REVIEW

SPRED proteins and their roles in signal transduction, development, and malignancy

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The roles of SPRED proteins in signaling, development, and cancer are becoming increasingly recognized. SPRED proteins comprise an N-terminal EVH-1 domain, a central c-Kit-binding domain, and C-terminal SROUTY domain. They negatively regulate signaling from tyrosine kinases to the Ras–MAPK pathway. SPRED1 binds directly to both c-KIT and to the RasGAP, neurofibromin, whose function is completely dependent on this interaction. Loss-of-function mutations in SPRED1 occur in human cancers and cause the developmental disorder, Legius syndrome. Genetic ablation of SPRED genes in mice leads to behavioral problems, dwarfism, and multiple other phenotypes including increased risk of leukemia. In this review, we summarize and discuss biochemical, structural, and biological functions of these proteins including their roles in normal cell growth and differentiation and in human disease.

Ras proteins are binary molecular switches that cycle between GTP-bound on and GDP-bound off states. Accumulation of active Ras-GTP in response to growth factors is understood in some detail where the translocation of guanine exchange factor (GEF), such as SOS, to the plasma membrane stimulates the nucleotide exchange on Ras from GDP to GTP (Buday and Downward 1993). GTP-bound Ras proteins activate the MAPK kinase cascade by binding and recruiting Raf to the membrane (Terrell et al. 2019). This cascade regulates a variety of biological processes such as cell proliferation, differentiation, and survival. Ras proteins also bind activate PI 3' kinases, RalGDS proteins, and possibly other effector pathways (Simanshu et al. 2017). Conversion of Ras-GTP back to the inactive form, and maintenance of Ras in the inactive form prior to restimulation is mediated by GTPase-activating proteins (GAPs), such as neurofibromin and RASA (p120 RasGAP).

Ras signaling is tightly controlled; hyperactive Ras signaling results in diseases such as cancer, developmental disorders (known collectively as RASopathies) and learning disabilities (Simanshu et al. 2017). To maintain tight control of Ras activity, numerous signals and interactions regulate the duration, magnitude and context of Ras activation. SPRED proteins, and their better-known relatives, proteins of the Sprouty family, are negative regulators of Ras signaling. Although SPRED and Sprouty proteins share a conserved C-terminal Sprouty (SPR) domain, the mechanisms by which they regulate Ras signaling are distinct.

The *SPROUTY* gene was first identified as a negative regulator of FGF signaling and tracheal branching in *Drosophila* (Hacohen et al. 1998). The role of Sprouty proteins as negative regulators of Ras–MAPK signaling is conserved from *Drosophila* to humans (Hacohen et al. 1998; Casci et al. 1999). Sprouty proteins inhibit Ras activation by preventing the membrane localization of SOS, a Ras GEF, by binding and sequestering GRB2, an adaptor protein that links the phosphorylated receptor tyrosine kinase (RTK) with SOS (Fig. 1). Activated RTKs, such as FGFR, EGFR, and VEGFR, phosphorylate a conserved phosphotyrosine in Sprouty, which is recognized by the SH2 domain found on GRB2 and competes for GRB2 binding with SOS (Hanafusa et al. 2002). Sprouty proteins are down-regulated by dephosphorylation of the phosphotyrosine by the phosphatase Shp2 (Hanafusa et al. 2004) or engagement with c-Cbl, an E3 ubiquitin-ligase, which results in polyubiquitination and proteasomal degradation (Hall et al. 2003; Mason et al. 2004). In addition to GRB2 binding, Sprouty may also regulate Ras signaling by binding Raf, which inhibits Raf kinase activity (Fig. 1; Yusoff et al. 2002).

Sprouty-related SPRED proteins were identified in a yeast two-hybrid screen to identify proteins that interact with RTKs c-Kit and c-Fms (Wakioka et al. 2001). This screen identified SPRED1 as a binding partner for the intracellular kinase domain of c-Kit in its inactive state.

[**Keywords:** Legius syndrome; NF1; Ras–MAPK; SPROUTY; signal transduction]

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SPRED isoforms

each member of the SPRED family may have a selective capacity and function for the regulation of Ras-MAPK signaling.

EVH-1 domain

The N-terminal region of SPRED family members is comprised of an enabled/VASP homology-1 (EVH-1) domain (Fig. 2A), one of the four distinct interaction domains among SH3-WW, GYF, and EVH-1—that recognize proline-rich sequences (Peterson and Volkman 2014). The EVH-1 domain has been found in ~630 human proteins (Peterson and Volkman 2014). This domain spans ~115 residues in length and is invariably located at the N terminus. EVH-1 domains are found in multidomain scaffolding proteins that mediate multiprotein complex assembly associated with modulating the actin cytoskeleton or signal transduction in postsynaptic compartments (Ball et al. 2002). Despite having low sequence homology, EVH-1

Figure 2. Domain and sequence alignments of human SPRED and Sprouty proteins. (A) Domain structure of SPRED and Sprouty proteins. SPRED proteins share a N-terminal EVH-1 domain and C-terminal sprouty domain. SPRED1 and SPRED2 share a central c-Kit-binding domain. Sprouty proteins share a conserved tyrosine and C-terminal sprouty domain. (B) Sequence alignment of SPRED proteins. (Red) EVH-1 domain, (blue) KBD, (green) SPR, (highlighted in yellow) conserved residues.

