The value of plasma osteopontin levels as a predictive factor of disease stage and recurrence in patients with bladder urothelial carcinoma: A prospective study

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Received 14 June 2011; accepted 17 August 2011
Available online 26 September 2012

KEYWORDS
Bladder;
Osteopontin;
Urothelial carcinoma

Abstract This study was performed in order to determine the value of plasma osteopontin (OPN) levels as a predictive factor of disease stage and recurrence in patients with bladder urothelial carcinoma (UC). Data from 50 patients diagnosed to have bladder UC after transurethral resection of bladder tumor (TURBT) from 2009 to 2010 were evaluated prospectively. Blood tests were performed before and after TURBT, and plasma OPN levels were measured using enzyme-linked immunosorbent assay. Differences in OPN levels according to clinicopathologic variables were analyzed statistically. Significant differences in plasma OPN levels were observed between groups with and without muscle invasion (89.16 vs. 67.08 ng/mL, p = 0.041). Comparison according to tumor grade found no significant difference between high and low grade groups (p = 0.115). Mean plasma OPN levels decreased after TURBT without statistical significance (p = 0.571). Between groups with recurrence and those without recurrence, OPN levels of the group with recurrence were higher without statistical significance (p = 0.161). Comparison of plasma OPN levels according to performance of radical cystectomy (RC) showed significant differences; patients who underwent RC showed higher levels of plasma OPN (95.58 vs. 70.37 ng/mL, p = 0.030). Comparison according to T stage after RC showed significant differences in OPN levels (T1: 67.45, T2: 86.60 and T3: 95.23 ng/mL, respectively, p = 0.006). The group with lymph node invasion showed significantly higher levels of OPN, compared to the group without invasion (153.24 vs. 68.03 ng/mL, p = 0.017). Preoperative plasma OPN levels correlated to muscle invasion of bladder UC and pathological stage after RC.

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http://dx.doi.org/10.1016/j.kjms.2012.04.014
Introduction

Clinical stage and pathologic grade are usually the primary prognostic factors in urothelial carcinoma (UC) of the bladder [1]. However, even in patients with the same stage or grade, there have been differences in the biological potential [2,3]. Therefore, in order to provide further prognostic information relevant to a particular UC of the bladder in a specific patient, and to allow for more appropriate therapies for individuals, novel biomarkers are under continuous investigation [4]. Unfortunately, there has been no reliable biomarker for diagnosis, prediction of prognosis, or selection of appropriate treatment modalities for UC of the bladder.

Osteopontin (OPN) is an integrin-binding glycoprotein secreted by many normal tissues, and is found in blood, urine, and breast milk [4]. OPN is involved in both normal and pathological processes, and is thought to have a role in promotion of tumor cell survival, invasiveness, and progression [5,6]. Thus, increased OPN expression correlates with tumorigenic transformation in a variety of stromal and epithelial cell lines [7]. Clinical studies have linked OPN to tumorigenesis, progression, and metastasis in various human tumors, including breast, colon, gastric, lung, ovarian, and prostate cancer [8]. In addition to its expression in tissues, overexpression of OPN in the blood of patients with a variety of other cancers, including breast, colon, hepatic, and hormone-refractory prostate cancers has been reported [9–11]. There is also the possibility of correlation between plasma OPN levels and disease stage and prognosis in patients suffering from UC of the bladder. However, only a few studies have been conducted to investigate the value of plasma OPN levels in UC of the bladder [4]. We therefore hypothesized that, in patients with UC of the bladder, plasma OPN level might be predictive of disease stage, as well as recurrence of disease. Objectives of this study were therefore to evaluate this hypothesis in a pilot study for assessment of the value of plasma OPN levels as a predictive biomarker for UC of the bladder.

