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Serum tissue inhibitor of metalloproteinase-2 (TIMP-2) and matrix metalloproteinase-2 in complex with the inhibitor (MMP-2:TIMP-2) as prognostic markers in bladder cancer

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Abstract

Objectives: To examine whether the circulating TIMP-2 or MMP-2:TIMP-2 complex associate with disease-free or cause-specific survival in bladder cancer patients.

Design and methods: Levels of circulating TIMP-2 and MMP-2:TIMP-2 complex from 50 patients and 44 healthy volunteers were measured by ELISA and compared with the clinical data.

Results: In cancer patients the levels of TIMP-2 and MMP-2:TIMP-2 complex were significantly lower than in healthy volunteers ($p=<0.0001$). Low TIMP-2 levels and low MMP-2:TIMP-2 complex levels correlated significantly with poor prognosis ($p=0.0032$, $p=0.0022$, respectively). The 5-year cause-specific survival rate was 67% and 60% in patients with a high serum level for TIMP-2 and MMP-2:TIMP-2 versus 18% and 20% in those with low levels of TIMP-2 and MMP-2:TIMP-2 complex.

Conclusions: The results indicate that TIMP-2 and MMP-2:TIMP-2 complex associate with favorable clinical course and could be used as a novel prognostic indicators in bladder cancer.

Keywords: Bladder neoplasm; Gelatinases; Matrix metalloproteinase; Matrix metalloproteinases inhibitor

Introduction

Bladder carcinoma is among the four most frequent cancers among male in European countries [1]. The incidence has been increasing in recent years [2]. Mortality is increasing in Europe as a whole but there are large variations between the European countries [2]. The overall 5-year survival rate is 66%. Superficial bladder carcinoma has much better prognosis; up to 80% survive for 5 years. The risk of recurrence and progression is, however, high [1]. It is obvious that more prognostic biological markers would be needed to help make the decisions on how to treat the patients most successfully.

Metalloproteinases are a family of zinc-dependent endopeptidases that are known to participate in the process of tumor cell invasion and metastasis. Gelatinases (MMP-2 and MMP-9) are able to degrade wide variety of extracellular matrix proteins among others the type IV collagen, which forms the backbone protein structure of the basement membrane. Therefore, their role in promoting the invasion and metastasis is important and quite well established [3,4]. Moreover, MMPs have also been implicated in tumorangiogenesis [5]. TIMPs (tissue inhibitors of metalloproteinases) regulate the MMP activities in the tissue and are therefore in charge of maintaining the balance between extracellular matrix (ECM) deposition and degradation [6]. Four different TIMPs have been identified until now: TIMP-1, TIMP-2, TIMP-3 and TIMP-4.

In previous studies, expression changes of MMPs and TIMPs have been detected in tumor tissue, urine and in blood serum in bladder cancer including for example some studies analyzing MMP-1 and MMP-3 in tissue and urine samples of bladder carcinoma [7,8]. Different studies have also shown association between expression of MMP-7, MMP-11, MMP-13 or MMP-28 and tumor invasion and metastasis in urothelial carcinoma [9–12]. Most interest has been shown to study MMP-2 and MMP-9 expression in bladder cancer [12–17].

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It has been recently reported that TIMP-1, TIMP-2 and TIMP-3 are highly expressed both in normal tissue and in tumor tissue, with increased expression in high-grade tumors in urothelial carcinoma [12]. In a previous report, similar patterns have also been reported by Grignon et al. [18]. Several reports have postulated the MMP/TIMP ratio as a significant tool in predicting either tumor recurrence or progression in bladder cancer, concentrating on MMPs 1, 2 and 9 and TIMPs 1 and 2 [19]. Gakiopoulou et al. found that TIMP-2 is involved in regulation of apoptosis and is associated with an adverse prognosis in patients with TCC of the bladder [20].

Stage is recognized as an independent prognostic factor in bladder cancer. The survival between patients with the same stage is, however, variable. Moreover, it is not known whether some patient groups with this cancer type would benefit from adjuvant therapy. In this study, we have searched for new, easily evaluable prognostic factors to use, for instance for the planning and targeting the therapy in bladder carcinoma.

Methods

Pre-treatment serum samples from 50 consecutive voluntary patients were collected during the routine diagnostic workout. These patients were referred to the Department of Clinical Oncology and Radiotherapy of Oulu University Hospital between the years 1996 and 1999. The patients were treated according to the local protocol and were followed for minimum of 4 years. The study protocol was approved by the local ethical committee (7/2002) and the National Authority for Medical Affairs, Finland (5180/32300/02).

