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Increased numbers of gastric-infiltrating mast cells and regulatory T cells are associated with tumor stage in gastric adenocarcinoma patients

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Abstract. Mast cells (MCs) and regulatory T cells (Tregs) are the important components of the inflammatory infiltrating leukocytes in most malignant tumors. Our study was designed to investigate the infiltrating correlation between MCs and Tregs and clarify their prognostic significance in gastric cancer (GC). A total of 60 fresh GC tissues were collected and tumor-infiltrating leukocytes were isolated by gradient centrifugation. Tryptase and Foxp3 were used as markers for MCs and Tregs, respectively. The expression of tryptase and Foxp3 was determined in tumor-infiltrating leukocytes using flow cytometry. The expression of tryptase and Foxp3 were positively correlated. The increased infiltration of MCs correlated significantly with advanced stage of GC. The infiltration of MCs into the tumor may increase the number of Tregs. Tryptase is a promising marker to stratify GC patients into different risk groups.

Introduction

Gastric cancer (GC), a common malignancy of the digestive tract worldwide, remains largely incurable (1,2). It is one of the leading causes of tumor-associated mortality in a number of developing Asian countries despite declining morbidity and mortality among patients with GC in the majority of developed countries (3,4). Tumor-induced immunosuppression, which hinders the cytotoxic responses of T lymphocytes and natural killer cells, is a principal problem to be solved in cancer immunotherapy (5,6). There are various immunosuppressive strategies employed by tumors in which regulatory T cells (Tregs) play a pivotal role (7).

Tregs are a distinct lymphocyte lineage with immunosuppressive potential in maintaining immunological tolerance. The transcription factor Foxp3 is a unique marker for Tregs and is indispensable for their development (8,9)

Mast cells (MCs) are a group of long-lived heterogeneous cells originating from the bone marrow. They were recognized by their modulating activities in inflammation and angiogenesis until recent investigations revealed their roles in shaping adaptive immune responses (10). MCs have been found to accumulate in the tumor microenvironment through the SCF/c-kit signaling pathway, leading to the aggravation of inflammation and immunosuppression in the tumor microenvironment (11).

The complex reciprocal correlation between MCs and Tregs determines the functions of both cell types. This interaction of MCs with Tregs dictates the intensity of tumor-associated inflammation and thus either promotes or inhibits tumor growth (10).

In numerous experimental models and human specimens, it has been observed that the increase in the number of MCs occurs in a number of tumors (12). However, there is little evidence of a clear correlation between the number of MCs and Foxp3 expression in human GC. The purpose of this study was to assess the infiltrating correlation between MCs and Tregs in GC.

Patients and methods

Patients and specimens. Tumor samples were obtained from 60 patients with pathologically confirmed gastric adenocarcinoma from the Department of Gastrointestinal Surgery, Union Hospital (Wuhan, China). None of the patients accepted anticancer therapy prior to surgical resection. Clinical stages were classified according to the 7th UICC TNM staging system. Fresh GC tissues were used for the isolation of tumor-infiltrating leukocytes, as previously described (13). The clinical characteristics of all patients are summarized in Table I. The research protocol was approved by the Institutional Review Board of Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China). Written and oral consents were obtained from the patients prior to the collection of samples.

Flow cytometry analysis. Tumor-infiltrating leukocytes were stained extracellularly with specific antibodies against human

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Table I. Clinical characteristics and the stages of the patients.

Characteristics	Number (%)
Gender (male/female)	39/21 (65/35)
Age, years (range)	30-75
TNM stage	
I	10 (16.7)
II	15 (25)
III	20 (33.3)
IV	15 (25)

Figure 2. Correlation between MCs and Tregs. MCs were positively correlated with Tregs (R=0.898, P<0.01). Patients with more MCs had more Tregs. The percentage of tryptase expression = the percentage of tryptase in tumor tissue - the percentage of tryptase in normal tissue. The percentage of Foxp3 expression = the percentage of Foxp3 in tumor tissue - the percentage of Foxp3 in normal tissue. MC, mast cell; Treg, regulatory T cell.
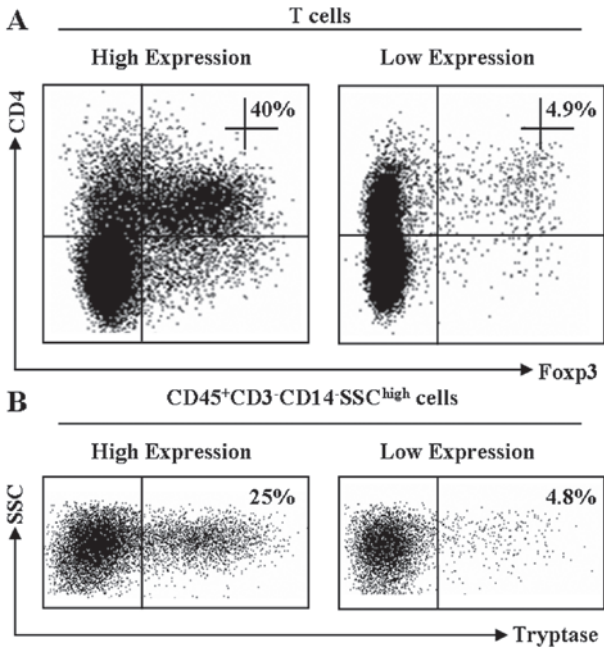

