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BMP-2 Protein Expression in Clear Cell Renal Carcinoma

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Abstract

Introduction. Bone morphogenetic protein-2 (BMP-2) is a member of BMP family of proteins. In human, it has role in skeletal repair and regeneration, as well as in heart formation. Kidneys of BMP-2 heterozygous null mutant mice have normal gross anatomy, but exhibit increased ureteric bud branching. Dysregulation of BMP-2 signaling has been suggested in carcinogenesis.
Methods. We determined BMP-2 protein expression in CCRC and in healthy renal tissue, and evaluated its prognostic significance after five years of follow-up. Twenty patients with localized clear cell carcinomas at the time of diagnosis were included in the investigation. Immunohistochemical staining for BMP-2 was evaluated semiquantitatively and the specimens were scored according to the distribution of positive cells.
Results. In normal tissue, the expression of BMP-2 was localized predominantly in tubular cells, with less intensive staining in glomerular mesangial cells. Other glomerular cells were BMP-2 negative. The cellular staining pattern for BMP-2 was both cytoplasmic and membranous. In 14 of 20 patients, loss of BMP-2 staining was observed in the malignant tissue. However, three out of six patients with positive BMP-2 staining died from disseminated malignant disease, one of them had concomitant acute myeloid leukemia.
Conclusions. According to our results, BMP-2 expression in CCRC may be associated with an adverse outcome with development of bone metastatic disease.

Key words: BMP-2, clear cell renal carcinoma, outcome, bone metastases, immunohistochemistry

Introduction

Bone morphogenetic protein-2 (BMP-2) is a member of BMP family of proteins. BMP-2 heterozygous null mutant mice die at embryonic day 7,5-9 with failure of proamnionic canal to close and abnormal development of the heart. In human, it has role in skeletal repair and regeneration, as well as in heart formation [1]. During kidney development, BMP-2 transcripts are expressed in condensed metanephric mesenchyme close to the tips of the ureteric bud [2]. Kidneys of BMP-2 heterozygous null mutant mice have normal gross anatomy, but exhibit increased ureteric bud branching. It is believed that BMP-2 inhibits branching morphogenesis at the tips of the branching ureteric bud [3]. Dysregulation of BMP-2 signaling has been suggested in carcinogenesis [4-10]. Clear cell renal carcinoma (CCRC) at the localized stage is considered as curable surgical disease. Still, almost 30% of patients who present with limited disease at the time of surgery develop metastasis within the next 3 years [11]. Numerous molecular markers have been investigated in terms of predicting disease progression, as well as potential therapeutic targets for CCRC. The clinicopathological significance of BMP-2 expression in human CCRC has not been investigated.
In the present study, we determined BMP-2 protein expression in CCRC and in healthy renal tissue, and evaluated its prognostic significance after five years of follow-up.

Materials and methods

Tissue samples were obtained at the Department of Urology, University Hospital Zagreb, Zagreb, Croatia, from 25 consecutive patients who underwent nephrectomy for renal cancer. Out of 25 tumor samples, there were 20 clear cell carcinomas that were further processed and these patients were included in the investigation. The study was approved by the investigators' Institutional Review Board.
The study included 12 male and 8 female patients ranged in age from 39 to 83 years (mean 63 years), 6 of them being smokers for more than 10 years. Tumor size ranged from 2 to 4,8 cm (average 2,5 cm). Renal cancer was incidentally found on routine examination in 6 patients. Presenting symptoms included haematuria in 6 patients, flank pain in 6 patients, while one patient presented with a palpable mass. Laboratory investigations at diagnosis demonstrated elevated sedimentation rate in 12 patients. Thrombocytopenia, anemia and erythrocytosis were found in 1 patient each. Elevated liver chemistries were found in two patients. Sixteen patients had one or more concomitant diseases including urolithiasis, diabetes mellitus, valvular heart disease and angina pectoris. One patient had

