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Imatinib ameliorates bronchiolitis obliterans via inhibition of fibrocyte migration and differentiation

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BACKGROUND: Imatinib, a tyrosine kinase inhibitor, has been proposed as a potential anti-fibrotic agent for fibroproliferative diseases, including bronchiolitis obliterans (BO). However, the underlying anti-fibrotic mechanisms of the agent remain unclear. We evaluated whether bone (BM)–derived progenitor cells, fibrocytes, might be a target of imatinib in the attenuation of BO.

METHODS: We used a murine BO model induced by heterotopic tracheal transplantation and assessed the origin of fibroblasts by using green fluorescent protein–BM chimeric mice. We also evaluated the effects of imatinib on luminal obstruction and fibrocyte accumulation. The effects of imatinib on fibrocyte migration and differentiation were assessed by culturing fibrocytes in vitro.

RESULTS: In the murine BO model, tracheal allografts showed epithelial injury and developed complete luminal occlusion 28 days after transplantation. Most of the mesenchymal cells that had accumulated in the tracheal allograft were derived from BM cells. Imatinib treatment ameliorated the airway luminal occlusion and significantly reduced the number of fibrocytes in the allografts. In vitro studies showed that imatinib inhibited migration of cultured blood fibrocytes via the platelet-derived growth factor/platelet-derived growth factor receptor axis. Imatinib also inhibited differentiation of fibrocytes via suppression of c-Abl activity that was essential for the differentiation of monocytes to fibrocytes.

CONCLUSIONS: Imatinib prevents airway luminal obstruction by inhibiting the migration and differentiation of fibrocytes. Fibrocytes may be a novel target in the prevention and treatment of BO.

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Bronchiolitis obliterans (BO) is a fibroproliferative disorder resulting in terminal small-airway occlusion that leads to an irreversible decline in lung function.[1] BO occurs in more than 50% of lung transplant recipients within 5 years of lung transplantation and remains the predominant cause of morbidity and death after lung transplantation.[2] BO responds poorly to corticosteroids or immunosuppressants. Lung transplant physicians and respirologists need to elucidate its pathogenesis and identify useful biomarkers for the diagnosis and severity of BO as well as effective interventions to prevent and treat BO.

Imatinib is a tyrosine kinase inhibitor of Bcr-Abl, c-Abl, c-Kit, and platelet-derived growth factor (PDGF) receptor (PDGFR).[3] Recent preclinical and clinical studies have

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suggested that imatinib is effective for the treatment of fibroproliferative disorders such as pulmonary fibrosis, lupus nephritis, and pulmonary hypertension.[4–8]

We previously reported 2 patients with BO after allogeneic hematopoietic stem cell transplantation who were successfully treated with imatinib.[9] Imatinib treatment stabilized the pulmonary function of both patients and prolonged their survival time. Several studies have also described the efficacy of imatinib in the treatment of BO in patients with chronic graft-versus-host disease.[10–12] In the rat BO model of heterotopic tracheal transplantation (HTT), imatinib inhibited the tracheal luminal occlusion 30 days after transplantation compared with vehicle-treated allografts.[13,14] Taken together, these data suggest that imatinib can be a useful agent for treating BO.

The precise mechanisms by which imatinib ameliorates BO are incompletely understood. On the one hand, imatinib inhibited the development of luminal occlusions in the rat BO model without suppressing inflammatory cells, suggesting direct anti-fibrotic effects of imatinib.[13] On the other hand, late administration of imatinib during the fibroproliferative phase of BO failed to prevent luminal obstruction.[14] These findings suggest that imatinib exerts its preventative effects on fibroproliferative lesion development in tracheal allografts only when it is administered during the inflammatory phase of BO and that the therapeutic target of imatinib may exist soon after tracheal transplantation.

