinguish in the clinical setting RO from CHRCC-EO.

Nuclear expression of LMP2

LMP2 was expressed less commonly in the nucleus but with a more revealing profile. All CHRCC-EO cases (7 of 7, 100%, Figures 2A) strongly LMP2 nuclear expression, as opposed to only 2 of 56 (4%, $p < 0.0001$, Figures 2B) ROs, 9 of 38 (24%, $p = 0.001$, Figure 2C) CHRCC, and 0 of 84 normal kidney controls (Table 1). The sensitivity and specificity of a positive nuclear LMP2 staining in distinguishing CHRCC-EO from RO were 100% and 98% respectively, suggesting that this staining can be useful in clinical scenarios where the H&E histologic appearance does not allow the distinction between these two tumor entities.

DISCUSSION

RO and CHRCC are thought to originate from intercalating cells of distal tubuli of the kidney and share a genetic and morphologic continuum. This makes a distinction purely based on H&E microscopic appearance difficult, especially between RO and CHRCC-EO. This distinction, however, is critical for patient care since the two tumors have markedly different biological courses: benign for RO and malignant for CHRCC. To aid in this distinction, numerous protein and DNA markers have been developed, achieving satisfactory results when used as a panel. For example, the combination of S100A1 positivity and focal CK7 expression distinguished RO from CHRCC with 91% sensitivity and 93% specificity (Carvalho et al., 2011). Similarly, Memeo and colleagues reported that the CK7-/KIT+/PAX2+ expression profile orients toward RO whereas CK7+/KIT+/PAX2- toward CHRCC (Memeo et al., 2007). Loss of chromosomes 1, 2, 6, 10, or 13 associates with CHRCC-EO, whereas complete or partial loss of chromosome 1 is typical of ROs. Gene expression profiles and high-throughput SNP genotyping were also shown to effectively distinguish CHRCC from RO (Toro et al., 2008). Nevertheless, additional biomarkers are desirable, especially if they can be used individually.

Our study first reports that the nuclear expression of LMP2 is accurate (100% specificity and 98% sensitivity) in distinguishing RO from its histological mimicker CHRCC-EO. The nuclear staining was universal in the CHRCC-EO and rare in ROs. It is important, however, to note that the small sample size of CHRCC-EO cases suggests caution and requires validation. Given the nuclear LMP2 positivity in about a quarter of conventional (not eosinophilic variant) CHRCC cases, it may be also advisable to combine LMP2 with additional biomarkers to improve the overall diagnostic accuracy RO, CHRCC-EO, and CHRCC. It will additionally be interesting to assess LMP2 nuclear expression in tumors displaying both oncocytoma and chromophobe RCC features (the so-called hybrid tumors), as described in patients with Birt-Hogg-Dube syndrome (Toro et al., 2008).

The mechanism and significance of nuclear LMP2 expression remain to be elucidated. The immunoproteasome is fundamental in immunology since it degrades cytosolic proteins into short peptides that are then going to be presented in the groove of MHC class I molecules. The nuclear expression could also be related to immune functions. Recently, it has been reported that the expression of the immunoproteasome in both liver cytosol and nucleus is directly proportional to the degree of chronic inflammation in patients with chronic active hepatitis or cirrhosis (French et al., 2011). But non-immune functions of the immunoproteasome are appearing in the literature, including relieve of oxidative stress, control of muscle mass, and regulation of tumor growth (Angeles et al., 2012). Like the constitutive proteasome, which has become an important pharmaceutical target for cancer therapies, see for example the use in multiple myeloma of Bortezomib (Velcade) an inhibitor of the $\beta 5$ subunit, the immunoproteasome is emerging as a novel therapeutic target for auto-inflammatory and neoplastic conditions (Angeles et al., 2012). It is possible that the nuclear expression of LMP2 in CHRCC-EO mediates non-immune functions and that specific LMP2 inhibitors could be useful in treating this form of cancer.

In summary, we report the expression of LMP2 in patients with renal oncocytomas and chromophobe renal cell carcinomas, and suggest its use as an aid in the differential diagnosis of these neoplasias.

Acknowledgments

The study was supported by NIH grant DK55670 to PC, and by the Joseph C. Eggleston research grant from the Johns Hopkins department of Pathology to GZ.

