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Expression of chemokines CCL5 and CCL11 by smooth muscle tumor cells of the uterus and its possible role in the recruitment of mast cells

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Abstract

Objective. Smooth muscle tumors of uterus have been reported to contain considerable *number* of mast cells, especially cellular leiomyoma. However, to our knowledge the mechanism by which mast cells *increased* in them is not known. The purpose of this study was to reveal the different mast cell subsets in smooth muscle tumors of uterus and to investigate the mechanism of local increase of mast cells.

Methods. Tissue sections from 85 uterine smooth muscle tumors were studied using immunohistochemical double labeling techniques, including 40 cases of ordinary leiomyomas, 30 cases of cellular leiomyomas and 15 cases of leiomyosarcomas. The sections were double immunostained for mast cell tryptase and chymase, mast cell tryptase and ki-67, mast cell tryptase and chemokines (i.e., CCL2, CCL5, CCL11, TGFβ), as well as tryptase and CCR3.

Results. MC_{TC}-type of mast cells was the predominant type in ordinary leiomyoma and cellular leiomyoma, whereas MC_T-type was *seldom* found in them. There was no MC_C in smooth muscle tumors. The total intratumoral number of mast cells in cellular leiomyoma group was significantly higher than that in both leiomyosarcoma and ordinary leiomyoma ($P<0.01$). Mast cells proliferation was rarely detected in smooth muscle tumors, as revealed by constant negative labeling of the proliferation marker Ki-67 in mast cells. Almost all mast cells (tryptase positive) in smooth muscle tumors were also CCL2, CCL5, CCL11 and TGFβ positive. Expressions of CCL5 and CCL11 in tumor cells in cellular leiomyoma were all significantly higher than that in both ordinary leiomyoma and leiomyosarcoma ($P<0.01$). While the expression of TGFβ in tumor cells in cellular leiomyoma was not significantly different from that in ordinary leiomyoma, expression of CCL2 was not observed in smooth muscle tumor cells. There were positive correlations between CCL5 and the number of mast cells ($r_s=0.801$, $P<0.01$) and between CCL11 and the number of mast cells ($r_s=0.744$, $P<0.01$) in smooth muscle tumors as well. The vast majority of the mast cells in cellular leiomyoma were CCR3 positive.

Conclusions. Using the monoclonal anti-mast cell tryptase antibody could detect all mast cells in smooth muscle tumor. The increased intratumoral mast cell counts in cellular leiomyoma might be the result of mast cells recruitment from the peripheral blood rather than local mast cells proliferation. CCL5 and CCL11, which are expressed by smooth muscle tumor cells, are possibly responsible for the recruitment of mast cells in uterine cellular leiomyoma. Whether they combine to CCR3 expressed by mast cells need further study.

Keywords: Chemokines; CCL5; CCL11; Mast cells; Uterine smooth muscle tumors

Introduction

Mast cells (MCs) are multifunctional effector cells of the immune system [1–5]. They circulate in the blood as immature progenitor cells that migrate into the tissue, where they differentiate into mature mast cells. Mature tissue mast cells produce and release a wide variety of mediators, which can be categorized into preformed secretory granule-associated mediators, lipid-derived mediators and cytokines. Some of these mediators stimulate angiogenesis, promote progression and facilitate metastasis [6–9], while some of them induce smooth

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muscle cell apoptosis or inhibit tumor cells associated with T-cell-mediated cytotoxicity [10,11]. In humans, mast cells are subtyped according to the enzyme content in the granules. Mast cells having tryptase exclusively in their granules are defined as MC_T-type mast cells; in contrast, MC_{TC}-type mast cells have both tryptase and chymase. In the study of mast cell subsets in human rejected kidneys, Yamada et al. [12] have discovered another type of mast cell, which only has chymase (MC_C type).

Smooth muscle tumors of the uterus are the most common gynecologic neoplasms and occur in a variety of histologic subtypes, such as cellular, bizarre and epithelioid leiomyomas [13–15]. According to WHO classification, a cellular leiomyoma is defined as "a leiomyoma that is significantly more cellular than the adjacent myometrium with little mitotic activity and no nuclear atypia". They are regarded as a histological variant of otherwise ordinary leiomyomas. These tumors generally display a benign clinical behavior, even when they are high cellular, with some mitotic activity. Coagulation necrosis, nuclear atypia and mitotic rate of the neoplastic cells are the features used in distinguishing leiomyosarcomas from cellular leiomyomas. Although it rarely happens, it is useful when there are problems in distinguishing between a cellular leiomyoma and a leiomyosarcoma because of inaccurate and inconsistent definitions in diagnostic criteria [16] and of the assessment of mitotic count and nuclear atypia, which might differ from one pathologist to another, due to different microscopic field size, degree and type of fixation. However, the distinction between leiomyosarcomas and cellular leiomyomas is of crucial clinical importance for the treatment and prognosis of the patients. Various ancillary techniques, such as immunohistochemistry, have been evaluated to improve diagnostic accuracy. One of the recently investigated parameters, mast cells, has shown significant differences in the degree of expression in uterine leiomyosarcomas and benign smooth muscle tumors. It has been reported by Orii et al. [13] that the number of MCs in cellular leiomyoma was significantly higher than in leiomyosarcoma, and they suggested that the number of MCs might be useful as part of a multivariate approach to the differential diagnosis of leiomyosarcoma from cellular leiomyoma. In addition, our previous study showed that the MC counts were significantly higher in cellular leiomyoma than in endometrial stromal sarcoma [17]. These studies, which were based only on an anti-MC tryptase antibody, did not investigate MC subsets.

