Increased understanding leads to increased complexity: Molecular mechanisms of pulmonary arterial hypertension

Victor A. Ferraris, MD, PhD

From the Department of Surgery, University of Kentucky, Lexington, Ky.

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Address for reprints: Victor A. Ferraris, MD, PhD, University of Kentucky, A301 Kentucky Clinic, 740 S Limestone, Lexington, KY 40536-0284 (E-mail: Ferraris@earthlink.net).

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Salusin- β is a bioactive peptide that was discovered by means of computer-assisted search of full-length, enriched, human complementary DNA libraries.¹ This search found a previously unidentified 20-amino acid peptide translated from messenger RNA of the *TOR2A* gene group. Expression of the *TOR2A* messenger RNA is ubiquitous, and reactive salusin- β can be detected in many human specimens, including plasma and urine. In the original description of the actions of salusin- β , intravenous administration of salusin- β in rats was reported to cause rapid and profound hypotension and bradycardia. Salusins increase intracellular calcium, upregulate a variety of genes, and induce cell mitogenesis.¹ Subsequently, salusin- β was found to accelerate inflammatory responses in vascular endothelium by stimulating the transcription factor nuclear factor κ light-chain enhancer of activated B cells (NF- κ B).²

The manuscript by Xu and coauthors³ in this issue of *The Journal of Thoracic and Cardiovascular Surgery* is a busy discussion of the effects of salusin- β on a rat model of pulmonary arterial hypertension (PAH) induced by monocrotaline. Salusin- β is notoriously difficult to measure in living tissues. Xu and coauthors³ performed a robust series of experiments aimed at defining the impact of salusin- β on inflammation-related PAH. They used standard methods to define the effect of salusin- β on the development of PAH. Their experiments are well constructed and seemingly do not contain any significant flaws. Through a combination of in vitro and in vivo experiments, they describe at least 5 separate studies relating to salusin- β and PAH as follows:

1. Identify salusin- β expression in macrophages and vascular endothelial cells in the lungs of monocrotaline-treated rats.
2. Use polyclonal antibodies to block the in vivo effects of salusin- β in their rat model.
3. Measure the direct hemodynamic effects of salusin- β in monocrotaline-treated rats.
4. Assess the effect of salusin- β on the transcription factor NF- κ B, in monocrotaline-treated rats with PAH.

Activities associated with nuclear factor κ B.

Central Message

Complex relationships of induction of pulmonary arterial hypertension may include salusin- β -induced activation of nuclear factor κ B transcription factor.

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5. Salusin- β stimulated endothelial cell adhesion, accelerated tube formation, and upregulated cytokine expression in cultured human PAH cells in vitro.

Their appropriate conclusions from these experiments are that salusin- β is likely a contributor to the inflammatory changes associated with development of PAH, primarily through activation of NF- κ B.

NF- κ B is a protein complex found in the cytoplasm that can migrate to the nucleus to activate gene transcription. NF- κ B is found in almost all animal cell types and controls transcription of DNA, cytokine production, and cell survival. It is involved in cellular responses to multiple stimuli, including stress, cytokine stimulation, free radicals, and bacterial or viral antigens (<https://en.wikipedia.org/wiki/NF-κB>). Incorrect regulation of NF- κ B has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development.

NF- κ B consists of either heterodimers or homodimers formed by the members of the NF- κ B family (Figure 1). In mammalian cells, there are 5 NF- κ B family members that have various functions, including forming dimers that bind to nuclear DNA (*RelA* and *p50* in Figure 1). Some forms of NF- κ B produce inhibitors of NF- κ B actions (*IκB*). In most quiescent normal cells, the NF- κ B dimers are squelched in the cytoplasm by associating with *IκB* proteins. There are 7 members of the *IκB* family that can potentially inhibit NF- κ B function in the cytoplasm. *IκB* is a major molecular target for blocking NF- κ B activation and