Table 1. *Chromosomal localization*

	Human	Mouse
<i>SPRED1</i>	15q13.2	2E5
<i>SPRED2</i>	2p14	11A3-A4
<i>SPRED3</i>	19q13.13	7A3

Chromosomal localization was identified by an NCBI database search for "human" and Kato et al. 2003 for "mouse."

domains consist of two perpendicular antiparallel β -sheets followed by a C-terminal α -helix (Peterson and Volkman 2014). Structural studies with the SPRED1 EVH-1 domain from *Xenopus tropicalis* found that the polyproline-binding groove is narrower than in other EVH-1 domains (Harmer et al. 2005). The only known interactor for SPRED EVH-1 is neurofibromin, which does not contain proline-rich sequences within the interaction region (Harmer et al. 2005; Stowe et al. 2012). Together, this demonstrates a new binding mechanism for SPRED EVH-1 that does not require proline-rich sequences similar to other EVH-1 interactors (Hirata et al. 2016), and presents a new mode of protein–protein interaction that may exist in other proteins containing an EVH-1 domain. Finally, although the three SPRED proteins bind neurofibromin, the SPRED3 EVH-1 has a much weaker binding affinity compared with SPRED1 and SPRED2 (Hirata et al. 2016). Unlike SPRED1 and SPRED2, sequence alignment of SPRED proteins shows that SPRED3 has a smaller N terminus and contains a three-amino-acid insertion in the β 4 strand (Yan et al. 2020); this corresponds to decreased sequence identity between SPRED1 and SPRED3 (57%), compared with SPRED2 (67%). These differences in amino acid sequence likely cause minor changes in tertiary structure of SPRED3 EVH-1 domain and may be responsible for weaker neurofibromin binding and ERK suppression activity of SPRED3. Indeed, several residues in SPRED1 (L32, T86, and T88), which directly interact with neurofibromin via nonbonded contacts and hydrogen bond are not conserved in SPRED3 (Yan et al. 2020).

KBD domain

The central region of SPRED contains the c-Kit-binding domain (KBD) (Fig. 2A). This domain spans 50 residues and was initially identified through a yeast two-hybrid screen for tyrosine kinase-binding proteins (Wakioka et al. 2001). This interaction domain is unrelated to any previously identified tyrosine kinase interaction domains such as SH2 and PTB domain (Wakioka et al. 2001). Although SPRED2 KBD only shares 51% identity with SPRED1 KBD, SPRED2 also binds to c-Kit via this region. In contrast, the corresponding domain of SPRED3 shares <20% identity with SPRED1 KBD (Fig. 2A). Arginine 247 of SPRED1 is required for c-Kit binding (Kato et al. 2003), but in SPRED3 the equivalent residue (240) is a glycine. As a result, SPRED3 does not bind or become phosphorylated by c-Kit, unlike SPRED1 and SPRED2 (Kato et al. 2003). Moreover, it is not known whether SPRED proteins can also interact with other RTKs besides c-Kit; perhaps the KBD is a new in-

teraction domain that mediates binding with RTKs, allowing RTKs to regulate SPRED function.

SPR domain

The C-terminal region of SPRED proteins consists of the Sprouty (SPR) domain (Fig. 2A), a conserved cysteine-rich domain analogous to that found in Sprouty proteins (Fig. 2C). Despite attenuation of Ras signaling by apparently distinct mechanisms, membrane localization is consistently important for efficient inhibition by both SPRED and Sprouty. Sprouty proteins translocate to membrane raft/caveolae following growth factor stimulation (Impagnatiello et al. 2001; Lim et al. 2002). Caveolae represent a subset of lipid rafts that are implicated in signal transduction-related events due to the presence of signaling proteins such as RTKs (Bastiani and Parton 2010). Membrane localization of Sprouty proteins may be a consequence of their interaction with a membrane-associated scaffold protein, caveolin-1, and/or through palmitoylation (Impagnatiello et al. 2001). Similar to Sprouty, SPRED proteins localize in lipid rafts/caveolae where they interact with caveolin-1 (Nonami et al. 2005). SPRED1 and SPRED3 also interact with a palmitoyl acetyltransferase, HIP14, and coexpression leads to palmitoylation of SPRED (Butland et al. 2014). Additionally, SPRED1 associates with B-Raf in the cytoplasm, where B-Raf/C-Raf dimerization induces SPRED1 membrane translocation to the plasma membrane (Siljamäki and Abankwa 2016). The SPR domain of SPRED is important for effective Ras inhibition by translocating neurofibromin to the plasma membrane where it can interact with Ras (Stowe et al. 2012). SPRED mutants with deletion of the SPR domain fail to localize to the plasma membrane and fail to inhibit ERK phosphorylation (King et al. 2005). Moreover, pathogenic SPR mutations in SPRED1 bind neurofibromin but fail to recruit it to the plasma membrane (Stowe et al. 2012; Hirata et al. 2016). Altogether, these data show that the SPR domain is necessary to down-regulate Ras signaling by recruiting neurofibromin to the plasma membrane compartment in which it can interact with Ras. RASA1/p120RasGap does not interact with SPRED proteins, as discussed below. This GAP is recruited to the plasma membrane through interactions between its SH2 domains and phospho-tyrosines on activated RTKs, such as PDGFR and EphA2 (Tong et al. 2003). The mechanisms by which other GAPs are recruited to Ras in the plasma membrane remain to be determined.

