Heme oxygenase-1 expression is associated with tumor aggressiveness and outcomes in patients with bladder cancer: a correlation with smoking intensity

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Heme oxygenase (HO)-1 is upregulated in malignancies and, in turn, regulates other cancer-related factors. Although HO-1 expression has been associated with cigarette smoking under various pathologic conditions, little is known about their association in patients with bladder cancer. HO-1 expression was assessed in 215 formalin-fixed bladder cancer specimens by immunohistochemistry. Microvessel density, lymph vessel density (LVD), proliferation index (PI), and expression of the vascular endothelial growth factor (VEGF)-A, -C, and -D, cyclooxygenase (COX)-2, matrix metalloproteinase (MMP)-2, and MMP-9 were investigated by similar methods. Multivariate analyses were performed to evaluate the pathologic role and predictive value of HO-1 expression. Our results demonstrated that HO-1 expression was positively associated with T stage, lymph node metastasis, and grade. HO-1 expression was also positively correlated with PI, LVD, and expression levels of VEGF-D, COX-2, MMP-2, and MMP-9 ($P < 0.001$). In addition, multivariate analyses showed that HO-1 expression positively correlated with smoking intensity. Positive HO-1 expression was a significant predictor of subsequent metastasis ($P = 0.008$) and poor cause-specific survival ($P < 0.001$). Similarly, multivariate analyses showed that HO-1 expression was a predictor of cause-specific survival (hazard ratio = 3.13, $P = 0.013$). In conclusion, pathologic changes of HO-1–related factors were dependent on smoking intensity. Smoking upregulated HO-1 expression, and HO-1 was associated with malignant behavior of bladder cancer. Cancer cell proliferation, lymphangiogenesis, and expression levels of VEGF-D, COX-2, and MMP-2 played important roles in these HO-1–related effects. The clinical correlations of HO-1 were regulated by a complex mechanism that depended on smoking intensity. (Translational Research 2014;164:468–476)

Abbreviations: CI = confidence interval; COX = cyclooxygenase; HO = heme oxygenase; HPF = high-power field; HR = hazard ratio; IQR = interquartile range; IRS = immunoreactive score; LVD = lymph vessel density; MIBC = muscle invasive bladder cancer; MMP = matrix metalloproteinase; MVD = microvessel density; OR = odds ratio; PI = proliferation index; SD = standard deviation; UC = urothelial cancer; VEGF = vascular endothelial growth factor

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AT A GLANCE COMMENTARY

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Background

Heme oxygenase (HO)-1 is up-regulated in malignancies and, in turn, regulates various cancer-related factors and molecules. In addition, HO-1 expression has been associated with cigarette smoking. However, little is known about its smoking-related function in bladder cancer.

Translational Significance

In this manuscript, we demonstrated that HO-1 was up-regulated by smoking and was associated with malignant behavior including cancer cell proliferation, lymphangiogenesis, and expression of VEGF-D, COX-2, and MMP-2. These pathological roles of HO-1 in bladder cancer were regulated by a complex mechanism that depended on smoking intensity.

INTRODUCTION

Angiogenesis and lymphangiogenesis are important for tumor growth and cell dissemination in nearly all solid tumors including urothelial cancer (UC).[1,2] In fact, tumor-associated angiogenesis and lymphangiogenesis are closely correlated with survival in patients with UC.[3] Moreover, cancer cell proliferation and invasion crucially mediate outcomes in these patients.[4,5] Vascular endothelial growth factors (VEGFs) are well-known mediators of angiogenesis and lymphangiogenesis under pathologic conditions. In the past, VEGF-A was thought to be important for angiogenesis, whereas VEGF-C was thought to be important for lymphangiogenesis. However, recent studies have demonstrated that VEGFs are not limited to these specific pathologic roles. VEGF-A has been associated with lymphangiogenesis,[6] and VEGF-C can stimulate lymphangiogenesis under various pathologic conditions.[7] In addition, VEGF-D expression has been positively associated with lymphangiogenesis in cancer tissues.[3] Cyclooxygenase (COX)-2 is a well-known angiogenesis-related molecule.[8] COX-2 also functions in tumor growth, progression, and survival in patients with bladder cancer.[9] Furthermore, matrix metalloproteinase (MMP)-2 and MMP-9 are closely associated with cancer cell invasion and metastasis in various cancers.[10,11] Elucidating the regulatory mechanism of these cancer-related factors is essential for identifying predictive markers and potential therapeutic targets in bladder cancer.

Cigarette smoking is an important risk factor for UC, especially in the developed world.[12] Interestingly, the cancer-related molecules mentioned previously have also been associated with smoking.[13-15] There is a general agreement that smoking positively correlates with tumor aggressiveness in many malignancies. However, it remains unclear how smoking regulates the expression of VEGFs, COX-2, and MMPs in patients with bladder cancer.

In the present study, we focused on heme oxygenase (HO)-1 for the following reasons: (1) HO-1 regulates the expression of VEGF-A, COX-2, and MMPs under various pathologic conditions[16-18]; (2) HO-1 is associated with features of tumor aggressiveness, including angiogenesis and cell proliferation[19-21]; and (3) cigarette smoking induces HO-1 expression in a variety of cancer cells.[17,22] Therefore, the main aim of this study was to clarify the changes of pathologic significance and predictive roles of HO-1 expression with respect to smoking status in patients with bladder cancer. Furthermore, we tested the hypothesis that smoking intensity is correlated with angiogenesis, lymphangiogenesis, and cancer cell proliferation via the regulation of VEGF-A, VEGF-C, VEGF-D, COX-2, MMP-2, and MMP-9 in human bladder cancer tissues.

MATERIALS AND METHODS

Patients and tissue samples. Two hundred fifteen consecutive patients who were diagnosed with bladder cancer were reviewed retrospectively. Our study included 166 men (77.2%) and 49 women (22.8%), and their mean age at diagnosis was 68.6 years (standard deviation [SD], 11.6; median, 70). Patients who had received any preoperative therapy, such as chemotherapy and/or radiotherapy, were excluded from the study. In addition, tumors with squamous cell carcinoma and/or adenocarcinoma were excluded regardless of pure and a part of tumor from this study. All histologic diagnoses were performed using formalin-fixed and paraffin-embedded specimens. Staging and grade was assessed in accordance with the 2002 tumor-node-metastasis classification and the 2004 World Health Organization grading system. A single pathologist performed all pathologic examinations. The median follow-up period was 60.9 months (range, 3–256 months). We explained the purpose, methods, and right to refuse all patients in writing, and we obtained informed consent in writing. The study protocol, including methods of informed consent and protection of personal information, was approved by the Human Ethics Review Committee of Nagasaki University Hospital.