Targeting GRK2 by gene therapy for heart failure: benefits above β-blockade

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Heart failure (HF) is a common pathological end point for several cardiac diseases. Despite reasonable achievements in pharmacological, electrophysiological and surgical treatments, prognosis for chronic HF remains poor. Modern therapies are generally symptom oriented and do not currently address specific intracellular molecular signaling abnormalities. Therefore, new and innovative therapeutic approaches are warranted and, ideally, these could at least complement established therapeutic options if not replace them. Gene therapy has potential to serve in this regard in HF as vectors can be directed toward diseased myocytes and directly target intracellular signaling abnormalities. Within this review, we will dissect the adrenergic system contributing to HF development and progression with special emphasis on G-protein-coupled receptor kinase 2 (GRK2). The levels and activity of GRK2 are increased in HF and we and others have demonstrated that this kinase is a major molecular culprit in HF. We will cover the evidence supporting gene therapy directed against myocardial as well as adrenal GRK2 to improve the function and structure of the failing heart and how these strategies may offer complementary and synergistic effects with the existing HF mainstay therapy of β-adrenergic receptor antagonism.

INTRODUCTION

Heart failure (HF), being a common denominator of acute and chronic heart diseases, still has a poor prognosis despite considerable progress in pharmacological treatment and new interventional and surgical therapeutic options. Accordingly, HF remains one of the leading causes of mortality and is a major financial burden to health-care systems in industrialized countries. Current guidelines recommend therapies for HF that are predominantly based on pharmacotherapy, including β-adrenergic receptor (βAR) blockers, angiotensin-converting enzyme inhibitors, diuretics and cardiac glycosides. These approaches are complemented by electrophysiological (that is, cardiac resynchronization therapy in the presence of interventricular dyssynchrony) and surgical interventions such as left ventricular assist devices or cardiac transplantation. Despite the successes that some of these treatment options have seen and the advanced understanding of molecular mechanisms underlying HF, the true efforts for improving global cardiac function and halting the progression of HF are still limited. The reason for this is that most therapeutic interventions only mitigate symptoms without directly addressing the underlying molecular cause. On the molecular level, HF is characterized by alterations of neurohormonal adrenergic signaling, Ca^{2+} dysregulation, cell death and diastolic dysfunction.

A hallmark abnormality of failing myocardium is the imbalance of the adrenergic system, especially deranged βAR signaling, including alterations of its downstream signaling cascade.[1] This includes the upregulation of the G-protein-coupled receptor (GPCR) kinase 2 (GRK2) that can cause enhanced desensitization of βARs as well as other GPCRs on the sarcolemmal membrane of cardiomyocytes.[1,2] In fact, as outlined below, GRK2 appears to be a nodal mediator of the chronic enhancement of the sympathetic nervous system (SNS) that begins acutely after cardiac injury to stimulate compromised myocardium to increase inotropy and cardiac output.[1–3] Chronic circulating and tissue elevations of the sympathetic catecholamines epinephrine and norepinephrine appear to drive elevations of GRK2 not only in myocardium but also in SNS tissues including the adrenal gland, which results in dysfunctional adrenergic signaling in both organs with the net result being the perpetuation of this vicious cycle that can continue to drive disease progression.[3] As detailed below, at the level of the SNS, enhanced GRK2 activity overly desensitizes adrenal α_2ARs, limiting the negative feedback of catecholamine release while myocardial GRK2 elevations uncouple βARs.[3] Therefore, GRK2 is critical at two different levels of the neurohormonal adrenergic axis promoting this negative cycle.

Because of this evidence for GRK2 being a potential player in HF pathogenesis, attempts have been made over the last two decades to determine if GRK2 is a viable molecular therapeutic target for HF-therapy in both the heart and adrenal gland. Gene therapy approaches for GRK2 inhibition will be the focus of this review. Touching on the role of the SNS in HF, we will define the pathophysiological role of myocardial and adrenal GRK2 in HF and detail the promising gene therapeutic interventions targeting GRK2. This is an exciting time for potential HF gene therapy as it has been gaining momentum in the past couple of years with the first clinical trials being undertaken such as the CUPID (Calcium upregulation by percutaneous administration of gene therapy in cardiac disease) trial, a milestone in HF treatment with overexpression (viral-mediated

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cardiac delivery) of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) aimed at improving sarcoplasmic Ca^{2+} handling,[4] and a trial using adenylyl cyclase type VI overexpression.[5] These trials have certainly paved the way for future HF gene therapeutic interventions. Indeed, we will present below the promising approach of inhibiting GRK2 activity with a peptide inhibitor known as the βARKct (carboxyl-terminus of the β-adrenergic receptor kinase), which has been overwhelmingly positive in preclinical testing. We will present strategies directed against myocardial and adrenal GRK2 as potential effective novel HF therapies, and we will discuss potential complementary effects with pharmacological β-blockade.