Thus far, to the best of our knowledge, MC subsets in human smooth muscle tumors of the uterus have not been reported. To answer the question of whether anti-MC tryptase antibody can adequately detect all MCs in smooth muscle tumors of the uterus, in the present study, we used double immunohistochemical staining for tryptase and chymase to reveal MC subsets. To analyze whether the increased numbers of MC in smooth muscle tumors are the result of MC local proliferation or are due to increased recruitment of MCs or MC precursors from the peripheral blood, or both reasons [18], the tissue sections were double labeled with the anti-Ki-67 (MIB-1) and anti-tryptase to assess the relationship between MCs increase and MCs proliferation. To understand better the mechanism for MC recruitment, in parallel, we investigated the expression profiles of the MC-attractant chemokines: chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 5 (CCL5), chemokine (C-C motif) ligand 11 (CCL11) and transforming growth factor beta (TGFβ) in smooth muscle tumors and clarify MCs recruitment-related chemokines.

Materials and methods

Case selection

From pathology files between 1998 and 2005, we selected 85 patients diagnosed with smooth muscle tumors of the uterus who were not receiving any type of hormonal or drug therapy at the time of surgery. Hematoxylin and eosin-stained slides were reviewed by two of the authors (B.H.G. and M.X.) who had no access to results of immunohistochemical analysis to confirm the histological diagnosis, which was made according to accepted criteria [13,15,17] with some modifications. Briefly, ordinary leiomyoma (OL) was defined as a tumor showing typical histological features with a mitotic index (MI) [obtained by counting the total number of mitotic figures (MFs) in 10 high-power fields (HPFs)] of <5 MFs per 10 HPFs. Cellular leiomyoma (CL) was defined as a tumor with significantly increased cellularity (>2,000 myoma cells/HPF) and an MI <5, but without cytologic atypia. Leiomyosarcoma (LMS) was diagnosed in the presence of an MI >10 with either diffuse cytologic atypia or coagulative tumor cell necrosis, or both. Of 85 smooth muscle tumors, 40 were diagnosed as OL, 30 were cellular leiomyoma and 15 were LMS. The age range for OL was 28–52 years old (median, 37); for CL, 26–60 years old (median, 42); and for LMS, 29–63 years old (median, 49). Informed consent was obtained from all subjects. This study was approved by the Ethics Committee of Wenzhou Medical College. All specimens were fixed in 10% buffered formalin (pH 7.4) and paraffin-embedded within 24 h. Serial sections, 4 μm thick, were taken from the tissue blocks and processed for immunohistochemical studies.

Double staining for chymase and tryptase

To determine the subsets of the MCs, sections of tissue were double stained for two proteases that are known to be present in MCs. After deparaffinized and rehydrated, sections were pretreated in 10 mM citrate buffer, at pH 6.0, and autoclaved for 15 min at 120 °C. The sections were then soaked in absolute methanol containing 0.3% hydrogen peroxide for 30 min to remove endogenous peroxidase activity and incubated with 1.5% non-immune goat serum for 20 min to suppress non-specific binding. The sections were incubated with mouse monoclonal antibody CC1 (1:500, NeoMarkers, USA) or with non-immunized mouse serum (as a control) at 4 °C overnight. After rinsing with 0.05 M Tris–HCl (pH 7.4), the slides were subsequently incubated with biotin-conjugated goat antimouse immunoglobulin (Ig) G antibody at room temperature for 20 min. Then, after being washed again with TBS, the sections were incubated with streptavidin–alkaline phosphatase conjugate solution (Zymed, CA, USA) for 20 min. Subsequently, the sections were treated with Fast Blue BB for 20 min. The alkaline phosphatase substrate–chromogen mixture was prepared by dissolving 2 mg Naphthol-AS-MX-Phosphat (Sigma) in 0.2 ml dimethylformamide and then adding 9.8 ml TBS (pH 8.2). Immediately before this preparation was used, Fast Blue BB Base (10 mg, Sigma) and levamisole (1 M, 0.2408 mg, Sigma) were added and filtered onto slides.

The sections were incubated for 30 min with double staining enhancer and digested with trypsin for 10 min at 37 °C. They were exposed again to non-immune goat serum. Subsequently, the sections were incubated with anti-tryptase monoclonal antibody (AA1, 1:200, NeoMarkers, USA) or with non-immunized mouse serum (as a control) at room temperature for 60 min. After rinsing with phosphate-buffered saline (PBS), the sections were treated with peroxidase-conjugated anti-mouse IgG for 20 min and then streptavidin–peroxidase conjugate solution for 20 min. The sections were exposed to AEC to visualize the tryptase. Finally, the sections were counterstained with Mayer hematoxylin and mounted with aqueous mounting solution. Tryptase was detected in the cytoplasm as a red color. In contrast, chymase was visualized in the cytoplasm as a strong blue stain. A double positive result was expressed in purple.