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previously been treated for acute myeloid leukemia with allogenic bone marrow transplantation.
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 site were collected.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues (3-4 μm) were deparaffinised in xylene and then rehydrated through graded alcohol. Endogenous peroxidase activity was blocked with 0,3 % hydrogen peroxide for 10 min. The sections were blocked with 20% normal rabbit serum for 30 min prior to 1 h of incubation with primary antibody (mouse monoclonal BMP-2 antibody, Abcam, UK). The slides were washed twice in Tris-buffered saline and incubated with biotinylated rabbit-antimouse antibody (DAKO, Glostrup, Denmark) diluted 1:500 in blocking serum. The detection of antibody reaction was carried out with a standard streptavidin-biotin complex (Dako, Glostrup, Denmark). Negative control sections were processed in an identical manner after omitting the primary antibody and showed no staining.

Evaluation of immunohistochemistry

Immunohistochemical staining for BMP-2 was evaluated semiquantitatively and the specimens were scored according to the distribution of positive cells. Immunostaining results were graded according to the following protocol: 3+, more than 50 % of cells positive; 2+, 50-75% of cells positive; 1+, 10-49 % of cells positive; and 0, if < 10 % of cells demonstrated positive staining.
The cellular localization and pattern of immunoreactivity were examined in a blinded fashion independently by two investigators.

Statistical analysis

SAS for Windows, version 9.1 (SAS Institute, Cary, USA) was used to perform statistical calculations. The chi-square test was used to evaluate the association between BMP-2 expression and the clinicopathologic parameters. P values <0.05 were considered statistically significant.

Results

Expression of BMP-2 in normal kidney tissue

Expression of BMP-2 was investigated in specimens of normal kidney by using immunohistochemistry (Figure 1). Tissue samples were collected from the site most distant from the tumor. In normal tissue, the expression of BMP-2 was localized predominantly in tubular cells, with less intensive staining in glomerular mesangial cells. Other glomerular cells were BMP-2 negative (Figure 1). The cellular staining pattern for BMP-2 was both cytoplasmic and membranous. All healthy samples exhibited positive BMP-2 staining with average score 2.77 (range 2-3).

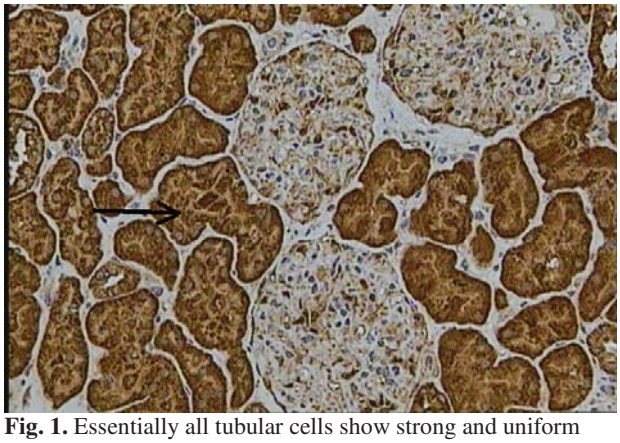

Fig. 1. Essentially all tubular cells show strong and uniform cytoplasmic positivity for BMP2 (arrow)

Expression of BMP-2 in clear cell renal carcinoma

Next, we examined BMP-2 expression in a series of clear cell renal carcinoma specimens. In 14 of 20 patients, loss of BMP-2 staining was observed (Figure 2).

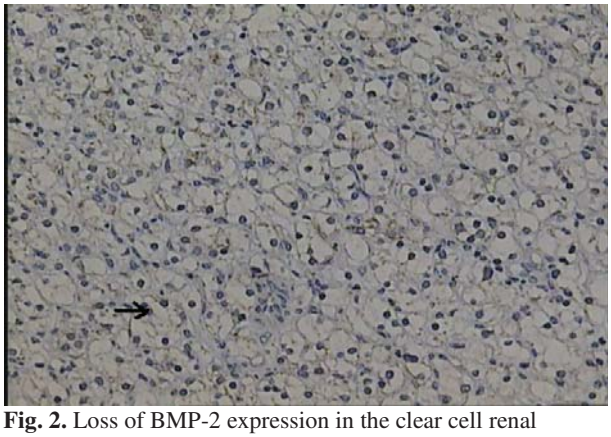

Fig. 2. Loss of BMP-2 expression in the clear cell renal carcinoma (arrow)

Six samples exhibited positive BMP-2 staining. It is interesting that 5 out of 6 patients with positive BMP-2 staining in malignant tissue presented with macrohaematuria, compared with occurrence of macrohaematuria in only 1 out of 14 patients with negative BMP-2 staining in the malignant tissue ($p<0.05$). BMP-2 expression did not correlate with other presenting symptoms.