NEUROHORMONAL ADRENERGIC IMBALANCES IN HF

HF, characterized by cardiac dysfunction and hemodynamic overload, leads to activation of compensatory neurohormonal signaling that becomes maladaptive chronically.[1] Systemic and local catecholamine levels are increased, initially intended to maintain cardiac function. As introduced above, the chronic activation of the SNS and catecholamine increases is mediated by the upregulation of adrenal GRK2 in HF, which leads to a loss of the negative feedback loop in chromaffin cells through α_{2C}ARs as these receptors that normally inhibit catecholamine secretion become desensitized and downregulated because of heightened GRK2 activity.[6] In HF, circulating catecholamines are related to chronic adverse activation of the adrenergic system and subsequent negative effects in the heart, like activation of reactive oxygen species.[7] Indeed, plasma norepinephrine levels are known to be significantly correlated to HF mortality.[8] In addition to the AR changes seen in the SNS, the density and signaling of ARs in myocardium are significantly altered in HF because of chronic catecholamine exposure and the actions of GRK2.[1–3] In cardiac myocytes, β_1- and β_2-ARs are desensitized and β_1ARs additionally downregulated, leading to an overall loss in contractile stimulatory capacity of compromised myocardium through defective cyclic AMP signaling and Ca^{2+} handling.[9] GRK2 levels and activity are elevated in the failing heart as first reported almost 20 years ago.[10] Its increase in both myocardium and the adrenal gland following rising catecholamine levels act to further depress cardiac function and contribute to further rise in plasma catecholamine levels, fueling this vicious cycle (see Figure 1). Therefore, GRK2 has significant importance in HF signaling as it mediates the adrenergic rush, contributing to HF development and progression at two separate levels: (1) in the adrenal gland catecholamine secretion is increased, contributing to the systemic adrenergic rush (see the section 'GRK2 and the adrenal chromaffin cell'); and (2) in cardiac myocytes, GRK2 uncouples βAR signaling, demanding a further rise in plasma catecholamines thought to maintain contractility (see the section 'GRK2 and the cardiomyocyte'). These circumstances fuel the vicious cycle of neurohormonal adrenergic imbalances in HF and, thus, render myocardial and adrenal GRK2 an excellent target for future HF therapy.

GRK2: A NODAL CULPRIT IN HF

GRK2 and the cardiomyocyte

The cytosolic protein GRK2 is one of the seven members in this family of serine/threonine kinases and is ubiquitously expressed but the most abundant GRK in mammalian heart. It is increasingly clear that GRK2 plays a key regulatory physiological role in the heart.[1–3] The structure of the 78 kDa protein GRK2 comprises a well-conserved, central catalytic domain (~270 aa) flanked by an amino-terminal domain (NT ~ 185 aa) and a variable-length carboxyl-terminal domain (CT ~ 105–230 aa; see Figure 2). The NT of GRK2 is thought to mediate receptor recognition and intracellular membrane anchoring.[11] Furthermore, it harbors the RGS domain (regulator of G-protein signaling homology domain), which is thought to be responsible for the regulation of GPCR signaling independent of phosphorylation.[12,13] The CT region of GRK2 contains a pleckstrin homology domain (PH) that contains sites responsible for binding to the βγ-subunits of dissociation (that is, activated) G proteins ($G_{\beta\gamma}$), which targets this kinase to the membrane to enhance GPCR phosphorylation.[14,15] The PH domain also helps anchor GRK2 to the membrane through binding with PIP2 (phosphatidylinositol 4,5-bisphosphate).[16]

In the heart, fine-tuning of adrenergic signaling by GRK2 is pivotal to maintain adequate cardiac output in the healthy myocardium. However, data have accumulated showing GRK2 to be responsible for the disturbed AR signaling observed in the injured and failing heart.[1–3] The molecular cause of this situation is increased GRK2 activity in the injured cardiac myocyte, which attenuates βAR catecholamine sensitivity by receptor desensitization and downregulation.[10,17] Indeed, it is upregulated in patients with HF and it has also been observed in several animal models.[10,18–21] Moreover, enhanced GRK2 expression has been linked to hypertension,[22] cardiac hypertrophy[23] and post-myocardial infarction (MI).[24] In consideration of the fact that GRK2 levels often increase before manifested clinical HF,[25] and normalize again with advanced β-adrenergic signaling and ventricular function,[26,27] GRK2 not only represents a potential biomarker of cardiac function,[28] but is also an attractive therapeutic target for HF treatment.[29,30]

