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Invited Review

INVITED REVIEW

Pathogenetics of the RASopathies

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Abstract

The RASopathies are defined as a group of medical genetics syndromes that are caused by germ-line mutations in genes that encode components or regulators of the Ras/mitogen-activated protein kinase (MAPK) pathway. Taken together, the RASopathies represent one of the most prevalent groups of malformation syndromes affecting greater than 1 in 1,000 individuals. The Ras/MAPK pathway has been well studied in the context of cancer as it plays essential roles in growth, differentiation, cell cycle, senescence and apoptosis, all of which are also critical to normal development. The consequence of germ-line dysregulation leads to phenotypic alterations of development. RASopathies can be caused by several pathogenetic mechanisms that ultimately impact or alter the normal function and regulation of the MAPK pathway. These pathogenetic mechanisms can include functional alteration of GTPases, Ras GTPase-activating proteins, Ras guanine exchange factors, kinases, scaffolding or adaptor proteins, ubiquitin ligases, phosphatases and pathway inhibitors. Although these mechanisms are diverse, the common underlying biochemical phenotype shared by all the RASopathies is Ras/MAPK pathway activation. This results in the overlapping phenotypic features among these syndromes.

Introduction

The RASopathies are a group of medical genetic syndromes that are caused by germ-line mutations in genes that encode components, both positive and negative regulators, of the Ras/mitogen-activated protein kinase (MAPK) pathway (1). These syndromes, which share many overlapping phenotypic characteristics include neurofibromatosis type 1 (NF1), Noonan syndrome (NS), NS with multiple lentigines (NSML), Legius syndrome, Costello syndrome (CS), cardio-facio-cutaneous syndrome (CFC), capillary malformation-arteriovenous malformation syndrome (CM-AVM) and autosomal dominant intellectual disability type 5 (Table 1). Together, the RASopathies represent a common group of developmental malformation syndromes affecting >1 in 1000 individuals. The Ras/MAPK pathway plays a vital role in both development and cancer. Ras proteins are small guanosine nucleotide-bound GTPases that comprise a critical signaling hub within the cell. Ras is activated through a multitude of mechanisms including growth factors binding to receptor tyrosine kinases (RTK). The binding of growth factor causes RTK autophosphorylation and interaction with the adaptor protein growth factor receptor-bound protein 2 (GRB2). GRB2 is bound to son-of-sevenless (SOS), which is then recruited to the plasma membrane. SOS proteins are guanosine nucleotide exchange factors (GEFs) that increase the Ras nucleotide exchange rate of GDP for GTP, resulting in an increase of Ras in the active GTP-bound form. Activated Ras leads to the activation of Raf (ARAF, BRAF and/or CRAF the multi-protein family of Raf). Raf phosphorylates and activates mitogen-activated protein kinase kinase 1 (MEK1) and/or MEK2 (MAPK kinase 2), which in turn phosphorylates and activates the terminal MAPK, extracellular signal-regulated kinase (ERK), ERK1 and/or ERK2. Phosphorylated ERK1/2 are the ultimate effectors and exert their function on a large number of downstream molecules, both nuclear and cytosolic (2). Although Ras signals to multiple intracellular pathways, the central dominant pathogenetic denominator to all of the RASopathies

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Table 1. The RASopathies

Syndrome	Gene	Chromosome location	Protein function	Clinical phenotype
Autosomal dominant intellectual disability,type 5	*SYNGAP1*	6p21.3	RasGAP	Typically nondysmorphic to mild dysmorphic craniofacial features, moderate to severe intellectual disability, global developmental delay with behavioral issues, autism spectrum disorder, ophthalmologic findings, hypotonia, seizures.
Capillary malformation-AV malformation	*RASA1*	5q14.3	RasGAP	Nondysmorphic craniofacial features, multifocal capillary malformations which may be associated with arteriovenous malformations and fistulae.
Cardio-facio-cutaneous	*BRAF*	7q34	Kinase	Dysmorphic craniofacial features, congenital heart defects, failure to thrive with short stature, ophthalmologic abnormalities, multiple skin manifestations including progressive formation of nevi; variable neurocognitive delay; hypotonia, may be predisposed to cancer
	MAP2K1	15q22.31	Kinase	
	MAP2K2	19p13.3	Kinase	
	KRAS	12p12.1	GTPase	
Costello	*HRAS*	11p15.5	GTPase	Dysmorphic craniofacial features, congenital heart defects, failure to thrive with short stature, ophthalmologic abnormalities, multiple skin manifestations including papilloma; variable neurocognitive delay; hypotonia; predisposition to cancer.
Legius	*SPRED1*	15q14	Negative Regulator	Café-au-lait maculae, intertriginous freckling, normal to mild neurocognitive impairment, macrocephaly; unclear predisposition to cancer.
Noonan	*PTPN11*	12q24.1	Phosphatase	Craniofacial dysmorphic features, congenital heart defects, short stature, undescended testicles, ophthalmologic abnormalities, bleeding disorders, normal to mild neurocognitive delay; predisposition to cancer.
	SOS1	2p22.1	RasGEF	
	RAF1	3p25.1	Kinase	
	KRAS	12p12.1	GTPase	
	NRAS	1p13.2	GTPase	
	SHOC2	10q25.2	Scaffolding	
	CBL	11q23.3	Ubiquitin ligase	
	RRAS	19q13.33	GTPase	
	RIT1	1q22	GTPase	
	RASA2	3q23	RasGAP	
	SOS2	14q21.3	RasGEF	
	MAP3K8	10p11.23	Kinase	
	SPRY1	4q28.1	Inhibitor	
	MYST4	10q22.2	Acetyltransferase	
	LZTR1	22q11.21	Adaptor	
	A2ML1	12p13.31	Protease inhibitor	
Noonan with multiple lentigines	*PTPN11*	12q24.1	Phosphatase	Same as Noonan syndrome but may develop multiple skin lentigines as individuals gets older; unclear predisposition to cancer.
	RAF1	3p25.1	Kinase	
Neurofibromatosis 1	*NF1*	17q11.2	RasGAP	Café-au-lait maculae, intertriginous freckling, neurofibromas and plexiform neurofibromas, iris Lisch nodules, osseous dysplasia, optic pathway glioma, normal to mild neurocognitive delay; predisposition to cancer

is Ras/MAPK pathway activation (Table 2). However, each syndrome results from mutations in specific genes associated with the Ras/MAPK pathway and distinct mutations within each of these genes affect Ras signaling through different molecular mechanisms. Therefore, we have examined the RASopathies based on the pathogenetics in relation to Ras signaling.

