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ANATOMICAL PATHOLOGY

Leptin and its receptor: can they help to differentiate chromophobe renal cell carcinoma from renal oncocytoma?

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Summary

One of the challenges in differentiating chromophobe renal cell carcinoma (chRCC) from benign renal oncocytoma (RO) is overlapping morphology between the two subtypes. The aim of this study was to investigate the usefulness of expression of leptin (Ob) and its receptor (ObR) in discriminating chRCC from RO. Sections from paraffin-embedded, formalin-fixed tumour nephrectomy specimens of 45 patients, made up of 30 chRCC (15 eosinophilic variant and 15 non-eosinophilic variant) and 15 RO, were used in this study. Samples (30) of clear cell RCC (ccRCC), the most common histological subtype, were used to verify staining patterns found by others in our cohort of Australasian patients. Matched morphologically normal non-cancer kidney tissues were included for each specimen. Sections were batch-immunostained using antibodies against Ob and ObR. Stained sections were digitally scanned using Aperio ImageScope, and the expression pattern of Ob and ObR was studied. In this cohort, male to female ratio was 2:1; median age was 64 (45–88 years); and median tumour size was 3.8 cm (range 1.2–18 cm). There were 47 (62.7%) T1, seven T2, 20 T3 and one T4 stage RCC. Two patients with ccRCC presented with metastases. Nuclear expression of Ob was significantly higher in RO compared with chRCC. The increased nuclear expression of Ob in RO compared with chRCC may be a useful aid in the difficult histological differentiation of RO from chRCC, especially eosinophilic variants of chRCC.

Key words: Renal cell carcinoma; chromophobe; oncocytoma; immunohistochemistry; leptin; leptin receptor; kidney.

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INTRODUCTION

Diagnostic molecular biomarkers which can accurately distinguish benign from malignant renal tumours can improve accuracy of pre-operative biopsies. The aim of this study was to investigate the expression and expression patterns of leptin (Ob) and leptin receptor (ObR) across various renal cell carcinoma (RCC) subtypes, and to determine whether Ob/ObR can improve upon current immunohistochemistry (IHC) clinical diagnostic panels for chromophobe RCC (chRCC) and renal oncocytoma (RO). Improving the sensitivity of IHC markers has the potential benefit of reducing unnecessary surgery, thereby preserving nephron mass and subsequently reducing risk of chronic kidney disease with its associated cardiovascular mortality. The histopathological analysis of certain subtypes of renal tumours is difficult when morphological features overlap. The distinction of malignant chRCC from benign RO is one such diagnostic dilemma that can pose significant difficulties to pathologists.[1,2] Accurate diagnosis of the pathological specimens is crucial to dictate further surveillance and potential management for chRCC compared with RO cases, where an expectant approach is sufficient. While select biomarkers, such as cytokeratin 7 (CK7), have shown promise in their ability to distinguish between RO and chRCC, there is a lack of consensus among pathologists regarding cut-off thresholds to distinguish between these tumour phenotypes.[3] Furthermore, when it comes to eosinophilic variants of chRCC, it remains challenging to distinguish these tumours from RO, even with CK7 staining.[3] Therefore, novel and reproducible effective biomarkers which can aid in the differential diagnoses of chRCC, including eosinophilic variants from RO are still needed.

Ob is a hormone secreted by adipose cells that helps to regulate energy balance by inhibiting hunger. Obesity is a known risk factor for RCC.[4,5] Some studies suggest that Ob plays a role in carcinogenesis through cell proliferation, angiogenesis, apoptotic inhibition and proinflammatory effects.[6–8] Ob acts through its receptor (ObR), a single-transmembrane-domain receptor of the cytokine receptor family. Increased Ob/ObR signalling is a risk factor for RCC[9] and promotes renal cancer cell invasion and metastasis.[10–12] These reports are typically based on clear cell RCC (ccRCC), the most common subtype of RCC. The aim of this study was

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to investigate the usefulness of Ob and/or ObR, as potential biomarkers to differentiate chRCC and RO. The secondary aim was to establish Ob and ObR expression in ccRCC, the most common histological subtype in our cohort of Australasian patients.

MATERIALS AND METHODS

Patient samples

Archived paraffin blocks from human renal tumour tissue, from 2009 to 2014, were obtained from Aquesta Pathology, Toowong, Australia. Ethics approval for scientific use of archived pathology samples was obtained from Aquesta Pathology Ethics Committee (protocol 14/02). Seventy-five specimens were from patients who underwent RCC tumour nephrectomy (radical or partial) at various hospital centres in Brisbane. Each of the sections from these blocks had morphologically normal non-cancerous kidney as well as the tumour, which were evaluated by a highly trained uropathologist and diagnosis was achieved using H&E staining (Fig. 1). Ratio of males:females was 2:1, with a median age of 64 years (range 45–88), in concordance with more RCC in males than females,[13] and most patients being in the 50–60 year age group. The median renal tumour size from this series was 3.8 cm (range 1.2–18). The 75 specimens comprised 30 ccRCC, 30 chRCC (15 eosinophilic variant and 15 non-eosinophilic variant) and 15 RO. Although the ultimate aim was identifying IHC biomarkers that differentiated chRCC and RO, ccRCC samples were included as the most common RCC, with distinct morphological features from chRCC and RO, and for which there were previous publications showing that Ob and ObR are expressed in ccRCC.[1,2,6–8] The low number (15 cases) of RO was because RO account for only approximately 5% of all adult renal tumours[14] and fewer samples were available from Aquesta Pathology for RO in this time period. Using the pathological stage at presentation, there were 47 patients (62.7%) in pT1, seven in pT2, 20 in pT3 and one pT4 stage RCC. Two patients with ccRCC presented with metastases. The trend of patients presenting with smaller confined tumours in T1 stage is due to increasing detection rates for incidental renal tumours from widespread availability of radiological imaging; similar to other published series.[15] These data are summarised in Table 1.