One of the cellular components involved in the fibroproliferative lesion is a bone marrow (BM)–derived mesenchymal progenitor cell, the fibrocyte. Fibrocytes represent 0.1% to 0.5% of the circulating leukocytes that differentiate from monocyte precursors and coexpress the leukocyte marker CD45 and mesenchymal markers such as collagen I.[15] Fibrocytes act as precursors of fibroblasts that participate in tissue remodeling and also the secretion of inflammatory cytokines, chemokines, and growth factors that contribute to the pathogenesis of fibrotic disorders.[16–18] Increased numbers of fibrocytes were identified in the alveolar parenchyma of BO patients who underwent lung transplantation.[19] The number of peripherally circulating fibrocytes was also increased with advancing BO stage, suggesting that fibrocytes may play an important pro-fibrotic role in the development of BO.[20]

We hypothesized that in the BO model of HTT, imatinib ameliorated BO by inhibiting fibrocytes by unknown mechanisms. To test this hypothesis, we examined the causal role of fibrocytes in the development of BO by using green fluorescent protein (GFP)–chimeric mice. In this model, we evaluated the effect of imatinib on the airway obstruction of transplanted tracheas and the effect on fibrocytes in vivo. Furthermore, to investigate the mechanisms underlying fibrocyte regulation by imatinib, we conducted an in vitro assessment of the targets of imatinib (i.e., c-Abl, c-Kit, and PDGFR).

Methods

Murine BO model

The Kanazawa University Advanced Science Research Center Institute for Experimental Animals approved the animal studies. Male 7- to 10-week-old BALB/c, C57BL/6 wild-type mice, and GFP-positive transgenic (GFP-Tg) mice on a C57BL/6 background were purchased from Sankyo Laboratory Service Corporation (Toyama, Japan). The HTT model was prepared as previously described.[21,22] A trachea from a BALB/c (isograft) or a C57BL/6 (allograft) was transplanted into a BALB/c mouse.

Imatinib was provided by Novartis (Basal, Switzerland). This solution was diluted with 0.9% saline and injected intraperitoneally once daily (final dosage, 10 mg/kg/day).

GFP-BM chimeras, in which BM was replaced by GFP-marked BM cells, were also prepared as previously described.[23] The HTT was performed 2 months after BM transplantation. Allogenic HTT was performed by transplanting tracheas from BALB/c to GFP-Tg, GFP-Tg to BALB/c, and BALB/c to GFP-BM chimeras. Transplanted grafts were isolated on the indicated days after transplantation.

Tracheal tissues were washed in phosphate-buffered saline (PBS), fixed in 10% formaldehyde, and embedded in paraffin or embedded directly in Tissue-Tek optimal cutting temperature compound (Sakura, Alphen aan den Rijn, The Netherlands) and snap-frozen in liquid nitrogen. Frozen tissues were serially cut into 14-μm-thick cross-sections and stored at –80°C until used.

Histopathologic analysis

Tracheal graft sections were stained with hematoxylin and eosin. The degree of airway injury and luminal occlusion was examined as previously described.[21] ImageJ 1.49 software (National Institutes of Health, Bethesda, MD) was used for the evaluations.

Culturing murine or human fibrocytes

Human peripheral blood was collected from healthy adult volunteers after informed consent was obtained under the guidance of the Kanazawa University Graduate School of Medicine Ethical Committee. Fibrocytes were harvested and cultured as previously described.[24,25] Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from murine or human blood by using LymphoPrep (Progen Biotechnik). Human monocytes were isolated using a Dynabeads FlowComp Human CD14 Kit (Life Technologies). We prepared serum-free medium that consisted of Roswell Park Memorial Institute 1640, 1% non-essential amino acid, 1% pyruvate, 1% ITS-3 (500 μg/ml bovine serum albumin, 10 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenite, 5 μg/ml linoleic acid, and 5 μg/ml oleic acid), and 1% penicillin/streptomycin. We also prepared serum-containing medium consisting of Dulbecco Modified Eagle's Medium, 10% heat-inactivated human AB serum (Sigma-Aldrich), and penicilli/streptomycin. PBMCs or $CD14^+$ cells were cultured in 96-well plastic plates or 8-well glass slides.

Immunostaining

Paraffin-embedded tissues were incubated with rabbit anti-GFP antibody in the experiments using GFP chimeric mice. Frozen tissues/cells were fixed in 4% paraformaldehyde, blocked with 2% skim milk in 0.2% Triton X-100/PBS, and were stained with the primary antibodies. These sections were stained using specific antibodies, followed by appropriate secondary antibodies and Hoechst 33342. The inflammatory cells observed in sections were expressed as the number of positively staining cells per tracheal graft cross-section in 10 separate, high-power (original magnification ×400) fields. The densities of endothelial cells and pericytes were defined as the percentage of pixels covered by each individual staining marker. The cellular localization of fluorescently labeled proteins was viewed under fluorescent microscopy (Zeiss).