In addition to membrane localization, the SPR domain plays a role in SPRED and Sprouty dimerization (Hanafusa et al. 2002; King et al. 2005). SPRED1, SPRED2, and SPRED3 have been found to heterodimerize, although SPR deletion mutants cannot (King et al. 2005). However, the affinity, context, and consequences of dimerization are unclear. Furthermore, membrane localization and Ras inhibition in Δ SPR mutants were rescued by the addition of CAAX motif (Stowe et al. 2012), a consensus sequence known to undergo a series of post-translational modifications that allow proteins to associate with membranes (Wright and Philips 2006). It was previously shown that

the CAAX motif induces Ras dimerization (Nan et al. 2015). Interestingly, neurofibromin was recently identified as a high-affinity dimer in cells (Sherekar et al. 2020) and suggests that these signaling complexes are regulated by dimerization, although the precise mechanisms remain to be elucidated.

Protein interactions

Neurofibromin

Neurofibromin interacts with SPRED through the EVH-1 domain (Stowe et al. 2012). Neurofibromin negatively regulates Ras signaling through its function as a GAP to accelerate the hydrolysis of GTP (Martin et al. 1990; Xu et al. 1990). This interaction is necessary for the recruitment of neurofibromin to the plasma membrane where it can down-regulate Ras activity (Stowe et al. 2012) and provides a satisfying explanation for the phenotypic overlap between loss of SPRED1 function in Legius syndrome and loss of function of neurofibromin in NF1, discussed later.

The crystal structure of the neurofibromin GAP-related domain (GRD) (PDB:6V65) consists of a module of two subdomains: the central domain (GAPc), which is necessary and sufficient to bind and inactivate Ras, and the extra domain (GAPex), which is composed of the N-terminal and C-terminal residues flanking the central GAP domain (Fig. 3B; Yan et al. 2020). The neurofibromin-GAPc primarily interacts with RAS. Meanwhile, the neurofibromin-GAPex region forms two crossing α -helix coils at one end outside the GAPc region, and is required for binding to the SPRED1 EVH-1 domain (Fig. 3A,B; Dunzendorfer-Matt et al. 2016). Although the GAPex region has been shown to be dispensable for GAP activity in vitro (Hirata et al. 2016), the GAPex region is the major interaction domain for SPRED1 binding (Dunzendorfer-Matt et al. 2016; Sherekar et al. 2020). The N-terminal residues of neurofibromin GAPex forms a salt bridge and hydrogen bonds with residues in SPRED1 EVH-1 domain. Meanwhile, the C-terminal residues of neurofibromin GAPex have a less direct interaction with SPRED1 and instead act as structure-supporting module to maintain the overall architecture of neurofibromin GAPc and GAPex domains through hydrophobic interactions and a weak hydrogen bond. Besides the GAPex, the GAPc region also interacts with SPRED1 EVH-1 and contributes to complex stabilization mainly through hydrophobic interaction (Yan et al. 2020). Furthermore, the SPRED1 region forming the neurofibromin-SPRED1 interphase in the crystal structure was found to be highly flexible in NMR studies (Führer et al. 2017). LS mutations such as R24Q (Sumner et al. 2011), G30R (Sumner et al. 2011), and T102R (Messiaen et al. 2009) disrupt electrostatic interactions with neurofibromin, and result in decreased or undetectable binding (Führer et al. 2017; Fig. 3A,B; Yan et al. 2020). Likewise, several NF1 patient mutations that map to the GAPex region resulted in reduced binding with SPRED1 EVH-1 domain (Fig. 3D; Hirata et al. 2016). These LS and NF1 mutations are located at the neurofibromin-SPRED1 interface and form direct interac-

tions between neurofibromin and SPRED1 (Fig. 3E; Yan et al. 2020).

c-Kit

Both SPRED1 and SPRED2 bind c-Kit and are phosphorylated on tyrosine residues in response to growth factor stimulation with SCF, PDGF, and EGF. The binding site for c-Kit was mapped to a 50-amino-acid region (codon 233–285) in SPRED1 subsequently designated the “c-Kit-binding domain” (Wakioka et al. 2001). Despite this, little is known about the consequence and context of c-Kit binding to SPRED, perhaps interaction with c-Kit contributes to SPRED membrane localization and allows growth factor-dependent regulation of SPRED membrane translocation.

