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An Assessment of the Therapeutic Landscape for the Treatment of Heart Disease in the RASopathies

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Abstract

The RAS/mitogen-activated protein kinase (MAPK) pathway controls a plethora of developmental and post-developmental processes. It is now clear that mutations in the RAS-MAPK pathway cause developmental diseases collectively referred to as the RASopathies. The RASopathies include Noonan syndrome, Noonan syndrome with multiple lentigines, cardiofaciocutaneous syndrome, neurofibromatosis type 1, and Costello syndrome. RASopathy patients exhibit a wide spectrum of congenital heart defects (CHD), such as valvular abnormalities and hypertrophic cardiomyopathy (HCM). Since the cardiovascular defects are the most serious and recurrent cause of mortality in RASopathy patients, it is critical to understand the pathological signaling mechanisms that drive the disease. Therapies for the treatment of HCM and other RASopathy-associated comorbidities have yet to be fully realized. Recent developments have shown promise for the use of repurposed antineoplastic drugs that target the RAS-MAPK pathway for the treatment of RASopathy-associated HCM. However, given the impact of the RAS-MAPK pathway in post-developmental physiology, establishing safety and evaluating risk when treating children will be paramount. As such insight provided by preclinical and clinical information will be critical. This review will highlight the cardiovascular manifestations caused by the RASopathies and will discuss the emerging therapies for treatment.

Keywords

Noonan syndrome; Congenital heart disease; RASopathies; RAS-MAPK signaling

Introduction

The RAS/mitogen-activated protein kinase (MAPK) pathway is an evolutionarily conserved signaling cascade that links extracellular stimuli to intracellular signaling responses [1–5]. The RAS/MAPK pathway regulates a wide range of fundamental cellular events, such as growth, differentiation, survival, and senescence [6, 7]. The RAS/MAPK pathway plays

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a primary role in the development of hypertrophic cardiomyopathy (HCM) (Fig. 1) [8–11]. HCM is a pathophysiological process that is driven by extracellular stimuli, such as biomechanical stress and neurohumoral factors that remodel myocardial structures leading to cardiac hypertrophy [12–15]. Hypertrophic stimuli are sensed by cardiomyocytes through a variety of cell membrane receptors, including integrins for mechanical sensing, as well as G-protein-coupled receptors (GPCR) and receptor tyrosine kinases (RTKs) [16, 17]. The RAS/MAPK pathway serves to integrate signals from a variety of extracellular sources that are involved in cardiac hypertrophy. The activation of the RAS/MAPK pathway modulates the levels and activities of cardiac-specific transcription factors involved in cardiomyocyte growth, such as GATA binding protein 4 (GATA4), nuclear factor of activated T cells (NFAT), and myocyte enhancer factor 2 (MEF2), leading to re-expression of fetal cardiac genes, including increases of β-myosin heavy chain (β-MHC) and atrial natriuretic factor (ANF) expression and a decrease in the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) expression [9, 11, 18]. Several mouse models have been generated to explore the RAS/MAPK cascade in HCM. The inhibition of MAPK kinases (MEK1/2), dominant-negative RAF1 mutations, and knockouts of RAF1/ERK1/2 have been shown to attenuate HCM responses [19–23]. Collectively, these findings demonstrate that the RAS/MAPK pathway plays a causal role in HCM progression.

Abnormal activation of the RAS/MAPK pathway has been implicated in the pathogenesis of various human diseases, including cancer and neurodegenerative diseases [24–27]. Germline mutations that hyperactivate RAS/MAPK signaling have been identified and linked to a class of developmental diseases that are known as the RASopathies [28–30]. The RASopathies include Noonan syndrome (NS, Online Mendelian Inheritance of Man (OMIM)# 163,950) and Noonan syndrome with multiple lentigines (NSML, OMIM# 151,100), neurofibromatosis type 1 (NF1, OMIM# 162,200), Costello syndrome (CS, OMIM# 218,040), and cardiofaciocutaneous syndrome (CFCS, OMIM# 115,150) [28, 29]. Although each condition exhibits a unique phenotype, patients with distinct mutations in components of the RAS/MAPK pathway present with substantial overlapping clinical features, such as craniofacial dysmorphology, cardiac malformations, cutaneous-musculoskeletal abnormalities, neurocognitive inability, and increased risk of cancer [29, 31].

Much research has been conducted towards understanding the mechanisms of how germline mutations in the RAS/MAPK pathway contribute to the pathophysiological manifestation of the RASopathies. To date, induced-pluripotent stem cells and animal models for the RASopathies have been used to define these mechanisms [32, 33]. The inherent complexities of the RASopathies limit the utility of these models, particularly in their translation to potential therapeutic strategies. However, certain RASopathy-associated conditions such as HCM can be studied in these model systems as they provide a valuable tool in which to establish mechanistic causality. Additionally, since RASopathy-associated cancer and HCM bear the most impactful consequences on patient mortality, addressing the validity of models to inform therapy is pressing. The goal of this review will be to focus on the cardiac abnormalities in animal models that represent RASopathy-associated HCM and how these models can be leveraged to provide information towards potential therapies.