GRK2 and the adrenal chromaffin cell

Beside its essential role in the desensitization process of myocardial GPCRs such as βARs, GRK2 is involved in the regulation of plasma catecholamine release from the adrenal gland and also by extensions of SNS ganglia. Specifically, the physiological function of adrenal α_{2c}ARs is to inhibit catecholamine release into the synaptic cleft, acting as a negative-feedback loop in this process. Analogous to dysfunctional βAR signaling in the cardiac myocyte, SNS hyperactivity in HF also causes the dysfunction of α_{2c}ARs, and we have shown that this is triggered by adrenal GRK2 upregulation in the adrenal medulla.[19] Enhanced GRK2 activity in the adrenal gland was seen in a mouse model of HF and also in post-MI rats, causing the local desensitization of α_{2c}ARs.[19] As a consequence, the inhibitory effect of α_{2c}AR on catecholamine release is abrogated, with the net effect being chronically elevated epinephrine and norepinephrine plasma levels contributing to the progression of HF. The hypothesis that GRK2 modulates chromaffin catecholamine secretion was further supported by experiments with adenoviral-mediated overexpression of GRK2 in rats as this caused an increase of plasma catecholamine levels.[31] Thus, elevated GRK2 expression levels and activity in the adrenal medulla appear to contribute greatly to plasma catecholamine elevation in HF, which certainly perpetuates the vicious SNS overdrive cycle in HF.[3,32]

PHARMACOLOGICAL VERSUS GENE THERAPY STRATEGIES TARGETING ADRENERGIC SIGNALING IN HF

Ideally, HF therapy would be designed to tackle the root of the underlying molecular signaling alterations and aimed at halting or reversing disease progression. State-of-the-art pharmacological treatment, including β-blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists and diuretics, do not directly address intracellular molecular signaling derangements and are therefore considered symptomatic approaches. This may be attributed to these drugs not being specifically engineered to affect molecular targets in cardiac myocytes. Direct manipulation would be desirable as these intracellular molecular signaling alterations clearly contribute

