NOGO could go far

By Michael J. Haas, Senior Writer

A team of U.S. and Canadian researchers has shown that blocking production of NOGO-B in pulmonary arteries could prevent the deleterious vascular remodeling seen in pulmonary arterial hypertension.[1] If future studies demonstrate that NOGO-B inhibitors could treat disease that already is established, the compounds could become the first disease modifiers for pulmonary arterial hypertension, which currently is treated with vasodilators that alleviate disease symptoms but do not address underlying causes.

In pulmonary arterial hypertension (PAH), increased proliferation and resistance to apoptosis in pulmonary vasculature—but not in systemic vasculature—results in obstruction of the pulmonary arteries, hypertension, right ventricular hypertrophy and eventual death.

Last year, researchers from the **University of Alberta** and **Metabolic Modulators Research Ltd.** reported in *Science Translational Medicine* that in mouse models of hypoxia-induced PAH, a metabolic shift from glucose oxidation to glycolysis in pulmonary arterial cells drove the proliferative, antiapoptotic phenotype seen in PAH.[2] However, the team did not identify the mechanism underlying the change in metabolism.

Evangelos Michelakis, who was corresponding author on both the 2010 study and the new paper, told *SciBX* the same metabolic shift occurs in nonhypoxic PAH models and human diseases such as idiopathic PAH, familial PAH associated with mutations in bone morphogenic protein receptor type II (BMPRII), scleroderma

