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

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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 (CHRCC-EO). A total of 56 RO, 38 classic CHRCC, and 7 CHRCC-EO cases, as well 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%) CHRCCs, and 7 of 7 (100%) CHRCC-EO cases. The nuclear expression of LMP2, however, was more informative. All CHRCC-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 CHRCCs. These results suggest that the nuclear LMP2 expression can be used in clinical scenarios where histological distinction between RO and CHRCC-EO remains challenging.

Introduction

Renal oncocytomas (RO) and the eosinophilic variant of chromophobe renal cell carcinoma (CHRCC-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). CHRCC 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 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-Ordonez 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. The 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 ($\alpha1$–$\alpha7$) that participate in the assembly and regulation of the proteasome. The two inner rings contain seven beta subunits ($\beta1$–$\beta7$) endowed with the proteolytic activity. In particular, $\beta1$ has caspase-like activity, $\beta2$ trypsin-like activity, and $\beta5$ chymotrypsin-like activity. When a cell is exposed to pro-inflammatory stimuli like interferon-gamma (IFNγ) 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 i$\beta1$ (LMP2), i$\beta2$ (LMP10 or PSMB10), and i$\beta5$ (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

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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 IFNγ 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, 2009a, 2009b). Using genetic and pharmacologic tools we have shown that this thyroid oncocytic transformation depends upon the overexpression 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 present 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 and 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 min in citrate buffer pH 6.0 for heat-induced antigen retrieval. Sections were then incubated for 30 min 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 min with the provided post-primary antibody, then washed and incubated for 8 min with polymer detection. Endogenous peroxidase was blocked by incubation with 3% hydrogen peroxide and color development obtained by the 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 the 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 that observed in normal kidney (8 of 84, 10%, Table 1). This staining ranged from low (18 of 56, 32%, Fig. 1A), to moderate (16 of 56, 29%, Fig. 1B), to high intensity (10 of 56, 18%, Fig. 1C), and was easily distinguishable from the scattered and weak staining found in a minority of normal controls (Fig. 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, were 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%, Fig. 2A) strongly LMP2 nuclear expression, as opposed to only 2 of 56 (4%, $P<0.0001$, Fig. 2B) ROs, 9 of 38 (24%, $P=0.001$, Fig. 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 CHRC-EO, whereas complete or partial loss of chromosome 1 is typical of ROs. Gene expression profiles

Table 1
Frequency of cytoplasmic and nuclear LMP2 staining in renal oncocytomas (RO), chromophobe renal cell carcinoma (CHRCC), eosinophilic variant of CHRCC (CHRCC-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%)
CHRCC	38	27 (71%)	9 (24%)
RO	56	44 (79%)	2 (4%)
CHRCC-EO	7	7 (100%)	7 (100%)