REFERENCES

- Abrahams NA, Ayala AG, Czerniak B. Chromophobe renal cell carcinoma with sarcomatoid transformation. *Ann Diagn Pathol*. 2003; 7:296–9. [PubMed: 14571432]
- Adley BP, Gupta A, Lin F, Luan C, Teh BT, Yang XJ. Expression of kidney-specific cadherin in chromophobe renal cell carcinoma and renal oncocytoma. *Am J Clin Pathol*. 2006; 126:79–85. [PubMed: 16753598]
- Angeles A, Fung G, Luo H. Immune and non-immune functions of the immunoproteasome. *Front Biosci*. 2012; 17:1904–16. [PubMed: 22201844]
- Carvalho JC, Wasco MJ, Kunju LP, Thomas DG, Shah RB. Cluster analysis of immunohistochemical profiles delineates CK7, vimentin, S100A1 and C-kit (CD117) as an optimal panel in the differential diagnosis of renal oncocytoma from its mimics. *Histopathology*. 2011; 58:169–79. [PubMed: 21323945]
- Caturegli P, Hejazi M, Suzuki K, Dohan O, Carrasco N, Kohn LD, Rose NR. Hypothyroidism in transgenic mice expressing IFN-gamma in the thyroid. *Proc Natl Acad Sci U S A*. 2000; 97:1719–24. [PubMed: 10677524]
- Demirovic A, Dzombeta T, Tomas D, Spajic B, Pavic I, Hudolin T, Milosevic M, Cupic H, Kruslin B. Immunohistochemical expression of tumor antigens MAGE-A3/4 and NY-ESO-1 in renal oncocytoma and chromophobe renal cell carcinoma. *Pathol Res Pract*. 2010; 206:695–9. [PubMed: 20591578]
- Eble, JN.; Sauter, G.; Epstein, JI. Pathology and genetics of tumours of the urinary system and male genital organs. WHO Classification of Tumours. IARC Press; Lyon: 2004.
- French BA, Oliva J, Bardag-Gorce F, French SW. The immunoproteasome in steatohepatitis: its role in Mallory-Denk body formation. *Exp Mol Pathol*. 2011; 90:252–6. [PubMed: 21256843]
- Gaczynska M, Rock KL, Spies T, Goldberg AL. Peptidase activities of proteasomes are differentially regulated by the major histocompatibility complex-encoded genes for LMP2 and LMP7. *Proc Natl Acad Sci U S A*. 1994; 91:9213–7. [PubMed: 7937744]
- Garcia E, Li M. Caveolin-1 immunohistochemical analysis in differentiating chromophobe renal cell carcinoma from renal oncocytoma. *Am J Clin Pathol*. 2006; 125:392–8. [PubMed: 16613342]
- Guaraldi F, Zang G, Dackiw AP, Caturegli P. Oncocytic mania: a review of oncocytic lesions throughout the body. *J Endocrinol Invest*. 2011; 34:383–94. [PubMed: 21301204]
- Hes O, Michal M, Sima R, Vanecek T, Brunelli M, Martignoni G, Kuroda N, Alvarado Cabrero I, Perez-Montiel D, Hora M, Urge T, Dvorak M, Jarosova M, Yang X. Renal oncocytoma with and without intravascular extension into the branches of renal vein have the same morphological, immunohistochemical, and genetic features. *Virchows Arch*. 2008; 452:193–200. [PubMed: 18066590]
- Huber EM, Basler M, Schwab R, Heinemeyer W, Kirk CJ, Groettrup M, Groll M. Immuno- and constitutive proteasome crystal structures reveal differences in substrate and inhibitor specificity. *Cell*. 2012; 148:727–38. [PubMed: 22341445]
- Kimura H, Kimura M, Westra WH, Rose NR, Caturegli P. Increased thyroidal fat and goitrous hypothyroidism induced by interferon-gamma. *Int J Exp Pathol*. 2005; 86:97–106. [PubMed: 15810981]
- Kimura HJ, Chen CY, Tzou SC, Rocchi R, Landek-Salgado MA, Suzuki K, Kimura M, Rose NR, Caturegli P. Immunoproteasome overexpression underlies the pathogenesis of thyroid oncocytes

