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VEGF-B Gene Therapy for the Heart: Proceed with Caution

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An unfortunate circumstance in biology is that the vascular endothelial growth factor type B (VEGF-B) was identified only after its homolog family member VEGF-A had already been discovered.[1] Should the opposite have occurred, VEGF-B would likely not have been named as such. Indeed, VEGF-B is a poor growth factor for endothelial cells and, unlike VEGF-A, it is not angiogenic in the strict sense, while its only known main receptor, VEGFR-1, is expressed in endothelial cells as well as in a vast series of other cell types. Little was known about the non-angiogenic functions of selective VEGFR-1 ligands until a study in 2008 showed marked anti-apoptotic effects of VEGF-B, both *in vitro* and *in vivo*.[2] Subsequent studies confirmed cell-protective functions of this factor in different cell types.

In this issue of *Molecular Therapy*, Lähteenvuo et al.[3] add a new piece to the puzzle of VEGF-B activities. By using an adenoviral vector to overexpress VEGF-B_{186}, they show that this factor induces sympathetic nerve sprouting in mouse and pig hearts via a VEGFR-1 signaling-independent mechanism. The integrity of cardiac innervation is very important for electrical stability, contractility, myocardial metabolism, and coronary function.[4] Therefore, the induction of cardiac re-innervation by the VEGF-B_{186} transgene should theoretically enhance post-ischemia cardiac recovery. Nonetheless, the authors report an association between a likely disordered nerve sprouting[5] and increased risk of ventricular arrhythmias and sudden cardiac death in pigs, at 6 days after infarction, unlike any other VEGFs tested in their previous studies. The authors should be commended for their rigorous approach based on retrospective analysis of 334 pigs and statistics, more usually utilized in the clinical studies realm. Such careful evaluation revealed an intrinsic risk in cardiac VEGF-B_{186} gene therapy, whereas preclinical studies, strictly prospective and necessarily based on smaller sample sizes, usually tend to overemphasize beneficial effects of experimental therapies, paying little attention to untoward events.

Does the observation by Lähteenvuo et al.[3] raise a red flag on the therapeutic utilization of VEGF-B gene transfer in the heart? Not necessarily. If the goal is angiogenesis, VEGF-A or VEGF-D are more established therapeutic factors. Despite the fact that VEGF-A binds with 10-fold greater affinity to VEGFR-1 than to its angiogenic receptor VEGFR-2, it does not appear to be arrhythmogenic. However, if the goal is rather the direct protection of cardiomyocytes and other cell types in the ischemic or non-

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ischemic failing heart, then strong evidence suggests that the 167 aa isoform of VEGF-B (VEGF-B_{167}) might be an equally effective and safer candidate. VEGF-B_{167} is the most abundant VEGF-B isoform and is expressed in brown fat, myocardium, and skeletal muscle.[6] Mice knocked out for this factor develop normally but exhibit a mild cardiac phenotype, with dysfunctional coronary vasculature, impaired recovery from cardiac ischemia, and, most notably, decreased heart size.[7] Our own work using adeno-associated virus (AAV)-mediated gene delivery of VEGF-A_{165} and VEGF-B_{167} in infarcted rat hearts revealed that both factors produced a marked improvement in cardiac function by promoting cardiac contractility, preserving viable cardiac tissue, and preventing remodeling of the left ventricle over time.[8] However, VEGF-B_{167} was more effective in preserving cardiac mass, even in the absence of a significant induction of angiogenesis. Two subsequent studies by our group in a dog model of tachypacing-induced dilated cardiomyopathy showed that AAV-VEGF-B_{167}, injected directly in myocardium or infused in coronary arteries, nearly halted cardiac structural and functional abnormalities and delayed the onset of decompensated heart failure.[9,10] Lähteenvuo et al.[3] rightly observe that tachypacing might mask possible ventricular arrhythmias. However, our ongoing clinical trial in dogs with severe spontaneous heart failure is not revealing unexpected increases in sudden death after VEGF-B_{167} gene delivery to the heart and, on the contrary, some canine patients monitored for months have displayed a surprising prolongation of life expectancy (unpublished data).

Over the last several years, there has been considerable interest in understanding the role of VEGF-B in the heart. Despite not being angiogenic in the strict sense, transgenically overexpressed VEGF-B was shown to induce overgrowth of the epicardial coronary vessels and of their main branches.[6] In addition, this factor was reported to regulate metabolism by promoting endothelial uptake and transcytosis of fatty acids from the bloodstream into various tissues, including myocardium,[11] and also to deplete endothelial plasma membrane cholesterol, with consequent reduction in glucose uptake.[12] Of interest, under normal conditions, VEGF-B appears to be highly expressed by cardiomyocytes themselves, from which it is secreted and binds to the surrounding extracellular matrix. Endothelium-secreted heparinase can mobilize this extracellular pool of VEGF-B, thus enabling it to exert pro-survival and protective activities.[13] This protective mechanism appears to be severely attenuated in the setting of uncontrolled diabetes mellitus, which could explain the development of diabetic cardiomyopathy.[13]

How VEGF-B exerts its beneficial function on cardiomyocytes remains an open matter. VEGFR-1 is expressed by cardiomyocytes in a functional form at levels that are lower than those of endothelial cells but still sufficient to exert a protective effect.[7,8] In addition, this factor was also shown to influence myocardium through the activation of endothelial cells in the coronary vasculature.[14]

What are the molecular mechanisms underlying possible differences between the cardiac effects of VEGF-B_{186} versus VEGF-B_{167}? They are both specific ligands of VEGFR-1 and, similar to VEGF-A_{165} and PlGF, bind neuropilin receptor-1 (Nrp-1),[15] the receptor that seems to mediate the neurotrophic effect in the heart in the Lähteenvuo et al.[3] study. However, VEGF-B_{167} and VEGF-B_{186} bind Nrp-1 with different modalities involving, respectively, a heparin-binding domain and proteolytic processing. Importantly, VEGF-A_{165} and VEGF-B_{167} tend to remain stably attached to heparin sulfates, while VEGF-B_{186} and PlGF are more freely soluble and diffusible, probably functioning also as paracrine factors.

To what extent these subtle molecular differences might explain the different arrhythmogenic potential of the two VEGF-B isoforms in the absence of VEGFR-1 binding remains a matter for interesting, future investigation. This appears to be an important field of research, since the marked beneficial effect of VEGF-B on the heart continues to be very attractive for the development of clinically effective gene therapy for heart failure.

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