

ANATOMICAL PATHOLOGY

PTEN expression in renal cell carcinoma and oncocytoma and prognosis

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Summary

Aims: Deletion or inactivation of the tumour suppressor gene PTEN (phosphatase and tensin homologue deleted from chromosome 10) contributes to tumorigenesis in a variety of human carcinomas. The present study evaluated PTEN expression in renal cell carcinomas and oncocytomas.

Methods: A tissue microarray from 493 specimens including renal cell carcinomas ($n=440$), oncocytomas ($n=21$) and tumour-negative renal tissue ($n=32$) from patients ($n=461$) was incubated with an anti-PTEN antibody for subsequent analysis of PTEN expression. Furthermore, the effect of PTEN expression on the survival of renal carcinoma patients was evaluated.

Results: Renal cell carcinomas, and even more pronouncedly oncocytomas, expressed PTEN predominantly in the cytoplasm. In contrast to oncocytomas, PTEN expression was typically decreased in renal cell carcinoma subtypes. PTEN expression in sarcomatoid renal cell carcinomas was comparable to that in non-sarcomatoid subtypes. The PTEN expression pattern had no significant influence on prognosis.

Conclusions: Renal tumours (renal cell carcinomas and oncocytomas) express PTEN protein predominantly in the cytoplasm. A reduction in PTEN expression appears to be an early step in renal cell carcinogenesis. However, the PTEN expression pattern of renal cell carcinomas apparently is not prognostic for patient survival.

Key words: Renal cell carcinoma, oncocytoma, PTEN expression, cytoplasmic, nuclear, membranous, tissue microarray, survival, carcinogenesis.

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INTRODUCTION

Mutations of the tumour suppressor gene phosphatase and tensin homologue deleted from chromosome 10 (PTEN) and alterations in PTEN protein expression are present in a variety of human carcinomas.^{1,2} The phosphatase PTEN inhibits phosphatidylinositol-3-kinase (PI3K)-dependent activation of protein kinase B (AKT) and therefore is involved in cell proliferation and survival. Deletion or inactivation of PTEN results in uncontrolled AKT activation, which contributes to tumour development and progression by inhibiting apoptosis.³ Mechanisms causing PTEN inactivation include: (1) germline and somatic mutations, (2) deletions, and (3) promoter hypermethylation.^{4–8}

In renal cell carcinomas, somatic mutations and deletion of PTEN-flanking markers have been reported to cause decreased PTEN protein expression.^{2,9–15} These findings suggest that alterations in PTEN expression may predispose to renal cell carcinoma formation. Similarly, Western blot analysis of oncocytomas showed PTEN bands to differ from those of normal renal tissue.¹⁰

However, conclusive evidence showing alterations in PTEN protein expression to predispose for development of renal cell carcinoma or oncocytoma is still awaited. Moreover, to date most studies have focused mainly on screening for PTEN gene mutations and not on analysis of PTEN expression.¹⁰ Therefore, the present study immunohistochemically evaluated PTEN expression in renal cell carcinoma and in oncocytoma microarray specimens.

MATERIALS AND METHODS

A tissue microarray from paraffin embedded renal cell carcinoma ($n=440$), oncocytoma ($n=21$) and adjacent tumour negative renal tissue ($n=32$) specimens was constructed. In detail, for each case four core tissue biopsies (diameter 0.6 mm) from representative tumour regions and from tumour negative renal tissue were arrayed on nine recipient blocks. Each recipient block hosted up to 10 tumour negative cores randomly distributed between tumour positive cores, as well as three to four consecutive tumour negative cores at the end of the last line of tissue cores. Deparaffinised microarray sections (2 µm thick) were autoclaved and placed in a citrate buffer (10 mM, pH 6.0) before being incubated with a rabbit anti-PTEN antibody ab2979 (dilution 1:50; Abcam, UK) and subsequently with a secondary anti-rabbit antibody (dilution 1:500; Dako, Denmark). Each incubation step took 30 min at room temperature. Subsequently, immunohistological staining was performed with a commercially available kit (ABC-Kit Vectastain; Vector, USA). The sections were counterstained with haematoxylin. According to the manufacturer's guidelines, prostate cancer specimens served as positive control.

Immunohistochemical staining results were independently scored by two blinded pathologists (HH and KR). When the evaluation of the two observers differed, the specimens were re-evaluated on a discussion microscope and assessed as mentioned above. In detail, as compared with tumour negative renal tissue, the same cytoplasmic staining intensity was scored '2', decreased was scored '1' and the absence of cytoplasmic staining was scored '0'. Additionally, the presence of nuclear staining was scored '1', while its absence was scored '0'.