- and primary hypothyroidism: studies in humans and mice. *PLoS One*. 2009a; 4:e7857. [PubMed: 19924240]
- Kimura HJ, Rocchi R, Landek-Salgado MA, Suzuki K, Chen CY, Kimura M, Rose NR, Caturegli P. Influence of signal transducer and activator of transcription-1 signaling on thyroid morphology and function. *Endocrinology*. 2009b; 150:3409–16. [PubMed: 19325004]
- Kuroda N, Toi M, Hiroi M, Shuin T, Enzan H. Review of renal oncocytoma with focus on clinical and pathobiological aspects. *Histol Histopathol*. 2003; 18:935–42. [PubMed: 12792905]
- Liu L, Qian J, Singh H, Meiers I, Zhou X, Bostwick DG. Immunohistochemical analysis of chromophobe renal cell carcinoma, renal oncocytoma, and clear cell carcinoma: an optimal and practical panel for differential diagnosis. *Arch Pathol Lab Med*. 2007; 131:1290–7. [PubMed: 17683191]
- Mazal PR, Exner M, Haitel A, Krieger S, Thomson RB, Aronson PS, Susani M. Expression of kidney-specific cadherin distinguishes chromophobe renal cell carcinoma from renal oncocytoma. *Hum Pathol*. 2005; 36:22–8. [PubMed: 15712178]
- Memeo L, Jhang J, Assaad AM, McKiernan JM, Murty VV, Hibshoosh H, Tong GX, Mansukhani MM. Immunohistochemical analysis for cytokeratin 7, KIT, and PAX2: value in the differential diagnosis of chromophobe cell carcinoma. *Am J Clin Pathol*. 2007; 127:225–9. [PubMed: 17210525]
- Muchamuel T, Basler M, Aujay MA, Suzuki E, Kalim KW, Lauer C, Sylvain C, Ring ER, Shields J, Jiang J, Shwonek P, Parlati F, Demo SD, Bennett MK, Kirk CJ, Groettrup M. A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. *Nat Med*. 2009; 15:781–7. [PubMed: 19525961]
- Navon A, Ciechanover A. The 26 S proteasome: from basic mechanisms to drug targeting. *J Biol Chem*. 2009; 284:33713–8. [PubMed: 19812037]
- Osunkoya AO, Cohen C, Lawson D, Picken MM, Amin MB, Young AN. Claudin-7 and claudin-8: immunohistochemical markers for the differential diagnosis of chromophobe renal cell carcinoma and renal oncocytoma. *Hum Pathol*. 2009; 40:206–10. [PubMed: 18799195]
- Perez-Ordóñez B, Hamed G, Campbell S, Erlandson RA, Russo P, Gaudin PB, Reuter VE. Renal oncocytoma: a clinicopathologic study of 70 cases. *Am J Surg Pathol*. 1997; 21:871–83. [PubMed: 9255250]
- Renshaw AA, Henske EP, Loughlin KR, Shapiro C, Weinberg DS. Aggressive variants of chromophobe renal cell carcinoma. *Cancer*. 1996; 78:1756–61. [PubMed: 8859189]
- Romero P, Corradin G, Luescher IF, Maryanski JL. H-2Kd-restricted antigenic peptides share a simple binding motif. *J Exp Med*. 1991; 174:603–12. [PubMed: 1714934]
- Stec R, Grala B, Maczewski M, Bodnar L, Szczylik C. Chromophobe renal cell cancer--review of the literature and potential methods of treating metastatic disease. *J Exp Clin Cancer Res*. 2009; 28:134. [PubMed: 19811659]
- Toro JR, Wei MH, Glenn GM, Weinreich M, Toure O, Vocke C, Turner M, Choyke P, Merino MJ, Pinto PA, Steinberg SM, Schmidt LS, Linehan WM. BHD mutations, clinical and molecular genetic investigations of Birt-Hogg-Dube syndrome: a new series of 50 families and a review of published reports. *J Med Genet*. 2008; 45:321–31. [PubMed: 18234728]

Highlights

- Distinguishing renal oncocytoma from chromophobe renal cell carcinoma can be challenging
- LMP2 is an immunoproteasome subunit overexpressed in thyroid oncocytic lesions
- LMP2 nuclear expression was prominent in chromophobe renal cell carcinoma
- LMP2 nuclear expression was rare in renal oncocytoma
- LMP2 is a novel marker to aid in the differential diagnosis of these two conditions

Figure 1. Cytoplasmic LMP2 immunostaining in renal oncocytomas. Examples of low (panel A), moderate (panel B), and high intensity (panel C) staining. A normal, non-neoplastic kidney is indicated in panel D. The inset shows a control without addition of the LMP2 antibody. All magnifications are $\times 160$.

Figure 2.

Nuclear LMP2 immunostaining, and matched hematoxylin and eosin stain, in the eosinophilic variant of chromophobe renal cell carcinoma (CHRC-EO) (panels A and B), renal oncocytoma (RO) (panels C and D), and classic chromophobe renal cell carcinoma (CHRC) (panels E and F) (magnification $\times 160$)

Table 1

Frequency of cytoplasmic and nuclear LMP2 staining in renal oncocytomas (RO), chromophobe renal cell carcinoma (CHRC), eosinophilic variant of CHRC (CHRC-EO), and normal kidney controls (N= 84).

	Total cases	N (%) positive for cytosolic LMP2	N (%) positive for nuclear LMP2
Normal kidney	84	8 (10%)	0 (0%)
CHRC	38	27(71%)	9 (24%)
RO	56	44 (79%)	2 (4%)
CHRC-EO	7	7 (100%)	7 (100%)