Protein expression pattern

The mild phenotypes of both *SPRED1* and *SPRED2* knockout mice (discussed below) suggest some redundancy between the two proteins. RNA expression profiling shows that *SPRED1* and *SPRED2* have overlapping expressions and are both widely expressed across human tissues (Fig. 4A; The Genotype Tissue Expression Project, <https://www.gtexportal.org/home>). Protein expression profiling through immunohistochemistry staining of various human tissue sections shows similar expression distribution (Fig. 4B; The Human Protein Atlas, <https://www.proteinatlas.org>). In contrast, RNA expression profiling shows that, unlike *SPRED1* and *SPRED2*, *SPRED3* expression is limited to the brain, pituitary gland, and breast (Fig. 4A; The Genotype Expression Project, <https://www.gtexportal.org/home>; Kato et al. 2003).

In addition, expression patterns of SPRED proteins change during development. Comparing protein levels in fetal and adult mouse tissue lysates, SPRED1 is expressed in fetal liver, brain, and heart, whereas SPRED2 is not detected in these fetal tissues (Engelhardt et al. 2004). This suggests that SPRED1 may be more functionally important during fetal development. This is in agreement with the observations that germline mutations in *SPRED1* result in the developmental disorder Legius syndrome (LS) as discussed below, whereas mutations in *SPRED2* have never been reported in this disease.

Transcriptional regulation

Ras signaling pathways control gene expression through phosphorylation and regulation of transcription factors, coregulatory proteins, and chromatin proteins (Whitmarsh 2007). Levels of Ras–MAPK activation are tightly controlled by ERK-regulated transcriptional regulation of negative feedback regulators (Pratilas et al. 2009; Dry et al. 2010). For example, *SPROUTY1*, *SPROUTY2*, and *SPROUTY4* expression is known to be positively regulated by ERK signaling resulting from RTK activation (Ozaki et al. 2001; Packer et al. 2009). Recently, *SPRED1* and *SPRED2* expression has been shown to be positively

Figure 3. Model for neurofibromin membrane recruitment through SPRED1 interaction. (A) SPRED1 EVH-1 domain directly binds to the GAPex domain of neurofibromin GRD. SPRED1 KBD interacts with c-Kit. SPRED1 SPR is membrane anchored through palmitoylation. Neurofibromin GAPex interacts with Ras. (B) Overall structure of the complex formed by GMPPNP-bound KRAS (green), neurofibromin GRD (blue), and SPRED1 EVH-1 (pink) (PDB:6V65). (C) Ortholog alignment of SPRED1 EVH domain with LS mutations that disrupt SPRED1-neurofibromin binding in red. (D) Ortholog alignment of neurofibromin GAPex with NF1 mutations that disrupt SPRED1-neurofibromin binding in red. (E) Mapping of pathogenic mutations in SPRED1 (pink) and NF1 (green) on the neurofibromin-SPRED1 complex (PDB:6V65).

regulated by Ras–MAPK signaling in melanoma and GIST cell lines (Xie et al. 2018). Pharmacological inhibition of the Ras pathway, either with MEK, c-Kit, or RAF inhibitors, resulted in decreased *SPRED* expression 6 h after treatment and remained down-regulated at 24 h (Packer et al. 2009; Pratilas et al. 2009; Xie et al. 2018). In these cells, *SPRED* expression is regulated by ETV1, a member of the Ets family of transcription factors. ETV1 is overexpressed in melanoma (Jané-Valbuena et al. 2010) and gastrointestinal stromal tumors (Ran et al. 2015). ETV1 transcription activity is stimulated by Ras–MAPK activation where Erk1-mediated phosphorylation regulated ETV1 protein stability (Janknecht 1996; Bosc et al. 2001; Oh et al. 2013). Finally, sequencing of tumor tissues and cancer cell lines demonstrated a correlation between BRAF V600E or KRAS G12 mutations with high *SPRED1* mRNA expression (Cerami et al. 2012; Figshare, https://figshare.com/articles/dataset/DepMap_20Q2_Public/12280541).

