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Urologic Oncology: Seminars and Original Investigations ▮ (2018) ▮▮▮–▮▮▮

UROLOGIC ONCOLOGY

Seminars Article

Myeloid-derived suppressors cells (MDSC) correlate with clinicopathologic factors and pathologic complete response (pCR) in patients with urothelial carcinoma (UC) undergoing cystectomy

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Received 25 July 2017; received in revised form 29 January 2018; accepted 27 February 2018

Abstract

Background: Myeloid derived suppressor cells (MDSC) are heterogeneous immunosuppressive cells with potential predictive and prognostic roles in cancer. The association between MDSC, clinicopathologic factors, and pathologic response in patients with bladder urothelial carcinoma (UC) was explored.

Methods: Peripheral blood or tissue were collected from patients with UC undergoing definitive surgery. MDSCs levels were measured in peripheral blood mononuclear cells and fresh tumor tissue. MDSCs were identified by flow cytometry and defined as total MDSC (T-MDSC) CD33+/HLADR−. From this population, 3 subsets were identified: polymorphonuclear-MDSC (PMN-MDSC) defined as CD33+/HLADR−/CD15+/CD14-, monocytic-MDSC (M-MDSC) defined as CD33+/HLADR-/CD15-/CD14+, and immature-MDSC (I-MDSC) defined as CD33+/HLADR−/CD15−/CD14−. MDSC populations were presented as % of live nucleated blood cells. Spearman correlations (r) and Wilcoxon rank sum test were used to assess correlations between MDSC populations, clinicopathologic factors, and pathologic complete response (pCR).

Results: 85 patients scheduled to undergo cystectomy from February 2015 through Dec 2016 were included. All patients had blood drawn for analysis and 23 patients had residual tumor tissue collected for analysis at the time of surgery. Of these 85, 74 (87%) were men with a median age at diagnosis of 68 (range: 44–87). Pure UC was the most common histology (75%); 28 (35%) patients had prior treatment with intravesical therapy and 36 (42%) were treated with neoadjuvant chemotherapy, primarily gemcitabine plus cisplatin ($n = 24$). On surgical pathology, 18 (21%) of the patients had pCR, 11 (13%) had positive lymph nodes, and 20 patients (24%) had lymphovascular invasion. Statistically significant associations were found between circulating MDSC levels and pCR rates ($P < 0.01$), absolute neutrophil-lymphocyte ratio ($P = 0.008$), and histology ($P = 0.01$). Tumor % M-MDSCs were negatively associated with lymphovascular invasion ($P = 0.04$). There were no significant correlations between peripheral blood mononuclear cells and tumor MDSC subtypes.

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https://doi.org/10.1016/j.urolonc.2018.02.018

Conclusions: Blood and tissue MDSC levels correlate with several clinicopathologic factors and may predict for pCR. Future studies are needed to highlight the role of MDSC in predicting long-term outcomes and to determine the clinical implications of these findings.

Keywords: Bladder cancer; Urothelial carcinoma; Myeloid-derived suppressor cells; MDSC; Tumor immunology; Biomarkers

Introduction

Bladder cancer is the most common cancer of the urinary tract with urothelial carcinoma (UC) as its most common histologic type [1]. Approximately 20% to 40% of patients have muscle-invasive urothelial carcinoma (MIUC) either at initial diagnosis or following progression from a previously non–muscle-invasive tumor state [2]. Radical cystectomy and bilateral pelvic lymph node (LN) dissection remains the standard treatment for most patients with nonmetastatic MIUC, and neoadjuvant cisplatin-based chemotherapy improves long-term survival compared to patients undergoing surgery alone [3].

The clinical and pathological features at the time of cystectomy can be prognostic for disease recurrence and long-term outcomes for patients with MIUC. In particular, the presence of lymph node (LN) involvement, higher tumor stage, and lymphovascular invasion (LVI) are poor prognostic factors [4,5]. Conversely, a complete pathological response (pCR) following cystectomy predicts for improved progression-free survival and overall survival (OS) [6–9]. To date, limited biomarkers exist to predict surgical pathological outcomes at the time of cystectomy [10]. Such data could be useful in a variety of settings including potentially favoring a bladder-sparing definitive therapy or guide future surveillance approaches instead of radical cystectomy for patients who have a pCR to neoadjuvant chemotherapy [11–15].

Myeloid derived suppressor cells (MDSCs) are a phenotypically diverse population of bone marrow-derived cells that play an important role in tumor progression based on their immunosuppressive and proangiogenic properties [16]. Circulating MDSCs have been shown to correlate with stage, tumor burden, treatment response, and clinical outcomes in a variety of cancers [17–19]. Circulating MDSC subsets have been previously shown to be highly proliferating and activate a host of proinflammatory cytokines in patients with bladder cancer [20]. In this study, the correlation between blood and tissue MDSCs, clinicopathologic features, and neoadjuvant treatment outcomes in patients with MIUC undergoing radical cystectomy was investigated.

Materials and methods

Patients

Patients with UC of the bladder who were scheduled to undergo cystectomy were all consented on an IRB approved protocol (Cleveland Clinic IRB 14–1222, approval date February 12, 2015) for blood and tissue collection prior to surgery. All surgical patients were considered for inclusion regardless of prior intravesical therapy or neoadjuvant chemotherapy. Patients with metastatic disease at the time of surgery were excluded from the analysis, with the exception of patients with only regional pathological LN involvement, defined as the pathological stage at the time of cystectomy. The institutional standard is to perform extended LN dissections. As all Ta and CIS tumors included for analysis were high-grade, they are summarized together.

Laboratory assays

MDSCs levels were measured in fresh peripheral blood mononuclear cells (PBMC) and in fresh tumor tissue. PBMCs were purified from unfractionated blood by Ficoll separation. Tumor samples were digested to single cell suspensions using the Miltenyi Tumor Dissociation Kit following manufacturer's instructions. Cell viability of $>90\%$ was determined by tryphan exclusion before staining took place. Purified PBMCs and tumor cell suspensions were incubated with mAb directed against CD33, HLADR, CD15, or CD14 (BD Biosciences). After incubation samples were acquired using a LSR-Fortessa Cell Analyzer (BD Biosciences), and data was analyzed using FlowJo software. MDSC populations were presented as percentage of viable cells. Neutrophil and lymphocytes were measured as part of standard complete blood count.

Total MDSCs (T-MDSC) were defined as CD33+/HLADR- cells. Out of this population, 3 MDSC subtypes were identified: polymorphonuclear MDSCs (PMN-MDSC) defined as CD33+/HLADR-/CD15+/CD14- cells, monocytic MDSCs (M-MDSC) defined as CD33+/HLADR-/CD15-/CD14+, immature MDSCs (I-MDSCs) defined as CD33+/HLADR-/CD15-/CD14-. T-MDSC were also reported. Given the significant complexity of choice and identification of MDSC subsets, these specific subsets were chosen based on general consensus of the identification of PMN-, M-, and I-MDSCs in the literature as well as our institution′s prior experience and publications with these MDSC subsets [19,21,22]. Gating strategy for both PBMCs and tumor cell suspensions is depicted in Fig. 1.

Statistical considerations

Categorical data were summarized as frequency counts and percentages, continuous data as medians and ranges.