Cardiac Abnormalities in RASopathies

Congenital heart disease (CHD) is the most frequently diagnosed congenital disorder in newborns, afflicting about 1% of live births [34, 35]. CHD is defined as a structural abnormality that arises from improper formation of the heart and/or major blood vessels [36]. Several factors, such as chromosomal abnormality, genetic syndrome, and environmental factors, are known to be associated with CHD [37]. The RASopathies represent a striking example of CHD's that emanate from genetic anomalies [28]. The most frequent RASopathy-associated CHD conditions are pulmonary valve stenosis (PVS), atrial septal defect (ASD), ventricular septal defect, atrioventricular canal defect, left ventricular outflow tract obstruction (LVOTO), patent ductus arteriosus (PDA), and Tetralogy of Fallot [38–40]. HCM is often one of the major clinical features amongst the cardiovascular defects observed in RASopathy patients [41]. The manifestation of HCM in RASopathies is considered as a secondary category, distinct from that of CHD, in accordance with the American Heart Association classification system [42]. Thus, causative mechanisms that give rise to CHD may be distinct from those associated with the post-developmental mechanisms that promote HCM. This nuance is of particular importance when considering strategies to treat HCM. Thus, understanding the nature of the RASopathy-associated signaling pathways that drive post-developmental HCM will be essential for identifying new opportunities for the treatment of this life-threatening manifestation of cardiovascular disease.

Noonan Syndrome

Noonan syndrome (NS) is one of the most common autosomal dominant developmental disorders with an estimated prevalence of 1 in 1000–2500 [43]. NS patients are characterized by short stature, facial dysmorphism, skeletal abnormality, CHD, bleeding diathesis, lymphatic conditions, and intellectual disability [44]. The cause of NS has been attributed to numerous gene mutations involved in the activation of the RAS-MAPK pathway including *PTPN11*, *SOS1*, *KRAS*, *NRAS*, *RAF1*, *BRAF*, *RIT1*, and *LZTR1* [45–54]. Approximately, 50% of NS patients have been linked to mutations in the *PTPN11* gene, which encodes the SH2 domain-containing protein tyrosine phosphatase 2 (SHP2) [45, 55]. SHP2 comprises of two SH2 domains, a PTP domain and a C-terminal tail [56, 57], and is required for the propagation of the RAS-MAPK pathway [58–61]. The SH2 domains serve to direct protein–protein interactions with its upstream phosphotyrosyl targets, and the phosphatase domain catalyzes substrate-specific dephosphorylation [57]. Interestingly, NS-associated *PTPN11* missense mutations tend to cluster around the interface between the N-terminus SH2 domain and the protein tyrosine phosphatase domain [45]. A combination of genetic, biochemical, and structural studies indicates that NS-associated *PTPN11* mutations disrupt the inactive "closed" conformation resulting in an "open" conformation that acquires increased catalytic activity and SH2 domain binding affinity [45]. Other genes in the RAS-MAPK pathway cause NS with reduced prevalence as compared with mutations in the *PTPN11* gene. These genes include mutations in *SOS1* (~ 20%), *KRAS* (~ 2%), *NRAS* (~ 1%), *RIT1* (~ 8%), *RAF1* (~ 8%), *BRAF* (1 ~ 2%), and *LZTR1* (~ 8%) [52, 62, 63] (Fig. 1). Up to 80% of NS patients have some form of CHD, including pulmonary valve stenosis (PVS) (50 ~ 60%) and atrial septal defect (ASD) (6 ~ 10%). However, other cardiovascular

defects, such as ventricular septal defect, atrioventricular canal defect, and aortic coarctation, occur less frequently, and presentation of HCM can occur in up to 20% of cases [39, 41, 62, 64]. Genotype-cardiac phenotype association analyses reveal that distinct genetic mutations lead to different manifestations of CHD in NS [62]. While NS-associated *PTPN11* and *SOS1* mutations are predominately associated with pulmonic valve stenosis and atrial septal defects [65, 66], other NS-specific genes, such as *RAF1* and *RIT1*, are linked to HCM [49, 67].

Noonan Syndrome with Multiple Lentigines (NSML)

Noonan syndrome with multiple lentigines (NSML) is a very rare RASopathy that shares many clinical features with NS [68, 69]. NSML is an autosomal dominant genetic disorder and affects males and females in equal numbers [70]. NSML is characterized by the presence of multiple lentigines or café-au-lait spots, electrocardiographic abnormalities, ocular hypertelorism, congenital heart defects, cryptorchidism, short stature, pulmonary stenosis, and deafness [69, 71]. Up to 85% of NSML cases exhibit mutations in the *PTPN11* gene [72, 73]. Other genes in the RAS-MAPK pathway, such as *RAF1*, *BRAF*, and *MAP2K1*, have also been identified to be mutated in NSML patients; however, the prevalence of mutations in these genes is unknown [49, 51, 74, 75]. The mutations identified in the NSML-associated *PTPN11* gene cluster in the protein tyrosine phosphatase domain result in impaired SHP2 catalysis and an open conformation [45, 76]. It has been proposed that the “open” conformation of NSML-associated *PTPN11* mutations contributes to propagating aberrant NSML signaling in a manner that is largely phosphatase activity independent [77, 78]. However, it has yet to be formally excluded that the residual phosphatase activity in NSML-associated SHP2 mutants contributes to disease-associated signaling. Up to 80% of NSML patients display cardiac defects, and HCM is frequently observed in up to 70% of NSML cases [79]. HCM is the main life-threatening problem in NSML patients, and rapid progression in the severity of HCM in early infancy has been reported [80, 81]. Electrocardiographic anomalies, such as Q wave, prolonged QT interval, and repolarization abnormalities can be associated with HCM in NSML patients [68]. Pulmonary valve stenosis (PVS) and left ventricular outflow tract obstruction (LVOTO) are also observed in NSML patients [68, 79].