GTPase

HRAS

HRAS is a highly conserved gene located on 11p15.5 and encodes the Harvey rat sarcoma viral oncogene homologue, HRAS, which is a hydrolase enzyme that can bind and hydrolyze guanosine triphosphate (GTP). It is a part of a large family of hydrolases called GTPases. Heterozygous activating germ-line mutations in *HRAS* cause CS (3,4). Overall, the vast majority of HRAS mutations in CS result from a missense amino acid substitution for glycine at position 12 or 13, with >80% of CS individuals having a p.G12S substitution, followed by the second most common, p.G12A. These substitutions disrupt guanine nucleotide binding and cause a reduction in intrinsic and GTPase-activating protein (GAP)-induced GTPase activity resulting in Ras remaining in the active state leading to increased effector activity including MAPK activity (5–7).

Table 2. Functional characterization of genes associated with RASopathies

Class	Gene	Protein name	Protein function	Pathogenetic mechanism
GTPase	*HRAS*	HRAS: Harvey rat sarcoma viral oncogene homologue	Hydrolyzes GTP) and activates Raf by recruiting to the cell membrane	Activating mutations
	KRAS	KRAS: the V-Ki-Ras2 Kirsten rat sarcoma viral oncogene homolog	Hydrolyzes GTP and activates Raf by recruiting to the cell membrane	Activating mutations
	NRAS	NRAS: neuroblastoma Ras viral (V-Ras) oncogene homolog	Hydrolyzes GTP and activates Raf by recruiting to the cell membrane	Activating mutations
	RRAS	RRAS: Related Ras viral R-Ras) oncogene homologue	Hydrolyzes GTP and activates Raf by recruiting to the cell membrane	Activating mutations
	RIT1	RIT1; Ras-like protein in tissue	Hydrolyzes GTP and participates in Ras/MAPK and p38 signaling	Activating mutations
RasGAP	*NF1*	Neurofibromin	Binds activated G-proteins and stimulate their GTPase activity switching the active GTP-bound Ras to the inactive GDP-bound form	Loss of function
	RASA1	RASA1: p120-RasGTPase-activating protein	Binds activated G proteins and stimulate their GTPase activity switching the active GTP-bound Ras to the inactive GDP-bound Ras	Loss of function
	RASA2	RASA2: Ras p21 protein activator 2	Binds activated G proteins and stimulates GTPase activity switching the active GTP-bound Ras to the inactive GDP-bound Ras	Loss of function
	SYNGAP1	SynGAP: Synaptic Ras GAP	Neuron-specific RasGAP that binds activated G proteins and stimulates GTPase activity switching the active GTP-bound Ras to the inactive GDP-bound Ras	Loss of function
RasGEF	*SOS1*	SOS1: Son of sevenless homologue 1	RasGEF that stimulates the conversion of Ras from the inactive GDP-bound form to the GTP-bound active form	Activating mutations
	SOS2	SOS2: Son of sevenless homologue 2	Ras-GEF that stimulates the conversion of Ras from the inactive GDP-bound form to the GTP-bound active form	Activating mutations
Scaffolding	*SHOC2*	SHOC2: Homologue of suppressor of clear (SOC-2) in *Caenorhabditis elegans*	Binds GTP-Ras and mediates protein phosphatase 1 translocation to the cell membrane.	Activating mutation
Ubiquitin Ligase	*CBL*	CBL: casitas B-lineage lymphoma	E3 ubiquitin ligase that inhibits Ras activity by targeting phosphorylated substrates for proteasome degradation	Loss of function
Phosphatase	*PTPN11*	SHP2: Tyrosine-protein phosphatase non-receptor type 11; Src Homology 2	Non-receptor protein tyrosine phosphatase that in its active form, increases downstream Ras activity	Activating mutations

(continued)

Table 2. (continued)

Class	Gene	Protein name	Protein function	Pathogenetic mechanism
Kinase	*BRAF*	BRAF: v-Raf murine sarcoma viral oncogene homolog B	Serine/threonine protein kinase that activates MEK1 and/or MEK2 by phosphorylation	Activating mutations
	RAF1	CRAF: v-Raf-1 murine leukemia viral oncogene homolog 1	Serine/threonine protein kinase that activates MEK1 and/or MEK2 by phosphorylation	Activating mutations
	MAP2K1	MEK1: Mitogen-activated protein kinase kinase 1	Threonine/tyrosine kinase that activates ERK1 and/or ERK2 by phosphorylation	Activating mutations
	MAP2K2	MEK2: Mitogen-activated protein kinase kinase 2	Threonine/tyrosine kinase that activates ERK1 and/or ERK2 by phosphorylation	Activating mutations
	MAP3K8	MAP3K8: Mitogen-activated protein kinase kinase kinase 8	Serine/threonine protein kinase which can activate both the Ras/MAPK and JNK pathways.	Activating mutation
SproutyRelatedprotein	*SPRED1*	SPRED1: Sprouty-related EVH1 domain containing 1	Negative regulator of Ras by inhibiting phosphorylation of Raf. Also, SPRED1 binds to the RasGAP, NF1, inducing the membrane localization of NF1 which in turn inhibits Ras	Loss of function
	SPRY1	SPRY1/Sprouty1	Negative regulator of Ras/MAPK pathway signaling	Loss of function
Acetyltransferase	*MYST4*	MYST4: Histone Acetyltransferase (Monocytic Leukemia-4)	Epigenetic modification of DNA by transferring an acetyl group from acetyl-CoA to histone proteins.	Loss of function
Adaptor protein	*LZTR1*	LZTR1: Leucine-zipper-like transcriptional regulator 1	Unknown	Unknown
Protease inhibitor	*A2ML1*	A2ML1: Alpha-2-macroglobulin-like 1	Protease inhibitor that binds lipoprotein receptor-related protein 1, which is an upstream activator of the Ras/MAPK pathway	Unknown

KRAS

The KRAS gene is located on chromosome 12p12.1 and consists of five coding exons of which exon 4 is alternatively spliced. This gene encodes the V-Ki-Ras2 Kirsten rat sarcoma viral oncogene homolog, KRAS protein, either KRAS4A or KRAS4B. Like HRAS, the KRAS protein is a GTPase, which converts GTP into GDP. Activating heterozygous *KRAS* mutations cause NS and CFC (8,9). Functional studies of novel KRAS mutants reveal that these mutations activate the MAPK pathway (10). Biochemical analyses of mutations have demonstrated a reduced intrinsic GTPase activity of Ras compared to the wild-type protein resulting in a decrease in Ras inactivation and, therefore, increased signaling of the MAPK pathway. In addition, further biochemical analyses have shown some germ-line mutants have normal GTPase activity but are mutated and cause GTPase activation independent of GEF binding (9).