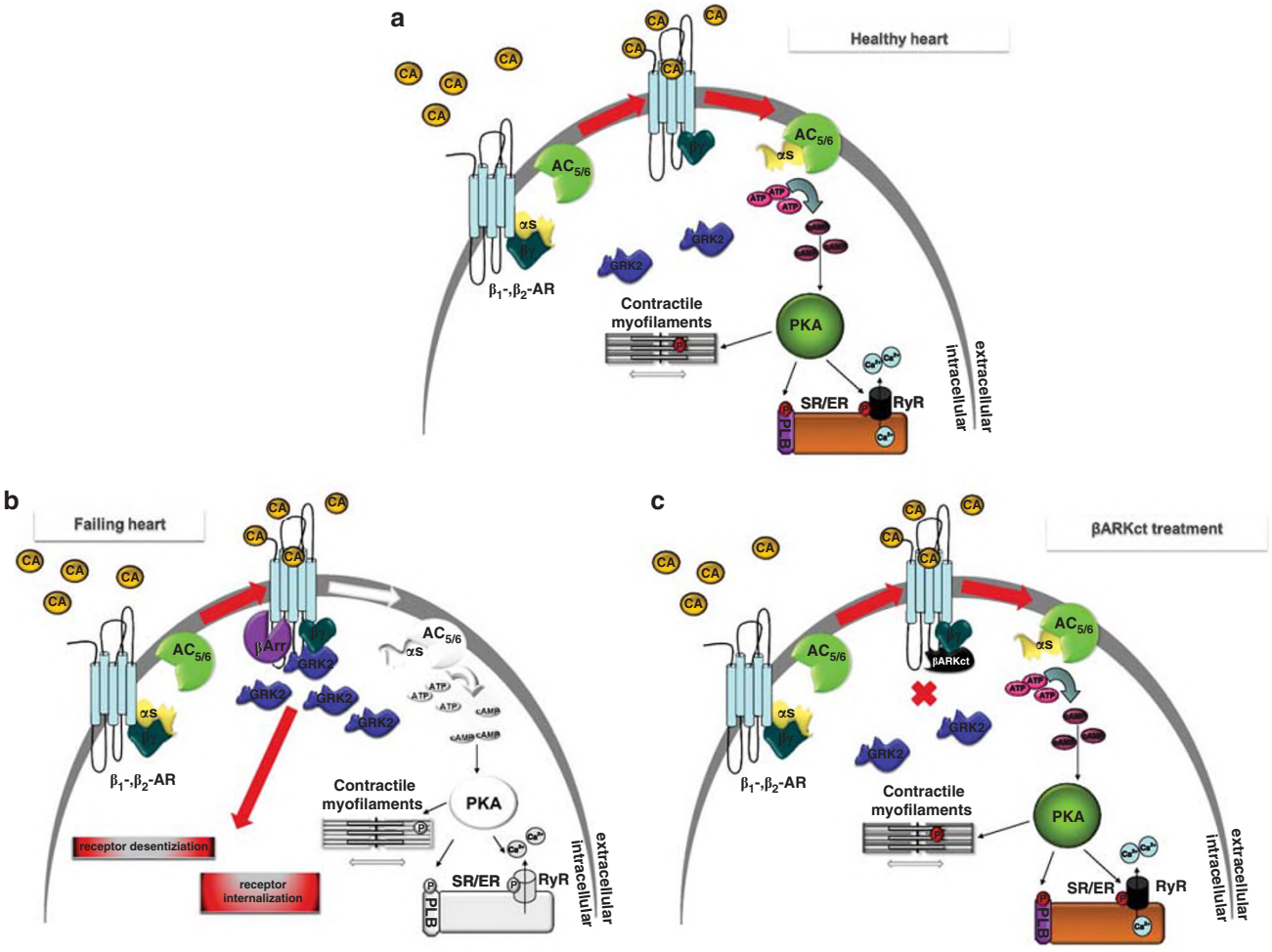

Figure 1 Cardiac βAR signaling and regulation by GRK2. (**a**) β-adrenergic signaling in the healthy heart. Catecholamine binding to sarcolemmal βARs results in the activation of the G protein, Gs, and dissociation of the Gαs subunit. Activated Gαs in turn activates adenylyl cyclises (ACs) in the myocyte, which mediate the conversion of ATP into the second messenger cAMP, which activates protein kinase A (PKA), leading to enhanced contractility through Ca^{2+}. (**b**) Adrenergic signaling in HF. Elevated catecholamine levels induce an initially strong activation of βARs and also induce the upregulation of GRK2, which targets βAR desensitization and a loss of responsiveness. GRK2 targeting to activated βARs is a G-protein-dependent event as sites within the carboxyl-terminus of GRK2 bind dissociated βγ-subunits of G proteins ($G_{\beta\gamma}$). The $G_{\beta\gamma}$-GRK2 targeting and phosphorylation of βARs induces the recruitment of the final piece in desensitization, β-arrestins, which block further G-protein coupling and also mediate receptor internalization. (**c**) Restored adrenergic signaling with βARKct treatment. βARKct is a peptide derived from the last 194 amino acids (carboxyl-terminus) of GRK2 and it blocks $G_{\beta\gamma}$ binding and subsequent GPCR phosphorylating activity of GRK2. Although in the untreated failing heart, GRK2-mediated phosphorylation leads to receptor desensitization and downregulation, βARKct expression interferes with this process and allows for the normalization of the receptor activity and levels and its G-protein coupling. With a more normal responsiveness, it appears that SNS activity is lowered, breaking the vicious adrenergic cycle present in HF.

to cardiac deterioration. However, engineering compounds for specific manipulation of intracellular signaling is chemically challenging. Additionally, the systemic distribution of pharmacological drugs mediates unwanted side effects as they influence global signaling pathways or inhibit membrane-bound receptors in organs other than the heart. Therefore, in addition to standard pharmacological therapy for effects that are currently viewed as effective symptom-wise, there is great potential for novel molecular approaches. The growing dissection of pathobiochemical molecular pathways and closer definition of intracellular culprits involved in HF development and progression and the advanced search for new molecular targets all go hand in hand to promote the development of an effective and specific new therapeutic approach complementing the established ones. Gene therapy constitutes a promising potential novel therapeutic strategy for direct manipulation of intracellular mechanisms in cardiac myocytes.[30]

Concerning gene therapy, significant efforts have been undertaken to develop molecular tools such as mini-peptides/proteins, which can be used to inhibit protein or enzyme function, or RNA interference (small interfering RNA/microRNA) for downregulation of genes. Alternatively, gene transfer can be used to replace or supplement downregulated proteins or enzyme targets. These approaches in combination with modern viral vectors and cardiac-specific promoters are all orchestrated to effectively target intracellular molecular signaling defects and render gene therapy a rather interesting tool in potentially complementing established HF therapies.[30] Of course, cardiac gene therapy is still in its clinical infancy and a lot of effort is put into further optimization, with the ultimate goal of correcting key underlying signaling abnormalities inside the failing cardiac myocyte. Below, we highlight one gene therapeutic strategy, GRK2 inhibition, and how this target may be utilized in established pharmacological approaches (βAR blockade), both aimed at intercepting

the chronic adrenergic vicious cycle in HF. We will dissect the knowledge on β-blockade and shed light on molecular gene therapeutic approaches targeting GRK2 in the heart and adrenal gland; we will explore differences and set accents on potential synergisms.

β-blockers: a solid rock in HF treatment

Blocking excessive catecholamine stimulation of inotropic βARs is the strategy behind pharmacologic β-blockade, which represents the current mainstay of HF therapy.[33] This excessive SNS activity results in sustained βAR activation and consequent desensitization and downregulation. Ventricular remodeling is reinforced by progressive catecholamine-induced myocyte cell death, resulting in cardiac deterioration, and changes in ventricular mass promote ventricular dilation and failure.[33,34] Although β-blockers only indirectly address key molecular signaling alterations within the cardiac myocyte, their clinical use substantially improved HF prognosis, and they are thus considered a solid rock in HF treatment. Interestingly, molecular alterations observed with sustained therapeutic effects of β-blockade in HF include resensitization of βARs, normalization of GRK2 levels and activity and Gα levels. As a consequence, intracellular systems like the cardiac βAR-G-protein(s)-adenylyl cyclase system are enhanced by β-blockers. This is because of the fact that β-blockers cause the upregulation of cardiac βARs (which are downregulated in HF) as well as the normalization of both Gi-protein activity and GRK activity (which are enhanced in HF).[35] Therefore, in situations when catecholamines overcome the β-blocker (that is, exercise), cardiac βAR signaling is improved, which can contribute to less SNS activity, thus breaking the vicious cycle indirectly (see below). Importantly, these molecular effects are associated with improved hemodynamics and overall cardiac performance.[36–40] However, the full extent of whether these molecular changes are the true therapeutic mechanisms of action of βAR antagonists in HF is not fully understood as there are some specific differences in β-blocker classes used (discussed below).[41]