Costello Syndrome (CS)

Costello syndrome (CS) is a very rare disorder (1:300,000 to 1:1,250,000) that is caused by germline *HRAS* gene mutations (Fig. 2). CS patients present with craniofacial abnormalities, growth retardation, skin abnormalities, cardiac defects, and cancer predisposition [29, 82]. The nucleotide changes of glycine at codon 12 to serine or alanine, which impair GTP hydrolysis, result in H-RAS remaining in the active state, as observed in most of the CS patients [83]. Diverse cardiac abnormalities including HCM, valve anomalies, septal defects, and arrhythmias have been reported in CS patients [83–85]. HCM is particularly high in CS with reports of upwards of 65% [86]. The progression of HCM is variable where in the most severe cases surgical intervention through septal myectomy is conducted.

Cardiofaciocutaneous Syndrome (CFCS)

Cardiofaciocutaneous syndrome (CFCS) is a rare RASopathy that has many overlapping clinical features with CS, such as short stature, CHD, and facial dysmorphism, but very distinct in presentation because of the profound skin and hair anomalies [29, 87, 88]. Heterozygous mutations of four genes in the RAS-MAPK pathway are associated with CFCS, *BRAF* (~ 75%), *MAP2K1/MAP2K2* (~ 25%), and *KRAS* [89, 90] (Fig. 2). Cardiac defects in CFCS are similar (~ 75%) in prevalence to those of Noonan syndrome (NS) and CS with pulmonic valve stenosis predominating in presentation [29, 41]. HCM is present in ~ 40% of patients with CFCS; once again as seen with other RASopathies, the severity of heart disease varies.

Neurofibromatosis Type 1 (NF1)

Neurofibromatosis type 1 (NF1) is an autosomal dominant condition with an incidence of 1 in 2000 to 4000 [91, 92]. NF1 patients are diagnosed by clinical features, such as café-au-lait spots, intertriginous freckling, iris Lisch nodules, neurofibromas, optic pathway gliomas, and distinctive bony lesions [93]. NF1 is caused by a nonfunctional mutation of the *NF1* gene, which encodes the neurofibromin (NF) protein [94–96] which is a GTPase-activating protein (GAP) that facilitates GTP hydrolysis [97]. Loss-of-function mutations of *NF1* result in an increase of the active GTP-bound Ras and subsequent hyperactivation of downstream signaling pathways, including the MAPK and mammalian target of rapamycin (mTOR) pathways [98, 99] (Fig. 2). NF1 patients develop cardiac and vascular diseases, vasculopathy, and hypertension [100]. It was reported that pulmonary stenosis and atrial septal defects were found in 0.4 ~ 6.4% of NF1 cases [101]. HCM is not a prominent feature in NF1, and it has been reported only in an extremely small percentage of NF1 patients; indeed, in a retrospective study of over 2000 NF1 patients, Lin et al. did not find a single case of HCM [102].

Mouse Models and Clinical Trials for the Treatment of RASopathies-Associated Cardiac Disorders

As highlighted, the RASopathies give rise to complex clinical presentations from growth retardation, cancer predisposition, and neurodevelopmental disabilities to cardiovascular disease. The most severe clinical presentation of the RASopathies are those of the cardiovascular system. Amongst the cardiovascular disorders with mutations in non-sarcomeric proteins, HCM in the RASopathies presents with different manifestation as compared with other types of HCM caused by mutations in sarcomeric proteins [42]. Notably, RASopathy-associated HCM presents much earlier in life and often before other pediatric sarcomeric forms of HCM. As reported by Wilkinson et al., NS children who present with HCM and heart failure before 6 months of age have an extremely poor prognosis [103]. Therefore, in these most severe cases, early treatment will be most desirable. In order to begin evaluating the efficacy of potential treatments, primarily mouse models of the RASopathy diseases have been utilized (Table 1) [33]. Additionally, in some cases, experimental trials in children have been performed under certain exceptional circumstances. The following sections will highlight studies in preclinical animal models

and in human trials to evaluate the effectiveness of small molecules for the treatment of RASopathy-associated HCM.