Subtyping of renal cell carcinomas was performed according to the WHO classification.¹⁶ Nevertheless, the highly aggressive sarcomatoid renal cell carcinoma, which can occur by metaplastic transformation of all renal cell carcinoma subtypes,¹⁷ was regarded as a separate variant.

Survival was calculated from the date of diagnosis until the date of death or last follow up.

Statistical analysis

Descriptive statistics were used for data analysis. The statistical computer package SPSS 11.0.0 for Windows XP Professional (SPSS, USA) was used for statistical analysis. PTEN expression in the various tumour subtypes was compared by means of the Mann–Whitney U Test. Kaplan–Meier curves and the log rank test were used to analyse the effect of PTEN expression (e.g., unchanged or decreased) on the survival of renal cell carcinoma positive patients. Spearman's correlation coefficient was calculated. A *p* value <0.05 was considered significant.

RESULTS

A total of 493 specimens, including renal cell carcinomas (*n*=440), oncocytomas (*n*=21) and adjacent tumour-negative renal tissue (*n*=32) from patients (*n*=461; male/female, 259/202) aged 64 ± 12 years were investigated. Because of technical problems, 32 tumour positive cases had to be excluded from further analysis, achieving 429 tumour positive cases (Tables 1, 2).

All tumour negative renal tissue specimens showed comparable but only cytoplasmic PTEN expression exclusively in tubular epithelial cells. In tumour positive renal tissue, nuclear PTEN expression was also found, but never without concomitant cytoplasmic PTEN expression. In detail, nuclear PTEN expression was found in less than 10% of all specimens and only in some subtypes of renal cell carcinoma (clear cell, sarcomatoid, and chromophobe; Table 1). Cytoplasmic PTEN expression was found in all oncocytoma and in 75% of renal cell carcinoma specimens (Table 2; Fig. 1). PTEN expression was typically decreased

in renal cell carcinoma (64%), but largely unchanged in oncocytoma (80%) specimens (Table 2). PTEN expression (e.g., same, decreased, absent) in sarcomatoid renal cell carcinomas was comparable with that in non-sarcomatoid subtypes (e.g., clear cell, papillary, chromophobe, Bellini; Table 2). No correlation between PTEN expression (nuclear and cytoplasmic) and stage as well as grade was found.

The overall survival time of the present study's renal cell carcinoma patients was 142.3 months (95% confidence interval 136–149). However, neither localisation (nuclear or cytoplasmic) nor degree (normal, decreased or absent) of PTEN expression had a significant impact on the survival of those patients, irrespective of the renal cell carcinoma subtype (Fig. 2).

DISCUSSION

Renal cell carcinomas, and even more pronouncedly oncocytomas, expressed PTEN predominantly in the cytoplasm. In detail, as compared with tumour negative renal tissue, cytoplasmic PTEN expression was mostly decreased in renal cell carcinoma, but was largely the same in oncocytoma specimens.

The tumour suppressor gene PTEN is located on chromosome 10q23 and is mutated in a variety of human cancers.^{1,2} Since PTEN indirectly inhibits the activation of AKT, a decrease in PTEN prompts increased AKT activity, which fosters tumour development and progression.¹⁸

Although mutations of the PTEN gene are infrequently (less than 17%) found in renal tumours,^{9,11–13} decreased PTEN protein expression has been observed.¹⁰ Analysis of PTEN expression in renal cell carcinomas and oncocytomas in the present study focused on PTEN's intracellular localisation (e.g., cytoplasmic, nuclear or membranous) and the intensity of PTEN expression compared with that in tumour negative renal tissue.

With regard to intracellular localisation, nuclear PTEN expression was found in only 6% of renal cell carcinomas. In contrast, cytoplasmic PTEN expression was present in 75% of renal cell carcinomas and in all cases of oncocytoma and normal renal tissue.

Interestingly enough, membranous PTEN expression was present in neither the tumour positive nor the tumour negative renal tissue specimens analysed. This latter finding stands somewhat in contrast to the findings of Brenner *et al.*, who reported membranous PTEN expression in tumour negative renal tissue (in proximal and to a lesser degree in distal tubular epithelial cells) while confirming the absence of membranous PTEN expression in renal cell carcinomas and oncocytomas.¹⁰ In general, any loss of membranous PTEN expression allows increased AKT activity and thereby promotes tumorigenesis.¹⁰