Three different subclasses of β-blockers were defined depending on their properties. First, nonselective β-blockers like propranolol antagonize the catecholamine activation of both β_1AR and β_2AR expressed in the heart.[33] The second type of β-blockers (for example, metroprolol and bisoprolol) have much greater affinity for β_1AR than for β_2AR and are thus considered 'cardioselective'.[33] The third type of β-blockers provide a broader anti-adrenergic effect than type 2 blockers because of the fact that its members, carvedilol and bucindolol, possess vasodilatory properties by blocking vascular αARs as well as the ability to reduce cardiac and systemic adrenergic drive.[33] Several large prospective, randomized, placebo-controlled trials have provided evidence for sustained therapeutic clinical effects of β-blockers.[33] This includes demonstration of improved quality of life and, even more importantly, reduction of cardiovascular mortality and risk of sudden death.

It is important to point out that caution is still needed for the long-term use of β-blockade in HF patients as there is risk associated with their usage. Unwanted systemic side effects include bradycardia (due to depression of the cardiac conduction system), hypotension, dizziness, fatigue and aggravation of depression.[42] Furthermore, dose is a critical factor for therapeutic success and has to be titrated individually for each HF patient. Finally, not all HF patients are suited for β-blocker treatment, opening a demand for new therapeutic approaches. Thus, a powerful complementing approach would potentially enhance and sustain the therapeutic effects and potentially allow dose reduction, minimizing unfavorable effects. As pointed out below, GRK2 inhibition appears to be such a strategy and is in line with providing complementation to β-blockade.

Myocardial GRK2 as a HF gene therapy target

The central role of GRK2 in the vicious cycle of catecholaminergic and receptor desensitization/downregulation was extensively discussed above. Several reports have been published over the past two decades in several species showing that GRK2 inhibition can prevent and/or reverse HF development. This approach has been primarily done with the βARKct as a peptide inhibitor of GRK2.[42–48] A recent report has also shown similar effects with a small molecular inhibitor of GRK2 that, interestingly, appears to act as the βARKct in targeting $G_{\beta\gamma}$ binding.[45] The βARKct was designed by us in the 1990s for targeted inhibition of the GRK2–Gβγ interaction.[14] The molecular mechanism of action of the βARKct is competitive inhibition of the GRK2–GPCR (notably, βARs) interaction. In numerous HF animal models, the cardioprotective potential and HF reversal properties of βARKct has been assessed. In various genetic mouse models of HF, transgenic expression of the βARKct has been used to prevent HF development.[43,49,50] In addition, viral delivery of βARKct (adenovirus and adeno-associated virus) has demonstrated successful inhibition of GRK2 activity connected with decreased βAR desensitization without disrupting normal signaling and leading to HF prevention or reversal in rats and rabbit models of HF.[40,44,45] Taking advantage of genetic HF models, Blaxall *et al.*[48] demonstrated that the therapeutic βARKct effect is accompanied by normalization of cardiac gene expression.[48] Importantly, Williams *et al.*[51] could demonstrate improved single myocyte contractility on isolated failing human cardiomyocytes following adenovirus-mediated gene transfer of βARKct.

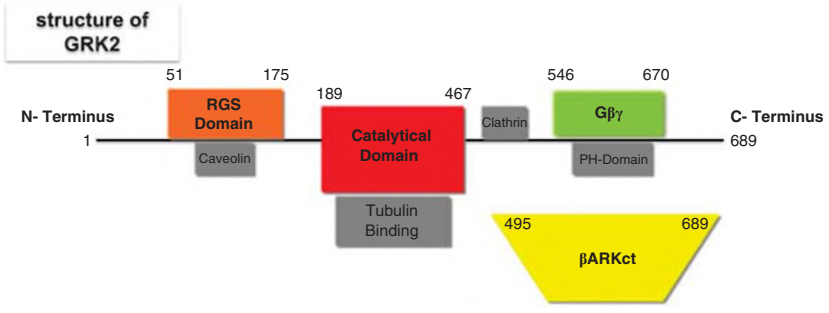

Figure 2 GRK2 and its functional domains. Depicted are the GRK2 domains involved in its protein–protein interactions and domains implicated to have regulatory roles for GRK2 activity against GPCRs and signaling. Concerning the subject of gene therapy in this review, the critical domain is the carboxyl-terminus, which contains the $G_{\beta\gamma}$ binding and PH domain for GRK2 membrane targeting. The βARKct is used as an inhibitor of GRK2 activation by competing for $G_{\beta\gamma}$ binding.