RAS-MAPK Pathway Inhibitors

The RAS-MAPK pathway is an established target for the treatment of a variety of different cancers [104–106]. A number of small molecules inhibiting the RAS-MAPK pathway have been developed and evaluated for cancer therapy [105]. Therefore, RAS-MAPK inhibitors if successfully repurposed could provide benefit for the treatment of HCM caused by certain types of RASopathies. The most effective approach to determine the efficacy of small molecule inhibitors for the treatment of HCM associated with the RASopathies has been the use of genetically engineered mouse models. Animal models that have been generated to study the RASopathies have elegantly employed knockin strategies to introduce the mutant RASopathy allele into mice. A knock in of the NS-associated Son-of-Sevenless homolog 1 (*Sos1*) E846K ($Sos1^{E846K}$) mutation into mice recapitulated several major phenotypes of NS, including cardiac defects [107]. Prenatal or postnatal treatment of the MEK inhibitor, PD0325901, successfully rescued embryonic lethality, growth, and cardiac defects in NS-associated $Sos1^{E846K}$ mice [107] (Fig. 2). The $Kras^{V14I}$ NS-associated mutant mouse model also exhibited NS-like phenotypes that included growth retardation and HCM [108]. Interestingly, although prenatal treatment of the NS-associated $Kras^{V14I}$ mutant mice with PD0325901 rescued HCM, when administered postnatally, HCM was not rescued [108]. These observations suggest that the MEK-ERK axis in context of the $Kras^{V14I}$ NS-associated mutant might engage distinct pathways postnatally for the progression of HCM. Given the perils of treating pregnant mothers with MEK inhibitors, the failure of PD0325901 to reverse HCM postnatally is disappointing, and it dramatically limits options for the treatment of HCM for the $Kras^{V14I}$ NS-associated mutant. A cautionary note is raised here as it relates to the pathways engaged during development that cause HCM and those that may be engaged postnatally that are involved in either maintaining and/or driving the progression of the disease.

The use of the MEK inhibitor in reversing HCM in the mouse model of NS represented by a knockin mutation of the $Raf1^{L613V}$ allele has been demonstrated [109]. The activating mutant of *Raf1*, L613V, when knocked into mice causes short stature, facial dysmorphia, and HCM [109]. Postnatal treatment of the NS-associated $Raf1^{L613V}$ mutant mice with the MEK inhibitor completely rescued HCM with accompanying improvement in cardiac functionality [109]. Collectively, these findings from genetically engineered mouse models of NS illustrate the potential utility of the MEK inhibitor in treating HCM and possibly other RASopathy-related manifestations that lead to enhanced ERK activation. In support of this, a study using trametinib, a highly selective reversible allosteric inhibitor of MEK1/2 activity, was found in an extended use trial to reverse progressive HCM in an NS infant harboring either RIT^{S35T} or RIT^{F82L} mutation [110]. Upon trametinib treatment, an amelioration of the increased ventricular mass was observed, accompanied by reduced outflow tract obstruction and improved parameters of heart failure [110] (Fig. 2). These very promising studies give hope to the use of MEK inhibitors for the treatment of RASopathies that are driven by gain-of-function properties of the RAS-MAPK pathway. However, it is also evident that certain RASopathy mutants will not be responsive to MEK inhibition, and a full understanding

of the genotype-to-MEK-responsive signaling relationship needs to be understood utilizing these mouse models in more extensive preclinical testing.

Phosphatidylinositol 3′-Kinase-AKT Pathway Inhibition

It is now well established that the phosphatidylinositol 3′-kinase (PI3K)-AKT pathway positively regulates cardiac tissue mass [111, 112]. AKT activation through the mammalian target of rapamycin (mTOR) increases protein synthesis and prevents muscle atrophy and apoptosis, resulting in cardiomyocyte hypertrophy [113–115]. It suggests that the AKT/mTOR pathway is a therapeutic target for the treatment of HCM in certain RASopathies in which AKT is hyperactivated. The increased AKT activity in the heart of NSML-associated $Pptn11^{Y279C/+}$ mice prompted investigations into whether attenuating AKT activation could reverse HCM [116]. Indeed, pharmacological studies showed that postnatal treatment of $Pptn11^{Y279C/+}$ mice with rapamycin, a mTOR inhibitor, prevented the development of HCM and associated increase in cardiomyocyte hypertrophy [116] (Fig. 2). Similarly, direct AKT blockade using ARQ092, an allosteric AKT inhibitor, demonstrated that intraperitoneal injection of this drug postnatally into NSML-associated $Pptn11^{Y279C/+}$ mice also reversed HCM [117] (Fig. 2). In another example of an extended use case, one patient with a $PTPN11^{Q510E}$ mutation that represents a NSML RASopathy who exhibited severe HCM was treated with a mTOR inhibitor, everolimus [81] (Fig. 2). It was found that everolimus treatment improved heart failure and decreased the levels of the HCM marker, brain natriuretic peptide. Unlike in the $Ptpn11^{Y279C/+}$ mice, everolimus treatment in this patient did not reverse the HCM, which could imply that earlier intervention prior to irreversible cardiac remodeling would have been more effective. Nevertheless, these data, albeit in a single patient, are encouraging and provide support for more extensive clinical trials to be conducted where both timing and dosing of the interventional therapy can be evaluated.

