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GRK5: big-hearted target?

By Michael J. Haas, Senior Writer

A team of U.S. and U.K. researchers has uncovered a new role for G protein–coupled receptor kinase 5 in pathological cardiac hypertrophy, providing a first mechanistic target for this indication.[1] It behaves more like an histone deacetylase kinase than a G protein–coupled receptor kinase, activating histone deacetylase 5 and triggering a signaling cascade that results in cardiac hypertrophy.

The finding, reported in the *Proceedings of the National Academy of Sciences*, may make it possible to treat hypertrophy with kinase inhibitors. In contrast to current therapeutic strategies that address more general conditions such as hypertension or ischemia, which can lead to cardiac hypertrophy, this research provides the potential to treat the condition directly at its mechanistic root.

However, the effectiveness of such a therapeutic strategy will depend on whether the function of G protein–coupled receptor kinase 5 (GRK5) is unique in hypertrophy or is redundant with other histone deacetylase kinases (HDAC kinases).

The team of scientists from **Thomas Jefferson University** and **University College London** was led by Walter Koch, VP of research in medicine, professor of medicine and director of the Center for Translational Medicine at Thomas Jefferson.

"GRK5 has been shown to be increased in heart failure," Koch told *SciBX*. "Consequently, it made sense to study it in hypertrophy. Our work shows that myocytes with high GRK5 levels are primed for hypertrophy."

Failure of mighty-hearted mice

Left ventricular hypertrophy (LVH)—also known as pathological or maladaptive cardiac hypertrophy—is a condition in which the heart grows and accumulates scar tissue in response to stress, such as hypertension and injury. This enlargement compromises heart function over time and leads to heart failure. There are no approved therapeutics to treat hypertrophy.

GRK5, a membrane-bound protein expressed in most tissue types, regulates signaling and function in myocytes.[2] It is also known to be upregulated in pathological cardiac conditions such as heart failure, but how it contributed to those conditions was unknown.[3,4]

"There are two GRKs in the heart—GRK2 and GRK5," Koch said. "Previously we have focused on GRK2, which has a pathological role in heart failure, but we were also interested in hypertrophy."

Last year, Koch and colleagues at Thomas Jefferson University described how GRK2 signaling accelerates heart failure in a heart whose function is already compromised.[5]

In an unpublished study conducted in 2003 by Koch while at **Duke University Medical Center**, mice that overexpressed GRK5 were subjected to aortic banding, a surgical procedure that creates resistance to blood flow out of the heart, thus putting stress on the organ. Koch's group found that about 75% of these mice died of heart failure, compared with about 10% of mice having normal GRK5 expression.

Ex vivo analysis revealed excessive hypertrophy in the GRK5-overexpressing mice, he said.

Koch said that prior to aortic banding "the hearts of the GRK5-overexpressing mice are slightly larger than normal but have no loss of function. The mice are healthy and normal until they are stressed."

In 2004 a team at University College London led by Julie Pitcher, senior lecturer of pharmacology and coauthor on the *PNAS* paper, showed that GRK5 contains a nuclear localization sequence that allows it to translocate from the membrane to the nucleus of myocytes in response to G protein–coupled receptor (GPCR) signaling. Previous work by others had also shown increased nuclear localization of GRK5 in a rat model of cardiac hypertrophy.

Together these results led Pitcher to suggest that GRK5 may have a role in modulating gene expression linked directly to hypertrophy.[6]

Based on these two studies, Koch's team hypothesized that nuclear GRK5 played a direct role in pathological hypertrophy via transcriptional regulation of genes responsible for this condition.

To investigate their hypothesis, the team first repeated Koch's aortic banding experiment in GRK5-overexpressing mice and obtained similarly high rates of heart failure and mortality. *Ex vivo* analysis showed both a significant increase in heart size and a greater accumulation of GRK5 in the nucleus of myocytes relative to what was seen in mice with normal GRK5 expression.

By contrast, mice that overexpressed a mutant form of GRK5 that lacked the ability to cross the myocyte nuclear membrane showed no increase in heart size or heart failure in response to aortic banding—suggesting that GRK5's nuclear function was indeed critical to hypertrophy.

Next, *in vitro* experiments demonstrated that stress-induced GPCR signaling in myocytes activated guanine nucleotide binding protein, q polypeptide (G_q; GNAQ), thereby triggering upregulation of GRK5, which then translocated to the myocyte nucleus. In the nucleus, GRK5 activated histone deacetylase 5 (HDAC5)—a class II HDAC—which then translocated to the cytosol, where it activated myocyte enhancing factor 2 (Mef2; D-Mef2), a transcription factor known to upregulate expression of hypertrophic genes.

The team said its results collectively demonstrate that GRK5 is a class II HDAC kinase that plays a critical role in the development of cardiac hypertrophy in response to cardiac stress.

Koch acknowledged that the effects of GRK5's role in hypertrophy are probably exaggerated in his team's mouse model because the mice

overexpress GRK5. Nonetheless, the findings should be relevant in animals with normal GRK5 expression because hypertrophic stimuli result in the nuclear localization of GRK5 in such animals as well, he said.

Best heart target

Researchers contacted by *SciBX* agreed that Koch's team had identified an important mechanism in the development of cardiac hypertrophy, but they disagreed over whether the findings would easily translate into a therapeutic to treat the condition directly.

