Oral Examination for
the Degree of Doctor of Philosophy

The Role of EBP50 in Regulating Endothelial-To-Mesenchymal Transition in Pulmonary Hypertension

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Molecular Pharmacology
Zoom Dissertation Defense
Tuesday, April 7, 2020
3:00 PM

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Abstract

Pulmonary hypertension (PH) is a cardiopulmonary disease that manifests in a chronic elevation of mean pulmonary arterial pressure, pulmonary vascular remodeling, and right ventricular hypertrophy, and arises from a combination of various factors including endothelial dysfunction. Among the many signs of endothelial dysfunction in PH, increased production of vasoconstrictive signaling molecules and growth factors, proinflammatory shift, and endothelial-to-mesenchymal transition (EndMT) are increasingly recognized as important events potentiating endothelial reprogramming and impaired vascular homeostasis. Despite the critical need to identify molecular players that regulate endothelial reprogramming and the process of EndMT in PH, progress in that direction has been limited.

By utilizing lung samples from PH patients as well as three experimental rodent models of PH, we show that expression of the scaffolding PDZ protein ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50, NHERF1) is downregulated in PH pulmonary vessels and isolated pulmonary vascular endothelial cells. Our in vitro studies indicate that PH-relevant downregulation of endothelial EBP50 expression can be induced by hypoxia and inflammatory cytokine interleukin-1 beta (IL-1β) in a time-dependent manner. Phenocopy of EBP50 reduction using siRNA was found to exert regulatory control over EndMT transcription factors Snail, Zeb1 and Slug, and mesenchymal phenotype transition in pulmonary endothelial cells, and to regulate endothelial cell proliferation and barrier function. Further, the downregulation of EBP50 appeared to potentiate hypoxia-driven EndMT transcription factor Slug upregulation. In vivo studies on $EBP50^{+/-}$ mice demonstrated that downregulation of EBP50 exacerbates hemodynamic manifestations of chronic hypoxia PH.

In our work, we identify EBP50 as a key regulator of EndMT in PH whose expression is downregulated in PH patient pulmonary endothelium as well as animal models of PH, and whose partial deletion exacerbates PH disease manifestations in rodents. Taken together, our findings support the importance of this protein in PH and highlight the rescue of EBP50 levels in this disease as a promising therapeutic approach.

Publications

Gorelova A, Berman M, Alsuraimi A, Riva P, Negi V, Bertero T, Goncharov D, Kudryashova T, DosunmuOgunbi A, Nouraie S, Baust J, Sebastiani A, Straub A, Goncharova E, Mora A, Chan SY, and Al Ghouleh I. 2020. **The Role of Ezrin-Radixin-Moesin-Binding Phosphoprotein 50 in Regulating Endothelial-To-Mesenchymal Transition in Pulmonary Hypertension.** *ATVB, submitted*

Gorelova A, Berman M, Al Ghouleh I. 2020. **Endothelial-to-Mesenchymal Transition in Pulmonary Arterial Hypertension** (Invited Review). *ARS, submitted*

Al Ghouleh I, Meijles DN, Mutchler S, Zhang Q, Sahoo S, **Gorelova A**, Amaral JH, Rodríguez AI, Mamonova T, Song GJ, Bisello A, Friedman PA, Cifuentes-Pagano M-E, Pagano PJ. **Binding of EBP50 to Nox Organizing Subunit p47*phox* is Pivotal to Cellular Reactive Species Generation.** PNAS; 2016 Sep 6;113(36): E5308-17.

Selected Poster Presentations

May 2019 – American Thoracic Society International Conference, Dallas, TX: *New insights into endothelial-to-mesenchymal transition in pulmonary hypertension: potential role for EBP50*

February 2019 – Pulmonary Vascular Research Institute Annual World Congress on Pulmonary Vascular Disease, Barcelona, Spain: *New insights into endothelial-to-mesenchymal transition in pulmonary hypertension: potential role for EBP50*

September 2017 – American Heart Association Council on Hypertension, San Francisco, CA: *Novel Role of Axl kinase in Endothelial Cell Proliferation and Pulmonary Arterial Hypertension*

Most Recent Invited Oral Presentations

Gorelova, A. 2020. New insights into endothelial-to-mesenchymal transition in pulmonary hypertension: potential role for EBP50. *American Heart Association Fellows Research Day* (Pittsburgh, PA)

Funding Sources and Awards

William Cotswold Foundation Fellowship, 2018-2019
Louis Ignarro Cardiovascular Pharmacology fellowship, 2017-2018
Louis Ignarro Cardiovascular Pharmacology fellowship, 2016-2017

Future Plans

Anastasia accepted a science writing fellowship from the Creative Nonfiction Foundation (Pittsburgh, PA). In the summer, she will begin her training as an American Association for the Advancement of Science (AAAS) Mass Media Fellow.