Figure 1. Numbers of Tregs and MCs are increased in tumor tissues. Single-cell suspensions of normal (right) and tumor (left) tissues were prepared from fresh human GC stained with relevant antibodies. Tregs and MCs were analyzed by flow cytometry. The specimens were from 60 patients with GC (see Table I and Materials and methods). The results of one patient are shown. (A) Tregs (Foxp3) in T cells (gated CD4 cells) from the tumor were analyzed by flow cytometry. Numbers on the plots are the proportions of Foxp3^{+} cells in the CD4^{+} cells. (B) MCs in CD45^{+}CD3^{-}CD14^{-}SSC^{high} cells. Numbers on the plots are the proportions of tryptase^{+} cells in the CD45^{+}CD3^{-}CD14^{-}SSC^{high} cells. MC, mast cell; Treg, regulatory T cell; GC, gastric cancer.

Figure 3. Correlation between number of MCs and stage of GC in the total set of subjects. The percentages of tryptase expression are separated into four groups by the TNM stage. The short bar indicates the mean value in each group. MC, mast cell; GC, gastric cancer.

CD45, CD3 and CD4 (BD Biosciences, Franklin Lakes, NJ, USA), fixed and permeabilized with Perm/Fix solution (eBiosciences, San Diego, CA, USA) and finally stained intracellularly with anti-Tryptase (Millipore, Billerica, MA, USA) and anti-Foxp3 (eBiosciences). Tryptase and Foxp3 were used as the markers for MCs and Tregs, respectively. Samples were acquired on an LSR II (BD Biosciences) and data were analyzed with DIVA software (BD Biosciences).

Statistical analysis. Results are expressed as mean ± SEM. Spearman's rank correlation coefficient test and ANOVA were carried out as indicated. P<0.05 was considered to indicate a statistically significant result. All analyses were performed using SPSS v12.0 software (Chicago, IL, USA).
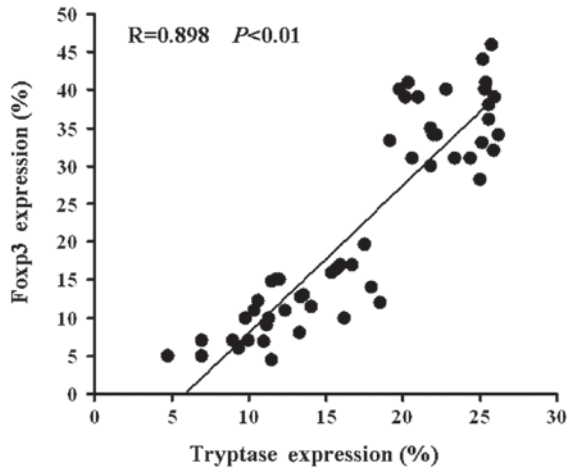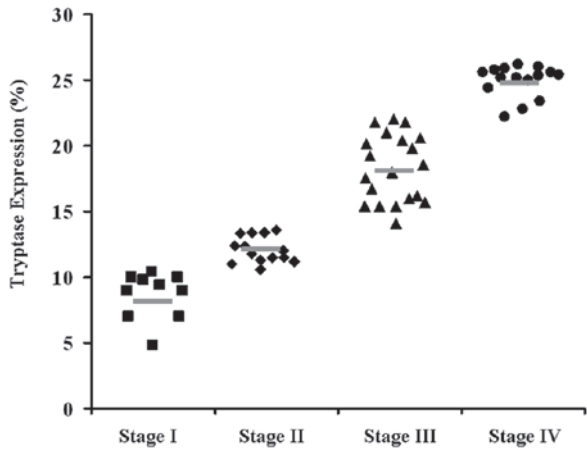
Results

Tryptase and Foxp3 expression were positively correlated in human GC. Representative dot plots for Foxp3 and tryptase expression are shown in Fig. 1. Tryptase and Foxp3 expression were positively correlated (R=0.898, P<0.01; Fig. 2). Correlations were determined by Spearman's rank correlation coefficients.

