Pathology (■ 2018) ■(■), pp. 1–8

ANATOMICAL PATHOLOGY

Nuclear factor-kappa B subunits and their prognostic cancer-specific survival value in renal cell carcinoma patients

Keng Lim Ng[1,2,3,*], Ning Yi Yap[2,*], Retnagowri Rajandram[2,3,4], David Small[3], Jayalakshmi Pailoor[5], Teng Aik Ong[2], Azad Hassan Razack[2], Simon Thomas Wood[1], Christudas Morais[3], Glenda Carolyn Gobe[3]

[1]*Department of Urology, Princess Alexandra Hospital, Brisbane, Qld, Australia;* [2]*Department of Surgery, University of Malaya, Kuala Lumpur, Malaysia;* [3]*Centre for Kidney Disease Research, University of Queensland, Brisbane Australia;* [4]*University of Malaya Cancer Research Institute, University of Malaya, Kuala Lumpur, Malaysia;* [5]*Department of Pathology, University of Malaya, Kuala Lumpur, Malaysia;* [*]*these authors contributed equally*

Summary

Better characterisation and understanding of renal cell carcinoma (RCC) development and progression lead to better diagnosis and clinical outcomes. In this study, expression of nuclear factor-kappa B (NF-κB) subunits: p65 (RelA), p105/p50, p100/p52, and cRel in RCC tissue were compared with corresponding normal kidney, along with tumour characteristics and survival outcome. Ninety-six cases of RCC with paired normal kidney were analysed. Clinicopathological data, demographics and survival data were available. Immunohistochemistry (IHC) for NF-κB subtypes was analysed using the Aperio digital pathology system for overall cellular expression and localisation. The prognostic cancer-specific survival value of the subunits in RCC patients was analysed. Approximately 50% of patients had clinical stage T1, with 22 patients having metastases at presentation. RCC subtypes were: clear cell (n = 76); papillary (n = 11); chromophobe (n = 5); clear cell tubulopapillary (n = 3); and one multilocular cystic RCC. Median follow up was 54.5 months (0.2–135), with 28 deaths at time of analysis. NF-κB p65 had higher overall and nuclear expressions, with lower overall and nuclear expressions of p50, p52 and cRel in RCC compared with normal kidney. Higher expressions of p65 (nuclear), p52 (overall and nuclear) and p50 (overall) correlated significantly with worse cancer-specific survival. This is the first large series of analysis of expression of NF-κB subunits in RCC. Especially with regards to the less studied subunits (p52, p50, cRel), our results allow a better understanding the role of NF-κB in RCC development and progression, and may pave the way for future targeted NF-κB subunit specific therapies.

Key words: Cancer specific survival; nuclear factor-kappa B; renal cell carcinoma.

Received 27 January, revised 28 February, accepted 7 March 2018
Available online: xxx

INTRODUCTION

Renal cell carcinoma (RCC) constitutes 90–95% of kidney malignancies and the worldwide incidence is increasing.[1] The most commonly encountered RCC subtypes are clear cell (70–80%), papillary (10%), chromophobe (5%) and collecting duct RCC (1%). Nephrectomy for localised RCC remains the best mode of management and offers excellent prognosis. However, metastatic RCC is resistant to cell apoptosis induced by chemotherapy and radiotherapy.[2] Based on the understanding that most clear cell RCC (ccRCC) have von Hippel–Lindau (VHL) gene aberrations, the current therapies for metastatic RCC include tyrosine kinase inhibitors (TKI) and mammalian target of rapamycin (mTOR) inhibitors which target the pVHL (VHL protein)-mediated defective pathways. However, most patients eventually develop resistance to these therapies. The molecular mechanisms of tumour pathogenesis and resistance to therapy are still not well elucidated in RCC.[3]