Although the inhibition of myocardial GRK2 via cardiac βARKct expression in both genetic mouse models of HF and by virus-mediated myocardial gene transfer has proven to be beneficial, the above studies have not involved animals larger than rabbits. We have recently completed a study in a preclinical large animal model (farm pigs) aimed at investigating whether GRK2 inhibition with the βARKct represents a feasible therapeutic approach for HF.[46] In this study, taking advantage of our established ischemic cardiomyopathy pig model,[52] we found that AAV6-βARKct delivery via retrograde coronary venous perfusion could improve left ventricular dysfunction, and this therapeutic effect was accompanied by normalization of neurohormonal signaling and repression of adverse cardiac remodeling and fetal gene expression.[46] This study is important as the pig HF model more closely reflects human pathophysiology and is thus a prerequisite for planning clinical testing (Figure 3).

Importantly, the specific targeting of GRK2 by these molecular approaches has been confirmed as the primary mechanism, as recent studies by us in cardiac-specific GRK2 knockout mice show that HF is prevented following MI.[53] Moreover, when GRK2 knockout is induced in the hearts of mice after HF is present, we have found an active reversal of left ventricular dysfunction including reverse remodeling.[53] These results clearly indicate that lowering GRK2 in the heart is therapeutic in HF. Taken together, all data gathered regarding myocardial GRK2 inhibition show that this is a valid and promising novel molecular approach for treating HF with sustained therapeutic effects. Most studies—including studies with βARKct expression in HF pigs—have shown a reversal of βAR dysfunction including receptor upregulation and a normalization of signaling; however, no doubt, there are effects of the βARKct that go beyond resensitization of cardiac βARs and these effects are currently being explored by us and others.[54,55]

Adrenal GRK2 as a novel HF gene therapy target

As discussed above, HF is associated with enhanced catecholamine secretion from the adrenal glands because of α_{2c}AR desensitization by GRK2. This central role in the critical elevation of plasma catecholamine levels contributing to HF development and progression renders adrenal GRK2 an interesting target for HF treatment. The question therefore is whether targeted GRK2 inhibition in the adrenal gland might reduce sympathetic activation and prevent HF. In this regard, adrenal-restricted GRK2 knockout mice presented with a significant reduction in catecholamine levels and improvement of cardiac performance in post-MI HF.[20] Methods to direct viruses to the adrenal medulla have also been developed to attempt βARKct-mediated gene therapy in the adrenal gland to inhibit GRK2-mediated α_{2c}AR desensitization.[55] Importantly, in an initial study directed toward a true gene therapeutic approach, adenoviral-mediated βARKct gene transfer to the adrenal gland of a genetic line of HF mice returned increased catecholamine release back to normal levels, which was accompanied by improved cardiac performance and βAR signaling.[19] Moreover, a rat MI model with adenovirus-mediated expression of βARKct in the adrenal medulla showed decreased plasma catecholamine levels positively influencing hemodynamic parameters and ejection fraction.[19] These are promising data underlining the potential benefits of targeting adrenal GRK2 by gene therapy and with it the sympathetic adrenergic drive involved in HF development and progression. However, the studies published to date have only investigated rodent HF models, and clinically relevant large animal models are certainly warranted in order to further evaluate the true potential of adrenal GRK2 inhibition via gene therapy.

Potential synergism of cardiac and adrenal βARKct gene therapy

As described above, GRK2 appears to be a key culprit in HF pathogenesis within two organs that work together to pathologically stress the heart. GRK2 inhibition in cardiac muscle and adrenal gland influences cardiac performances on two different levels. Whereas myocardial GRK2 inhibition improves hemodynamic parameters and cardiac performance by influencing intracellular signaling pathways, adrenal GRK2 inhibition normalizes systemic catecholaminergic

