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LETTER TO THE EDITORS

Fibroblasts of recipient origin contribute to airway fibrosis in murine tracheal transplantations

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Dear Sirs,

Bronchiolitis obliterans (BO) is a major limitation in the long-term success of lung transplantation. BO is characterized as fibrotic obliterations in small airways [1]. Fibroblasts are the key players in fibrosis. They produce extracellular matrix components, and those deposition results in fibrosis. The source of fibroblasts in transplanted organs is an unresolved question. Determining the origin of airway fibroblasts is considered to be a key step in establishing ways to prevent fibrosis.

According to previous reports [2–4], three possible sources of fibroblasts in transplanted organs are recipient bone marrow cells [2], regional fibroblasts in the grafts [3], or transitioned donor cells that had undergone epithelial to mesenchymal transition (EMT) [4]. In this study, we investigated whether fibroblasts in rejected airways originated from donor or recipient cells using orthotopic tracheal transplantation (OTT) and heterotopic tracheal transplantation (HTT) mouse models with transgenic C57Bl/6 mice that ubiquitously expressed green fluorescent protein (GFP) (B6-Tg(GFP)). Subepithelial fibrosis in OTT allografts [5] and intraluminal fibrosis in HTT allografts [6] are observed on or after the 28th day.

All animals received humane care in compliance with the 'Guide for Animal Experimentation, University of Tokyo, revised 2007' and the 'Act on Welfare and Management of Animals' published by Japanese ministry. All of the mice were purchased from Japan SLC, Inc.

BALB/c and B6-Tg(GFP) female mice were used as donors or recipients. The heterotopic and orthotopic tracheal transplantations were performed under the operating microscope as previously reported [5,6]. For HTT, the donor tracheas were placed into the subcutaneous space of the anterior neck area.

For OTTs and HTTs, the following strain combinations were used: transplantations from BALB/c to B6-Tg(GFP) mice, those from B6-Tg(GFP) to BALB/c mice, and from BALB/c to BALB/c as a syngeneic control. All grafts harvested on the 28th day were frozen within an optimal cutting temperature compound (Sakura Finetek Japan, Tokyo, Japan). The hematoxylin and eosin (H&E) stains were performed regularly after formalin fixation. Fibroblasts in allografts were characterized using anti-mouse α-SMA Cy3-conjugated antibody (C6198; Sigma-Aldrich, St. Louis, MO, USA). The emergence of fibroblasts suggested the occurrence of fibrosis. For GFP staining, an anti-mouse GFP fluorescein isothiocyanate-labeled antibody (A21311; Life technologies, Carlsbad, CA, USA) was used. After fixation and blocking, the sections were incubated with diluted conjugated antibodies (α-SMA 1:200, GFP 1:200). Additionally, DAPI (D1306; Life technologies, Carlsbad, CA, USA) was used as a nuclear counterstain. The images were acquired using a microscope (BIOREVO-9000; Keyence, Osaka, Japan). The percentages (GFP co-localized area)/(subepithelial or intraluminal α-SMA expression area) were calculated using Image J Software (version 1.4.3.67; National Institute of Health, USA) for the OTTs and HTTs. The results are displayed as a box plot that was created with SPSS 11.0 (Dr. SPSS II for Windows, standard version; SPSS Inc., Chicago, IL, USA).

In both OTT groups, subepithelial thickening and the emergence of fibroblasts occurred on the 28th day, and the reproducibility was similar in each group. For transplanted group from BALB/c to B6-Tg(GFP), the subepithelial α-SMA-positive cells were also GFP positive (Fig. 1a). In contrast, the subepithelial α-SMA-positive cells were GFP negative for the group transplanted from B6-Tg(GFP) to BALB/c (Fig. 1b). The proportion of GFP co-localization with the subepithelial αSMA staining is shown in Fig. 1e.

Partial luminal obliterations were observed in both HTT groups on the 28th day. For transplanted group from BALB/c to B6-Tg(GFP), the majority of the α-SMA-positive cells in the graft lumens were GFP positive (Fig. 1c). In contrast, another allogeneic group, the luminal α-SMA-positive fibroblasts were GFP negative (Fig. 1d). The calculated colocalized percentages for the luminal fibrosis groups

