Human Pathology (2014) **45**, 674–682

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Human PATHOLOGY

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Original contribution

Enhanced stromal syndecan-1 expression is an independent risk factor for poor survival in bladder cancer☆,☆☆

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Received 22 July 2013; revised 3 October 2013; accepted 6 October 2013

Keywords:
Bladder cancer;
Syndecan-1;
CD138;
Serum;
Prognosis

Summary In this study, we assessed the changes and prognostic relevance of syndecan-1 (SDC1) tissue and serum levels in bladder cancer (BC). SDC1 levels were analyzed in 213 samples (119 paraffin-embedded and 79 serum samples of BC patients and 15 controls) using immunohistochemistry and enzyme-linked immunosorbent assay. Results were correlated with clinicopathological characteristics and follow-up data, as well as previously determined serum levels of angiogenic factors (basic fibroblast growth factor, endostatin, angiostatin, angiopoietin, vascular endothelial growth factor, Tie2 and MMP-7). SDC1 staining was present in the cell membrane of normal bladder epithelium and non–muscle-invasive BC cells but was absent in a significant proportion of muscle-invasive carcinomas ($P < .001$). In contrast, stromal SDC1 expression was enhanced in muscle-invasive compared to non–muscle-invasive BCs ($P = .001$). Serum concentrations of the SDC1 ectodomain were higher in muscle-invasive BCs compared to controls or non–muscle-invasive carcinomas ($P < .001$ each). Lymph node–positive cases had the highest SDC1 serum concentrations ($P < .001$). SDC1 expression in stromal cells was independently associated with survival (hazard ratio = 2.034, 95% confidence interval 1.176-3.519, $P = .011$). SDC1 serum concentrations correlated with those of endostatin and matrix metalloproteinase 7. Loss of SDC1 in tumor cells and the parallel increase of serum SDC1 ectodomain concentration in high-stage, high-grade BCs suggest the involvement of SDC1 shedding in BC progression. In addition, high preoperative SDC1 serum levels may help to identify patients with lymph node metastases, supporting therapeutic decision-making. Presence of SDC1 in tumor stroma is an independent risk factor for patient survival and may therefore be used to select patients for more aggressive therapy.

1. Introduction

Bladder cancer (BC) is the most common malignancy of the urinary tract, with an estimated 357 000 new cases and 145 000 deaths annually [1]. More than 70% of newly diagnosed BCs are superficial at first presentation and are

☆ Disclosure: The authors have no conflict of interest.

☆☆ Funding: This work was supported by German National Federal Ministry of Education and Research (0313659B).

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http://dx.doi.org/10.1016/j.humpath.2013.10.036

curatively treated by transurethral resection with or without intravesical therapy. Patients with non–muscle-invasive BC have a high (~70%) recurrence rate but a low (~15%) progression rate resulting in a generally good prognosis with a 5-year survival rate of over 95%. Patients diagnosed with muscle-invasive BC (~30%) are usually treated with radical cystectomy with or without perioperative systemic chemotherapy. These patients are at high risk for metastatic tumor progression and cancer-related death with an overall 5-year survival estimated at only 50% to 60% [2]. Better insight into the molecular processes underlying bladder carcinogenesis, progression, and metastasis is needed to identify new prognostic and predictive markers and targets for therapy.

Syndecans are transmembrane proteoglycans capable of carrying heparan sulfate and chondroitin sulfate chains on their extracellular domains. Syndecan-1 (SDC1), also called CD138, is typically expressed in epithelial cells and mediates cell-cell and cell–extracellular matrix interactions [3]. The ectodomain of SDC1 is able to bind a variety of soluble and insoluble ligands, such as extracellular matrix components, growth factors, proteinases and other bioactive molecules and is therefore involved in the regulation of several cellular processes such as cell growth, migration and angiogenesis [3–6]. Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are 2 prominent proangiogenic factors bound by SDC1, which acts as an affinity coreceptor concentrating and presenting ligands to the high-affinity cell surface receptors [7,8].

The extracellular domain of SDC1 bearing the core protein and heparan sulfate chains can be proteolytically cleaved from the cell surface by a highly regulated shedding mechanism [9]. Released extracellular domain of SDC1 can be detected in cell culture supernatants and serum of both healthy and cancerous individuals [10]. Enhanced circulating SDC1 levels have been associated with poor survival of myeloma and lung cancer patients [10,11].

SDC1 has been shown to play a significant role in the maintenance of epithelial cell morphology [12]. Accordingly, loss of epithelial SDC1 expression was found to be associated with high tumor aggressiveness and poor prognosis in a number of epithelial cancers such as carcinomas of the head and neck, mesothelioma, non–small cell lung cancer, and hepatocellular carcinoma [13–16]. In addition, the appearance of stromal expression of SDC1 has been associated with poor prognosis in a variety of cancers [17–19].

The role of SDC1 in BC is poorly understood. The only study that analyzed SDC1 in BC tissues found high SDC1 expression in high-stage and high-grade BCs but did not assess its prognostic significance [20]. Furthermore, the serum levels of SDC1 ectodomain in BC have not been evaluated yet. Therefore, we analyzed tissue expression and serum concentrations of SDC1 in BC patients using immunohistochemistry and enzyme-linked immunosorbent assay (ELISA), respectively. We correlated these data with clinicopathological characteristics and follow-up data of BC patients. Furthermore, we assessed whether serum levels of SDC1 are correlated with those of angiogenic factors such bFGF, VEGF, Tie2, endostatin, angiostatin, angiopoietin-1 and -2, and matrix metalloproteinase 7 (MMP-7).

2. Patients and methods

2.1. Clinical samples

This study included a total of 213 subjects (198 BC patients and 15 healthy controls). Paraffin-embedded tissue samples of 119 patients were analyzed by SDC1 immunohistochemistry, and serum samples of 79 BC patients and 15 age-matched healthy controls were assessed by ELISA. Tissue and serum samples were obtained from patients who underwent surgical treatment for BC at the Department of Urology at the University Hospital of Essen between 1991 and 1994; all cases were reclassified by one pathologist (H.R.) according to the 2004 World Health Organization (WHO) classification of urothelial neoplasms [21]. The criteria for study enrollment were histopathological diagnosis of urothelial carcinoma of the bladder, exclusion of other tumor diseases and chemotherapy prior to bladder surgery, availability of sufficient tumor/serum sample and the potential to follow-up. The ethics committee of the University Hospital Essen approved the study protocol. Serum samples were collected before surgery, aliquoted, and stored at −80°C until analysis.

2.2. Measurement of serum SDC1 levels

SDC1 serum levels were quantified by using a sandwich ELISA (Diaclone CD138; Gene-Probe, San Diego, CA; cat. no. 950.640.096) according to the manufacturer's instructions.

2.3. Correlation between serum SDC1 levels and other serum markers

We previously analyzed serum levels of different factors in a partly overlapping patient cohort. Serum levels of angiopoietin-1, angiopoietin-2, Tie2 (tyrosine kinase with immunoglobulin and EGF factor homology domains 1), VEGF, angiostatin, endostatin, bFGF, and MMP-7 have been already reported in previously published studies [22–24].

2.4. Correlation between tissue SDC1 expression and other proteins

We correlated SDC1 tissue expression with those of previously analyzed tissue proteins such as MMP-7, endostatin, Tie2 and CD44 [24,25].