Genetic models

Mouse models

SPRED1^{−/−} mice were generated by deleting exons encoding for KBD and SPR domains. These mice were generally healthy and fertile despite having lower body weight and a

shortened face (Inoue et al. 2005). Mice exhibited increased pigmentation in ears, paw pads, and tails (Tadokoro et al. 2018). Mice lacking *SPRED1* demonstrated cognitive impairments in hippocampus-dependent visuo-spatial memory (Denayer et al. 2008). *SPRED1*^{−/−} mice displayed learning deficits in the Morris water maze and T-maze tests (Denayer et al. 2008). In cognitively more demanding stages of the T-maze, *SPRED1*^{+/−} mice performed at an intermediate level between wild-type and *SPRED1*^{−/−} littermates (Denayer et al. 2008). The cognitive and synaptic plasticity phenotype in *SPRED1*^{−/−} mice were remarkably similar to *NF1*^{+/−} mice, where treatment with statins, which interfere with Ras membrane anchoring and activation, acutely rescued the learning and synaptic plasticity defects (Cui et al. 2008). Similarly, learning difficulties have been reported in Legius syndrome (Brems et al. 2007; Pasmant et al. 2009) and NF1 patients (Costa et al. 2002; Hyman et al. 2005), where deficits visuo-spatial and visual constructive skills are considered hallmarks of NF1. *SPRED1*^{−/−} mice also exhibited allergen-induced airway eosinophilia indicating that *SPRED1* may negatively regulate IL-5-mediated eosinophil proliferation, possibly by inhibiting Ras–MAPK signaling (Inoue et al. 2005).

SPRED2^{−/−} mice were generated using a gene trap inserted between exons 4 and 5 of *SPRED2*. These mice appeared healthy and fertile despite having reduced body

Figure 4. RNA and protein expression of SPRED proteins. (A) RNA expression of SPRED1, SPRED2, and SPRED3 across tissues from The Genotype Tissue Expression (GTEx) Project (<https://www.gtexportal.org/home>). (B) Protein expression of SPRED1 and SPRED2 across tissues from The Human Protein Atlas (<https://www.proteinatlas.org>).

length and weight, and had smaller skeletons compared with wild-type littermates (Tuduce et al. 2010). *SPRED2*^{-/-} mice were smaller from birth and remained smaller throughout their lifetime, consistent with achondroplasia (dwarfism), a condition caused by activating mutations in *FGFR3*. FGF and *FGFR3* signaling play major roles in the regulation of bone growth in both mice and humans. Indeed, *FGFR3*-induced Ras–MAPK activity is a negative regulator of bone growth (Colvin et al. 1996). Loss of *SPRED2* in mice chondrocytes resulted in increased ERK1/2 phosphorylation in response to FGF, which likely inhibited chondrocyte differentiation and consequently bone growth (Bundschiu et al. 2005). In another *SPRED2*^{-/-} mouse model, generated by deleting exons encoding for KBD and SPR domain, adult mice appeared healthy with no apparent abnormalities in most organs (Nobuhisa et al. 2004). However, *SPRED2* loss resulted in a significant increase in megakaryocytes, granulocytes, and hematopoietic cells (Nobuhisa et al. 2004). These results suggest that *SPRED2* regulates hematopoiesis in the aorta-gonad-mesonephros by regulating c-Kit-mediated Ras–MAPK signaling during hematopoiesis.

The relatively mild phenotype of both *SPRED1* and *SPRED2* knockout mice may be due to redundancy when they are coexpressed in various organs. Double knockout of *SPRED1* and *SPRED2*, generated by backcrossing *SPRED1*^{+/-} and *SPRED2*^{+/-} animals, resulted in embryonic lethality (Taniguchi et al. 2007a). *SPRED1*^{+/-};

SPRED2^{+/-} double heterozygotes were healthy and fertile. *SPRED1*^{+/-}; *SPRED2*^{-/-} were smaller, consistent with the dwarfism phenotype of *SPRED2* knockout mice. *SPRED1*^{-/-}; *SPRED2*^{+/-} were born at severely sub-Mendelian frequencies, but several natal mice appeared sick and often died within a few months from uncharacterized causes. Most *SPRED1*^{-/-}; *SPRED2*^{-/-} embryos died due to severe subcutaneous hemorrhage and edema between E12.5 to E15.5. This phenotype resembles mouse embryos lacking Syk, SLP-76, or PLCγ-2, which are hematopoietic intracellular signaling proteins important for separation of lymphatic vessels from blood vessels (Abtashian et al. 2003). Together, these suggest that lymphatic vessel development is abnormal in *SPRED1/2* double-knockout embryos. In vitro, lymphatic endothelial cells from *SPRED1/2* double knockout embryos proliferated more than wild-type cells. These lymphatic endothelial cells also exhibited stronger ERK activation following VEGF-C stimulation compared with wild type (Taniguchi et al. 2007a). Together, these results suggest that *SPRED1* and *SPRED2* are critical for the separation of lymphatic vessels from the parental vein by regulating VEGFR-3 induced Ras activation. Interestingly, lymphatic abnormalities commonly occur in patients with RASopathies, which further highlights the role of Ras–MAPK signaling in lymphatic vessels (Sevick-Muraca and King 2014).

Despite having a shared SPR domain, *SPRED* knockout mouse models have a distinct phenotype from *SPROUTY* knockout mouse models, except shared features with *SPRED2* and *SPROUTY4* knockout, both of which develop dwarfism (summarized in Table 2).