Materials and methods

The study protocol was reviewed and approved by the institutional review board of the Korea University hospital (IRB No.: GRO891-003). From January 2009 to March 2010, blood samples were collected from 50 consecutive patients (male: 44, female: 6) with UC of the bladder, before transurethral resection of bladder tumor (TURBT), at the Korea University Guro Hospital oncology clinic and Ansan Hospital urology clinic. The inclusion criteria of the present study limited the participants to those with newly diagnosed UC of the bladder, and patients were excluded if they had coexisting malignancy of another organ, past history of urolithiasis, or a recent urinary tract infection, as OPN levels might be affected by these conditions [8,12,13]. Patients who agreed to participate in this study were followed up after TURBT, and monthly history and physical examination, urinalysis, and urine cytology were included in periodic follow-up. Abdominopelvic computed tomography (AP-CT) and chest X-ray were performed every 6 months. Cystoscopy was performed every 3 months. Pathological stage was assigned using TNM staging and grading criteria [14,15], and clinical stage was also assigned using reports from imaging studies, including chest X-ray, AP-CT, and bone scans. Bladder recurrence was defined as transurethral resection or biopsy of the bladder lesion confirmed by pathology during follow-up periods after initial TURBT. Extra-bladder recurrence included local recurrence and distant metastasis (beyond the regional lymph nodes) diagnosed by AP-CT and bone scan. Blood tests for measurement of plasma OPN levels were performed two times—i.e., before TURBT and 3 months after TURBT. Blood was centrifuged at 600 × g for 15 minutes in order to obtain plasma. Plasma OPN levels were measured using the enzyme-linked immunosorbent assay, as described by previous investigators [9,10,16,17]. Plasma OPN levels were analyzed statistically, as follows: paired t-test for comparison of pre- and post-TURBT, independent t-test for comparison according to pathologic variables (muscle invasiveness, grading and lymph node invasion), one-way analysis of variance for comparison according to urine cytology and pathological T-stage, and Kaplan–Meier analysis for comparison of recurrence-free survival (RFS) between subgroups; p < 0.05 was considered to indicate statistical significance. The SPSS software version 14.0 (SPSS Inc., Chicago, IL) was used for statistical analysis.

Results

The average age of patients was 63.2 years (range 30–89 years); the mean follow-up period was 19.9 months (range 12–26 months), and no deaths occurred during the study period. Among 50 patients, 28 (56%) had more than one comorbidity, including hypertension, diabetes, and cardiovascular disease. Twenty-four (48%) were smokers or ex-smokers. Baseline characteristics of the investigated patients are summarized in Table 1.

Comparison of plasma OPN levels according to the results of urine cytology before TURBT showed no significant difference between the four subgroups (negative, atypical cell present, suspicious of malignancy and malignant cell present group; p = 0.497). Among 50 patients, pathologic results for nine (18%) patients after TURBT showed muscle invasion and there were significant differences in plasma OPN levels between the groups with and without muscle invasion (p = 0.041). With respect to tumor grade after TURBT, pathologic results for 18 (36%) patients showed that high-grade and plasma OPN levels did not appear to differentiate between patients with high grade and those with low grade tumors (p = 0.115). Plasma OPN levels showed a decreasing trend after TURBT, without significant difference, compared with preoperative OPN levels (p = 0.571). After TURBT, 16 patients received intravesical BCG instillation, and none of the patients received intravesical chemotherapy. Recurrence after TURBT was noted in 13 (26%) of these patients, and between groups with recurrence and those without recurrence after TURBT, OPN levels of the group with recurrence showed higher levels; however, no statistically significant difference was observed between the two groups (p = 0.161). In the present study, 15 (30%) patients...
underwent radical cystectomy (RC) during the follow-up period. Of the total, nine patients with muscle invasion and three patients with high-grade T1 disease after TURBT underwent immediate RC, and three patients with high-grade T1 disease who received intravesical BCG instillation after TURBT eventually underwent RC after recurrence of bladder TCC. Comparison of plasma OPN levels according to performance of RC showed significant difference ($p = 0.030$); patients who underwent RC showed higher levels. Comparison according to T stage after RC showed significant differences in OPN levels ($p = 0.006$); the higher the T stage, the higher the OPN level. In addition, among 15 patients who underwent RC, five patients showed lymph node metastases and they showed significantly higher levels of OPN, compared to the group without lymph node invasion ($p = 0.017$). Results of our study are summarized in Table 2.

The mean plasma OPN level of 50 patients was 77.48 ± 33.76 ng/mL (range 31.31–246.84 ng/mL) and the median was 67.20 ng/mL. We divided patients into two groups according to the median: patients with lower OPN levels (group I) and those with higher OPN levels (group II).

Comparison of RFS rate using Kaplan–Meier curves showed nonsignificant difference in RFS between the two groups ($p = 0.744$) (Fig. 1).

