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Identification of endothelial progenitor cells in pulmonary vascular lesions of rats with Pulmonary Hypertension following immunomodulatory treatment

Hyun Ji, Daniela Farkas, Donatas Kraskauskas, Laszlo Farkas
Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, VCU, Richmond, VA

Rationale
Pulmonary hypertension (PH) is a progressive devastating disease that is defined as a mean pulmonary pressure (mPAP) of 25mmHg or greater at rest (Bazan 2015). Pulmonary arterial hypertension (PAH) is a subtype of PH (WHO Class I) in which the pre-capillary pressure is elevated while the PA wedge pressure is normal. Some characteristics of the pulmonary vasculature in PAH include medial hypertrophy, intimal proliferation and adventitial thickening (Bazan 2015). In its severe form, the pulmonary arterial lumen is obliterated by proliferating primitive cells with endothelial cell (EC) markers, which are likely endothelial progenitor cells (EPCs), in response to EC apoptosis. Several progenitor cell markers have been described in growing vasculature, such as c-kit and Nestin (Fang, 2012). Despite decades of research, PAH is still associated with significant mortality in patients. Increased inflammation and altered immunity may represent part of an abnormal response to vascular injury in PAH, but there is a fundamental gap in the understanding of immunomodulatory therapies in PAH. Toll-like receptor (TLR) 3 is a receptor of the innate immune system that recognizes double stranded RNA. Prior studies have shown that the activation of TLR3 by TLR3 agonist polyinosinic:polycytidylic acid (Poly[I:C]) antagonizes the TLR4-mediated pathway that induces alcoholic liver injury in mice (Byun 2013). Preliminary studies of animal models treated with Poly[I:C] prevented PAH. The clinical translational relevance of these studies lies in the availability of a clinical grade analogue for Poly[I:C] that is currently under clinical investigation as part of immunotherapeutic approaches for colorectal cancer and breast cancer.

We hypothesized that treating PAH rats with Poly[I:C] will reduce the overgrowth of EPC’s found in the pulmonary vasculature, and thus reduce the severity of the disease.

Methods
- SuHx model (SU5416 and chronic hypoxia rat PAH model) was employed as published previously (Taraseviciene-Stewart L et al. FASEB J 2001). SU4516 is an inhibitor of vascular endothelial growth factor receptors, which results in progressive severe PAH with luminal obliteration.
- SuHx animals received 10mg/kg-1 poly[I:C] three times a week or saline by intraperitoneal injection.
- For the 3 week model, the animals were euthanized for tissue harvest at day 21 after invasive hemodynamic measurements. For the 6 week model, the animals were euthanized for tissue harvest at day 42 days. Naive age-matched animals were housed under normoxic conditions.
- IF images were obtained with Olympus IX70 fluorescence microscope.
- Immunofluorescence stainings were performed according to established protocols. Data was analyzed using GraphPad Prism.
- Cell marker expression was investigated by Flow cytometry from lung single cell suspensions with BD FacsCanto and analyzed in FlowJo.

Results

Figure 1.
(A), (B). Representation of von Willibrand factor (vWF, green) and Nestin (red stain, orange arrow) immunohistochemistry on SuHx rats treated with poly[I:C] for 3 weeks and 6 weeks, respectively. (C) Representation of the control rats vasculature immunohistochemistry stained with vWF and Nestin.

Figure 2.
(A) Nestin+ cells in small pulmonary vasculature. (B) C-kit+ cells [n/vessels] in small pulmonary vasculature.

* P<0.0132 and ** P<0.0012. n = 3.

Figure 3.

Figure 4.
Representative flow cytometry dot plots of c-kit+ VEGFR2+ EPC in lung single cell suspensions of SuHx rats treated with Poly[I:C] or vehicle.

Conclusions
- Our findings indicate that treatment with poly[I:C] in the 6 week SuHx model reduced the overgrowth of EPCs marked with c-kit found in the pulmonary vasculature.
- Flow cytometry indicate that treatment with poly[I:C] in the 6 week model also decreases the overall number of c-kit+ and VEGFR+ cells in SuHx model tissue.
- These findings suggest that poly[I:C] may be more useful as a therapeutic rather than preventative measure.

References