Drosophila models

Only one homolog of *SPRED* has been identified in *Drosophila*. Habituation deficits were reported in flies with neuron-specific knockdown of *SPRED* (Fenckova et al. 2019). Habituation is a form of learning that allows an organism's initial response to gradually decline following frequently repeated stimuli (Kimmel 1973). In humans, habituation deficits have been reported in behavioral disorders (Swartz et al. 2013; Ethridge et al. 2016). This phenotype relates to the behavioral deficits observed in *SPRED1* knockout mice and Legius syndrome patients. Furthermore, knockdown of neurofibromin or expression of constitutively active Ras mutant recapitulated habituation deficiencies resulting from *SPRED1* loss. Additionally, *SPRED* is involved in photoreceptor cell specification in *Drosophila* eye development (Demille et al. 1996); however, the role of *SPRED* in *Drosophila* development has not yet been elucidated.

Disease

Developmental disorders

Legius syndrome (LS) is an autosomal dominant condition characterized by multiple café au lait spots, axillary freckling, macrocephaly, and Noonan-like craniofacial

Table 2. *SPRED* and *Sprouty* mouse models

Genotype	Phenotype	References
<i>SPRED1</i> ^{-/-}	Shortened face, lower body weight, and allergen-induced airway eosinophilia from IL-5-mediated eosinophil proliferation	Brems et al. 2007
<i>SPRED2</i> ^{-/-}	Spatial learning and memory defects from impaired hippocampal functions	Denayer et al. 2008
	Pigmentary abnormalities and shortened face	Tadokoro et al. 2018
	Dwarfism from increased FGFR signaling in chondrocytes	Bundschi et al. 2005
	Increase proliferation of megakaryocytes, granulocytes, and hematopoietic cells	Nobuhisa et al. 2004
<i>SPRED1</i> ^{-/-} <i>SPRED2</i> ^{-/-}	Embryonic lethal between E12.5–E15.5 due to severe subcutaneous hemorrhage and edema	Taniguchi et al. 2007a
<i>SPROUTY1</i> ^{-/-}	Kidney and ureteric bud defects from improper branching morphogenesis	Basson et al. 2005, 2006
<i>SPROUTY2</i> ^{-/-}	Viable with shortened life span (median survival 3–4 wk) and hearing loss from perturbations in organ of Corti cytoarchitecture	Shim et al. 2005
<i>SPROUTY1</i> ^{-/-} <i>SPROUTY2</i> ^{-/-}	Viable with shortened life span and severe gastrointestinal phenotype characterized by enteric nerve dysplasia due to GDNF hyperactivity	Taketomi et al. 2005
	Highly disorganized palatal rugae from disrupted submandibular gland epithelial development	Economou et al. 2012; Knosp et al. 2015
<i>SPROUTY4</i> ^{-/-}	Dwarfism and polysyndactyly from hyperactivation of FGF signaling	Taniguchi et al. 2007b
<i>SPROUTY2</i> ^{-/-}	Embryonic lethal by E12.5 with craniofacial, limb, and lung morphogenesis abnormalities	Taniguchi et al. 2007b, 2009
<i>SPROUTY2</i> ^{+/-} <i>SPROUTY4</i> ^{-/-}	Tusk-like incisor in the lower jaw due to presence of enamel on lingual surface	Boran et al. 2009; Klein et al. 2008

dysmorphia in some individuals. Other frequently observed features are learning and behavioral problems (Brems et al. 2007; Pasmant et al. 2009). The disorder is caused by loss-of-function mutations in the *SPRED1* gene. The clinical phenotype of LS patients resembles a mild version of neurofibromatosis type I (NF1). NF1 is caused by loss-of-function mutations in the *NF1* gene. Some of the phenotypes associated with NF1 are due to haploinsufficiency, such as developmental problems and learning disorders. Others are caused by loss of the wild-type allele to generate clonal growth of café au lait macules and benign neurofibromas, some of which often progress to malignant tumors of the peripheral and central nervous system (Riccardi 1992). Both NF1 and LS are RASopathies; syndromes caused by germline mutations in genes coding for proteins that regulate the Ras–MAPK pathway resulting in hyperactive signaling (Tidyman and Rauen 2009).

Sequencing of LS syndrome patients identified that most *SPRED1* mutations result in protein truncations, although missense mutations and deletions also occur (Fig. 5A; Brems et al. 2007; Brems and Legius 2013). Biochemical analysis of some EVH-1 missense mutations showed disruption of the interaction with neurofibromin, while SPR mutations fail to localize to the membrane (Stowe et al. 2012; Hirata et al. 2016; Führer et al. 2017). However, missense mutations also map to regions of *SPRED1* whose functions in regulating Ras are not yet clear (Fig. 5B; Sumner et al. 2011).