NRAS

The *NRAS* gene encodes neuroblastoma Ras viral (V-Ras) oncogene homolog (NRAS) and is located on chromosome 1p13.2. NRAS, like HRAS and KRAS, are the best studied of the Ras family of oncogenes and, like HRAS and KRAS, is a GTPase. Mutations in *NRAS* have been found in a very small number of individuals with the clinical phenotype of NS (11). Mutations have been identified within or near the switch II region of NRAS and are thought to interfere with GTPase function which results in enhanced phosphorylation of downstream MAPK effectors.

RRAS

The *RRAS* gene is located on chromosome 19q13.33 and encodes the GTPase related Ras viral oncogene homolog, RRAS, that exhibits 50–60% homology to the Ras proteins. RRAS is associated with several diverse cellular processes, including neuronal axon guidance, angiogenesis, cell adhesion and migration. Rare germ-line mutations in the *RRAS* gene have been identified in individuals with a NS phenotype (12). *In vitro* functional analyses of a RRAS p.G39dup mutant protein show a reduced intrinsic GTPase activity. Additionally, over-expression of RRAS variant proteins in COS-7 cells increased Ras/MAPK pathway signaling, consistent with other RASopathy-causing gene mutations.

RIT1

The *RIT1* gene is located on chromosome 1q22 and encodes RIT1 (Ras-like protein in tissues), which is a member of a novel branch of Ras-related GTPase proteins in the Ras family. RIT1 shares approximately 50% structural homology with Ras, but lacks a C-terminal lipidation site. RIT1 is important in neuronal development and function (13,14). Heterozygous missense mutations in RIT1 have been identified in individuals who have an NS phenotype (15). Most of the RIT1 mutations are in the switch I or II regions and are predicted to result in a constitutively active Ras protein. Functional analyses of RIT1 mutants identified in RASopathies have demonstrated increased signaling of the Ras/MAPK pathway (15,16).

RasGAP

Neurofibromin

The *NF1* gene is located on chromosome 17q11.2. *NF1* is a large gene with 60 exons covering approximately 350 kb and encodes the protein neurofibromin. Neurofibromin is a GTPase-activating protein, also known as a RasGAP, which is a family of Ras regulatory proteins that can bind activated G proteins and stimulate their GTPase activity switching the active GTP-bound Ras to the inactive GDP-bound form. This results in the negative regulation of activated Ras. Neurofibromatosis type 1 is an autosomal dominant genetic syndrome caused by various types of mutations in the *NF1* gene resulting in loss-of-function of neurofibromin causing haploinsufficiency within the cell (17–19). This, in turn, reduces RasGTPase activity and, therefore, results in an overall increase in active GTP-bound Ras.

RASA1

RASA1, like *NF1*, encodes a RasGAP, specifically the p120-RasGTPase-activating protein (p120-RasGAP). The N terminus contains a Src (sarcoma) homology region, and the C terminus contains a pleckstrin homology domain and the RasGTPase-activating domain. Like neurofibromin, RASA1 switches the active GTP-bound Ras to the inactive GDP-bound form and is, therefore, a negative regulator of the Ras/MAPK signal transduction pathway. Heterozygous-inactivating mutations in *RASA1* cause the autosomal dominant CM-AVM (20). This is a unique RASopathy in that the major feature of this syndrome is the multi-focality of the vascular malformations. Haploinsufficiency of p120-RasGAP causes a reduction in the hydrolysis of Ras-GTP and, therefore, increases Ras/MAPK pathway signaling.

RASA2

RASA2 is located on chromosome 3q23 and encodes the RasGAP protein Ras P21 Protein Activator 2, RASA2 which is a negative regulator of the Ras/MAPK pathway. Novel missense-causing mutations in the *RASA2* gene have been identified in individuals with a NS phenotype. The three mutations identified affect two different amino acid residues Y326 and R511 of which both are in the conserved RASA2 GAP domain (p.Y326C, p.Y326N and p.R511C). The p.R511C substitution is in the Ras interaction site and is predicted to act as a dominant negative competitor for Ras binding. Therefore, the mutations are assumed to result in loss of function. Over-expression of RASA2 mutant proteins cells result in prolonged Ras/MAPK signaling following epidermal growth factor stimulation. Moreover, missense mutations in RASA2 have been identified in human melanomas in addition to other cancers (21).

SYNGAP1

SYNGAP1 is a gene located on chromosome 6p21.3 and encodes SYNGAP1 (synaptic Ras GTPase-activating protein 1). SYNGAP1 is a recently described RasGAP thought to be expressed only in neurons and is a major component of the postsynaptic density found associated with excitatory N-methyl-d-aspartate (NMDA) receptors at synapses (22). Its GAP domain is homologous to that of p120 RasGap and neurofibromin, two RasGAPs known to be associated with RASopathies. Germ-line mutations in *SYNGAP1* have been identified to cause autosomal dominant

intellectual disability type 5, which is considered a non-syndromic form of intellectual disability (23). *De novo* heterozygous frame shift mutations have been identified in individuals with nondysmorphic to mild dysmorphic features, global developmental delay, behavioral issues (aggressive behavior, sleep disturbances and hyper-excitability), autism spectrum disorder, seizures and moderate to severe intellectual disability (24). We consider SYNGAP1 mutations to cause a unique RASopathy.

RasGEF

SOS1

SOS1 is located on chromosome 2p22.1 and consists of 23 exons encoding son of sevenless homolog 1, SOS1. SOS1 is a Ras-specific guanine exchange factor (RasGEF). RasGEF proteins are responsible for stimulating the conversion of Ras from the inactive GDP-bound form to the GTP-bound active form. Heterozygous autosomal dominant SOS1 missense mutations have been identified in NS (25,26). The majority of *SOS1* mutations are located in codon residues that are responsible for stabilizing the SOS1 protein in an inhibited conformation. Therefore, alteration of these residues disrupts the autoinhibition of SOS1 RasGEF activity causing gain-of-function and a subsequent increase in active Ras.