**Discussion**

OPN is an adhesive glycoprotein, first identified in bone, and subsequently discovered in kidney, liver, brain, and many cells of epithelial linings [18]. The glycoprotein structure has multiple function domains [1]. The N-terminal region binds to integrins, leading to cell attachment and activation of matrix-degrading proteases [1]. The OPN–integrin complex mediates tumor cell invasion and migration, induces angiogenesis, and activates osteoclasts, facilitating bone metastases [1]. It also stimulates
levels (lower (group I) and higher (group II) plasma osteopontin (OPN) difference in recurrence-free survival between patients with adhesion [18,19]. According to Yoon et al. [20], interleukin 12 and interferon expression, thus altering host-immunity [1]. The C-terminal region binds to CD44, activating the pathways of cell survival, chemotaxis, homing, and adhesion [18,19]. According to Yoon et al. [20], expression of OPN in UC of the bladder showed a significant relationship with the invasiveness of the disease. In other studies of the function of OPN in UC of the upper urinary tract and their findings, Ke et al. [1] reported that higher OPN expression was a potential biomarker for prediction of patient survival.

Few studies on the value of plasma OPN in UC of the urinary tract have been conducted, and only one pilot study has reported on UC of the bladder. According to Ang et al. [4] in their evaluation of the value of OPN in UC of the bladder in 2005, OPN appears to be associated with disease stage and to reflect tumor burden, as for other tumor systems [9,10]. However, they did not limit participants to newly diagnosed UC of the bladder and checked the plasma OPN level at just one time point, without follow-up; therefore, the prognostic value of OPN was not adequately evaluated.

In the present study, we limited participants to newly diagnosed UC of the bladder and checked the plasma OPN level at more than one time—i.e., preoperatively and postoperatively. In addition, we followed up the patients for more than 1 year, in order to evaluate the association between preoperative plasma OPN level and recurrence. However, in the present study, several main limitations exist, i.e., the relatively small number of patients and short follow-up period, as well as the lack of investigation of tissue OPN. In addition, the lack of control levels of plasma OPN in the present study was also a limitation. Despite these limitations, we were able to confirm that the possibility and value of preoperative plasma OPN levels correlated with the tendency to represent muscle invasion, which has a decisive effect on the treatment strategy for UC of the bladder. In addition, we found that preoperative plasma OPN levels correlated with both the T stage and lymph node involvement in the pathologic results of RC, demonstrating that the higher the preoperative plasma OPN levels, the more severe the pathological and clinical stage. With respect to the correlation between plasma OPN levels and grade, although statistical significance was not confirmed, our results showed a trend toward increase of plasma OPN levels in the group with high grade bladder UC. The detailed function of OPN in tumor progression is very complex and is not yet completely known [1]. Many downstream signals, including P13K/Akt, NF-κB, MMP-2, and EGFR, are regulated by OPN and have been known to play important roles in tumor migration and invasion [19].

As well as the utility of plasma OPN to provide information on prognosis, stratifying patients for adjuvant therapies, prediction of recurrence and survival after treatment is also valuable. In analysis of RFS between low and high OPN groups, our results showed no significant difference between the two groups. With respect to the reason for this result, as mentioned above, the relatively short follow-up period and small population sample could contribute to the results. In addition, significant difference was observed in plasma OPN levels according to muscle invasion after TURBT; therefore, most of the high OPN group patients tended to have a higher T stage, and, in cases of higher T stage, immediate RC was preferred; therefore, immediate RC could contribute to elimination of the possibility of recurrence in the higher T stage group. Although there was no significant difference in preoperative plasma OPN levels according to the recurrence of bladder UC in our study, if longer follow-up with more patients is available, we can obtain more accurate information on the value of preoperative plasma OPN as a predictive marker for recurrence and survival in patients of bladder UC. In addition, we expect the possibility of the use of plasma OPN in monitoring responses to treatment in patients with bladder UC. Although postoperative plasma OPN levels were not checked in all patients and the statistical significance was not established, plasma OPN levels tended to decrease after surgery in our study; therefore, if consecutive measurement of plasma OPN levels after surgery and during regular follow-up is possible, we can assess the changes in plasma OPN levels at the time of recurrence and evaluate the value of the plasma OPN as a monitoring tool for patients of bladder UC.

Conclusion

Preoperative plasma OPN levels were correlated to muscle invasion. In addition, plasma OPN levels appear to increase with disease burden in bladder UC. The implications of these findings may correlate with the value and usefulness of plasma OPN as a promising marker for predicting risk and clinical prognosis in bladder UC. Therefore, larger and more extended studies of plasma OPN in patients with bladder UC are needed.

Acknowledgments

The present research was funded by the research fund of the Korea Health Industry Development Institute in 2009.
References