Previous studies have reported cytoplasmic but also nuclear expression of PTEN for various tumours, such as thyroid tumours, melanomas and breast cancer, but also for tumour negative tissue, such as breast, pancreas and thyroid gland.^{19–22} However, it is important to know that the cellular staining localisation also depends to a certain degree on the type of PTEN antibody used. In detail, PTEN antibody clone 28H6 (Novocastra, UK) typically shows a predominant nuclear staining pattern, whereas

TABLE 1 Nuclear PTEN expression in oncocytoma and renal cell carcinoma

	Nuclear PTEN expression		
	Total analysed <i>n</i>	Positive <i>n</i> (%)	Negative <i>n</i> (%)
Oncocytoma			
Male/female (<i>n</i> =11/9)	20	0	20 (100)
Renal cell carcinoma			
Histological type			
Renal cell carcinoma	409	25 (6.0)	384 (94.0)
Clear cell type	324	23 (7.1)	301 (92.9)
Papillary	44	0	44 (100)
Sarcomatoid	18	1 (5.6)	17 (94.4)
Bellini	1	0	1 (100)
Chromophobe	22	1 (4.5)	21 (95.5)
Gender			
Female	178	13 (7.3)	165 (92.7)
Male	231	11 (4.8)	220 (95.2)
Nuclear grade			
I	65	8 (12.3)	57 (87.7)
II	173	10 (5.8)	163 (94.2)
III	143	5 (3.5)	138 (96.5)
IV	28	2 (7.1)	26 (92.9)
Stage			
I	247	20 (8.1)	227 (91.9)
II	70	0	70 (100)
III	88	5 (5.7)	83 (94.3)
IV	4	0	4 (100)

TABLE 2 Cytoplasmic PTEN expression in renal oncocytoma and renal cell carcinoma

	Cytoplasmic PTEN expression			
	Total analysed <i>n</i>	Same <i>n</i> (%)	Decreased <i>n</i> (%)	Negative <i>n</i> (%)
Oncocytoma	20	16 (80)	4 (20)	0
Renal cell carcinoma				
Histological type				
Renal cell carcinoma	409	45 (11.0)*	262 (64.0)*	102 (25.0)*
Clear cell type	324	29 (8.9)*†	203 (62.8)*	92 (28.3)*‡
Papillary	44	12 (27.3)*	30 (68.2)*	2 (4.5)
Sarcomatoid	18	1 (5.6)*	11 (61.1)*	6 (33.3)*
Bellini	1	0	1 (100)	0
Chromophobe	22	3 (13.6)*	18 (81.8)*	1 (4.5)
Gender				
Female	178	23 (12.9)	110 (61.8)	45 (25.3)
Male	231	22 (9.5)	153 (66.2)	56 (24.3)
Nuclear grade				
I	65	5 (7.7)	38 (58.5)	22 (33.8)
II	173	20 (11.6)	109 (63.0)	44 (25.4)
III	143	17 (11.9)	97 (67.8)	29 (20.3)
IV	28	3 (10.7)	19 (67.9)	6 (21.4)
Stage				
I	247	26 (10.5)	164 (66.4)	57 (23.1)
II	70	7 (10.0)	43 (61.4)	20 (28.6)
III	88	11 (12.5)	54 (61.4)	23 (26.1)
IV	4	0	4 (100)	0

Cytoplasmic PTEN expression compared with tumour negative renal tissue.

*Significant when compared with oncocytoma ($p \leq 0.05$).

†Significant when compared with papillary renal cell carcinoma ($p \leq 0.05$).

‡Significant when compared with chromophobe renal cell carcinoma ($p \leq 0.05$).

PTEN antibody clone 10P03 (Neomarkers, USA), PTEN antibody clone 6H2.1 (Cascade Bioscience, USA) and polyclonal PTEN antibody (Zymed, USA) show a predominant cytoplasmic staining pattern.²³ Abcam's

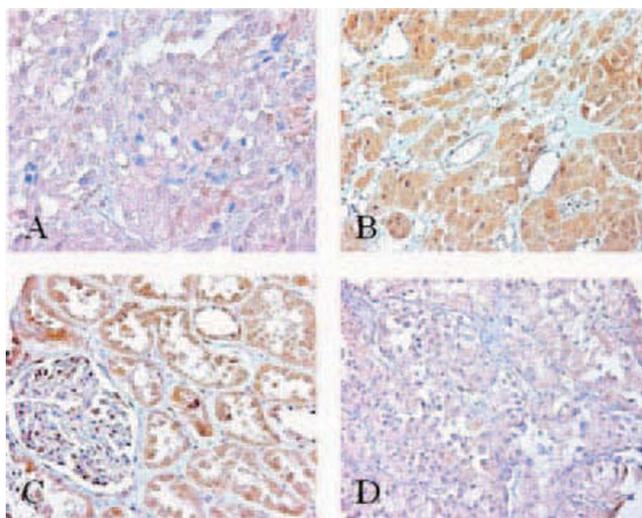


Fig. 1 (A) PTEN expression in sarcomatoid type renal cell carcinoma with decreased cytoplasmic staining and positive nuclear staining. (B) Strong cytoplasmic PTEN staining pattern in renal oncocytoma and (C) in tubular epithelial cells in normal renal tissue beside virtually absent staining in glomerula. (D) Decreased cytoplasmic staining pattern in clear cell carcinoma ($\times 400$).

