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EDITORIAL COMMENT

Location-specific and Kinase-Independent GRK5 Function in Heart*

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The family of G-protein coupled receptor kinases (GRKs) were discovered originally as critical modulators of G-protein coupled receptor (GPCR) signaling through targeted phosphorylation of the C-terminal fragment of their canonical 7-transmembrane receptors. GRK-mediated receptor phosphorylation is a key mechanism to desensitize cellular response to external stimuli by fine-tuning the cognate receptor activities and abundances.[1] Giving this pivotal role, GRKs have been actively investigated as molecular targets to treat a myriad of human diseases, in particular heart failure, neurological disorders, and cancer. However, over the years, the regulatory function of the GRK family and the underlying molecular mechanisms have been significantly broadened beyond the classic receptor signal modulation at cellular membrane and are now extended to different cellular compartments with direct roles in gene expression, cytosolic signaling, and metabolic regulation. Kinase-independent interaction and signaling modulation have also emerged as important mechanisms for GRK-mediated gene regulation and cellular functions.[2] Understandably, these added complexities can significantly affect GRK-targeted therapeutic strategies, which largely focus on kinase inhibition and GRK-GPCR interactions. In this issue of *JACC: Basic to Translational Science*, a new report by Marzano et al[3] from the Koch and Cannavo labs provides an interesting and potentially important new insight to GRK5, a member of the GRK family, regarding its kinase-independent, and compartment-specific functions in the context of cardiac physiology and the pathogenesis of heart failure.

GRK5 is highly enriched in cardiac tissue with a dynamic expression pattern associated with heart failure. Previous studies using genetic knockout or overexpression mouse models, mostly from the Koch lab, have established important roles for GRK5 in stress-induced cardiac hypertrophy, remodeling, and contractile dysfunction. Beyond the canonical role of GRK5 in adrenergic receptor signaling regulation, the Koch lab also found that GRK5 can translocate into nuclei on selected ligand stimulation. This translocation allows GRK5 to serve as a transcription activator of hypertrophic genes by targeted phosphorylation of histone deacetylases and direct protein-protein interaction with a key transcription factor, nuclear factor of activated T cells.[4] In addition to its kinase-dependent activity, GRK5 has also been implicated in nuclear factor-κB and p53-mediated signaling through direct protein-protein interaction to modulate cell viability in nonmyocytes. These complexities may contribute to the overall function of GRK5 in heart in the context of both acute response and long-term remodeling. Therefore, a major gap remains in our current knowledge of GRK5 function in the heart regarding its kinase-independent role and compartment-specific function in the heart.

In their report, Marzano et al[3] aim to provide clear in vivo evidence to determine the kinase-independent functions of GRK5 in intact mice.[3] They used a CRISPR/Cas9-based precision genome editing approach to generate a new mouse line where the lysine (K) 215 residue of the mouse GRK5 protein,

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FIGURE 1 GRK5 Kinase-independent Nuclear Activity in Heart

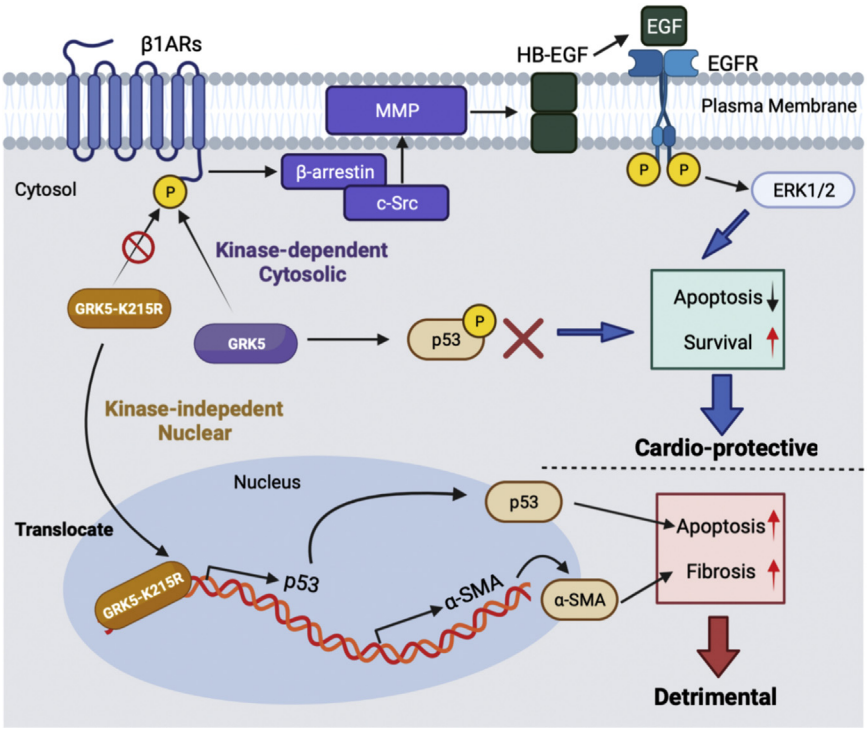

Illustration of kinase-independent nuclear activity of GRK5, a member of the G-protein coupled receptor kinase family, in the heart. EGF = epidermal growth factor; EGFR = epidermal growth factor receptor; ERK1/2 = extracellular signal-regulated kinases 1/2; HB-EGF = heparin-binding EGF-like growth factor; MMP = matrix metallopeptidase; P = phosphorylation; α-SMA = α-smooth muscle actin; β1ARs = β_1 adrenergic receptor antibodies.