Src Family Tyrosine Kinase Pathway Inhibition

Mutations in the *PTPN11* gene, which encodes for SHP2, give rise to up to 50% of the mutations in NS. Seminal work from the Neel group using knockin mutations of the $Ptpn11^{D61G}$ into mice was the first to demonstrate recapitulation of the cardiovascular defects observed in NS. Using these mouse models, it was discovered that SHP2 in both the $Ptpn11^{D61G/+}$ NS and $Ptpn11^{Y279C/+}$ NSML mice bound an upstream phosphotyrosyl-containing protein called protein-zero related (PZR), which anchors SHP2 to the plasma membrane. The identification of PZR was particularly interesting since it was hyper tyrosyl phosphorylated in both NS and NSML, suggesting that its increased tyrosyl phosphorylation levels were independent of SHP2 catalysis. Because of the sequence context required for the binding between SHP2 through its SH2 domains to PZR, it was subsequently shown that inhibitors of the Src family kinases (SFKs) that prevented the phosphorylation of PZR, and hence SHP2 binding, could serve to limit the contribution of SHP2 to RAS-MAPK signaling [118]. One such SFK inhibitor that was identified was an FDA-approved drug called dasatinib which had been shown to inhibit the SFKs and BCR-ABL in which it was used for the treatment of leukemia in both adults and children. It was found that extremely low doses of dasatinib in NS mice improved cardiac functionality [119] and in NSML mice reversed the development of HCM [120] (Fig. 2). Although HCM in NS patients is fairly rare, on certain genetic backgrounds, the $Ptpn11^{D61G}$ knockin mouse can develop HCM [119].

Prenatal and postnatal treatment with low-dose dasatinib in these mice also successfully rescued the HCM phenotype, and ex vivo experiments in isolated cardiomyocytes from these animals showed improved contraction coupling [119]. The realization that knockin mice representing either NS or NSML-associated *PTPN11* mutants could be treated with a low dose of dasatinib to improve cardiac functionality and reverse HCM postnatally has opened the door to the possibility that cancer drugs that target kinases, such as the SFKs that act upstream of RASMAPK and PI3K-AKT, could serve as a unifying therapy for the treatment of HCM and cardiac dysfunction in both NS and NSML. Given that very low doses of dasatinib may be effective at reversing HCM, the premise for moving into clinical trials to test this therapy appears solid and should be pursued.

Angiotensin-Converting Enzyme Inhibitors

A mouse model for Costello syndrome (CS) that represents the $HRas^{G12V}$ knockin was found to recapitulate many of the clinical features of this RASopathy. In particular, the CS model mice exhibited HCM, although whether this was a primary or secondary effect remains unclear given that these mice were hypertensive [108]. Angiotensin-converting enzyme (ACE), which converts angiotensin I (Ang I) to angiotensin II (Ang II), is a key regulator of the renin–angiotensin–aldosterone system that regulates blood pressure [121]. It has been shown that inhibiting ACE activity reduces left ventricular hypertrophy in patients with hypertension [122]. Consistent with this, an ACE inhibitor (captopril) ameliorated hypertension, left ventricular hypertrophy, and fibrosis in the hearts of CS model mice [123]. The applicability of this model of CS at this stage requires further study to properly inform the direction of future therapeutics for the treatment of HCM in Costello syndrome.

Conclusions and Perspectives

The goal of this review is to broadly highlight the key features of the RASopathies as a backdrop to discussing how the use of genetically engineered mouse models of the RASopathies have provided insight into the application of potential therapies to treat the cardiovascular manifestations of this disease. RASopathy-associated CHD and HCM can represent distinct clinical management paths with the latter being most amenable to pharmacological intervention. Given that HCM represents one of the most life-threatening clinical presentations of the RASopathies, addressing potential therapeutic strategies to target HCM is an important endeavor.

HCM represents the most prevalent cardiomyopathy variant of the associated RASopathies. The underlying pathogenetic mechanisms that cause RAsopathy-associated HCM remain unclear. In contrast, much more insight and progress towards understanding familial HCM caused by mutations in genes encoding sarcomeric proteins such as thin filamin proteins, thick filament proteins, titin, and Z-disc proteins has been made. The major pathogenic mechanisms that play a role in the progression of sarcomeric HCM include ion channel remodeling, alterations in myofilament Ca^{2+} sensitivity, microvascular dysfunction, and perturbations in energetics. The potential therapies for sarcomere-related HCM include Ca^{2+} desensitizers, beta-blockers, actin-myosin interaction inhibitors, small molecule myosin inhibitors, and CaMKII inhibitors [124]. Direct sarcomere modulators such as mavacamten

and omecamtiv mecarbil or CK-1827452 are the latest promising treatments for sarcomere-associated HCM [125].

As discussed, the RASopathies have been modeled in mice very successfully with virtually all the mouse models recapitulating the major clinical features of the disease, most notably HCM. This presents a tremendous opportunity for the development of preclinical studies to comprehensively identify therapeutic paths for the treatment of RASopathy-associated HCM. Several preclinical and clinical trials have been conducted for the treatment of various RASopathy-related disorders (Table 2). Given that HCM is amenable to pharmacologic intervention, we have begun to see efforts using trametinib as a therapy for the treatment of HCM in extended use cases (Table 2). Similarly, targeting the mTOR-AKT pathway with everolimus has provided some evidence for the utility of inhibiting this pathway for the treatment of NSML-associated HCM (Table 2). These cases which have one or two patients can hardly be classified as “clinical” trials but do provide valuable proof of principle in humans to the feasibility of these drugs for the treatment of RASopathy-associated HCM.