SOS2

SOS2 is located on chromosome 14q21.3 and consists of 23 exons encoding son of sevenless homolog 2, SOS2. SOS2 is a RasGEF and homologue to SOS1. The murine and human SOS1 and SOS2 proteins have an overall 65% amino acid identity; however, their functions do not seem to be identical as knockout Sos1 is an embryonic lethal in mouse models and a knockout Sos2 mouse model has no identifiable phenotype compared to wild-type controls (27,28). Heterozygous autosomal dominant SOS2 missense mutations have been reported in NS (29,30). The SOS2 mutations are located in the Dbl homology (DH) domain, responsible for maintaining SOS2 in an autoinhibited conformation. Functional studies of SOS2 mutants result in higher levels of GTP-bound Ras and, therefore, increased signaling of the Ras/MAPK pathway consistent with the known mechanism of the NS causative SOS1 mutations (30).

Scaffolding

SHOC2

SHOC2 is located on chromosome 10q25.2 and consists of nine exons encoding SHOC2, which is a homologue of suppressor of clear (SOC-2) in *Caenorhabditis elegans*, a protein whose primary structure consists almost entirely of leucine-rich repeats. SHOC2 functions as scaffold protein linking Ras to CRAF/RAF1. SHOC2 binds GTP bound Ras and mediates protein phosphatase 1 (PP1C) translocation to the cell membrane (31). This enables PP1C dephosphorylation that is required for CRAF/RAF1 translocation to the cell membrane and catalytic activity. A rare subset of NS individuals with a unique phenotypic feature of loose anagen hair is caused by a single missense mutation p.S2G (32). The mutation causes the abnormal addition of myristate to the N-terminal glycine of SHOC2 resulting in the aberrant translocation of SHOC2 to the cell membrane, prolonging PP1C dephosphorylation of CRAF/RAF1 and sustained MAPK pathway activation.

Ubiquitin ligase

CBL

CBL is located on chromosome 11q23.3 and consists of 16 exons encoding the tumor suppressor gene casitas B-lineage lymphoma, CBL. CBL is an E3 ubiquitin ligase, which is an enzyme that targets substrates for degradation by the proteasome. CBL mediates the association of ubiquitin with activated RTK which is necessary for receptor internalization and degradation and, therefore, acts as a negative regulator of Ras/MAPK signaling downstream of the RTK (33). A rare cause of NS includes mutations in CBL (34,35). Missense changes alter the RING finger domain or the linker connecting this domain to the N-terminal tyrosine kinase binding domain. Mutations in CBL reduce the turnover of activated RTK, therefore, increase MAPK activation.

Phosphatase

PTPN11

PTPN11 is the tyrosine-protein phosphatase non-receptor type 11 gene that consists of 16 exons and is located on chromosome 12q24.13. SHP2, the protein product of *PTPN11*, is a non-receptor protein tyrosine phosphatase (PTP) composed of N-terminal and C-terminal Src Homology 2 (SH2) domains and a catalytic PTP domain. The catalytic function of the protein is autoinhibited through a blocking interaction of the N-SH2 domain and the catalytic PTP domain (36). *PTPN11* is the predominant gene associated with NS (37). The mutations cluster in exons 3, 7, 8 and 13 that mainly affect residues involved in the interface between the N-SH2 and PTP domains affecting the stability of the catalytically inactive form interfering with the protein's ability to transition from the active to the inactive conformation (38,39). Novel heterozygous missense mutations in SHP2 can also cause NSML (40,41).

Kinase

BRAF

BRAF is located on chromosome 7q34, contains 18 exons and spans approximately 190 kb. *BRAF* encodes v-Raf murine sarcoma viral oncogene homolog B (BRAF), which is a serine/threonine protein kinase and is one of the many direct downstream effectors of Ras. BRAF's only currently known downstream effectors are MEK1 and MEK2. Heterozygous mutations in BRAF cause CFC with the most common mutations occurring in the cysteine-rich domain in exon 6 and in the protein kinase domain. *In vitro* functional analyses of BRAF mutation proteins have demonstrated that most have increased kinase activity (8,42). BRAF is a known oncoprotein with somatic mutations reported in several different types of malignancies including thyroid, lung, ovarian and colorectal.

CRAF/RAF1

CRAF, like BRAF, is a downstream effort of Ras and is a member of the Raf family of serine/threonine protein kinases. *RAF1* is the gene that encodes the v-raf-1 murine leukemia viral oncogene homolog 1 (CRAF/RAF1) and is located on chromosome 3p25.2. Like BRAF, CRAF also has three conserved regions and can phosphorylate to activate the dual specificity protein kinases MEK1 and MEK2, which, in turn, phosphorylate to activate the serine-/threonine-specific protein kinases, ERK1 and ERK2.

Mutations in this gene are associated with NS and NSML (43,44). The majority of *RAF1* mutations in NS cluster in two regions: in conserved region 2 flanking p.S259 and in conserved region 3, surrounding the activation segment. These mutations result in a gain-of-function because the phosphorylation of residues p.S259 and p.S621 is responsible for regulation of CRAF.

MEK1

The *MAP2K1* gene is located on chromosome 15q22.31 and spans approximately 104 kb over 11 exons. The *MAP2K1* gene encodes the MEK1, which is a threonine/tyrosine kinase and is a downstream effector of BRAF. MEK1 activates both ERK1 and ERK2 by phosphorylation. Missense mutations in MEK1 cause CFC (42). The vast majority are missense substitutions located in exons 2 and 3. Functional studies of CFC mutant proteins have shown that all are more active than wild-type MEK in stimulating ERK phosphorylation (42). Because of the initial identification of MEK1 mutations in CFC, MEK1 mutations have also been identified in cancer (45).