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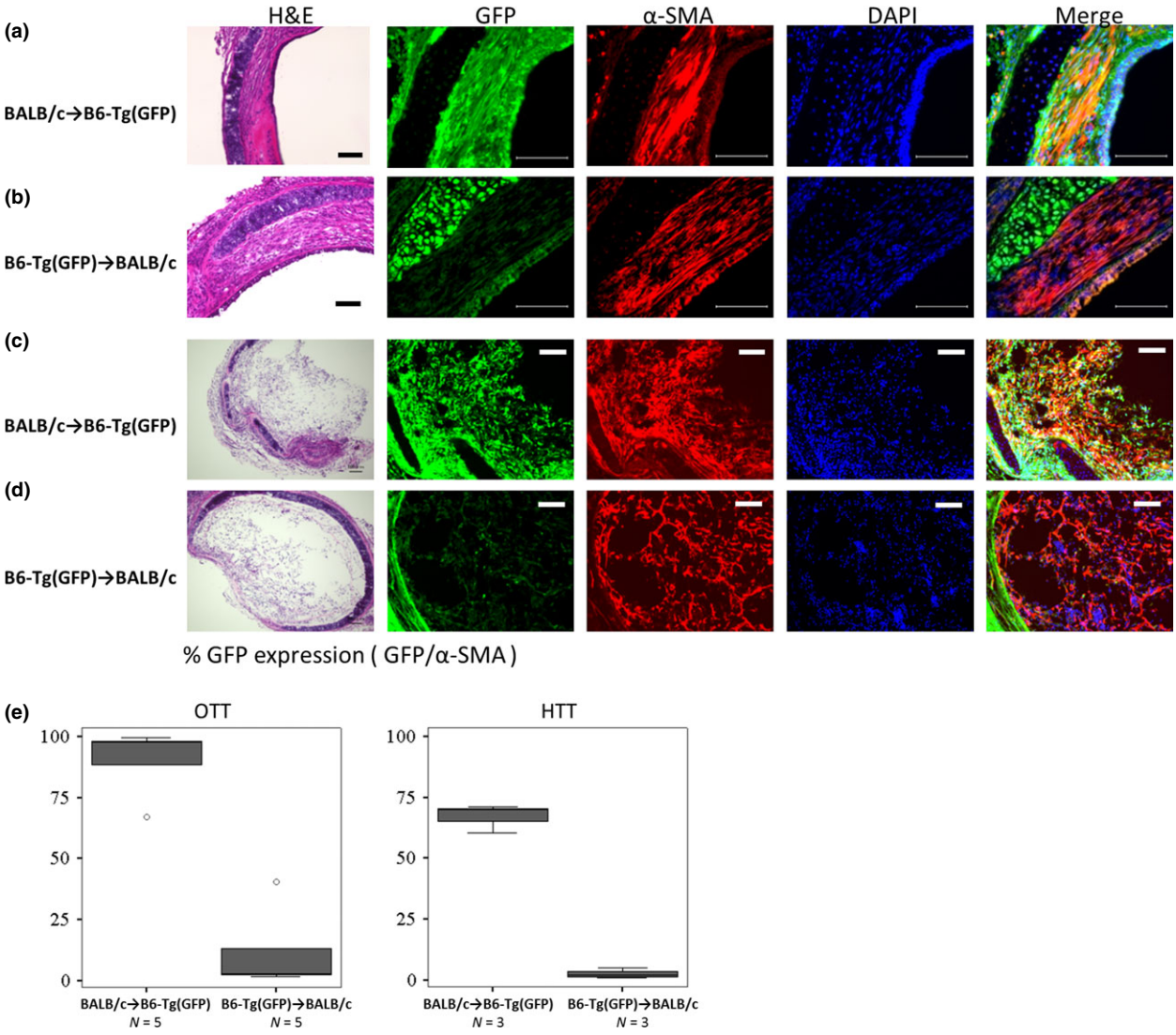

Figure 1 (a, b) In OTT allografts, subepithelial thickening and the emergence of fibroblasts were observed (H&E). The subepithelial α-SMA-positive cells were also GFP positive (a). In contrast, the subepithelial α-SMA-positive cells were GFP negative for group B (b). The proportion of GFP colocalization with the subepithelial αSMA staining is shown (e). (c, d) Partial luminal obliterations were observed in both groups C and D (H&E). For group C, the majority of the α-SMA-positive cells in the graft lumens were GFP positive (c). In contrast, the luminal α-SMA-positive myofibroblasts were GFP negative (d). The calculated colocalized percentages for the luminal fibrosis groups are shown (e). Each scale bar represents 100 μm.

are shown in Fig. 1e. No fibrotic changes were observed in syngeneic groups.

These results suggest that most fibroblasts in transplanted airways originate from recipient cells. In the orthotopic transplanted tracheas, prior to subepithelial fibrosis, the internal surface of the grafts is covered by recipient epithelial cells that may originate from both anastomoses [7]. Therefore, the possibility remains that fibroblasts originate from recipient epithelial cell EMT in the OTT model. On the other hand, in HTTs, this re-epithelialization does not occur. The same results in the HTTs and OTTs indicate that recipient bone marrow cells might be the primary fibroblast source.

Because the epithelium in the OTT allografts showed a dynamic morphological change before the occurrence of fibrosis and because it is believed to be a first target of immune injury [8–10], we first hypothesized epithelial cells that experienced immune-mediated injury could be primary players in transplanted airway fibrosis. In our previous OTT study [10], we observed transient mesenchymal marker (α-SMA) expression in epithelial cells with attenuation of an epithelial marker (E-Cadherin) during epithelial dynamic changes before subepithelial fibrosis. The results from this study seem to be in conflict with our previous results. This discrepancy may indicate a possibility that

fibroblasts potentially originate through a number of pathways, including the recruitment of circulating progenitors, the transition of airway epithelial cells, and the proliferation of resident fibroblasts.

The current study determined that the majority of the observed fibroblasts were of recipient origin. Recipient bone marrow fibroblast precursor cells could be primary targets for OB prevention. This study is the first histological report in animal models regarding the origin of fibroblasts.

Chihiro Konoeda, Jun Nakajima and Tomohiro Murakawa
Department of Thoracic Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
e-mail: murakawa-tky@umin.ac.jp

Conflict of interest

The authors have no conflict of interests to disclose.

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