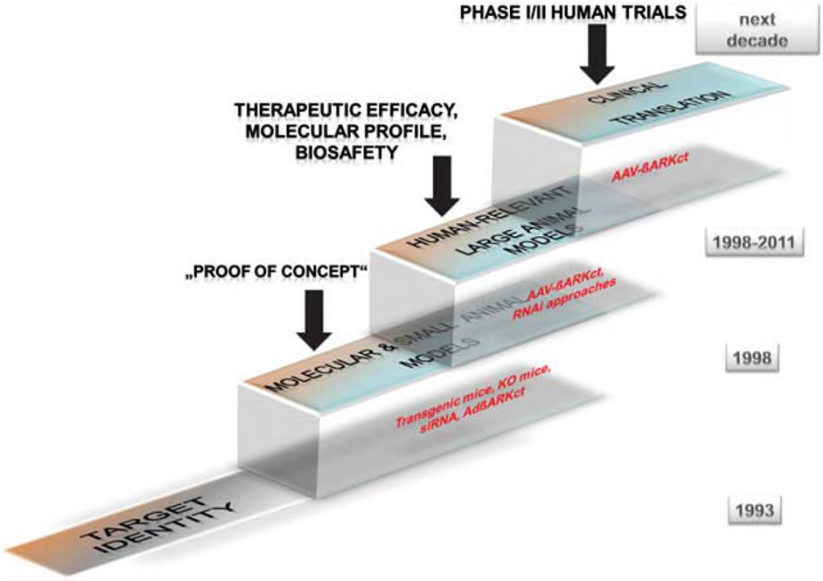

Figure 3 Path to translation of GRK2 targeted gene therapy inhibition. The different consecutive steps following target identification are depicted. The first 'proof of concept' studies mainly focus on *in vitro* and small animal models, paving the way for clinically relevant large animal models with the aim of proving therapeutic efficacy and assessing the molecular profile and biosafety. The last translational step brings the most successful approach to the clinic, initiating phase I/II clinical trials. For each step, the relevant models and therapeutic approaches regarding GRK2-aimed gene therapy are presented, as well as an approximate timeline of translation, which can now begin as we move forward in this decade.

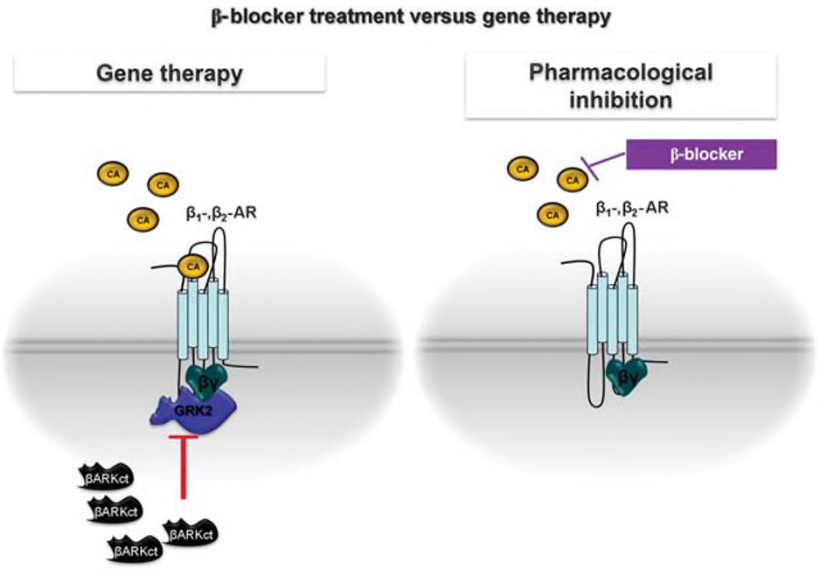

Figure 4 Schematic comparison of β-blocker treatment with βARKct-mediated gene therapy. Pharmacological manipulation of adrenergic receptor signaling with β-blockers approaches the deranged βAR system from the outside. This makes β-blocker treatment somewhat unspecific as general agonist inhibition occurs, which is not restricted to the target cell (the cardiomyocyte). In contrast, gene therapy allows for specific targeting of the cells involved (by use of a cardiac-restricted promoter after myocardial delivery). Intracellular pathways are directly manipulated and instead of general inhibition of the adrenergic receptor, it is possible to target certain/specific downstream signaling pathways. Shown above is a specific example using the βARKct as a GRK2 inhibitor against βAR desensitization; however, GRK2 also targets other proteins in myocytes that are inactivated by βARKct delivery.

overdrive. Both are well-established effects and with these two levels of action it can be assumed that the concomitant treatment with βARKct in the adrenal gland with βARKct treatment in the myocardium will have synergistic effects to correct the cardiotoxic pharmacodynamic and bioenergetic imbalances resulting from SNS hyperactivity and leading to myocardial failure. The prospects for this therapeutic strategy are promising; however, tests in preclinical models are warranted. This could also represent an advantage of any developed small-molecule inhibitor of GRK2 as it would presumably inhibit cardiac and adrenal GRK2 and this would lead to improved cardiac myocyte contractile function and direct sympatholytic effects.

Table 1 Advantages and disadvantages of βARKct versus β-blocker therapy

	Advantages	*Disadvantages*
β-blocker	Prevention and reversal of HF Improved hemodynamic parameters and cardiac performance Resensitization of βARs Normalization of GRK2 levels and activity Normalization of Gα levels	Short-term therapeutic benefits Personalized dosage needed Unwanted side effects Unspecific blocking of β-adrenergic receptor signaling pathway Vasoconstrictive influence by blockade of β_2-adrenoceptors
βARKct	Long-term therapeutic benefits Prevention and reversal of HF Improved hemodynamic parameters and cardiac performance Normalization of neurohormonal signaling Normalization of catecholamine levels Resensitization of βARs Cardiac-specific targeting of GRK2	No exit strategy so far Unspecific blocking of βγ subunit-mediated signaling (side effects)

Abbreviations: βAR, β-adrenergic receptor; βARKct, carboxyl-terminus of the β-adrenergic receptor kinase; GRK2, G-protein-coupled receptor kinase 2; HF, heart failure.
β-blockers are able to reduce sympathetic influences that normally stimulate chronotropy (heart rate), inotropy (contractility), dromotropy (electrical conduction) and lusitropy (relaxation).