Consistent with pigmentation abnormalities found in knockout mice (Tadokoro et al. 2018), café au lait macules occur in all LS patients. Development of café au lait macules requires a second-hit mutation on the wild-type *SPRED1* allele, such that the melanocytes are devoid of *SPRED1* (Brems et al. 2007). These findings are consistent

with café au lait macules in NF1 patients that also involve biallelic *NF1* loss (Schepper et al. 2008).

Mutations in *SPRED2* and *SPRED3* have not been reported to cause developmental disorders in humans. Due to the overlapping functions of *SPRED* family proteins, it is likely that loss of function of either *SPRED2* or *SPRED3* does not cause a developmental phenotype because the other family members compensate for this loss. However, the emerging use of high-throughput sequencing in clinical genetics could reveal syndromes caused by these genes. Perhaps *SPRED2* mutations could be associated with mild

Figure 5. *SPRED1* mutations found in Legius syndrome. (A) Frequency of each *SPRED1* mutation variants found in Legius syndrome patients (http://arup.utah.edu/database/SPRED1/SPRED1_welcome.php). (B) Missense mutations identified in patients with Legius syndrome.

achondroplasia since loss of *SPRED2* in mice results in dwarfism (Bundschu et al. 2005).

Cancer

Hyperactivation of Ras–MAPK signaling contributes to the proliferation, survival, metastasis, and many other traits characteristic of tumor cells. Therefore, negative regulators of this pathway have been observed to protect against tumorigenesis. SPRED and Sprouty proteins are putative tumor suppressors due to their ability to down-regulate Ras–MAPK signaling (Kawazoe and Taniguchi 2019). By binding and translocating neurofibromin to the plasma membrane, SPRED may play a critical role in regulating cellular levels of Ras-GTP in many contexts (Stowe et al. 2012). Hence, loss of SPRED in tumor tissue likely permits hyperactivation of Ras signaling, which contributes to driving tumor growth and invasion.

SPRED1 mutations occur in ~2% of cancers (TCGA). The most common alterations are deletions, truncations, and missense mutations (Fig. 6A). Currently, there are no known mutational hotspots for *SPRED1* (Fig. 6B); however, biochemical studies have shown that some EVH-1 and SPR missense mutations result in defective

neurofibromin binding or membrane localization (Dunzendorfer-Matt et al. 2016; Hirata et al. 2016). Furthermore, some of the *SPRED1* missense mutations in cancer have also been found in Legius syndrome. Similarly, there are no known mutational hotspots for *SPRED2* (Fig. 6C). Further biochemical characterization is necessary to determine the biological significance of these mutations. Finally, the lower frequency of *SPRED3* mutations in cancer may relate to its restricted tissue expression (Figs. 4A, 6D).

Individuals with Legius syndrome have a predisposition for pediatric leukemia resulting from loss of heterozygosity (Pasmant et al. 2009). Likewise, NF1 is associated with a predisposition to juvenile myelomonocytic leukemia (JMML) and acute lymphoblastic leukemia (ALL) (Stiller et al. 1994), since loss of SPRED1 binding partner, neurofibromin, also results in a similar hyperactivation of Ras signaling. Furthermore, *SPRED1* somatic mutations occurred in 2% of pediatric acute leukemias, and *SPRED1* expression was significantly decreased in pediatric AML and T-ALL (Pasmant et al. 2015). In those samples, decreased SPRED1 protein and RNA levels correlated to high ERK phosphorylation (Pasmant et al. 2015).

SPRED1 loss was also found as a driver of mucosal melanoma where 37% of mucosal melanomas examined

Figure 6. Mutations in SPRED proteins in human cancers. (A) Genetic alterations in SPREDs found in cancer (adapted from data from cBioPortal, <https://www.cbioportal.org>). (B) Truncating (top) and missense (bottom) mutations in SPRED1 found in cancer (TCGA; <https://portal.gdc.cancer.gov>). (C) Truncating (top) and missense (bottom) mutations in SPRED2 found in cancer (TCGA). (D) Truncating (top) and missense (bottom) mutations in SPRED3 found in cancer (TCGA).

showed *SPRED1* loss of function (Ablain et al. 2018). In most cases, melanomas were triple wild type for *BRAF*, *RAS*, and *NF1*. Notably, *c-KIT* activating alterations co-occurred in 30% of cases with *SPRED1* loss (Ablain et al. 2018). This suggests that *SPRED1* loss may cooperate with oncogenic events that activate the Ras–MAPK pathway.

In other cancers, although *SPRED1* is not mutated, *SPRED1* mRNA and/or protein levels are reduced (Yoshida et al. 2006; Kachroo et al. 2013; Wang et al. 2017; Jiang et al. 2018). In oral squamous cell carcinoma tumor tissues, *SPRED1* is significantly down-regulated due to the up-regulation of miR182 in malignant tissues that directly targets *SPRED1* (Wang et al. 2017). Likewise, *SPRED1* is significantly down-regulated in ER⁺ breast cancer due to the up-regulation of miR-196a that directly targets *SPRED1* (Jiang et al. 2018). In hepatocellular carcinoma, *SPRED1* and *SPRED2* were simultaneously down-regulated tumor tissue compared with noncancerous tissue (Yoshida et al. 2006). In prostate cancer, *SPRED2* expression levels were significantly reduced in tumor tissue relative to benign glands, and *SPRED2* expression was significantly down-regulated in grades 4 and 5 tumors (Kachroo et al. 2013). In all cases, down-regulation of *SPRED* correlated with increased ERK phosphorylation.