MEK2

The *MAP2K2* gene is located on chromosome 19p13.3 and spans approximately 34 kb and contains 11 exons. The *MAP2K2* gene encodes the MEK2. MEK2 is threonine/tyrosine kinase that, like MEK1, has the ability to phosphorylate and activate both ERK1 and ERK2. The MEK1 and MEK2 proteins have about 85% amino acid identity (46) but do not serve redundant purposes (47,48). Heterozygous missense mutations in MEK1 and MEK2 are present in approximately 25% of CFC individuals in which a gene mutation has been identified. Like MEK1, functional studies of MEK2 CFC mutant proteins have shown that all are activating (42,49).

MAP3K8

MAP3K8 is a gene located on chromosome 10p11.23 and has seven coding exons that encode the mitogen-activated protein kinase kinase kinase 8 (MAP3K8). MAP3K8 is a member of the serine/threonine protein kinase family tha can activate both the MAPK and the c-Jun N-terminal kinase pathways. A *de novo* missense mutation MAP3K8 p.L128V was identified in an individual with a NS phenotype (16). Functional analysis by overexpression of the mutant protein resulted in increased levels of phosphorylated ERK *in vitro*.

Sprouty-related protein

SPRED1

SPRED1 is located on chromosome 15q14 and encodes sprouty-related EVH1 domain containing 1, SPRED1. SPRED1 functions as a negative regulator of Ras by inhibiting phosphorylation of Raf (50). In addition, SPRED1 binds to the RasGAP NF1 that induces the membrane localization of NF1 and subsequent down-regulation of Ras through its RasGAP function (51). Heterozygous-inactivating mutations in *SPRED1* cause Legius syndrome, which is an autosomal dominant RASopathy that shares many phenotypic features with NF1 (52). The vast majority of SPRED1 mutations associated with Legius syndrome cause truncation of the protein, a loss of SPRED1 function and dysregulated Ras/MAPK pathway signaling.

SPRY1

SPRY1 is located on chromosome 4q28.1 and has one coding exon that encodes the sprouty RTK signaling antagonist 1 (SPRY1/Sprouty1). Sprouty1 is a negative regulator of Ras/MAPK pathway signaling; however, the mechanism by which Sprouty1 inhibits remains unclear. It is to thought to act at the level of the signaling from the RTK to Ras. A nonsense *de novo* mutation SPRY1 p.E79* has been reported in one individual in a 27 NS patient cohort (16). Functional studies need to be carried out to establish whether or not this *SPRY1* nonsense mutation results in increased Ras/MAPK pathway signaling.

Acetyltransferase

MYST4

MYST4/KAT6B is on chromosome 10q22.2, and transcript variants encoding different isoforms have been found for this gene. The *MYST4* gene, also known as *KAT6B*, encodes MYST histone acetyltransferase monocytic leukemia 4. Histone acetyltransferases modify DNA by transferring an acetyl group from acetyl-CoA to histone proteins on DNA. This epigenetic modification of DNA plays an important role in gene regulation. A translocation breakpoint 10q22.3 in a clinically diagnosed NS individual identified disruption of the *MYST4* gene (53). Functional studies using a patient-derived lymphoblastoid cell line causing MYST4 haploinsufficiency demonstrated an increase of Ras/MAPK pathway activity. The authors postulated that altered expression of multiple genes associated with Ras/MAPK pathway regulation may be responsible for the increase in pathway activation and the NS-like phenotype. However, more research is necessary to confirm this novel correlation.

Adaptor protein

LZTR1

LZTR1 is located on chromosome 22q11.21 and has 21 exons. The encoded protein is leucine-zipper-like transcriptional regulator 1 (LZTR1) that belongs to a functionally diverse family of proteins containing BTB-kelch domains that are thought to localize to the cytoplasmic surface of the Golgi membrane (54). Heterozygous missense mutations have been identified in individuals with a clinical diagnosis of NS (16,29). The mutations are in the highly conserved kelch domain and are predicted to disrupt protein function. These findings suggest that mutations in *LZTR1* may be responsible for a rare percentage of NS cases; however, it is not known if the identified mutations increase Ras/MAPK signaling. Somatic *LZTR1* mutations have been described in glioblastoma. In addition, germ-line *LZTR1* mutations are associated with schwannomatosis, one of the neurofibromatoses (55).

Protease inhibitor

A2ML1

A2ML1 is located on chromosome 12p13.31 and has 35 coding exons. The protein product alpha-2-macroglobulin-like 1 (A2ML1) is a secreted broad-range protease inhibitor (56). Rare *de novo* and autosomal dominant inherited mutations in *A2ML1*, which are predicted to impair protein function, have been associated with individuals who have a clinical diagnosis of NS (57,58). Biochemical studies of the A2ML1 mutations on Ras/

MAPK pathway signaling were examined by overexpression experiments. The mutations did not result in increased phosphorylated ERK levels; however, the authors postulated that A2ML1 is known to bind to lipoprotein receptor-related protein 1 activating of the Ras/MAPK pathway through its association with SHC domain proteins and CBL during recruitment to the plasma membrane (59,60). These findings suggest that rare *A2ML1* mutations may be associated with a RASopathy exhibiting a highly variable NS-like phenotype; however, further studies are needed to confirm the causality.

Conclusion

The RASopathies, which are caused by germ-line mutations in genes encoding components of the Ras/MAPK pathway, underscore the central role this pathway plays during development. It is well established that Ras exists as a complex family of GTPases and signals to a multitude of downstream effector pathways of which MAPK mediated by Raf is only one. These Ras effector pathways exhibit complex cross talk and feedback loops. Furthermore, Ras effectors have been shown to act synergistically, therefore the full impact of aberrant Ras signaling depends on the simultaneous activation of interactive downstream pathways and effectors (61). However, functional studies of RASopathy mutant proteins have demonstrated that the vast majority of mutations result in enhanced Ras/MAPK pathway signaling, the common pathogenetic feature of these syndromes. Because each of the RASopathies exhibit unique phenotypic features, even though the central molecular mechanism is Ras/MAPK pathway activation, the complexity of temporal-spatial signaling to other pathways is certain to play a significant role. The majority of observed RASopathy mutations affect components upstream of Ras resulting in aberrant Ras activation. Examples include mutations in *PTPN11* (SHP-2) that account for ~50% of NS cases and mutations in the RasGAP neurofibromin that cause NF1, two of the most common RASopathies. In contrast, mutations in Ras and downstream kinases are seen in the more rare RASopathies that may reflect intolerance for such mutations during development. Characterization of the nature of Ras/MAPK signal dysregulation is essential to understanding each RASopathy and elucidating the molecular mechanisms by which these novel gene mutations function, as well as how the mutant RASopathy protein affects signaling, is essential to understanding their pathogenetic etiology.

