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# Presence of HNP1-3 defensins in renal cell carcinoma

**KEYWORDS:** Renal cell carcinoma; Defensins

## INTRODUCTION

Defensins comprise a family of naturally occurring small peptides that exhibit antimicrobial activities as well as cytotoxicity to other various types of cells including tumour cells. There are two classes of human defensins:  $\beta$ -defensins are present in epithelial cells of lung, skin and urogenital tract, whereas  $\alpha$ -defensins are identified as human neutrophil peptides (HNP) in the granulocytes and HD5-6 in the intestinal Paneth cells.

## MATERIAL AND METHODS

In this study we investigated the presence and the origin of the HNP1-3 defensins in renal cell carcinoma (RCC). HNP1-3 defensins were detected *in vivo* in 31 RCC tissues by immunoperoxidase and triple immunofluorescence methods with specific antibodies DEF-3 (Bachem Biochemica, Heidelberg).

## RESULTS

Two different patterns of HNP1-3 staining in RCC tissue were observed. In the most of the analysed, particularly low grade RCC, the random focal patchy staining was seen. This type of staining was mostly found to be associated with granulocytes located between tumour cells, identified by neutrophil elastase staining in consecutive sections, indicating that defensin has been released by granulocytes in tumour tissue. However, in six tumours of high-grade malignancy all tumour cells were strongly and diffusely stained for HNP1-3 defensins. It is less likely that neutrophils, labelled by antibody to neutrophil esterase, participating in considerable number of tumour infiltrating cells, could release such a great amount of HNP 1-3 defensins that could impregnate RCC tissue. Triple immunofluorescence labelling with antibodies against HNP1-3, DAPI and neutrophil elastase or cytokeratin 8 confirmed the presence of defensins in association with neutrophils in most tumour tissue but also showed that HNP were clearly present in epithelial cells. There was co-expression of cytokeratin 8 and defensins in some tumour cells. However, we further investigated the possibility that RCC synthesised HNP1-3 defensins *in vitro*. All eight RCC lines analysed by FACS showed moderate to strong intracellular expression of  $\alpha$ -defensins after labelling with mAbs specific for HNP1-3. Analysis of RCC line A-498 by confocal scanning microscopy

revealed diffuse cytoplasmatic presence of  $\alpha$ -defensins. This is in contrast to the HL-60 and normal granulocytes used as control, where HNP defensins are confined to a specific cellular compartment indicating that defensins in tumour renal cells might exist as a propeptide. In addition, applying RT-PCR analysis, presence of HNP1-3 mRNA was detected in five RCC tissues and in two normal renal tissues, but these results could be interpreted as HNP mRNA from neutrophils also. However, the amplified fragments, which contained sequence coding for part of propeptide (position 112 to 242) as well as of mature peptides (position 243 to 326) of HNP 1-3, were found in five of eight tested RCC lines and in granulocytes of leukopheresis products used as control. And finally, the presence of HNP 1-3 peptides in RCC was demonstrated by the mass-spectrometric analysis of lysates of RCC line TW33. MALDI-MS analysis of an acidic cell extract from RCC line TW 33 revealed three prominent mass peaks at  $m/z$  3371.84, 3442.98 and 3486.93 that correlated to the expected average masses of mature HNP-1, -2, -3 peptides.

## CONCLUSIONS

Our results clearly revealed that  $\alpha$ -defensins HNP 1-3 are present in renal cell tissue as well as in renal cell lines. The presence of HNP1-3 defensins in renal carcinoma tissue is due to infiltrating polymorphonuclear leukocytes which could be present in different amounts among tumour cells, but also due to the expression of defensins by renal carcinoma cells.

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