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M2-macrophage infiltration and macrophage traits of tumor cells in urinary bladder cancer

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Abstract

Background: Tumor-associated macrophages (TAMs) constitute a subset of nonneoplastic cells in tumor stroma and influence cancer progression in solid tumors. The clinical significance of TAMs in urinary bladder cancer (UBC) is controversial.

Methods: We prospectively studied 103 patients with stage pT1–T4 UBC treated with cystectomy and pelvic lymph node dissection. Tumor sections were immunostained with M2-specific macrophage marker CD163 and proliferation marker Ki-67. The expression of these markers in cancer cells as well as macrophage infiltration (MI) in tumor stroma was analyzed in relation to clinical data and outcome.

Results: The mean rate of CD163 and Ki-67 expressed by cancer cells were 35% and 78%, respectively. With borderline significance, MI was associated with lower rate of lymph node metastasis ($P = 0.06$). CD163 expression in cancer cells was proportional to MI ($P < 0.014$). Patients with CD163-positive tumors and strong MI had significantly longer cancer-specific survival (CSS) (76 months), compared to patient with CD163-positive tumors and weak MI (28 months) ($P = 0.02$).

Conclusions: M2-specific MI tends to be inversely correlated with LN metastasis and improved CSS in UBC. MI might have protective impact in CD163-positive tumors. Expression of CD163 in cancer cells is significantly correlated with MI and might have a tumor promoting impact.

Keywords: Bladder cancer; Tumor-associated macrophages; Lymph node metastasis; Ki-67; CD163; Macrophage traits in tumor cells

1. Introduction

Muscle-invasive bladder cancer (MIBC) occurs in approximately 30% of all patients with UBC. Despite advances in diagnosis and treatment of UBC, the mortality rate has not improved substantially in the past 30 years [1,2]. UBC metastasize via lymphatic dissemination, and lymph node (LN) metastasis is associated with poor prognosis [3].

Tumor-associated macrophages (TAMs) are heterogeneous, terminally differentiated myeloid cells that constitute an important component of nonmalignant cells in tumor stroma and promote cancer growth and progression [4]. TAMs acquire M2-macrophage phenotype and exhibit a high degree of plasticity in response to various stimuli in the tumor microenvironment [5]. Accumulating evidence suggest that increased macrophage infiltration (MI) in several tumor types is correlated with advanced tumor stages and progression [6,7]. Nevertheless, the clinical significance of MI in tumor stroma is still controversial as other studies indicate that MI has antitumor activity and increases disease-free survival [8,9].

Macrophage traits in cancer cells are reported for several types of tumors (e.g., breast and colorectal cancers) and have been suggested to be the result of fusion between TAMs and cancer cells [6,10,11]. Such hybrid cells exhibit genetic and phenotypic characteristics originating from both maternal cells [12,13]. CD163 is a macrophage-specific

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transmembrane scavenger receptor and indicate M2-macrophage phenotype [14]. CD163 expression by breast and colorectal cancer cells is associated with early tumor recurrence and reduced survival time, which agrees with the concept that tumor cell × normal cell hybrids may exhibit a more malignant phenotype [15]. MI and CD163 expression by tumor cells have been described in UBC, but the published reports are based mainly on examination of tumor samples obtained after transurethral bladder resection and detection of macrophages by markers that are not entirely specific for M2-macrophages [16–18].

The aim of this study is to investigate the expression of macrophage antigen CD163 in tumor cells and intratumoural macrophage infiltration (MI) in UBC. We hypothesized that MI in UBC will result in an increased number of cancer cells with a macrophage phenotype, elevated cell proliferation, and more advanced tumors.

2. Materials and methods

2.1. Patient material and study design

Patients with stage pT1–4 UBC treated with radical cystectomy between 2005 and 2011 at the Department of Urology, University Hospital, Linköping, Sweden, were included. The study was approved by the Regional Ethics Committee in south-eastern Sweden (Ref no: Lu 01-48 and Lin 2010/311–31). All operations were performed with curative intent. Patients with preoperatively known distant metastases and previous radiotherapy of the pelvis were excluded from the study. Pelvic LN dissection (PLND) was performed on all patients, primarily as a standard dissection to the ureteric crossing. The patients were treated with adjuvant chemotherapy if metastatic LNs were detected or if an advanced T-category was found at postoperative pathological examination of removed tumor [19]. Five patients died due to postoperative complications within 1 month after surgery. These patients were excluded from survival analysis, as the mortality cannot be related to the biological course of UBC.

All the original sections of cystectomy specimens including urinary bladder tumors and LNs that were used in the initial routine clinical histopathological assessment were reevaluated by an experienced uropathologist (HO) blinded to clinical data and outcome. Primary tumors were graded according to the WHO 1999 and TNM 2010 classifications.

2.2. Immunostaining and antibodies

In previous studies, we have documented CD163 as a surrogate marker for fusion between cancer cells and TAM [10–12,20]. We have consistently chosen CD163 as a marker to detect macrophage phenotype in cancer cells as an indirect indicator of hybridization between these cells. Mouse antihuman monoclonal CD163 antibody (clone 10D6, Leica Biosystem, Germany) was used as a macrophage marker. Tumor cell proliferation was detected with monoclonal mouse antihuman Ki-67 antibody (clone Mib-1, Dako, Demark). Serial 5-μm sections from formalin-fixed paraffin-embedded tumor tissue blocks were stained according to DAKO Envision system described in previous studies [11]. Positive staining of tissue macrophages was used as internal positive control. As negative controls, the primary antibody was replaced with an isotype antimouse immunoglobulin G1 antibody.

2.3. Evaluation of immunostaining and MI

Immunostaining of tumor sections with CD163 was evaluated independently by 3 of the authors (FA, IS, and HO). Positive Ki-67 expression was defined by nuclear staining in cancer cells. The Ki-67 expression in tumor areas showing the highest density of stained tumor cells was determined by an initial scan at low magnification (×20). The number of positively stained nuclei was counted in 200 tumor cells at high magnification (×40) and was recorded as a percentage of the total number of cells.

The immunostaining of CD163 was defined as a granular cytoplasmic or a cytoplasmic and membranestaining pattern. TAMs and cancer cells were distinguished based on morphological criteria. Cells expressing CD163 with small and regular nuclei were recognized as M2-macrophages. TAM infiltration in tumor stroma was evaluated semiquantitatively over a whole section and was classified in 3 grades: no/low, moderate, or high (Fig. 1).

Cancer cells were enlarged and atypical, with pleomorphic, hypertrophic, and darker nuclei, and also showed a decreased cytoplasmic:nuclear ratio. A tumor was regarded as positive if it contained any CD163-positive cancer cells and was considered negative if it lacked such cells (Fig. 1). Interobserver agreement, calculated as Cohen kappa index, was $\kappa = 0.56$. The cases with discordant results were discussed between all investigators to reach consensus.

2.4. Statistical analysis

Statistical analyses were performed using SPSS statistics software, version 23 (IBM Corporation). Pearson's chi-square test was applied to evaluate the relationship between CD163 expression and MI grade in relation to clinical data and tumor characteristics. Comparison of clinical data, MI grade, and proportion of cancer cells expressing Ki-67 and CD163 was achieved by one-way analysis of variance (ANOVA) together with a post-hoc Bonferroni's test. Survival rates were estimated according to Kaplan-Meier and were based on cancer-specific survival (CSS) with a median follow-up of 23 months (range of 3-128 months). The statistical significance of differences between survival rates was determined by the log-rank test. $P < 0.05$ was considered statistically significant.