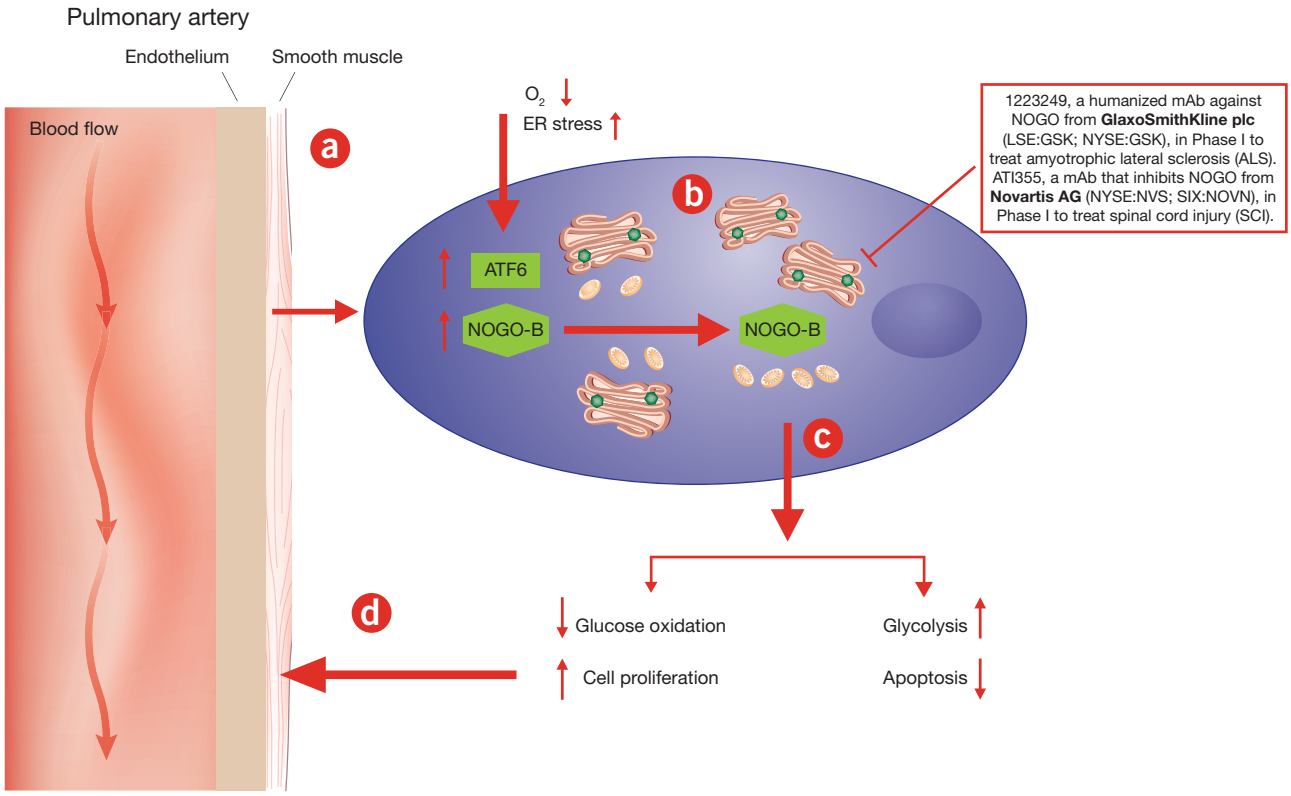

Figure 1. NOGO-B: on a stressful pathway to pulmonary arterial hypertension. In pulmonary artery smooth muscle cells, hypoxia induces endoplasmic reticulum (ER) stress and expression of activating transcription factor 6 (ATF6) [**a**], which upregulates the NOGO-B isoform of reticulon 4 (RTN4; NOGO; NOGO-A). Increased levels of NOGO-B disrupt the spatial proximity and normal interactions between the ER and mitochondria [**b**] to increase cytosolic glycolysis and decrease glucose oxidation [**c**], which promote cell proliferation and resistance to apoptosis [**d**], respectively, and lead to the deleterious vascular remodeling seen in pulmonary arterial hypertension (PAH).[2,10]

Axerion Therapeutics Inc. has reticulon 4 receptor (RTN4R; NGR) decoys that bind NOGO and two other NGR ligands in preclinical development to treat spinal cord injury (SCI).

associated with PAH and solid tumors.

Michelakis is a professor of medicine in the Division of Cardiology and vice chair of research in the Department of Medicine at the University of Alberta.

He noted that one factor shared by all of these diseases is increased endoplasmic reticulum (ER) stress. This led the group to look for a molecule or pathway linking stress in pulmonary arteries with ER function and vascular remodeling, he said.

Several lines of evidence pointed to NOGO-B—an isoform of reticulon 4 (RTN4; NOGO; NOGO-A)—as a likely candidate. For example, a 2006 study showed that NOGO plays a role in forming and stabilizing ER structure,[3] and a **Yale School of Medicine** group led by William Sessa showed that NOGO-B is highly expressed in lung tissue and regulates nonpathological vascular remodeling.[4–6]

Sessa is a professor of pharmacology at Yale.

Another Sessa-led study in 2010 showed that Nogo-b was downregulated in lung tissue but upregulated in pulmonary vasculature in mouse models of asthma,[7] which highlighted a NOGO-B-related mechanism that appeared to be specific to pulmonary blood vessels.

Given this background, a Michelakis-led team that included Sessa decided to investigate the role of NOGO-B in PAH.

The team found that NOGO-B levels were higher in plasma serum and pulmonary artery smooth muscle cells from PAH patients than from healthy controls.

In response to hypoxia, normal pulmonary artery smooth muscle cells from humans and mice upregulated NOGO-B. NOGO-B disrupted normal ER-mitochondrial function to induce the metabolic shift to glycolysis observed in PAH (*see* **Figure 1, "NOGO-B: on a stressful pathway to pulmonary arterial hypertension"**).

Lastly, in mouse models of chronic, hypoxia-induced PAH, homozygous *Nogo-b* knockouts did not develop arterial hypertension, right ventricular hypertrophy or other symptoms of the disease, and heterozygous knockouts developed less severe disease, compared with wild-type mice.

Data were reported in *Science Translational Medicine*.[1]

"We need therapeutic strategies that could change the proliferative phenotype in pulmonary hypertension, and this is one of the first mechanistic studies to address this," said Jeffrey Edelson, CMO of **Palatin Technologies Inc.** and principal of **AEQuanimitas Consulting LLC**. "The hypothesis is elegant and the science makes a strong argument for interfering with abnormal ER-mitochondrial function at the level of NOGO-B. This is fundamentally important to understanding the pathogenesis of PAH."

Palatin develops small molecule and peptide therapeutics for sexual dysfunction, congestive heart failure (CHF), obesity and metabolic syndrome.

Reversing course

The key question now is whether blocking NOGO-B will safely reverse established disease.

"While the association between *Nogo-b* status and disease severity in the mouse models shown in the study is compelling, my biggest reservation is that the study doesn't prove causality," Edelson said. "Thus, NOGO-B awaits further validation as a therapeutic target."

"Even if the NOGO-B-mediated stress response is indeed causal in PAH, it may not be reversible," he continued. "That is, once the stress response has induced cells to switch to glycolysis and the proliferative phenotype, inhibition of NOGO-B may not cause the system to stand down."