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References

1. Rauen, K.A. (2013) The RASopathies. *Annu. Rev. Genomics Hum. Genet.*, **14**, 355–369.
2. Yoon, S. and Seger, R. (2006) The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors*, **24**, 21–44.
3. Aoki, Y., Niihori, T., Kawame, H., Kurosawa, K., Ohashi, H., Tanaka, Y., Filocamo, M., Kato, K., Suzuki, Y., Kure, S., *et al.* (2005) Germline mutations in HRAS proto-oncogene cause Costello syndrome. *Nat. Genet.*, **37**, 1038–1040.
4. Estep, A.L., Tidyman, W.E., Teitell, M.A., Cotter, P.D. and Rauen, K.A. (2006) HRAS mutations in Costello syndrome: detection of constitutional activating mutations in codon 12 and 13 and loss of wild-type allele in malignancy. *Am. J. Med. Genet. A*, **140**, 8–16.
5. Gibbs, J.B., Sigal, I.S., Poe, M. and Scolnick, E.M. (1984) Intrinsic GTPase activity distinguishes normal and oncogenic ras p21 molecules. *Proc. Natl. Acad. Sci. U. S. A.*, **81**, 5704–5708.
6. McGrath, J.P., Capon, D.J., Goeddel, D.V. and Levinson, A.D. (1984) Comparative biochemical properties of normal and activated human ras p21 protein. *Nature*, **310**, 644–649.
7. Sweet, R.W., Yokoyama, S., Kamata, T., Feramisco, J.R., Rosenberg, M. and Gross, M. (1984) The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity. *Nature*, **311**, 273–275.
8. Niihori, T., Aoki, Y., Narumi, Y., Neri, G., Cave, H., Verloes, A., Okamoto, N., Hennekam, R.C., Gillessen-Kaesbach, G., Wieczorek, D., *et al.* (2006) Germline KRAS and BRAF mutations in cardio-facio-cutaneous syndrome. *Nat. Genet.*, **38**, 294–296.
9. Schubbert, S., Zenker, M., Rowe, S.L., Boll, S., Klein, C., Bollag, G., van der Burgt, I., Musante, L., Kalscheuer, V., Wehner, L.E., *et al.* (2006) Germline KRAS mutations cause Noonan syndrome. *Nat. Genet.*, **38**, 331–336.
10. Schubbert, S., Bollag, G., Lyubynska, N., Nguyen, H., Kratz, C.P., Zenker, M., Niemeyer, C.M., Molven, A. and Shannon, K. (2007) Biochemical and functional characterization of germ line KRAS mutations. *Mol. Cell. Biol.*, **27**, 7765–7770.
11. Cirstea, I.C., Kutsche, K., Dvorsky, R., Gremer, L., Carta, C., Horn, D., Roberts, A.E., Lepri, F., Merbitz-Zahradnik, T., Konig, R., *et al.* (2010) A restricted spectrum of NRAS mutations causes Noonan syndrome. *Nat. Genet.*, **42**, 27–29.
12. Flex, E., Jaiswal, M., Pantaleoni, F., Martinelli, S., Strullu, M., Fansa, E.K., Caye, A., De Luca, A., Lepri, F., Dvorsky, R., *et al.* (2014) Activating mutations in RRAS underlie a phenotype within the RASopathy spectrum and contribute to leukaemogenesis. *Hum. Mol. Genet.*, **23**, 4315–4327.
13. Shi, G.X. and Andres, D.A. (2005) Rit contributes to nerve growth factor-induced neuronal differentiation via activation of B-Raf-extracellular signal-regulated kinase and p38 mitogen-activated protein kinase cascades. *Mol. Cell. Biol.*, **25**, 830–846.
14. Shi, G.X., Cai, W. and Andres, D.A. (2013) Rit subfamily small GTPases: regulators in neuronal differentiation and survival. *Cell. Signal.*, **25**, 2060–2068.
15. Aoki, Y., Niihori, T., Banjo, T., Okamoto, N., Mizuno, S., Kurosawa, K., Ogata, T., Takada, F., Yano, M., Ando, T., *et al.* (2013) Gain-of-function mutations in RIT1 cause Noonan syndrome, a RAS/MAPK pathway syndrome. *Am. J. Hum. Genet.*, **93**, 173–180.
16. Chen, P.C., Yin, J., Yu, H.W., Yuan, T., Fernandez, M., Yung, C.K., Trinh, Q.M., Peltekova, V.D., Reid, J.G., Tworog-Dube, E., *et al.* (2014) Next-generation sequencing identifies rare variants associated with Noonan syndrome. *Proc. Natl. Acad. Sci. U. S. A.*, **111**, 11473–11478.

