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Original Article

Immunohistochemical Localization of Gremlin, a Bone Morphogenetic Protein Antagonist, in Human Clear Cell Renal Cancer

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Abstract

Introduction. Gremlin is a BMP-7 antagonist which was found to be involved in fibrosis of different organs and tissues. We examined expression of gremlin in human clear cell renal carcinoma.
Methods. Gremlin protein expression in localized human clear cell renal cancer (CCRC) obtained from 20 patients who underwent nephrectomy was determined by immunohistochemistry.
Results. All samples stained positive for gremlin. Immunostaining intensity for gremlin in healthy renal tissue ranged from 2 to 3 (average 2.6), while in renal clear cell carcinoma it ranged from 1 to 3 (average 2.0) (not significant). Among healthy kidney tissue samples we observed gremlin expression dominantly localized to tubular cells while glomeruli stayed negative. Positive staining was localized to cytoplasm of tubular cells. Gremlin staining was positive in all CCRC samples. Staining was localized in membrane of malignant cells. There was no statistically significant correlation between gremlin expression, tumor size, patients' characteristics and clinical symptoms at presentation.
Conclusion. Our results have shown gremlin protein expression in both malignant and healthy tissue. Gremlin expression was more intensive in healthy tissue. Further studies are needed to evaluate the role of gremlin in CCRC.

Keywords: clear cell renal cancer, gremlin, immunohistochemistry, expression

Introduction

Gremlin is a highly conserved 184 amino acid protein which belongs to the cysteine-knot protein superfamily [1,2]. It is an essential signal for kidney development [3]. Mice lacking gremlin gene die shortly after birth from uremia and from lung deformities [4].

The highest gremlin expression was found in areas of interstitial fibrosis and it was found to be colocalized with TGF-β [5]. TGF-β1 induced gremlin expression in renal proximal tubular cells during their transformation to fibroblast phenotype (epithelial-mesenchymal transition) [6]. Gremlin acts as bone morphogenetic protein antagonist [7].
In adult kidneys, gremlin was found to be overexpressed in different pathological processes including diabetic nephropathy [8], pauci-immune glomerulonephritis [9], and chronic allograft nephropathy [10].
In the study we investigated gremlin expression in human clear cell renal carcinoma.

Patients and methods

Tissue samples

Tissue samples were obtained at the Department of Urology, University Hospital Zagreb, Zagreb, Croatia, from 20 consecutive patients who underwent nephrectomy for renal cancer. Investigations were approved by the Hospital Ethic Committee. Besides the abdominal multi-slice computed tomography, all patients underwent bone scan and chest X-ray to exclude disease dissemination before surgery. Tumor samples and corresponding healthy parts taken from the normal tissue located as far as possible from the tumor mass were used for investigation.

Patients

There were 12 male and 8 female patients, mean age 63 years (range 39 to 83 years). Tumor size ranged from 2 to 4.8 cm (average 2.5 cm). All patients had T1N0M0 stage. Symptoms at diagnosis included haematuria (6 patients), flank pain (6 patients) and palpable mass in one patient. Malignancy was found on routine examination in 6 patients.

Immunohistochemistry

For light microscopy, kidney samples were fixed in 4%

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formalin, dehydrated and embedded in paraffin. Paraffin sections (3-4 μm) were deparaffinised in xylene and then rehydrated through graded alcohol and distilled water. Hydrogen peroxide (0.3 %) was used for 10 minutes to block endogenous peroxidase activity. Rabbit polyclonal Gremlin 1 antibody (Abcam, UK) was used for immunohistochemistry. Immunostaining was performed by the avidin biotin peroxidase complex method using LSAB+ kit (Dako, Glostrup, Denmark). The specificity was checked by omission of primary antibody and use of nonimmune sera.

Evaluation of immunohistochemistry

Results of immunohistochemistry were interpreted using a light microscope (Zeiss, Munchen, Germany). Gremlin immunostaining was semi quantitatively evaluated for intensity (0, negative; 1 weak; 2, moderate; 3, strong staining), as described previously [11]. Quantification was done twice by a pathologist.

Statistical analysis

SAS for Windows, version 9.1 (SAS Insititue, Cary, USA) was used to perform statistical calculations. $P<0.05$ was considered statistically significant.