βAR blockade and gene therapy targeting myocardial GRK2: potential synergisms

As shown in Figure 4, βARKct expression and subsequent GRK2 activity and βAR blockade do differ in how they target cardiac adrenergic signaling. Moreover, Table 1 lists potential advantages and disadvantages of βARKct targeting and β-blocker use. Interestingly however, the synergistic effects of βARKct expression and GRK2 inhibition with β-blocker usage in HF models have already been shown. First, in a mouse model of aggressive dilated cardiomyopathy due to cardiac-specific calsequestrin overexpression, not only did βARKct expression in the heart rescue the phenotype including a significant increase in lifespan, but also concurrent treatment with metoprolol revealed impressive synergistic effects on mortality and cardiac function.[50] More recently, a study in post-MI HF in rats showed considerable synergy between βARKct expression after AAV6 gene delivery to the heart and metoprolol.[40] Therefore, molecular GRK2 inhibition appears to complement the primary current pharmacological strategy aimed at normalization of βAR signaling. βARKct and β-blockade acting synergistically appears to be a paradox as βARKct and GRK2 inhibition would act to resensitize cardiac βAR whereas βAR antagonism blocks these receptors. Even if they become resensitized, that would abolish a βARKct effect. However, there are a couple of things to consider that make these two strategies actually synergistic. First, long-term βAR antagonism can promote receptor upregulation and GRK2 downregulation, which on molecular terms is the same response seen with GRK2 inhibition. Moreover, improving βAR signaling in response to an agonist challenge is

also a natural consequence of clinical βAR blocker use,[55] and this is also seen with βARKct expression. Furthermore, in studies with βARKct in rat and pig models of HF,[40,46] chronic βARKct expression in failing myocardium resulted in lower circulating catecholamine levels, showing that when βARs are normalized there is feedback to the SNS to lower its stimulation. This is also a property of β-blockers.[56] Therefore, these two strategies are more similar than different, and using them together apparently can improve HF rescue even more than each used alone, and GRK2 inhibition would also theoretically allow lower βAR antagonist dosages to see the same effect. This will have to be studied in future clinical trials.

Importantly, the data published to date on synergy between β-blockers and GRK2 inhibition also point toward the βARKct having crucial beneficial effects beyond cardiac βAR resensitization, which is no doubt the case and argue again in favor of using the two strategies together. One possibility is that as $G_{\beta\gamma}$ can also bind phosphatidylinositol 3-kinase, this targeted disruption by βARKct can add to the therapeutic effect, which has been supported by *in vivo* animal data.[57,58] Insight into this matter has been gained by comparing βARKct with truncated phosducin, which is another $G_{\beta\gamma}$-binding protein.[59] It is noteworthy that cytosolic cardiac phosducin exhibits a function similar to βARKct, and inhibits $G_{\beta\gamma}$ signaling after membrane recruitment; however, use of an amino terminal deletion mutant of phosducin shows that this protein can improve the contractility of myocytes without having an effect on βARs.[59] This observation suggests that the contractile effects of βARKct might be because of inhibition of additional $G_{\beta\gamma}$ pathways other than a βAR-mediated effect, although this has yet to be proven experimentally. In addition, Völkers *et al.*[54] recently demonstrated that a part of the positive inotropic effects of βARKct is attributable to the disruption of $G_{\beta\gamma}$-mediated inhibition of cardiac L-type Ca^{2+} channels, as βARKct expression in myocytes significantly improved Ca^{2+} cycling via augmentation of the L-type Ca^{2+} channel current. Another βAR-independent effect of the βARKct in the heart appears to be reversal of GRK2-mediated insulin resistance in myocytes,[55] which definitely needs to be explored as contributing to the therapeutic effects of GRK2 inhibition in HF. Overall, these effects of βARKct expression need to be further explored to understand the apparent synergy between GRK2 inhibition and βAR blockade in HF in order to delineate future clinical applications.

CONCLUSIONS

Modern HF therapies are symptom oriented, not directly addressing molecular signaling abnormalities. Dissection of pathways has improved our understanding of molecular signaling cascades like dysfunctional adrenergic signaling, which contributes to HF development and progression. GRK2 is a nodal mediator of the vicious cycle of adrenergic nemesis by enhancing catecholamine release from the adrenal medulla by desensitizing chromaffin α_{2c}ARs and by promoting cardiac dysfunction by desensitizing cardiac βARs. Therefore, GRK2 in the chromaffin cells of the adrenal medulla and in the cardiac myocyte are both potential targets for novel therapeutic interventions in HF. A gene therapy approach taking advantage of βARKct—disrupting the $G_{\beta\gamma}$–GRK2 axis—has revealed impressive therapeutic effects in various HF models after cardiac or adrenal delivery. With novel data describing βAR-independent effects of cardiac βARKct expression, potential complementary effects with pharmacological β-blockade might render gene therapy targeting GRK2 an attractive addendum to established HF therapeutic approaches.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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