17. Cawthon, R.M., Weiss, R., Xu, G.F., Viskochil, D., Culver, M., Stevens, J., Robertson, M., Dunn, D., Gesteland, R., O'Connell, P., *et al.* (1990) A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. *Cell*, **62**, 193–201.
18. Wallace, M.R., Marchuk, D.A., Andersen, L.B., Letcher, R., Odeh, H.M., Saulino, A.M., Fountain, J.W., Brereton, A., Nicholson, J., Mitchell, A.L., *et al.* (1990) Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science*, **249**, 181–186.
19. Viskochil, D., Buchberg, A.M., Xu, G., Cawthon, R.M., Stevens, J., Wolff, R.K., Culver, M., Carey, J.C., Copeland, N.G., Jenkins, N.A., *et al.* (1990) Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell*, **62**, 187–192.
20. Eerola, I., Boon, L.M., Mulliken, J.B., Burrows, P.E., Dompmartin, A., Watanabe, S., Vanwijck, R. and Vikkula, M. (2003) Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. *Am. J. Hum. Genet.*, **73**, 1240–1249.
21. Arafeh, R., Qutob, N., Emmanuel, R., Keren-Paz, A., Madore, J., Elkahloun, A., Wilmott, J.S., Gartner, J.J., Di Pizio, A., Winograd-Katz, S., *et al.* (2015) Recurrent inactivating RASA2 mutations in melanoma. *Nature Genetics*, **47**, 1408–1410.
22. Jeyabalan, N. and Clement, J.P. (2016) SYNGAP1: Mind the Gap. *Front. Cell. Neurosci.*, **10**, 32.
23. Hamdan, F.F., Gauthier, J., Spiegelman, D., Noreau, A., Yang, Y., Pellerin, S., Dobrzeniecka, S., Cote, M., Perreau-Linck, E., Carmant, L., *et al.* (2009) Mutations in SYNGAP1 in autosomal nonsyndromic mental retardation. *N. Engl. J. Med.*, **360**, 599–605.
24. Hamdan, F.F., Daoud, H., Piton, A., Gauthier, J., Dobrzeniecka, S., Krebs, M.O., Joober, R., Lacaille, J.C., Nadeau, A., Milunsky, J.M., *et al.* (2011) De novo SYNGAP1 mutations in nonsyndromic intellectual disability and autism. *Biol. Psychiatr.*, **69**, 898–901.
25. Roberts, A.E., Araki, T., Swanson, K.D., Montgomery, K.T., Schiripo, T.A., Joshi, V.A., Li, L., Yassin, Y., Tamburino, A.M., Neel, B.G., *et al.* (2007) Germline gain-of-function mutations in SOS1 cause Noonan syndrome. *Nat. Genet.*, **39**, 70–74.
26. Tartaglia, M., Pennacchio, L.A., Zhao, C., Yadav, K.K., Fodale, V., Sarkozy, A., Pandit, B., Oishi, K., Martinelli, S., Schackwitz, W., *et al.* (2007) Gain-of-function SOS1 mutations cause a distinctive form of Noonan syndrome. *Nat. Genet.*, **39**, 75–79.
27. Wang, D.Z., Hammond, V.E., Abud, H.E., Bertoncello, I., McAvoy, J.W. and Bowtell, D.D. (1997) Mutation in Sos1 dominantly enhances a weak allele of the EGFR, demonstrating a requirement for Sos1 in EGFR signaling and development. *Genes Dev.*, **11**, 309–320.
28. Esteban, L.M., Fernandez-Medarde, A., Lopez, E., Yienger, K., Guerrero, C., Ward, J.M., Tessarollo, L. and Santos, E. (2000) Ras-guanine nucleotide exchange factor sos2 is dispensable for mouse growth and development. *Mol. Cell. Biol.*, **20**, 6410–6413.
29. Yamamoto, G.L., Aguena, M., Gos, M., Hung, C., Pilch, J., Fahiminiya, S., Abramowicz, A., Cristian, I., Buscarilli, M., Naslavsky, M.S., *et al.* (2015) Rare variants in SOS2 and LZTR1 are associated with Noonan syndrome. *J. Med. Genet.*, **52**, 413–421.
30. Cordeddu, V., Yin, J.C., Gunnarsson, C., Virtanen, C., Drunat, S., Lepri, F., De Luca, A., Rossi, C., Ciolfi, A., Pugh, T.J., *et al.* (2015) Activating Mutations Affecting the Dbl Homology Domain of SOS2 Cause Noonan Syndrome. *Hum. Mutat.*, **36**, 1080–1087.
31. Rodriguez-Viciana, P., Oses-Prieto, J., Burlingame, A., Fried, M. and McCormick, F. (2006) A phosphatase holoenzyme comprised of Shoc2/Sur8 and the catalytic subunit of PP1 functions as an M-Ras effector to modulate Raf activity. *Mol. Cell*, **22**, 217–230.
32. Cordeddu, V., Di Schiavi, E., Pennacchio, L.A., Ma'ayan, A., Sarkozy, A., Fodale, V., Cecchetti, S., Cardinale, A., Martin, J., Schackwitz, W., *et al.* (2009) Mutation of SHOC2 promotes aberrant protein N-myristoylation and causes Noonan-like syndrome with loose anagen hair. *Nat. Genet.*, **41**, 1022–1026.
33. Dikic, I. and Schmidt, M.H. (2007) Malfunctions within the Cbl interactome uncouple receptor tyrosine kinases from destructive transport. *Eur. J. Cell Biol.*, **86**, 505–512.
34. Niemeyer, C.M., Kang, M.W., Shin, D.H., Furlan, I., Erlacher, M., Bunin, N.J., Bunda, S., Finklestein, J.Z., Sakamoto, K.M., Gorr, T.A., *et al.* (2010) Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. *Nat. Genet.*, **42**, 794–800.
35. Martinelli, S., De Luca, A., Stellacci, E., Rossi, C., Checquolo, S., Lepri, F., Caputo, V., Silvano, M., Buscherini, F., Consoli, F., *et al.* (2010) Heterozygous germline mutations in the CBL tumor-suppressor gene cause a Noonan syndrome-like phenotype. *Am. J. Hum. Genet.*, **87**, 250–257.
36. Hof, P., Pluskey, S., Dhe-Paganon, S., Eck, M.J. and Shoelson, S.E. (1998) Crystal structure of the tyrosine phosphatase SHP-2. *Cell*, **92**, 441–450.
37. Tartaglia, M., Mehler, E.L., Goldberg, R., Zampino, G., Brunner, H.G., Kremer, H., van der Burgt, I., Crosby, A.H., Ion, A., Jeffery, S., *et al.* (2001) Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat. Genet.*, **29**, 465–468.
38. Keilhack, H., David, F.S., McGregor, M., Cantley, L.C. and Neel, B.G. (2005) Diverse biochemical properties of Shp2 mutants. Implications for disease phenotypes. *J. Biol. Chem.*, **280**, 30984–30993.
39. Tartaglia, M., Martinelli, S., Stella, L., Bocchinfuso, G., Flex, E., Cordeddu, V., Zampino, G., Burgt, I., Palleschi, A., Petrucci, T.C., *et al.* (2006) Diversity and functional consequences of germline and somatic PTPN11 mutations in human disease. *Am. J. Hum. Genet.*, **78**, 279–290.
40. Digilio, M.C., Conti, E., Sarkozy, A., Mingarelli, R., Dottorini, T., Marino, B., Pizzuti, A. and Dallapiccola, B. (2002) Grouping of multiple-lentigines/LEOPARD and Noonan syndromes on the PTPN11 gene. *Am. J. Hum. Genet.*, **71**, 389–394.
41. Legius, E., Schrander-Stumpel, C., Schollen, E., Pulles-Heintzberger, C., Gewillig, M. and Fryns, J.P. (2002) PTPN11 mutations in LEOPARD syndrome. *J. Med. Genet.*, **39**, 571–574.
42. Rodriguez-Viciana, P., Tetsu, O., Tidyman, W.E., Estep, A.L., Conger, B.A., Cruz, M.S., McCormick, F. and Rauen, K.A. (2006) Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. *Science*, **311**, 1287–1290.
43. Pandit, B., Sarkozy, A., Pennacchio, L.A., Carta, C., Oishi, K., Martinelli, S., Pogna, E.A., Schackwitz, W., Ustaszewska, A., Landstrom, A., *et al.* (2007) Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. *Nat. Genet.*, **39**, 1007–1012.
44. Razzaque, M.A., Nishizawa, T., Komoike, Y., Yagi, H., Furutani, M., Amo, R., Kamisago, M., Momma, K., Katayama, H., Nakagawa, M., *et al.* (2007) Germline gain-of-function mutations in RAF1 cause Noonan syndrome. *Nat. Genet.*, **39**, 1013–1017.
45. Estep, A.L., Palmer, C., McCormick, F. and Rauen, K.A. (2007) Mutation analysis of BRAF, MEK1 and MEK2 in 15 ovarian