Suraj Shetty, lab head and project team leader of cardiovascular and metabolism research at **Novartis AG**'s Novartis Institute for Biomedical Research, said that "hypertrophy is a harbinger of adverse cardiac events like heart failure. Hence there has been a move to identify key targets involved in hypertrophy."

He added that nuclear factors involved in hypertrophy—such as the nuclear form of GRK5—are of particular interest because they could represent nodal points strategically located at the convergence of several different hypertrophic signaling pathways. "You want to be as downstream as possible to have the most impact," he said.

However, he noted that at least three other class II HDAC kinases are known: calcium/calmodulin-dependent protein kinase II (CAMKII), protein kinase D (PKD) and microtubule affinity-regulating kinase (MARK).

"There might be some redundancies among these kinases," Shetty said. "So the question is whether GRK5 is the key HDAC kinase responsible for mediating pathological hypertrophy" or whether the other kinases could compensate if GRK5 is inhibited, he said. "We would need to ascertain the relationship and redundancy among these kinases" before determining that GRK5 is a valid target, he said.

"If this pathway is relevant for stimulus such as high blood pressure and ischemia, then GRK5 could be a druggable target."

—Junichi Sadoshima, University of Medicine and Dentistry of New Jersey

"Certainly there are redundancies," Koch agreed. "But these kinases act differently depending on the type of stress and which receptor system is activated."

He added that it isn't yet known if GRK5 is part of other receptor pathways, a question that is the focus of an ongoing study.

Junichi Sadoshima, vice chair and professor of cell biology and molecular medicine and associate director of the Cardiovascular Research Institute at the **University of Medicine and Dentistry of New Jersey**, was more sanguine about GRK5's potential as a target.

"This paper is very interesting because I believe the signaling mechanism regulating HDAC translocations is really critical to pathological cardiac hypertrophy," he said.

"If this pathway is relevant for stimulus such as high blood pressure and ischemia, then GRK5 could be a druggable target," Sadoshima said.

Sadoshima also wanted to know how GRK5 is transported from the membrane to the nucleus. "If the molecule that associates with GRK5 to move it to the nucleus could be identified, that could be a good target," he said, and possibly preferable to GRK5 because of the known difficulties in targeting kinases.

Novartis's Shetty said whether this unknown transporter would be a good target would depend on how specific or general its function is. "If the chaperone molecule is a general factor involved in nuclear shuttling, then targeting it could have severe side effects," he said.

In 2007 Novartis began a Phase IV trial comparing Diovan valsartan plus the generic amlodipine to Cozaar losartan plus the generic hydrochlorothiazide for the treatment of pathological hypertrophy.

Novartis markets Diovan, an angiotensin II receptor antagonist, to treat hypertension. **Merck & Co. Inc.** markets Cozaar, also an angiotensin II receptor antagonist, for the same indication.

Direct benefit

Shetty said that treating pathological hypertrophy indirectly—by treating underlying conditions like hypertension—minimizes hypertrophy and protects against adverse cardiovascular events such as heart failure and arrhythmia. But he noted that growing evidence suggests that treating hypertrophy *per se* might confer similar protection.

According to Shetty, researchers had hypothesized that cardiac hypertrophy was a beneficial compensatory response to stress, and therefore inhibiting it directly would be detrimental. But that hypothesis has been challenged by short-term *in vivo* studies showing inhibition of pathological hypertrophy—induced in animals with otherwise normal blood pressure—is in fact beneficial.

"This raises the possibility that inhibition of pathological cardiac hypertrophy *per se* could have salutary effects," Shetty said. "Thus a concerted effort is underway to ascertain the long-term benefits of inhibition of hypertrophy *per se* in the context of heart failure."

Sadoshima believes direct treatment of hypertrophy might have several benefits.

"Regardless of the nature of the initial triggers—hypertension, ischemia, angiotensin and so on—many stimuli that induce pathological forms of hypertrophy utilize the HDAC-Mef2 pathway," he said. "Thus inhibitors of HDAC kinases would be effective in many forms of pathological hypertrophy and have broad application against many forms of heart failure."

By contrast, Sadoshima said blocking hypertrophic mechanisms upstream from GRK5 and HDAC—for instance, at the level of receptors or cytosolic kinases that have many cellular functions—could have undesirable side effects. "If you inhibit hypertrophy at final steps of signaling in cells, you can expect more targeted effects," he said.

Sadoshima also speculated that specific inhibitors of HDAC kinases might have fewer systemic side effects than inhibiting upstream targets, because class II HDACs are mostly expressed in terminally differentiated cell types.

Besides investigating whether GRK5's action on HDAC5 is redundant with other class II HDAC kinases, Koch said the team is attempting to elucidate the specific mechanism of binding between GRK5 and HDAC5.

"This would give insight into how HDAC5 is regulated by GRK5," he said. "If we know this, we could put small peptides into the nucleus to block that interaction."

Koch said another study by the team targets GRK5 with a GRK5-specific viral microRNA from adeno-associated virus (AAV).

Koch said the findings reported in *PNAS* are not patented, but added, "Once we have more data on targeting GRK5 specifically, maybe we will file a patent."

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