BMP-2 protein expression and survival

During the mean follow-up of five years 12 patients died. Average age of deceased patients was 78 years. Four of them had positive BMP-2 staining in malignant tissue. Three of the patients with positive BMP-2 staining died from disseminated malignant disease, one of them had concomitant acute myeloid leukemia. Two patients died from acute myocardial infarction. Cause of death of other patients is unknown, but they had no signs of disease dissemination.

Discussion

In addition to their roles in tissue morphogenesis, recent literature suggests that different members of the BMP family of proteins may be involved in human cancers. Thus, in recent years, BMP-2 has generated considerably attention in cancer biology. It was found in various human cancers including pancreatic cancer [9], fibrosarcoma, serous adenocarcinoma, prostate carcinoma [12], mucinous adenocarcinoma, fibrosarcoma, mesothelioma [7], glioma [13], ovarian cancer [8], oral carcinoma [14] and osteosarcoma [15]. It was found to be 8,9 fold upregulated in invasive human bladder cancer [16].

Besides the aberrant expression of BMP genes in tumorous tissues, an important factor of their action is expression of BMP receptors located on plasma membrane of the cell. Overexpression of BMP receptors may allow more ligand molecules to bind with receptors inducing abnormal cellular function. For example, BMP-2 is expressed in both osteosarcoma and malignant fibrous histiocytoma. However, BMP receptor could not be demonstrated by immunohistochemistry in malignant fibrous histiocytoma that may help to differentiate these two types of tumour, as well as explain why malignant fibrous histiocytoma does not ossify [17]. Enrichments of BMP2 in non-small cell lung cancer tumor cell enhance tumor growth in vivo [18,19], due to activation of second messengers SMAD 1 and 5 [20].

Bone morphogenetic proteins may demonstrate both stimulative and anti-proliferative action on tumor growth based on expression of their promoters or inhibitors, recaptors and second messengers.

BMP-2 is involved in heterotopic ossification in metastatic lesions from different types of malignant cells including urothelial bladder carcinoma [21], malignant melanoma [22] and gastric cancer [23,24]. It was found to enhance motility and invasiveness of prostate cancer cell lines [25] as well as migration and invasion of gastric cancer cells by activating the phosphatidylinositol 3-kinase pathway [6]. It may differently affect mesenchymal to epithelial and epithelial to mesenchymal transformation depending on the dosage in colon cancer cells [6].

Besides the role in cell proliferation, BMP-2 has generally being proposed to be an angiogenic factor [26,27], promoting neoangiogenesis when associated with VEGF (vascular endothelial growth factor) in lung cancer [4].

Increased expression of BMP-2 has recently been found by two independent investigators in cases of bone metastasis and muscle invasion of bladder urothelial carcinoma [28,29]. They conclude that BMP-2 correlates with bone metastases in bladder urothelial cancer.

Our data demonstrated that BMP-2 expression in malignant tissue was associated with development of metastatic disease and with mortality. Results are in line with published data dealing with BMP-2 expression in other malignancies [4-10, 21-30]. Over the last years, nephron sparing surgery has been used to treat majority of patients with small kidney cancers. However, even small cancers may spread throughout the body as demonstrated by our study. Further investigations with more patients with tumors of different stages and grades are necessary to define the role for BMP-2 in renal clear cell carcinoma.

Conclusions

BMP-2 may have different functions in tumor biology depending on the type of tissue or even on the type of cell, as well as conditions in the microenvironment. Current data suggest that BMP-2 may be involved mostly in metastasis, epithelial to mesenchymal transformation and invasion of cancers.

Conflict of interest statement. None declared.

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