In most cases, the RASopathies represent gain-of-function mutations in the RAS-MAPK pathway, which is probably the most “drugged” cancer-related pathway to date. Herein lies a tremendous opportunity to test the many FDA-approved RAS-MAPK pathway inhibitors against the RASopathy mouse models that have been developed to systematically identify the most promising inhibitor(s) to treat HCM. Similar studies using FDA-approved drugs could also be conducted with inhibitors for PI3K-AKT and SFK inhibitors with the RASopathy mouse models. An underdeveloped, albeit an equally important aspect that these mouse models are amenable to is represented in the form of addressing the issue of dosing. The repurposing of RAS-MAPK, PI3K-AKT, or SFK inhibitors at doses designed to kill cancer cells for the treatment of HCM in the RASopathies needs to be carefully evaluated. Although the basis for the RASopathies lies at its root pathogenetic causality, it can be argued that the pathophysiological outcome arises through disruption of pathways where the levels of signal flux dictates biological output. Thus, doses of drug required to kill a cancer cell, which accumulates a multitude of mutations that directly and indirectly propagate the malignancy, are likely to be different than the dose required to reset signaling homeostasis in a cell expressing a single RASopathy-associated mutation. Thus, the dose to establish normal signaling flux through the RAS-MAPK, PI3K-AKT, or SFK pathway and thus correct HCM could be substantially lower for a repurposed chemotherapeutic drug designed to kill a cancer cell and eradicate malignancy. Indeed, some success has been realized in this regard with the low-dose dasatinib studies targeting the SFKs where effects on HCM reversal are seen at up to 100-fold lower doses than that used to treat chronic myeloid leukemia in humans [119, 120]. If other FDA-approved RAS-MAPK, PI3K-AKT, and SFK inhibitors at their lowest effective dose can be tested in RASopathy models for their effects on HCM, it could lead to the identification of drugs that might have otherwise been considered too toxic to attempt clinical trials for the treatment of HCM. In general, identifying drugs that can be repurposed at much lower doses for the treatment of HCM than their prescribed dose for the treatment of cancer would be important for mitigating side effects especially considering these treatments will be used in very young children.

Finally, recent advances in genomics and computation also provide opportunities to uncover the underlying mechanisms of the RASopathies, which enable in silico drug repurposing as a potential therapeutic approach for the treatment of HCM [126]. It is also worthwhile considering the use of induced pluripotent stem cells as a model system for cell type-specific drug development that can be used in conjunction with the aforementioned RASopathy mouse models [32, 127–130]. The continued integration of these approaches will provide more specific insight into the mechanisms that will enable the development of therapeutics for the treatment of not just HCM but potentially other clinical manifestations that occur in RASopathy patients.

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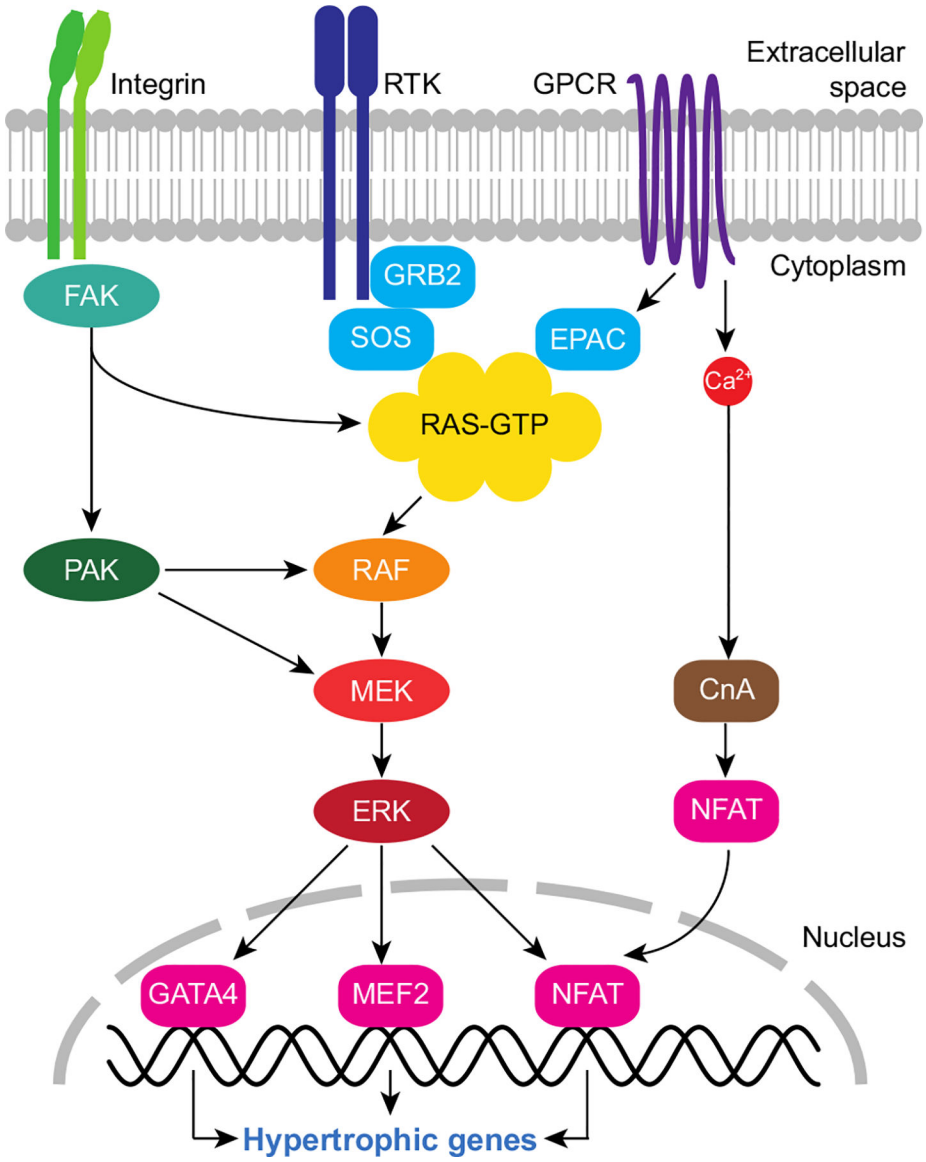