FIGURE 1. Mechanism of nuclear factor κ B action. In this figure, the nuclear factor κ B heterodimer (*RelA* and *p50* proteins) is used as an example. While in an inactivated state, nuclear factor κ B is located in the cytosol complexed with the inhibitory protein (*IκBα*). A variety of extracellular signals can activate the enzyme *IκB* kinase (*IKK*). *IκB* kinase in turn phosphorylates the *IκBα* protein (represented by *P* in the diagram), which results in dissociation of *IκBα* from nuclear factor κ B and eventual degradation of *IκBα*. The activated NF- κ B is then translocated into the nucleus, where it binds to specific sequences of DNA called response elements (*RE*). The DNA/NF- κ B complex then recruits other proteins, such as coactivators and RNA polymerase, which transcribe downstream DNA into messenger RNA (*mRNA*), which in turn is translated into protein, which results in a change of cell function. (From <https://commons.wikimedia.org/w/index.php?curid=3072083>.)

subsequent gene transcription (eg, bortezomib is a reversible proteasome inhibitor of the degradation of *IκB* and is US Food and Drug Administration approved for cancer treatment, especially multiple myeloma, when used in conjunction with other cancer drugs⁴).

Inflammatory stimuli can trigger the degradation of *IκB* proteins through a kinase in the cytoplasm, causing the release of NF- κ B followed by its translocation to the nucleus, where it modulates target gene transcription. Nuclear translocation of NF- κ B is a necessary component of gene activation by NF- κ B. Xu and coauthors³ found that salusin- β blockade inhibited monocrotaline-induced *IκB* degradation with subsequent decreased activation of NF- κ B in the lung tissue and intrapulmonary arterioles (see Figure 4 in the article of Xu and coauthors³). Further, they showed that salusin- β blockade inhibited the nuclear translocation of NF- κ B in cultured human pulmonary endothelial cells (see Figure 6, C and D, in the article of Xu and coauthors³).

The reason for describing the implications of NF- κ B regulation is to point out that they are complex. Tremendous progress has been made during the last 2 decades in unraveling the elaborate regulatory networks that control the

NF- κ B response. This has made the NF- κ B pathway a paradigm for understanding general principles of signal transduction and gene regulation. NF- κ B transcription factors are crucial players in an elaborate system that allows cells to respond to external stimuli, a process pivotal for survival and adaptation. A large number of diverse external stimuli lead to activation of NF- κ B, and the genes whose expression is regulated by NF- κ B play important and conserved roles in immune and stress responses.⁵⁻⁸ It is extremely simplistic to ascribe NF- κ B regulation to a single protein such as salusin- β . In their article, Xu and coauthors³ focus on salusin- β to the exclusion of other potential mediators and other signaling pathways. I realize that they intended to perform a preliminary study of salusin- β ; however, there is a very distinct possibility that other factors play an important role in NF- κ B activation-related PAH, perhaps even to the exclusion of salusin- β . Further, Xu and coauthors³ point out in their Discussion section that the effects of salusin- β on signaling in other inflammatory pathways are unknown.

The results described in the article by Xu and coauthors³ are intriguing, but further studies are needed to identify the

impact of altering salusin- β -induced NF- κ B activation as a therapeutic target for the treatment or prevention of PAH. Identification of drugs that can target salusin- β inhibition and that might have some use as therapeutic agents are important next steps. The work of Xu and coauthors³ is very preliminary but quite promising. Nonsteroidal anti-inflammatory drugs, including sulindac, aspirin, ibuprofen, indomethacin, and cyclooxygenase-2 inhibitors, are potential NF- κ B blockers. They function either by suppressing the inflammatory cell response to indirectly suppress NF- κ B or by directly suppressing NF- κ B at key points along the NF- κ B activation pathway. Whether any anti-inflammatory agents have effects on salusin- β function is uncertain, but investigation of this question surely must be an important next step. I look forward to future contributions from these authors regarding the impact of salusin- β on nuclear transcription.

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