Protease-activated receptor-4 deficiency does not protect against bleomycin-induced pulmonary fibrosis in mice

Protease-activated receptor (PAR)-4 is a thrombin receptor expressed in lung tissue. Previous *in vitro* data showed that PAR-4 triggers epithelial-to-mesenchymal transition, which is crucial in pulmonary fibrosis. These observations prompted us to investigate the role of PAR-4 in a murine model of bleomycin induced pulmonary fibrosis. We observed that wildtype and PAR-4 deficient mice developed fibrosis, but there were no differences in the severity of fibrosis or in the expression of fibrotic markers like collagen and fibronectin in PAR-4 deficient mice compared with wildtype controls. Overall, our results demonstrate that PAR-4 does not play a significant role during pulmonary fibrosis and question the importance of PAR-4 in fibrotic disease.

PAR-4 is a G-coupled receptor of the PAR family that is generally recognised as a thrombin receptor [1] although it can also be activated by trypsin, tissue kallikrein and cathepsin G. PAR-4 activation is best known to induce platelet aggregation, but more recent studies suggest that PAR-4 may also target other cell types thereby contributing to (patho)physiology. Indeed, thrombin stimulation of epithelial cells induces epithelial–mesenchymal transition (EMT), as evident from changes in cell morphology and expression levels of epithelial (E-cadherin) and myofibroblast (α-smooth muscle actin) markers [2]. Moreover, PAR-4 activation stimulates interleukin (IL)-6, IL-8, and prostaglandin E2 release from human respiratory epithelial cells. Considering the importance of both EMT and inflammation for fibrosis, it is thus tempting to speculate that PAR-4 would play a pro-fibrotic role and indeed several recent reviews [3, 4] specifically state that some of the pro-fibrotic effects of thrombin may be mediated through PAR-4. To prove or refute the importance of PAR-4 in fibrotic disease, PAR-4 deficient mice and wildtype controls were subjected to the well-established experimental model of pulmonary fibrosis [5]. To this end, mice were intratracheally inoculated with bleomycin sulfate (1 $mg \cdot kg^{-1}$ body weight in 45 μL of saline). 14 days later, mice were sacrificed and lungs