cancer cell lines: implications for therapy. *PloS One*, **2**, e1279.
46. Wu, J., Harrison, J.K., Dent, P., Lynch, K.R., Weber, M.J. and Sturgill, T.W. (1993) Identification and characterization of a new mammalian mitogen-activated protein kinase kinase, MKK2. *Mol. Cell. Biol.*, **13**, 4539–4548.
47. Brott, B.K., Alessandrini, A., Largaespada, D.A., Copeland, N.G., Jenkins, N.A., Crews, C.M. and Erikson, R.L. (1993) MEK2 is a kinase related to MEK1 and is differentially expressed in murine tissues. *Cell Growth Differ*, **4**, 921–929.
48. Alessandrini, A., Brott, B.K. and Erikson, R.L. (1997) Differential expression of MEK1 and MEK2 during mouse development. *Cell Growth Differ*, **8**, 505–511.
49. Anastasaki, C., Estep, A.L., Marais, R., Rauen, K.A. and Patton, E.E. (2009) Kinase-activating and kinase-impaired cardio-facio-cutaneous syndrome alleles have activity during zebrafish development and are sensitive to small molecule inhibitors. *Hum. Mol. Genet.*, **18**, 2543–2554.
50. Wakioka, T., Sasaki, A., Kato, R., Shouda, T., Matsumoto, A., Miyoshi, K., Tsuneoka, M., Komiya, S., Baron, R. and Yoshimura, A. (2001) Spred is a Sprouty-related suppressor of Ras signalling. *Nature*, **412**, 647–651.
51. Stowe, I.B., Mercado, E.L., Stowe, T.R., Bell, E.L., Oses-Prieto, J.A., Hernandez, H., Burlingame, A.L. and McCormick, F. (2012) A shared molecular mechanism underlies the human rasopathies Legius syndrome and Neurofibromatosis-1. *Genes Dev.*, **26**, 1421–1426.
52. Brems, H., Chmara, M., Sahbatou, M., Denayer, E., Taniguchi, K., Kato, R., Somers, R., Messiaen, L., De Schepper, S., Fryns, J.P., *et al.* (2007) Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. *Nat. Genet.*, **39**, 1120–1126.
53. Kraft, M., Cirstea, I.C., Voss, A.K., Thomas, T., Goehring, I., Sheikh, B.N., Gordon, L., Scott, H., Smyth, G.K., Ahmadian, M.R., *et al.* (2011) Disruption of the histone acetyltransferase MYST4 leads to a Noonan syndrome-like phenotype and hyperactivated MAPK signaling in humans and mice. *J. Clin. Investig.*, **121**, 3479–3491.
54. Nacak, T.G., Leptien, K., Fellner, D., Augustin, H.G. and Kroll, J. (2006) The BTB-kelch protein LZTR-1 is a novel Golgi protein that is degraded upon induction of apoptosis. *J. Biol. Chem.*, **281**, 5065–5071.
55. Piotrowski, A., Xie, J., Liu, Y.F., Poplawski, A.B., Gomes, A.R., Madanecki, P., Fu, C., Crowley, M.R., Crossman, D.K., Armstrong, L., *et al.* (2014) Germline loss-of-function mutations in LZTR1 predispose to an inherited disorder of multiple schwannomas. *Nat. Genet.*, **46**, 182–187.
56. Galliano, M.F., Toulza, E., Gallinaro, H., Jonca, N., Ishida-Yamamoto, A., Serre, G. and Guerrin, M. (2006) A novel protease inhibitor of the alpha2-macroglobulin family expressed in the human epidermis. *J. Biol. Chem.*, **281**, 5780–5789.
57. Vissers, L.E., Bonetti, M., Paardekooper Overman, J., Nillesen, W.M., Frints, S.G., de Ligt, J., Zampino, G., Justino, A., Machado, J.C., Schepens, M., *et al.* (2015) Heterozygous germline mutations in A2ML1 are associated with a disorder clinically related to Noonan syndrome. *Eur. J. Hum. Genet.*, **23**, 317–324.
58. van Trier, D.C., van Nierop, J., Draaisma, J.M., van der Burgt, I., Kunst, H., Croonen, E.A. and Admiraal, R.J. (2015) External ear anomalies and hearing impairment in Noonan Syndrome. *Int. J. Pediatr. Otorhinolaryngol*, **79**, 874–878.
59. Barnes, H., Larsen, B., Tyers, M. and van Der Geer, P. (2001) Tyrosine-phosphorylated low density lipoprotein receptor-related protein 1 (Lrp1) associates with the adaptor protein SHC in SRC-transformed cells. *J. Biol. Chem.*, **276**, 19119–19125.
60. Craig, J., Mikhailenko, I., Noyes, N., Migliorini, M. and Strickland, D.K. (2013) The LDL receptor-related protein 1 (LRP1) regulates the PDGF signaling pathway by binding the protein phosphatase SHP-2 and modulating SHP-2- mediated PDGF signaling events. *PloS One*, **8**, e70432.
61. Stephen, A.G., Esposito, D., Bagni, R.K. and McCormick, F. (2014) Dragging ras back in the ring. *Cancer Cell*, **25**, 272–281.