Table 1 Clinicopathological characteristics of this cohort of patients

Characteristics	
Patients	75
Period	2009–2014
Gender (male: female)	49: 26
Age, median years (range)	64 (18–88)
Size, median cm (range)	3.8 (1.2–18)
Subtype	
ccRCC	30
chRCC	30
RO	15
Stage	
T1	47 (62.7%)
T2	7 (9.3%)
T3	20 (26.7%)
T4	1 (1.3%)
Stage M1	2 (2.67%)
Fuhrman (ccRCC)	
Grade 2	63.3%
Grade 3	20.0%
Grade 4	16.7%

ccRCC, clear cell RCC; chRCC, chromophobe RCC; M1, metastasis; RO, renal oncocytoma.

Immunohistochemistry

The antibodies were from Santa Cruz Biotechnology (Dallas, USA). Paraffin sections (3 μM) were immunostained for Ob (anti-rabbit; #sc-842; 1:60) and ObR (anti-goat; #sc-1834; 1:50) using a Ventana Discovery ULTRA autostainer (Ventana Medical Systems, Roche, USA) and appropriate Ventana prediluted secondary antibodies plus an ultraView Universal diaminobenzidine hydrochloride (DAB) detection kit. The slides were counterstained with haematoxylin then dehydrated through ascending graded alcohols, cleared in xylene and mounted using DePex. Tissue array of human liver, kidney and gut was used for antibody optimisation and positive control. Negative controls without primary antibody were prepared for each batch stain. The sections were batch stained to allow comparison across slides.

Morphometry

Slides stained using IHC were scanned at ×20 with an Aperio ScanScope XT slide scanning system (Aperio Technologies, USA), and the digital images of the sections were analysed using Aperio ImageScope software (Leica Biosystems, Germany). Quantitative scoring of expression intensity and localisation of Ob and ObR was analysed with respect to overall, nuclear and membrane expression. Staining (positive pixels %) was scored according to the intensity and percentage of cells stained. The intensity output for the Aperio Positive Pixel Count v9 algorithm was determined as number of negative or positive pixels. Overall positive pixels (%) were calculated. The output for the Aperio Nuclear v1 algorithm was given as % pixels with 0, 1+, 2+ or 3+ staining intensity. Nuclear positive pixels (%) were calculated by adding the values for 2+ and 3+ staining. Nuclear expression in the tumour tissue was normalised against paired morphologically normal regions. These data were expressed as the percentage of overall normal values. The output for the Aperio Membrane v1 algorithm was determined as percentage of pixels with 0, 1+, 2+ or 3+ staining intensity. Membrane positive pixels (%) were calculated by adding the values for 2+ % and 3+ % staining.

All the intensity results (overall, nuclear and membrane) were calculated on Excel. These results of normal kidney (% change in overall, nuclear and membrane) and tumour (% change in overall, nuclear and membrane) were then tabulated and analysed with GraphPad Prism 6 (GraphPad Software). Graphs were generated to show the % expression change for tumour versus

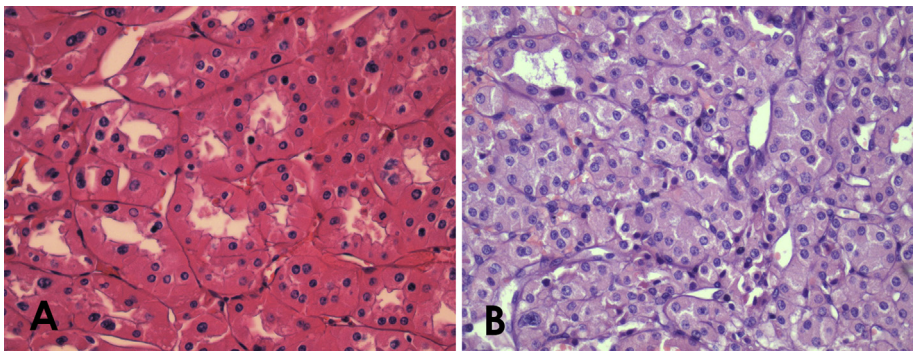

Fig. 1 Histopathology of chromophobe renal cell carcinoma and renal oncocytoma. (A) H&E stained section of an example of eosinophilic variant of chromophobe renal cell carcinoma, showing typical dense eosinophilic polygonal cells with prominent cell membranes. Nuclei tend to be irregular and wrinkled, and cells are sometimes binucleated. Perinuclear clearing can be prominent. (B) H&E stained section of an example of renal oncocytoma, showing large oncocytes with densely eosinophilic cytoplasm. Cells are round to polygonal and nuclei are round and monotonous. Nucleoli are small and inconspicuous.

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