Fig. 1.

Schematic of the RAS-MAPK pathway in HCM development. Ligand-stimulated membrane receptors (RTKs, GPCRs) and mechanical stress sensors (integrins) initiate HCM signal transduction through the RAS/MAPK pathway. Aberrant RAS/MAPK signaling converges on transcription factors that regulate gene expression in HCM pathogenesis

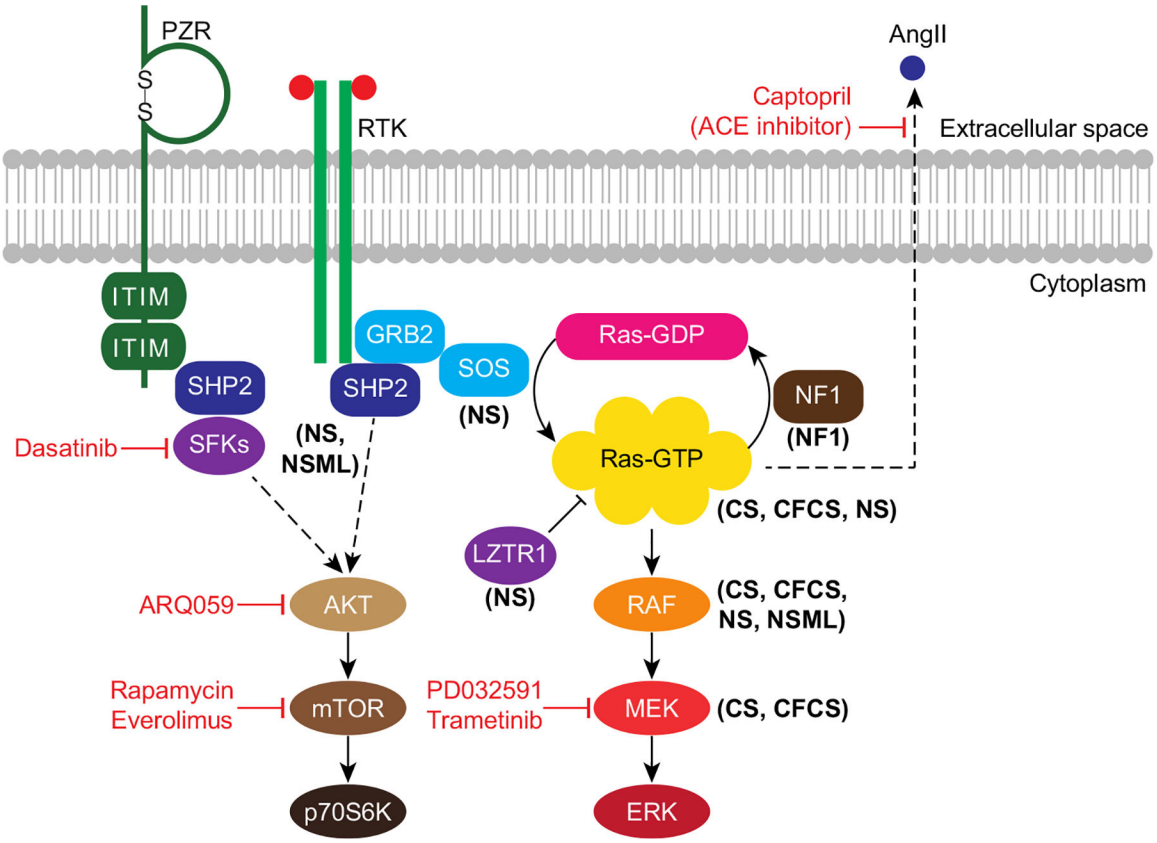

Fig. 2.