Tryptase and Foxp3 coexpression was associated with TNM stage in human GC. The development and progression of cancer are known to be regulated by various oncogenes and tumor suppressor genes. We analyzed 60 primary GC patients for the expression of tryptase by ANOVA. As shown in Fig. 3, a high level of tryptase expression was associated with advanced tumor stage, which is a marker of poor prognosis.

Discussion

Our study revealed that an increased number of MCs in GC patients is correlated with a higher frequency of Foxp3 expression. Numerous studies have documented an increased level of Foxp3 during GC progression (14,15). However, the role of MCs in GC remains unclear.

Ribatti *et al* (16) demonstrated that MC density correlates with progression of patients with gastric carcinoma. This means that the density of MCs is positively correlated with the development of the disease from stage I to stage IV. However, there have been reports of a protective role for MCs in human cancer. For instance, in a multivariate analysis of colorectal cancer patients, high counts of eosinophils and MCs predicted longer survival (17). MC tryptases activate the nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ); the expression of PPAR-γ is associated with improved clinical outcome in colon cancer (18). Our findings imply a complex correlation between the increased number of infiltrating MCs and advanced stages of GC patients.

These results suggest that higher numbers of MCs are associated with poor outcomes, which is similar to the results of previous studies reported in GC and other tumors (19-23). The present study not only provides support for the correlation between MCs and the stages of GC, but also focused on mechanisms other than angiogenesis, which has already been demonstrated (16). We studied the correlation between Tregs and MCs in order to provide an explanation of the influence of MCs on the stage of GC. Several studies have researched the immune suppression mechanism of MCs in tumors (11). However, to date, no study has directly demonstrated the correlation between the increased frequency of MCs and higher levels of Foxp3 in human GC. Our data demonstrate that MCs may affect the progression of GC, partially via interaction with Tregs. Studies in liver cancer reported similar results (32). Numerous researchers consider IL-9 to be critical factor in this interaction (24-26).

In several types of cancer, an increased level of Foxp3$^+$ Tregs has been detected in tumor tissues and peripheral blood, consistent with their presumed function in immunosuppression (27,28). Much concern has also been attached to the roles of Foxp3 in human GC. There is a link between the concentration of Tregs and patient survival in GC (29,30). Recently, a study partly explained the mechanisms of the weakened immune reactions in GC based on the overexpression of Foxp3 (31). Yuan *et al* (32) demonstrated a mechanism by which tumor-infiltrating Tregs with increased Foxp3 expression mediate immune suppression via COX-2/PGE2 production in the GC microenvironment. Furthermore, Tregs with higher levels of Foxp3 were able to suppress the proliferation of autologous CD4$^+$CD25$^-$T cells. The suppression of the effector T-cell response was reversed by COX inhibitors and PGE2 receptor-specific antagonists. In 2011, Yuan *et al* (33) performed further research on Tregs in GC and found that GC cells induce the development of Tregs via the production of TGF-β, by which the existence of cross-talk between the tumor and immune cells may regulate antitumor immune responses. Our research first confirmed that the expression of Foxp3 in tumor-infiltrating T lymphocytes was higher in the GC tissues compared with normal tissues. We also identified links between MCs and Foxp3, providing a new strategy of targeting Tregs and Foxp3. As mentioned, GC cells induce human CD4$^+$Foxp3$^+$ Tregs through the production of TGF-β (34), and as MCs secrete TGF-β, we speculate that one of the ways in which MCs affect Tregs is their secretion of TGF-β, thus explaining the correlation between MCs and Foxp3 at the molecular level, and providing a new support and research direction for the immunosuppressive effects of MCs.

In conclusion, our results reveal that the frequency of MCs and the level of Foxp3 are increased in tumors compared with normal tissues. The significant correlation between MCs and Foxp3 may be considered to support the hypothesis that MCs play a role in immunosuppression in GC and may be, at least partially, responsible for their prognosis. These results are significant and may provide promising clinical treatments for cancer, in at least in three aspects. First, these findings show the significance of MCs in GC and provide a probable mechanism by which MCs affect GC development, thus providing references for the application of MC-regulating drugs. Second, there are few studies concerning the correlation between MCs and Foxp3 and the present study linked them and provided new insights into the mechanism of immune suppression. Third, we confirmed the close correlation between MCs and Tregs, making a foundation for the further study of the detailed mechanisms. Therefore, further studies should be performed to explore the mechanism of the correlation between MCs, Foxp3 and GC.

Acknowledgements

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