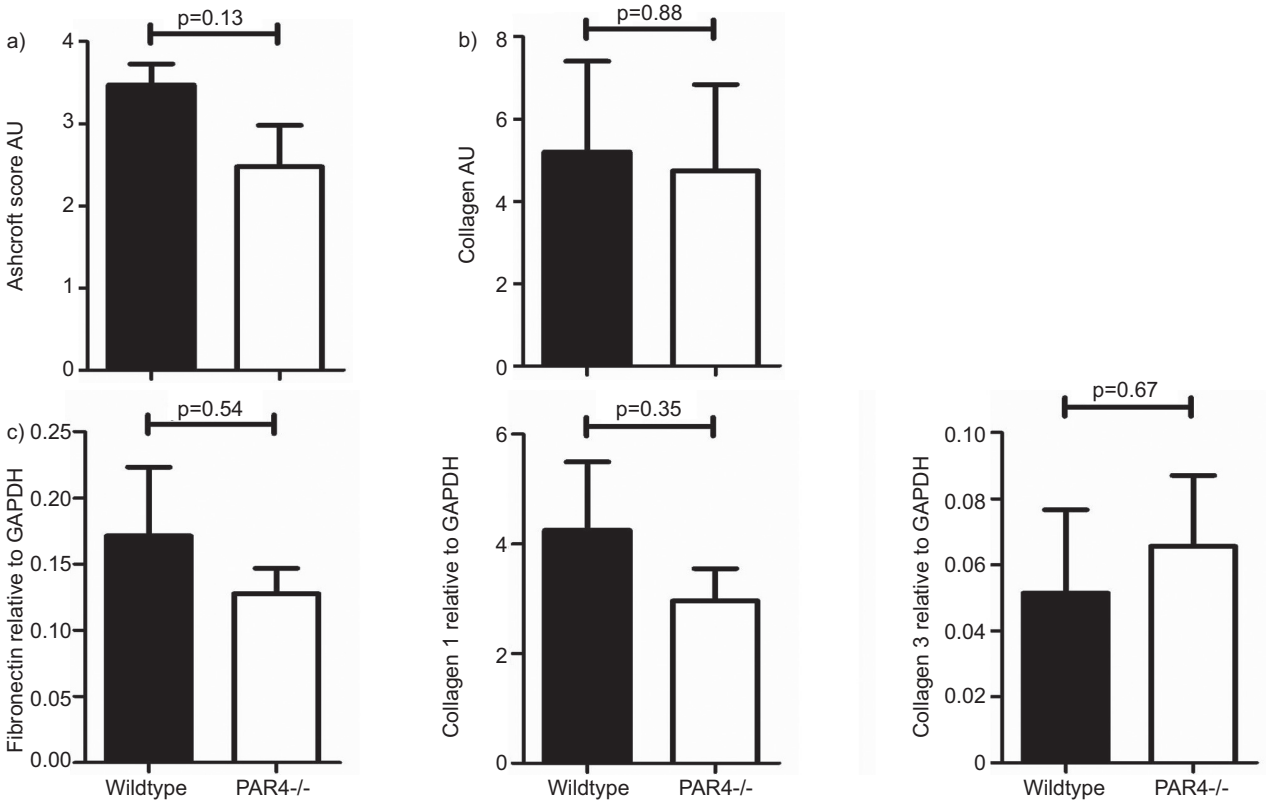

FIGURE 1. Protease-activated receptor (PAR)-4 does not regulate pulmonary fibrosis. a) Ashcroft score indicating the severity of fibrotic changes in wildtype and PAR-4 deficient mice 14 days after intratracheal injections of bleomycin. b) Lung collagen content as assessed by Masson's trichrome stainings in wildtype and PAR-4 deficient mice 14 days after intratracheal injections of bleomycin. c) Pro-fibrotic gene expression analysis in wildtype and PAR-4 deficient mice 14 days after intratracheal injections of bleomycin. AU: arbitrary units; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. Data are presented as mean±SEM, n=8 mice per genotype.

were removed to analyse fibrotic changes. As shown in figure 1a, the severity of fibrotic changes as indicated by the Ashcroft score was similar in wildtype and PAR-4 deficient animals (score of mean±SEM 3.46±0.25 and 2.48±0.50, respectively, p=0.13). In line, collagen deposition as assessed using Masson's trichrome stainings was also similar in wildtype and PAR-4 deficient mice (mean±SEM arbitrary units of 2.2±0.9 and 2.0±0.8, respectively, p=0.88; fig. 1b). Finally, we determined pro-fibrotic gene expression in the lungs of bleomycin treated mice and, as shown in figure 1c, no differences in fibronectin, collagen 1 and collagen 3 levels between wildtype and PAR-4 deficient mice were observed. Overall these data thus indicate that PAR-4 does not play a significant role in the regulation of pulmonary fibrosis. Although extrapolation of our data to other fibrotic disorders should be performed with great care, our data argue against an important role of PAR-4 in fibrotic disease and once more stress that *in vitro* data should not be over interpreted by claiming potential roles *in vivo*.

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Severe pulmonary hypertension leading to heart–lung transplantation and revealing breast cancer

To the Editors:

Pulmonary tumour embolism is considered to be a rare cause of pulmonary hypertension. The incidence, studied in autopsy series, varies from 3 to 26% of patients with solid tumour [1]. It occurs most frequently in breast, stomach, lung, liver, prostate and pancreas adenocarcinoma [1]. The literature shows a male predominance and an average age of 52.4 yrs [2]. The signs are those of any pulmonary hypertension, occurring either during the history of a known neoplasia or as the first manifestation of malignancy. Pre-mortem diagnosis is difficult to confirm and definitive diagnosis is usually made on an autopsy study. Several studies have reported pulmonary wedge aspiration cytology performed during a right heart catheterisation as a useful pre-mortem diagnostic tool [3]. Treatment consists of treating the neoplasia. Specific treatment for pulmonary hypertension has not been evaluated. Most frequently, evolution leads to refractory right cardiac failure and death [4]. In rare cases improvement has been reported after chemotherapy [5] or endarterectomy [6].

Two mechanisms can be involved in the development of pulmonary hypertension in tumour pulmonary embolism: the first one is by mechanical occlusion of the small pulmonary arteries by multiple neoplastic microemboli, the second one is through development of pulmonary tumour thrombotic microangiopathy (PTTM) as a result of endothelial interaction with tumoural cells. In PTTM, inflammatory and coagulation pathways are activated leading to vascular remodelling phenomena and diffuse narrowing of the pulmonary arteriolar system [7]. The role of tissue factor and vascular endothelial growth factor [8] as well as platelet-derived growth factor in the pathogenesis of PTTM has been highlighted by previous studies.

We report the case of a 49-yr-old female patient who was admitted on suspicion of pulmonary hypertension. This episode began 4 months ago following complaints of exercise-induced dyspnoea. A pulmonary angiographic computed tomography (CT) found no pulmonary embolism. An echocardiography showed a slight elevation of the systolic pulmonary artery pressure leading to the diagnosis of a possible diastolic dysfunction consecutive to a hypertensive cardiopathy. The patient's medical treatment for systemic hypertension was reinforced and no further explorations were conducted.

Due to disabling dyspnoea, the patient was hospitalised 4 months later. No signs of cardiac dysfunction were seen, the pulmonary auscultation was clear. Pulmonary embolism was eliminated by a pulmonary angiographic CT but rare ground-glass opacities were noticed. The echocardiography revealed signs of chronic cor pulmonale with dilatation of the right cavities and severe elevation of the systolic pulmonary artery pressure at 70 mmHg. The bronchoalveolar lavage