The transcription factor nuclear factor-kappa B (NF-κB) is upregulated in response to VHL aberrations. NF-κB upregulation has been implicated in many cancers, including renal cell carcinoma. The NF-κB family of proteins comprises five mammalian subunits: p65 (RelA), NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelB and cRel. The NF-κB subunits may form homo or heterodimers, and regulate inflammation, angiogenesis, immunity, cell proliferation and apoptosis.[2] NF-κB is sequestered in the cytoplasm in an inactive form, bound to one of the inhibitor of κB (IκB) molecules, such as IκB-α, IκB-β, IκB-γ, IκB-ε, p100, p102 or Bcl-3.[2] Upon degradation of IκB, NF-κB is released and translocated to the nucleus, where it binds to κB elements and activate processes involved in oncogenesis, for example proliferation, angiogenesis, metastasis and drug resistance.

The p65 subunit is the most well studied NF-κB subunit in cancers, including RCC. In RCC tissue, p65 expression is upregulated and constitutively activated.[4,5] In addition, both NF-κB p65 and p50 have both been correlated with apoptotic and proliferation markers in RCC.[6] Activation of NF-κB

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2018.03.003

Please cite this article in press as: Ng KL, et al., Nuclear factor-kappa B subunits and their prognostic cancer-specific survival value in renal cell carcinoma patients, Pathology (2018), https://doi.org/10.1016/j.pathol.2018.03.003

induces anti-apoptotic factors such as inhibitors of apoptosis (IAPs) or the anti-apoptotic Bcl-2 family of proteins.[7,8] NF-κB activity is enhanced in the absence of functional pVHL and furthermore, expression of NF-κB p65 is associated with vascular endothelial growth factor (VEGF) in clear cell RCC (ccRCC), the most common of the RCC.[5,9,10] Hence, the NF-κB family likely plays an important role in the development and progression of RCC. However, the prognostic implications of NF-κB in RCC are contradictory, based on previous research.[4–6,10–12]

Currently to our knowledge, there is no published literature on NF-κB2 (p100/p52), RelB and cRel expression in human RCC tissue samples, and their prognostic value has not been investigated. Additionally, most studies did not assess the survival outcome of RCC patients in relation to NF-κB expression. In this study, we aimed to report the expression of p65 (RelA), NF-κB1 (p105/p50), NF-κB2 (p100/p52), cRel in RCC tissue in comparison with the corresponding normal kidney. The association of these NF-κB subunits with the tumour characteristics and survival outcome was also evaluated.

METHODS

Patients and data collection

Ethics approval for retrospective and prospective tissue collection was obtained from the University Malaya Ethics Committee (Ref: 848.17). The present study examined 96 cases of formalin-fixed, paraffin-embedded RCC tissue with paired normal kidney from patients who had undergone nephrectomy in the University of Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia, from 2003–2013. The clinicopathological data and demographics of patients were collected from UMMC online database and patient medical records. Survival information was acquired from patient medical records or the National Registration Department, Malaysia. All data were handled with strict confidentiality.

Immunohistochemistry (NF-κB subunits)

Histology sections for each specimen were stained with haematoxylin and eosin (H&E) for general morphology and pathological analysis. For immunohistochemistry, primary antibodies were rabbit anti-human antibodies from Santa Cruz (USA): p50 (sc-7178, dilution 1:100), p52 (sc-298, 1:100), p65 (sc-372, 1:150), RelB (sc-226, 1:100) and cRel (sc-71, 1:400). Antibodies were diluted in 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS). Sections were cut onto Superfrost Plus slides (ThermoFisher, USA) and then antigen retrieval was performed in a decloaking chamber (Biocare Medical, USA) using ethylenediaminetetraacetic acid (EDTA)/Tris buffer (1 mM/0.01 M, pH 9.0) for 15 min at 105°C for p50, p52, RelB and cRel, or citrate buffer (0.1 M, pH 6) at 125°C for 5 min for p65. After antigen retrieval, non-specific binding of peroxidase or antibody was blocked with 3% H_2O_2 in TBS and Background Sniper (Biocare Medical, USA). The positive control for all antibodies was human tonsil and negative controls without primary antibodies were used for each batch stain. The detection kit used was MACH 1 Universal HRP-Polymer Detection (Biocare Medical, USA) with diaminobenzidine hydrochloride (DAB) as the chromogen. Sections were counterstained with haematoxylin, dehydrated in a series of ethanol, cleared in xylene and mounted in Depex with glass coverslips.

