Circulating myeloid-derived suppressor cells in patients with pancreatic cancer

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BACKGROUND: Myeloid-derived suppressor cells (MDSCs) are heterogeneous cell types that suppress T-cell responses in cancer patients and animal models, some MDSC subpopulations are increased in patients with pancreatic cancer. The present study was to investigate a specific subset of MDSCs in patients with pancreatic cancer and the mechanism of MDSCs increase in these patients.

METHODS: Myeloid cells from whole blood were collected from 37 patients with pancreatic cancer, 17 with cholangiocarcinoma, and 47 healthy controls. Four pancreatic cancer cell lines were cocultured with normal peripheral blood mononuclear cells (PBMCs) to test the effect of tumor cells on the conversion of PBMCs to MDSCs. Levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) and arginase activity in the plasma of cancer patients were analyzed by enzyme-linked immunosorbent assay.

RESULTS: $CD14^+/CD11b^+/HLA\text{-}DR^-$ MDSCs were increased in patients with pancreatic or bile duct cancer compared with those in healthy controls, and this increase was correlated with clinical cancer stage. Pancreatic cancer cell lines induced PBMCs to MDSCs in a dose-dependent manner. GM-CSF and arginase activity levels were significantly increased in the serum of patients with pancreatic cancer.

CONCLUSIONS: MDSCs were tumor related: tumor cells induced PBMCs to MDSCs in a dose-dependent manner and circulating $CD14^+/CD11b^+/HLA\text{-}DR^-$ MDSCs in pancreatic cancer patients were positively correlated with tumor burden. MDSCs might be useful markers for pancreatic cancer detection and progression.

(***Hepatobiliary Pancreat Dis Int 2016;15:99-105***)

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doi: 10.1016/S1499-3872(15)60413-1
Published online September 9, 2015.

KEY WORDS: **pancreatic cancer;**
myeloid-derived suppressor cells;
granulocyte-macrophage colony-stimulating factor;
arginase

Introduction

Pancreatic cancer is a highly aggressive disease that is usually at an advanced stage at diagnosis in most patients and therefore, the prognosis is extremely poor. It is the sixth most common cause of cancer-related mortality in China. Novel therapies, such as immunotherapy, are therefore urgently needed for treatment of this disease. Pancreatic cancer-associated antigens have spurred the development of vaccination-based treatment strategies. Although animal models have offered promising results, most clinical studies found only limited success,[1] which may be due to immune suppression mechanisms.[2] Abnormal accumulation of myeloid-derived suppressor cells (MDSCs) is thought to play a critical immunosuppressive role in tumor immune evasion and promotion.[3]

MDSCs expand during cancer, inflammation and infection in both preclinical models and human patients. In mouse models, MDSCs are described as $CD11b^+/Gr\text{-}1^+$ cells. More recently, however, MDSCs have been divided into monocytic and granulocytic subsets, reflecting differential expression of Ly6C and Ly6G markers.[4, 5] Although $CD15^+$ granulocytic MDSCs and $CD33^+$ MDSCs have been widely studied,[6-11] the origin of monocytic MDSCs in pancreatic cancer is still not clear. It was demonstrated that in patients with hepatocellular carcinoma, $CD14^+$ MDSCs induce T-cell anergy through multiple mechanisms.[12, 13] Tumor-secreted proinflammatory molecules induce MDSCs, leading to the hypothesis that inflammation promotes accumulation of MDSCs, which down-regulates immune surveillance and anti-tumor immunity and therefore, facilitates tumor growth.[14] A recent study[15] showed that monocytic MDSC subset has the immunoregulatory properties in many solid tumors and *in vitro* assays. Aberrantly expressed granulocyte-macrophage colony stimulating factor (GM-CSF) rep-

resents a key inflammatory component that facilitates monocytic MDSC accumulation.[16] MDSCs have a remarkable ability to suppress T-cell reactivity by production of arginase, reactive oxygen species, inducible NO synthase, IL-10, IL-6, transforming growth factor-β, and sequestration of cysteine.[17-21] The present study focused on the accumulation of monocytic MDSCs, their relevance to tumor burden and the possible mechanisms.