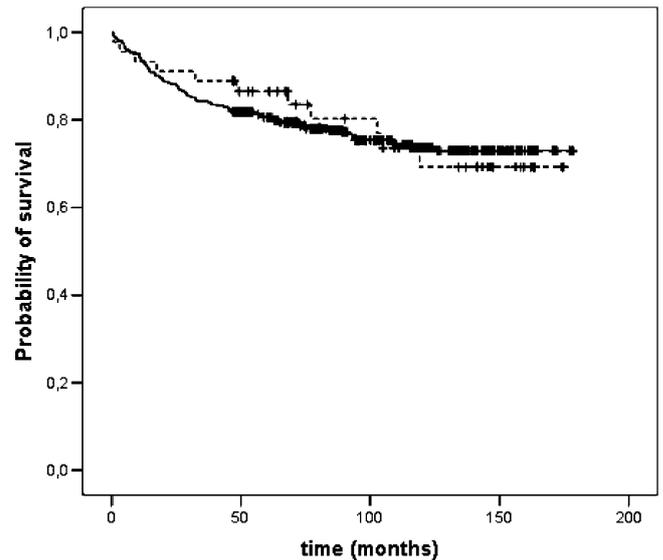


Fig. 2 Kaplan-Meier survival curves for renal cell carcinoma patients ($n=409$). Cytoplasmic PTEN expression, reduced ($n=364$; solid line) or normal ($n=45$; broken line), had no significant effect on survival ($p > 0.05$).

polyclonal rabbit anti-PTEN antibody showed a predominant cytoplasmic but also nuclear staining pattern as has previously been reported.²⁴ As for the intensity of cytoplasmic PTEN expression, the present study's renal cell carcinoma specimens showed reduced (64%) or absent (25%) cytoplasmic PTEN expression approximately twice as often as reported by others.^{25,26} A decrease in or even loss of PTEN, however, fosters tumour development and progression¹⁸ and can negatively affect the survival time of renal cell carcinoma patients by increasing AKT activity.²⁶⁻²⁸

In the present study, however, PTEN expression had no impact on patient survival, although it was significantly reduced in all renal cell carcinoma subtypes.

On a subtype level, PTEN expression in sarcomatoid renal cell carcinomas (e.g., the highly malignant endstage of possibly all renal cell carcinoma subtypes¹⁷) was comparable with that in non-sarcomatoid subtypes (e.g., clear cell, papillary, chromophobe and Bellini). Therefore, it is assumed that changes in PTEN expression pattern occur more likely at an early stage of carcinogenesis, and not only in the framework of a later increase in tumour malignancy.

In this context, a close-up on PTEN expression in oncocytomas could shed some light on when in carcinogenesis changes in PTEN expression occur (e.g., early or late). Generally, in oncocytomas PTEN protein was previously shown to produce a single band in Western blot analysis, whereas tumour negative renal tissue typically showed a PTEN protein double band.¹⁰ Western blot analysis was not part of the present study's protocol, which immunohistochemically focused on the PTEN expression pattern and intensity in oncocytomas. The PTEN decrease but not absence found was comparable with that given in other reports.¹⁰ The majority of the present study's oncocytomas (80%), however, showed a more or less unchanged PTEN expression compared with tumour negative renal tissue.

Oncocytomas are thought to be possibly the starting point of an adenoma-carcinoma sequence leading to chromophobe renal cell carcinoma.²⁹ Considering this, and following the hypothesis that any reduction in PTEN

expression fosters carcinogenesis,¹⁰ the found decrease of PTEN expression in the present study's chromophobe renal cell carcinoma specimens identifies changes in PTEN expression pattern as an early step in renal cell carcinogenesis.

In conclusion, we here show that renal tumours (e.g., renal cell carcinomas and oncocytomas) express PTEN protein predominantly in the cytoplasm. A reduction in PTEN expression appears to be an early step in renal cell carcinogenesis. The PTEN expression pattern of renal cell carcinomas, however, is apparently not prognostic for patient survival.

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