Morphometry

Stained slides were scanned in an Aperio ScanScope XT slide scanning system (AperioTechnologies, USA) at ×20 magnification. Digital images of the sections were viewed using Aperio ImageScope software (Leica Biosystems, Germany). Three random fields of the same size were selected for each RCC or paired normal kidney section. Analysis was carried out using the Positive Pixel Count v9 algorithm (for total staining intensity) and IHC Nuclear v1 algorithm (for nuclear staining) from the Aperio ImageScope software. Staining (positive pixels %) was scored according to the intensity and percentage of cells stained. The intensity of the staining calculated as overall expression was analysed by the Positive Pixel Count v9 algorithm of Aperio. Nuclear expression was analysed with the Nuclear v1 algorithm of Aperio. For survival analysis, the median positive pixel score was used to determine cut-off scores for 'high' or 'low' staining for each protein and calculated with Kaplan–Meier analysis.

Statistical analysis

Statistical analysis was performed using SPSS Statistics v20 (IBM, USA). Data analysis was carried out using Student's t-test or analysis of variance (ANOVA) to determine the difference in positive pixels (%) or staining intensity between groups. Survival curves were obtained from the Kaplan–Meier and survival differences between groups were evaluated using the log rank test. The Cox proportional hazards regression was used to analyse proteins that showed significance in the log rank test.

RESULTS

Table 1 shows clinical and pathological data from the 96 renal tumour specimens. Two-thirds of patients were males who underwent nephrectomy in the UMMC between 2003 and 2013 (male:female, approximately 2:1). Median age was 62 years (range 39–83) with median renal tumour size of 6 cm (1.5–17). Approximately half the patients presented with clinical stage T1, with 22 patients (22.9%) having metastatic RCC at presentation. As expected, the majority of the tumour pathological subtypes was ccRCC (n = 76), followed by papillary (pRCC) (n = 11), chromophobe (chRCC) (n = 5), clear cell tubulopapillary (ccpRCC) (n = 3) and one multilocular cystic RCC (mcRCC). Median follow up for these RCC patients was 54.5 months (0.2–135), with 28 deaths recorded at time of analysis.

Summary of the statistical analyses of NF-κB in RCC tissue is included in Table 2.

p65 subunit analysis

IHC staining characteristics of the p65 subunit in normal and renal tumour tissues are shown in Fig. 1. The overall and nuclear expression of p65 in ccRCC (Fig. 1A), papillary (Fig. 1B) and chromophobe (Fig. 1C) subtypes were significantly higher when compared with matched non-neoplastic regions. A representative image is shown in Fig. 1D. The papillary subtype showed the highest expression. However, the expression pattern did not correlate with clinical T stage, tumour grade or metastases. With regards to cancer-specific

Table 1 Clinical and pathological data

Patients/tumours	*n*
Total patients	96
Males: Females	67.7%: 32.3%
Median age, years (range)	62 (39–83)
Median tumour size, cm (range)	6 (1.5–17)
Subtype	
Clear cell RCC	76 (79.2%)
Papillary RCC	11 (11.5%)
Chromophobe RCC	5 (5.2%)
Multilocular cystic RCC	1 (1%)
Clear cell tubulopapillary RCC	3 (3.1%)
Stage	
T1	53 (55.2%)
T2	26 (27.1%)
T3	14 (14.6%)
T4	3 (3.1%)
Metastases (M1)	22 (22.9%)

RCC, renal cell carcinoma; T, tumour.

Please cite this article in press as: Ng KL, et al., Nuclear factor-kappa B subunits and their prognostic cancer-specific survival value in renal cell carcinoma patients, Pathology (2018), https://doi.org/10.1016/j.pathol.2018.03.003