Protective Role of Myelocytic Nitric Oxide Synthases in Hypoxic Pulmonary Hypertension in Mice

Masato Tsutsui¹, Takaaki Ogoshi², Takashi Kido², Sohsuke Yamada³, Ke-Yong Wang⁴, Yumiko Toyohira⁵, Hiroaki Shimokawa⁶, Nobuyuki Yanagihara⁶, Kazuhiro Yatera², Hiroshi Mukae⁷

¹Department of Pharmacology, Ryukyu Univ. Graduate School of Medicine, Japan, ²Department of Respiratory Medicine, Univ. Occup. & Environ. Health, Japan, ³Department of Pathology, Univ. Occup. & Environ. Health, Japan, ⁴Shared-Use Research Center, Univ. Occup. & Environ. Health, Japan, ⁵Department of Pharmacology, Univ. Occup. & Environ. Health, Japan, ⁶Department of Cardiovascular Medicine, Tohoku Univ. Graduate School of Medicine, Japan, ⁷Department of Respiratory Disease, Nagasaki Univ. Grad. Sch. Biomed. Sci., Japan

Background: All three nitric oxide synthases (nNOS, iNOS, and eNOS) are expressed under pulmonary hypertensive conditions. Although the role of all the NOSs in pulmonary hypertension (PH) has been examined in pharmacological studies with non-selective NOSs inhibitors, obtained results are inconsistent possibly due to non-specificity of the agents. We addressed this point in our mice lacking all the NOSs.

Methods and Results: Wild-type (WT), single nNOS-/-, iNOS-/-, eNOS-/-, and triple n/i/eNOSs-/- mice were exposed to hypoxia for 3 weeks. Hypoxic exposure significantly reduced survival rate only in the triple NOSs-/ genotype (P<0.05, n=14-34). Hypoxic exposure increased right ventricular (RV) pressure (RVP), RV hypertrophy (RVH), and medial thickening of small pulmonary arteries (MT) in all the genotypes. Notably, RVP were significantly worsened in the triple NOSs-/ genotype, and, to a lesser extent, in the eNOS-/ genotype, but not in the nNOS-/ or iNOS-/ genotype, as compared with the WT genotype, and RVH and MT were significantly deteriorated only in the triple NOSs-/ genotype (each P<0.05, n=5-10). In the triple NOSs-/ genotype exposed to hypoxia, the number of circulating bone marrow (BM)-derived vascular smooth muscle progenitor cells was significantly increased, and green fluorescent protein-transgenic BM transplantation (BMT) revealed contribution of BM cells to pulmonary artery remodeling (P<0.05, n=5). Importantly, triple NOSs-/-BMT significantly aggravated hypoxia-induced PH in the WT genotype, and WT-BMT significantly ameliorated hypoxia-induced PH in the triple NOSs-/- genotype (each P<0.05, n=5-6). RNA sequencing showed that, in the lungs of WT genotype with triple NOSs-/-BMT, as compared with those with WT-BMT, 69 and 49 mRNAs related to immunity and inflammation, respectively, were significantly up-regulated, suggesting the involvements of immune and inflammatory mechanisms in the aggravation of hypoxia-induced PH caused by the triple NOSs-/-BMT (each P<0.05, n=4).

Conclusions: These results provide the first evidence that genetic disruption of all NOSs, specifically in BM cells, markedly exacerbates hypoxic PH in mice, indicating the novel protective role of myelocytic NOSs in the pathogenesis of PH.