which is essential for its kinase activity, was replaced by an arginine (R). The mice carrying homozygous K215R mutant alleles showed the GRK5 mutant protein was expressed at normal levels but without its kinase activity. Marzano et al[3] first characterized baseline cardiac function in the GRK5-K215R mice without any external stresses and found that these mice developed spontaneous contractile dysfunction associated with elevated apoptosis and fibrosis. Furthermore, GRK5-K215R mutant mice developed accelerated heart failure and cardiac remodeling on pressure-overload, but without affecting the induction of hypertrophy. Based on recent observations made in GRK5 cardiac-specific knockout and transgenic overexpressing mice,[5] GRK5 activation in the heart is detrimental while GRK5 inactivation is expected to be cardioprotective. Therefore, the cardiac phenotype observed in the GRK5 kinase-dead mutant mice under basal as well as pathological conditions provided the first in vivo evidence that kinase-independent activity of GRK5 also has a significant contribution to its detrimental impact in the heart. It is very interesting to note that the functional outcome of GRK5 kinase-independent activity appears to be different from the outcomes observed in the GRK5 cardiac-specific knockout heart. Although the differences are consistent with distinct mechanisms for kinase-dependent versus kinase-independent activities of GRK5, we cannot exclude the potential impact from different strategies of genetic manipulation employed in these 2 studies, that is, global knockin[3] vs. cardiac specific knockout.[5]

To further dissect the specific mechanisms involved in GRK5 kinase-independent activity in the heart, Marzano et al[3] performed more studies in cultured H9c2 cells, a cardiomyocyte cell line. First, they showed that excluding GRK5-kinase dead mutant from nuclei lowered p53 expression, while trapping the same GRK5 mutant inside of nuclei led to higher p53 expression and more cell death. This observation led to the conclusion that GRK5 kinase-independent activity may have 2 opposite roles in cardiomyocytes for p53 regulation. On one hand, it can be cardioprotective by suppressing p53 expression in the cytosol. On the other hand, it can be detrimental by promoting p53

transcription in the nuclei. Beyond cardiomyocytes, Marzano et al[3] also showed that GRK5-K215R expression in fibroblasts resulted in myofibroblast phenotype transition based on marker gene inductions. Interestingly, the kinase-dead GRK5 mutant showed similar distribution in different cellular compartments as the wild-type GRK5 protein, but displayed a significantly higher DNA binding capacity to targeted genes. This result highlights the interaction between kinase-dependent and kinase-independent activities of GRK5 regarding its downstream gene regulation. Overall, these data offer some interesting new molecular insights to GRK5 kinase-independent function in both cardiomyocytes and fibroblasts where its nuclear expression induces p53 transcription and subsequent cell death (**Figure 1**).

Although both in vivo and in vitro phenotypic evidence is consistent with a kinase-independent role for GRK5 in cardiac pathogenesis, the current study raises additional interesting questions. It is intriguing to see that global inactivation of GRK5 kinase activity is sufficient to promote cardiac dysfunction and remodeling, whereas cardiac-specific knockout of the *GRK5* gene is largely cardioprotective. As Marzano et al[3] proposed, the differences can certainly be attributed to distinct features of GRK5 kinase-independent versus kinase-dependent activities. However, they may also be derived from the loss of GRK5 kinase activity for normal cardiac homeostasis, particularly in nonmyocytes. Comprehensive and cell-type specific dissection of GRK5 kinase-independent function in cardiomyocytes versus nonmyocytes will be needed to fully address this question. In addition, Marzano et al[3] observed that expression of kinase-dead GRK5 elicited p53 expression to a comparable level as the wild-type GRK5 under Gq-mediated induction, which is known to promote GRK5 nuclei-translocation. However, the GRK5-K215R mutant shows similar intracellular distribution as the wild-type protein but with higher DNA binding activity, implicating additional kinase-dependent modulation to GRK5 transcriptional activity at the DNA binding level. Furthermore, unlike the GRK5 wild-type protein, which has a potent effect on cardiac hypertrophy, the GRK5 kinase-dead mutant shows no impact on hypertrophy but a major role in p53-mediated apoptosis and fibrosis instead. Considering the fact the current study did not include any unbiased analysis for downstream targets or gene regulation, the molecular network responsible for GRK5 kinase-independent function, including interacting partners and downstream target genes, remains to be fully elucidated.

Several aspects of this study may have significant implications in future therapeutic development based on targeted inhibition of GRK5 in the context of heart failure. The kinase-independent activity of GRK5 is highly sensitive to cellular compartment. GRK5 in cytosol appears to suppress p53 expression, which may offer a cardioprotective effect. In contrast, GRK5 in nuclei can activate p53 expression, which leads to detrimental outcome. Therefore, targeted intervention of GRK5 needs to consider kinase-dependent versus kinase-independent activities, as well as cellular compartments in cytosol versus nuclei. These complexities add to the current challenges in GRK5-targeted therapy for heart failure or other diseases. Similar to the need for our knowledge to expand beyond the kinase-mediated regulation of GPCR activity, future strategies to develop GRK5-targeted therapies for heart failure should also be extended from kinase activity or kinase-GPCR interaction to include other components in its molecular network, such as pathways involved in GRK5 nuclear localization, cytosolic targeted protein degradation, and nuclear-targeted DNA binding and transcription regulation.

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