The patients' characteristics are shown in Table 1. Forty out of the 50 patients were males (80%). The median age was 70 years (range 47–80 years). The clinical stage was defined according to the 1997 International Union Against Cancer TNM classification [21]. Median time of follow-up after-treatment was 28 months. In this study the control group included 44 healthy volunteers, 27 of them were female and 17 were male. Their age ranged from 18 to 60 years, median age being 45 years.

Table 1
Patient's characteristics and serum levels of TIMP-2 and MMP-2:TIMP-2 complex in bladder cancer patients

Patient characteristics	*n* (%)	TIMP-2 (ng/mL)	MMP-2:TIMP-2 (ng/mL)
Patients	50	258 (198–347)	316 (153–889)
Men	40 (80)	263 (201–347)	335 (177–889)
Women	10 (20)	240 (198–296)	244 (153–329)
Median age (range)	70 (47–80)		
Stage (*n*)			
I	14 (28)	253 (204–345)	337 (177–771)
II	11 (22)	279 (203–344)	318 (189–889)
III	7 (14)	271 (237–347)	366 (220–591)
IV	18 (36)	245 (198–310)	281 (153–558)
Grade (*n*) [a]			
1	3 (6)	258 (241–270)	281 (240–316)
2	17 (35)	257 (209–347)	339 (177–889)
3	29 (59)	258 (198–344)	308 (153–630)

[a] Grade was available in only 49 cases.

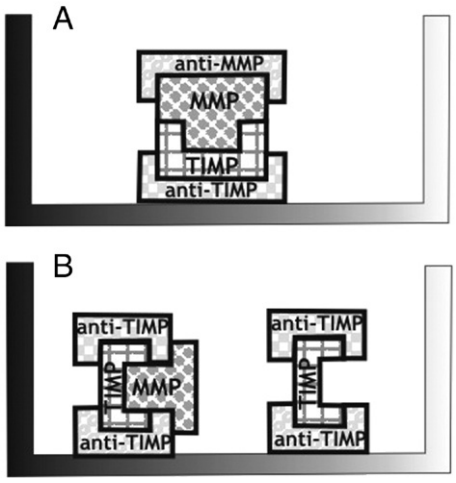

Fig. 1. Principles of ELISA methods: (A) for measuring MMP-2:TIMP-2 complex, the plate was coated with monoclonal anti-TIMP-2 antibody and the bound complex was detected with polyclonal antiMMP-2 antibody. (B) For measuring total TIMP-2, the plate was coated with monoclonal anti-TIMP-2 and the bound complex and free analyte was visualized with polyclonal anti-TIMP-2 antibody.

The treatment strategies for patients in this series were carried out according to the local protocol for treatment. The treatment line depended on the stage of the tumor and patient's age and health. When bladder cancer was superficial endoscopic resection was done and if needed an intravesicular BCG and/or chemotherapy was used. Seven patients in this study received only endoscopic treatment. In 34 cases representing a more invasive tumor a resection with radical radiotherapy (50–60 Gy) or cystectomy was performed. Seven out of the patients who were radically operated were treated with a pre- or postoperative chemotherapy. Nine patients were inoperable and were treated with radical radiotherapy (50–60 Gy), and four with pre- or postradiation chemotherapy. One patient got a palliative radiotherapy (40 Gy).

ELISA assays were performed on 96-well microtiter plates using 8 well stripes (Corning Inc., Corning, NY, USA) and standard protocols (Fig. 1). Prior to the analysis, samples had been stored at −80 °C (4 months to 5 years 2 months). Polyclonal chicken antibody was used as a second antibody. To visualize the peroxidase label OPD (*o*-phenylenediamine; Sigma, St Louis, USA) was used. The color formation was measured on 492 nm wavelength (Anthos 2001 microplate reader). Calculations were done using a Windows based control and evaluation software for Rosys Anthos microplate readers (Anthos Lastec Instruments, Wals, Austria). For the detection of the MMP-2:TIMP-2 complex the plate was coated with monoclonal anti-TIMP-2 antibody (code T2-101; SBA Sciences, Oulu, Finland).The bound complex was detected with a polyclonal anti-MMP-2 antibody (code DB-202; SBA S, Oulu, Finland).

For the detection of TIMP-2 the total protein was measured. This implies that the protocol quantitated simultaneously both the free and complex forms. The method was accomplished with a monoclonal antibody (code T2-101; SBA Sciences, Oulu, Finland), which recognizes both the free and complex forms of the analyte. The microtiter plate was coated with this