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>
> ***—Jeffrey Edelson, AEQuanimitas Consulting LLC***

Similarly, correcting the altered ER-mitochondrial phenotype might not fully reverse the structural and functional vascular abnormalities in PAH, he said. "We need to know more about the biology of this pathway to enable the evaluation of its potential as a therapeutic target to treat PAH," said Edelson.

He also noted that the team looked only at pulmonary arterial smooth muscle cells, even though the abnormalities seen in PAH also include changes in pulmonary arterial endothelial cells.

Thus Edelson wanted to see stronger preclinical proof of concept for the therapeutic benefit of blocking NOGO-B to treat PAH. Such experiments could include pharmacological inhibition of NOGO-B in the chronic, hypoxia-induced PAH model and studies in pulmonary endothelial cells.

Michelakis noted that other groups have seen the same metabolic switch and proliferative phenotype[8] or ER stress[9] in pulmonary endothelial cells from PAH patients that his team saw in pulmonary smooth muscle cells. Thus, he thinks the preclinical evidence already exists suggesting that NOGO-B inhibition would block the PAH phenotype in both types of cells.

Edelson also wanted to know more about the potential side effects of blocking NOGO-B and the ER-mitochondrial stress response in the systemic vasculature and other tissues.

He said future studies should investigate the cells and tissues in which the NOGO-B-mediated stress response occurs, the types of stress conditions that activate it and the effects of *Nogo* knockout on mice subjected to stresses unrelated to PAH, such as postoperative wound repair.

Michelakis acknowledged that additional work needs to be done to determine whether blocking NOGO-B can treat established PAH. "But we did see a gene dose–dependent effect of *Nogo* in the mouse knockouts, and this argues that the amount of the protein contributes to the disease state," he said.

Michelakis disagreed that NOGO-B inhibition might have significant side effects. "The knockout mice have no abnormal phenotype—for example, their wounds and other injuries heal normally—suggesting that NOGO-B is nonessential for normal function," he said.

Specifying subtypes

Another question is whether the paper's findings will apply to all pulmonary hypertension patients.

The **World Health Organization** has defined five subtypes of pulmonary hypertension that are based on etiology and/or associations with underlying disease. Group I includes idiopathic and familial PAH,

PAH resulting from viral infection and PAH associated with other vascular diseases such as scleroderma.

"Most drug development has focused on Group I because this group has a 3-year survival rate of 50% and thus a great unmet medical need for effective therapies," Edelson said.

However, because the team obtained its results in hypoxia-induced models of PAH, he thinks the findings might apply most directly to Group III, which includes PAH that is secondary to other lung diseases such as chronic obstructive pulmonary disease (COPD) and hypoxemic conditions such as sleep apnea. In these patients, "the clinical course of PAH is greatly affected by the underlying disease and it's not clear that treating PAH *per se* is a game changer," he said.

Michelakis agreed that his team's work is most applicable to hypoxia-related PAH but said the findings also might apply to other types of the disease. For instance, viral infections and BMPRII mutations associated with PAH are known to cause ER stress, he said. He also pointed out that the human plasma and tissues his team studied came from Group I—not Group III—patients.

Michelakis said the team is focusing primarily on blocking the activation of the axis between NOGO-B and its upstream transcription factor ATF6 (activating transcription factor 6). Specifically, the researchers are inhibiting chaperone molecules that mediate the response to ER stress upstream of ATF6.

"We have identified candidate chaperones and their inhibitors, and some of these small molecules can inhibit Nogo expression and reverse established PAH in both hypoxic and nonhypoxic animal models," Michelakis told *SciBX*.

He added, "We also have our eyes open for inhibitors of intracellular NOGO that we could test as PAH therapies. We have the tools and ideas, but we need compounds to test and aren't set up to do the kind of screening experiments needed to identify inhibitors."

GlaxoSmithKline plc and **Novartis AG** are developing anti-NOGO antibodies to treat CNS conditions. Michelakis said additional studies would have to determine whether such compounds—which bind extracellular NOGO to prevent activation of the NOGO receptor (reticulon 4 receptor; RTN4R; NGR)—would inhibit intracellular NOGO-B.

The team also is exploring the utility of NOGO-B as a diagnostic marker for PAH and a marker for treatment response to PAH therapies, Michelakis said.

The findings reported in the paper are unpatented.

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