Results

Immunohistochemical localization of gremlin

Expression of gremlin in tumor and healthy tissue was determined by immunohistochemistry.
All samples stained positive for gremlin. Immunostaining intensity for gremlin in healthy renal tissue ranged from 2 to 3 (average 2.6), while in renal clear cell carcinoma it ranged from 1 to 3 (average 2.0) (not significant).

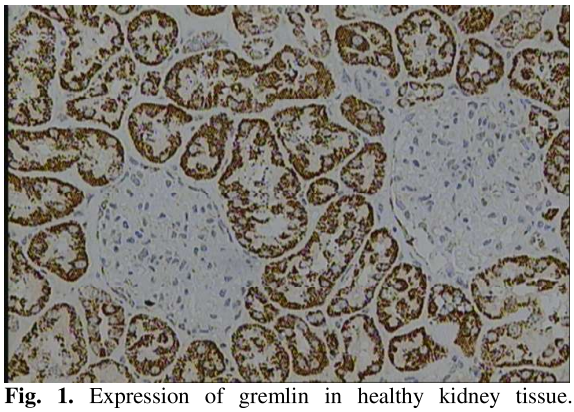

Fig. 1. Expression of gremlin in healthy kidney tissue. Positive staining of proximal tubular cells. Glomeruli are negative. (Original magnification x 200, gremlin stain)

Among healthy kidney tissue samples we observed gremlin expression dominantly localized to tubular cells while glomeruli stayed negative (Figure 1). Positive staining was localized to cytoplasm of tubular cells.

Gremlin staining was positive in all CCRC samples. Staining was localized in membrane of malignant cells (Figure 2).

Fig. 2. Expression of gremlin in human clear cell renal carcinoma. Positive expression in membrane of tumor cells. (Original magnification x 200, gremlin stain)

There was no statistically significant correlation between gremlin expression, tumor size, patients' characteristics and clinical symptoms at presentation.

Discussion

Although considered curable disease when discovered at the localized stage, almost 30% of patients who present with limited clear cell renal cancer at the time of surgery develop metastasis in next 3 years [12].
Numerous molecular markers have been investigated in terms of outcome prediction and potential therapeutic targets for CCRC. It is well known that genes involved in kidney development participate in the maintenance of structure and function of adult kidneys, and are involved in development of different pathological conditions. Bone morphogenetic protein-7 (BMP-7) is one of the most investigated genes which is essential for kidney development [13,14] and was found to inhibit tubular cell dedifferentiation, mesenchyme transformation, apoptosis, and to protect kidney from diabetic nephropathy, acute and chronic renal failure [15-18].
In our study we investigated gremlin expression in tissue samples of human clear cell renal carcinoma. We observed strong expression of gremlin in both healthy and malignant tissue. In healthy tissue it was predominantly localized to cytoplasm of tubular cells, while glomeruli stayed completely negative (Figure 1). Samples of malignant tissue were all positive for gremlin although with less intensive staining than corresponding healthy tissue (statistically not significant).
Gremlin was found to have pathogenic role in fibrosis of different organs and tissues [19]. It acts as a BMP-7 antagonist. While BMP-7 activity may block the extracellular matrix accumulation [20], reduce severity of injury in animal models of acute and chronic renal failure [21], ameliorate renal injury due to mesangialproliferation by suppression of mesangial cell mitosis [17] and may rescue podocytes from diabetic injury [22,23], inhibition of BMP-7 inhibitor (gremlin) may possibly help to preserve renal structure and function [24-27].

Gremlin promotes vascular smooth muscle cell proliferation [25], and was found to be overexpressed in the media layer of vessels in uremic rats and patients with vascular calcifications [26].
Gremlin is considered to be almost undetectable in healthy status, while its expression increases in different kidney disease models [28]. Contrary to this conclusion, our results have shown strong gremlin expression in healthy kidney tissue (Figure 1) in all samples. It is possible that gremlin expression may be induced by an ongoing tumor process within the same organ. It is also possible, that besides the role in fibrogenesis, gremlin has other still unknown functions. Further studies are needed to solve this dilemma.

Conclussion

Although performed on small number of patients, this is the first study which addressed immunohistochemical localization of gremlin in human clear cell carcinoma. Our results demonstrate that gremlin is expressed in malignant kidney tissue less intensively than in the corresponding healthy tissue. Further studies are needed to determine the role of gremlin in human clear cell renal carcinoma.

Conflict of interest statement. None declared.

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