Methods

Patients

Peripheral blood specimens were collected from 37 patients with pancreatic cancer (mean age: 57 years; range: 50-71) and 17 patients with cholangiocarcinoma (mean age: 55 years; range: 53-68) who were newly diagnosed at the Department of Biliary-Pancreatic Surgery, Affiliated Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, from May 2011 to October 2012 (Table). All cancer patients were grouped in accordance with the *American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 7th edition (2010)*. We collected 4 mL of venous blood from each patient in heparin-lithium-green top tubes (BD Biosciences, Bedford, MA, USA), before surgery, radiation, or any systemic chemotherapy. Forty-seven age-matched normal healthy volunteers served as controls. These studies were approved by the Ethics Committee of Tongji Medical College. Informed consent was obtained from all subjects.

Cell lines and cell culture

Pancreatic cancer cell lines were obtained from the Cell Repository, Chinese Academy of Sciences. BxPC-3 and SW-1990 were cultured in RPMI-1640 (Gibco, Life Technologies Corporation, Shanghai, China), Mia PaCa-2 and Panc-1 were in Dulbecco's Modified Eagle's Media (Gibco), supplemented with 10% FBS (Gibco), at 37 ℃ in a humidified 5% CO_2 incubator, and passaged 2-3 times per week by brief trypsinization.

Antibodies and flow cytometry analysis

All monoclonal antibodies used in the study were purchased from BD Biosciences. Staining was performed on fresh venous blood collected in heparin-lithium-green tubes. Briefly 100 μL of blood was mixed with 10 μL of each antibody or isotype control. Tubes were incubated at 4 ℃ away from light for 30 minutes. After incubation, each sample was mixed with 2 mL of red blood cell lysing buffer and incubated for 10 minutes twice. Samples were washed with BD Pharmingen Stain Buffer. Pellets were resuspended in 200 μL of the buffer. Samples were acquired and analyzed by flow cytometry using LSR II (BD Biosciences). Fc receptors were blocked by pre-incubating cells with 10 μL of Fc receptor blocker (Miltenyi Biotec, Gladbach, Germany). Freshly drawn whole blood from patients was then labeled with FITC-conjugated CD14, PE-Cy7-conjugated CD11b, and APC-conjugated HLA-DR monoclonal antibodies. After lysing the red blood cells, flow cytometry samples were taken. Target cells were mainly found among peripheral blood mononuclear cells (PBMCs; P1), which were then gated based on their $CD14^+$and $CD11b^+$ expressions (P2); the $HLA\text{-}DR^-$ cell fraction was determined from this population. In our study, MDSCs were thus defined as $CD14^+/CD11b^+/HLA\text{-}DR^-$, based on the study performed by Rodrigues et al.[15]

Table. Patient characteristics

Variables	Pancreatic cancer (*n*=37)	Resectable* (*n*=22)	Unresectable* (*n*=15)
Age (mean±SD, yr)	57±11	56±7	59±10
Gender (Male/Female)	20/17	12/10	8/7
Clinical TNM stage#			
I	2	2	0
II	6	6	0
III	19	13	6
IV	10	1	9
Tumor site			
Head, neck	26	16	10
Body, tail	11	6	5
Histological grade△			
Well/moderately	7	6	1
Poorly	5	3	2
Resection			
Yes	12	9	3
No	25	13	12

*: Resectable pancreatic cancer corresponds to AJCC stages I and II; unresectable to AJCC stages III and V. #: Stage according to the AJCC classification for pancreatic cancer. △: Of the 22 resectable cancer patients, 9 patients received curative resections eventually, 2 abandoned treatment in our hospital, and 3 received palliative operation; 3 of the 15 unresectable patients received curative resection as well.

Cell isolation

PBMCs were isolated from the peripheral blood of healthy controls and cancer patients by density gradient centrifugation. In brief, blood was collected in heparin-treated tubes, diluted with RPMI 1640 medium, and carefully layered onto a density gradient Ficoll-Hypaque (Hao Yang, Tianjin, China). After centrifugation, the PBMC band was aspirated and washed three times with ice-cold phosphate buffered saline containing 1% human serum.

MDSC induction *in vitro*

In 12-well plates, 1×10^5 healthy control PBMCs were separately incubated with four pancreatic cancer cell lines for 48 hours at 37 ℃ in AIM V serum-free media (Gibco).

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