The molecular basis of pulmonary arterial hypertension

Dr Sandra Predescu from Rush University focuses on investigating the molecular mechanisms underpinning pulmonary arterial hypertension (PAH). This lethal disease is caused by narrowing of the blood vessels, increasing blood pressure and ultimately leading to heart failure. The team has discovered that the N-terminal fragment of the protein called intersectin-1s (ITSN-1s), result of granzyme B cleavage, can activate molecular pathways which increase endothelial cell (EC) proliferation and pathological remodelling. By understanding the molecular basis of PAH, Dr Predescu aims to develop novel therapies to treat this devastating disease.

Pulmonary arterial hypertension (PAH) is a life-threatening disease that currently has no cure. The purpose of the pulmonary arteries is to carry de-oxygenated blood from the right side of the heart to the lungs for oxygenation. However, in PAH patients, the pulmonary arteries undergo a range of destructive vascular changes, including cell proliferation and pathological remodelling. The damage inflicted on the pulmonary arteries consequently causes these blood vessels to become much narrower, leading to an increase in blood pressure. Eventually, this can cause right-sided heart failure and death.

A hallmark of PAH is the formation of plexiform lesions, which are typically found close to the branching points in the small pulmonary arteries. Plexiform lesions are complex vascular formations that comprise dysfunctional endothelial cells (ECs). These abnormal ECs actively divide and are apoptosis resistant (controlled cell death). This means that plexiform lesions essentially obstruct the blood vessels and, thus, may contribute to the development of hypertension.

However, relatively little is known about the molecular mechanisms underpinning the formation of PAH-associated plexiform lesions. This motivated Dr Predescu and her team to focus their research efforts on furthering our understanding of PAH. Previous research conducted by Dr Predescu and her colleagues showed that the deficiency in this multi-modular protein called intersectin-1s (ITSN-1s) resulted in EC apoptotic death and was then followed by alterations in the endothelial phenotype which led to hyperproliferation and emergence of apoptotic-resistant and hyperproliferative ECs. Since these are key traits of PAH, the team decided to further investigate the potential role of ITSN-1s in PAH.

CLEAVAGE OF INTERSECTIN-1S

ITSN-1s belong to the family of adaptor proteins. These proteins mediate interactions between cell surface receptors and downstream signals in signal transduction pathways, leading to signalling specificity. As a result, adaptor proteins regulate many essential biological processes including endocytosis, proliferation, cytoskeletal organisation and cell differentiation. Significantly, the ITSN proteins couple endosmosis to nuclear events by shuttling between cytoplasm and nucleus.

Interestingly, the team discovered that inflammatory reactions associated with PAH affect the function of ITSN-1s. Inflammation attracts CD8 T-cells (a type of white blood cell) which secrete granzyme B (GrB), a cytotoxic serine protease that induces apoptosis. Research conducted by Dr Predescu and her team indicated that ITSN-1s is a substrate for GrB. GrB cleaves ITSN-1s at a specific, well-conserved site (EDQ207TGK) on its N-terminal side. This results in a decreased expression of full-length ITSN-1s and two biologically active cleavage products—the N-terminal fragment (EHITSN) and the C-terminal fragment (SH3A-EITSN). The team showed this experimentally by treating mice with lipopolysaccharide (LPS), which is a bacterial toxin that induces a strong inflammatory response, resulting in increased GrB release. These mice had reduced full-length ITSN-1s expression and a 2.8-fold decrease in c-Fos expression. These results showed a significant increase in ITSN-1s deficiency and the presence of the two protein fragments, supporting the previous findings.

Additionally, the team showed that this N-terminal fragment of ITSN-1s has EC proliferative potential. EC growth was significantly higher in cells transfected with the EHITSN compared to controls. In fact, the results indicated a 50% increase in the number of EHITSN-expressing ECs compared with controls. This led the team to further investigate the potential role of ITSN-1s in PAH.

HOW DOES EHITSN CAUSE EC PROLIFERATION?

The N-terminal fragment of ITSN-1s increases EC proliferation via activation of the p38 MAPK pathway. This is a fundamental cell signalling pathway that is involved in a wide array of cellular processes, including proliferation, inflammation, apoptosis and cell division. P38 MAPK activation results in the activation of Elk-1, a transcription factor. This binds to the promoter of the c-Fos gene, resulting in over-expression of the c-Fos protein, which facilitates EC proliferation. Results showed that ECs transfected with EHITSN exhibited 50% higher Elk-1 activation in comparison to the control. These cells also showed a 4.8-fold increase in c-Fos expression, in comparison to the control cells. Furthermore, to confirm these results, the team showed that treatment with a selective p38 MAPK inhibitor significantly reduces EC proliferation.

PAH SYMPTOMS IN ITSN-1S DEFICIENT MICE

By using ITSN-1s deficient mice, Dr Predescu and her team further investigated the effects of ITSN-1 deficiency on the formation of plexiform lesions and PAH. Gene silencing was used to disrupt the ITSN gene in mice, resulting in a reduced expression of ITSN-1s. This was achieved using small interfering RNA (siRNA). This is a double-stranded piece of RNA that is complementary to the ITSN mRNA. Essentially, the siRNA binds to the ITSN mRNA, promoting its degradation. This means that the ITSN mRNA cannot be translated and as a result there is a reduced production of the ITSN protein.

Dr Predescu and her colleagues then treated the ITSN-1s deficient mice with the EHITSN for 20 days. Results from immunohistochemistry and histological
Dr Predescu and her team recently developed a mouse model of pulmonary arterial hypertension / plexiform arteriopathy.

**Research Objectives**

Dr Predescu and her team recently developed a mouse model of pulmonary arterial hypertension / plexiform arteriopathy.

**Detail**

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Bio
Dr Sanda Predescu is professor in the Department of Internal Medicine at Rush University Medical Center in Chicago. She has a PhD degree in Molecular Pathology from the University of California San Diego. Her current research is focused on endothelial cell dysfunction in pulmonary hypertensive diseases. By doing so, they contribute to the understanding of the pathophysiological mechanisms underlying abnormal endothelial cell proliferation in pulmonary hypertension.

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**Collaborators**

• Dan Predescu, MD
• Monal Patel, PhD
• Brandon Carman, MS

**Personal Response**

How do the products of the cleaved protein ITSN-1s affect pulmonary arterial hypertension?

The interplay between the two biologically active ITSN-1s fragments has more subtle cellular effects by interfering with the uptake and transport (endocytosis, transcytosis) of small and large molecules up to 100 nm diameter. In PAH settings, to compensate for the deficient vesicular trafficking via clathrin-coated vesicles and caveolae, prolonged ITSN-1s deficiency and the presence of the two biologically active fragments cause the increased occurrence/trafficking of the alternative endocytic structures, repressed under normal conditions. By doing so, they contribute to the endocytic activity of dysfunctional ECs, their proliferation and overgrowth, and hence, the development and progression of the disease.