Schematic of the RAS-MAPK pathway and RASopathy-associated syndromes. Syndromes are denoted with the protein in the pathway encoded by the causative mutated gene (bold). Small molecules for the treatment of RASopathy-associated CHD and their target inhibition are labeled (red). NS, Noonan syndrome; NSML, Noonan syndrome with multiple lentigines; NF1, neurofibromatosis 1; CS, Costello syndrome; CFCS, cardiofaciocutaneous syndrome

Table 1

Summary of mouse models of RASopathies

Disease	Mutation	Targeting strategy	Cardiac phenotypes		Ref
			Developmental	***Post-developmental***	
NS	*Ptpn11* ^{D61G}	Global KI	VSD, ASD, DORV, valvular hyperplasia	Decreased contractility, myocardial disarray, fibrosis	[119, 131]
	Ptpn11 ^{D61Y}	EC-KI	ASD, VSD, DORV, valvular hyperplasia, myocardial thinning	N/A (embryonic lethal)	[26]
	Ptpn11 ^{Q79R}	MC-TG	VSD	Decreased contractility, congestive heart failure	[132]
	Ptpn11 ^{Q79R}	EC-TG	VSD, DORV, ECD	N/A (embryonic lethal)	[133]
	Ptpn11 ^{N308D}	Global KI	VSD, ASD, DORV, AVS, myocardial thinning	N/A (embryonic lethal)	[26]
	Sos1 ^{E846K}	Global KI	ASD, pericardial effusion	AVS, HCM	[107]
	Kras ^{V14I}	Global KI	HCM (hyperplasia)	HCM (hyperplasia), AVS	[108]
	Raf1 ^{U13V}	Global KI	N/A (no observation)	HCM (eccentric)	[109]
	Raf1 ^{U13V}	MC-TG	N/A (no observation)	HCM, impaired contractility	[134]
	Raf1 ^{U13V}	EC-TG	N/A (no observation)	HCM	[134]
	Rit1 ^{A57G}	Global KI	N/A (no cardiovascular abnormalities)	HCM (hyperplasia)	[135]
	Rit1 ^{M90I}	Global KI	N/A (no observation)	HCM	[136]
	Lztr1	Global KO	N/A (no observation)	HCM (eccentric)	[137]
	Lztr1	EC-KO	VHT, myocardial thinning	N/A (embryonic lethal)	[63]
NSML	*Ptpn11* ^{Y279C}	Global KI	VSD, AVS, PVS, ECD, HCM	HCM, progresses to chamber dilation	[116]
	Ptpn11 ^{Y279C}	EC-KI	ECD, VHT, myocardial thinning	HCM	[138]
	Ptpn11 ^{T468M}	Global KI	N/A (no observation)	HCM, progresses to chamber dilation	[139]
	Ptpn11 ^{Q510E}	MC-TG	N/A (no cardiovascular abnormalities)	VSD, HCM, myocardial disarray, fibrosis	[140]
CS	*Hras* ^{G12V}	Global KI	N/A (no observation)	HCM, AVS, fibrosis, systemic hypertension	[123]
CFCS	*Braf* ^{241R}	Global KI	VSD, PVS, ECD, VHT	N/A (embryonic lethal)	[141]
	Braf ^{L597V}	Global KI	N/A (no observation)	HCM	[142]
	Mek1 ^{Y130C}	Global KI	PVS	N/A (no cardiovascular abnormalities)	[143]
NF1	*Nf1* $^{+/-}$	Global KO	VSD, DORV, AVCD, ECD, myocardial disarray	N/A (embryonic lethal)	[144, 145]
	Nf1 $^{-/-}$	EC-KO	VSD, DORV, ECD, thinned myocardium, pericardial effusion	N/A (no observation)	[146]
	Nf1 $^{-/-}$	MC-KO	N/A (no observation)	HCM, fibrosis, progress to chamber dilatation	[147]

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KO, knockout; *KI*, knock in; *TG*, transgenic; *EC*, endocardium specific; *MC*, myocardium specific; *HCM*, hypertrophic cardiomyopathy; *VSD*, ventricular septal defect; *DORV*, double outlet right ventricle; *AVCD*, atrioventricular canal defect; *ECD*, endocardial cushion defect; *AVS*, aortic valve stenosis; *PVS*, pulmonary valve stenosis; *VHT*, ventricular hypertrabeculation

Table 2

Summary of preclinical and clinical trial studies for RASopathies

Syndrome	Drug	Target	Phase	Outcomes	Ref
NS	Somatropin	IGF-1	Clinical	Improves short stature	[148]
	Simvastatin/lovastatin	HMG-CoA reductase	Pre-clinical	Increases learning ability and cognitive function	[149]
	Trametinib	MEK	Clinical	Improves HCM	[110]
	Dasatinib	Src family kinases	Pre-clinical	Improves cardiac function	[119]
NSML	Dasatinib	Src family kinases	Pre-clinical	Improves HCM	[119, 120]
	Miransertib	AKT	Pre-clinical	Prevents heart failure	[117]
	Rapamycin	mTOR	Pre-clinical	Improves HCM	[116]
	Everolimus	mTOR	Clinical	Improves heart failure	[81]
NF1	Selumetinib	MEK1/2	Clinical	Reduces cell proliferation	[150]
	Mirdametinib	MEK	Clinical	Decreases tumor growth	[151]
	Methylphenidate	CNS stimulant	Clinical	Improves attention deficit	[152]
	Imatinib	BCR-ABL	Clinical	Reduces cancer progression	[153]