SPRED1 and *NF1* mutations tend to be mutually exclusive with gain-of-function *EGFR* mutations in lung adenocarcinoma (Collisson et al. 2014). In a model system expressing oncogenic *EGFR* L858R, *SPRED1* was highly phosphorylated at S105 (Yan et al. 2020), which is in close proximity to T102, which is mutated to arginine in LS (Messiaen et al. 2009). Mutation of *SPRED1* S105 resulted in disruption in neurofibromin-*SPRED1* binding and augmented cell proliferation in K562 cells (Yan et al. 2020). Furthermore, *SPRED1* S105 phosphorylation was found to be elevated in four additional *EGFR*-mutant cancer cell lines (PC9, U2OS, A431, and H1975) (Yan et al. 2020). To date, this is the only known mechanism by which RTK signaling directly regulates neurofibromin-*SPRED1* interaction. Thus, it raises the possibility that *SPRED1* function is normally regulated in response to signals to modulate Ras-GTP levels and downstream signal transduction.

Summary and future directions

SPRED proteins are crucial regulators of Ras–MAPK signaling. Although *SPRED* and Sprouty proteins share an SPR domain, each family regulates Ras through distinct yet complementary mechanisms. Sprouty limits GTP loading of Ras, while *SPRED* promotes Ras-GTP hydrolysis. Despite these findings, numerous unresolved questions remain regarding the function, regulation, interactions, and signaling control of *SPRED* proteins.

While the role of the EVH-1 and SPR domains in recruitment of neurofibromin to the membrane are clear, the role of the KBD in *SPRED* is not well understood. Specifically, what is the context of c-Kit binding to *SPRED*? Can *SPRED* bind other RTKs besides c-Kit? Is c-Kit binding

necessary for efficient Ras inhibition? Does *SPRED* binding inhibit c-Kit signaling, especially Ras activation? Furthermore, c-Kit signaling is important for melanocyte development. Café au lait macules and axillary freckling are features of Legius syndrome and *NF1* that affect melanocytes. Thus, is the interaction between *SPRED* and c-Kit what limits Legius syndrome phenotype to melanocytes, compared with other tissues, as in *NF1*?

The regulation of *SPRED* and neurofibromin binding needs to be further defined. Signals that regulate *SPRED* binding to the plasma membrane are not yet known. Sprouty proteins are regulated by phosphorylation and dephosphorylation of a conserved tyrosine. Hence, it seems likely that *SPRED* activity is regulated by phosphorylation, as well as dimerization and association with other protein partners. At present, little is known about the physiological functions of *SPRED3*. *SPRED3* has the least sequence similarity to other *SPRED* proteins and exhibits reduced binding to neurofibromin and does not bind c-Kit (Kato et al. 2003; Hirata et al. 2016).

Generation of *SPRED3* knockout mice is important to understand its biological function and further inform shared mechanisms of regulation for *SPRED* proteins. Understanding how *SPRED* proteins are regulated and in what signaling context will help the development of new therapies for RASopathies and cancers resulting from hyperactive Ras signaling.

Competing interest statement

F.M. is a consultant for the following companies: Aduro Biotech; Amgen; Daiichi Ltd.; Ideaya Biosciences; Kura Oncology; Leidos Biomedical Research, Inc.; PellePharm; Pfizer, Inc.; PMV Pharma; Portola Pharmaceuticals; and Quanta Therapeutics. F.M. is a consultant and cofounder for the following companies (with ownership interest, including stock options): BridgeBio; DNATRIX, Inc.; Olema Pharmaceuticals, Inc.; and Quartz. F.M. is Scientific Director of the NCI Ras Initiative at Frederick National Laboratory for Cancer Research/Leidos Biomedical Research, Inc.

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References

- Ablain J, Xu M, Rothschild H, Jordan RC, Mito JK, Daniels BH, Bell CF, Joseph NM, Wu H, Bastian BC, et al. 2018. Human tumor genomics and zebrafish modeling identify *SPRED1* loss as a driver of mucosal melanoma. *Science* **362**: 1055–1060. doi:10.1126/science.aau6509
- Abtahian F, Guerriero A, Sebzda E, Lu M-M, Zhou R, Mocsai A, Myers EE, Huang B, Jackson DG, Ferrari VA, et al. 2003. Regulation of blood and lymphatic vascular separation by signaling proteins SLP-76 and Syk. *Science* **299**: 247–251. doi:10.1126/science.1079477

- Ball LJ, Jarchau T, Oschkinat H, Walter U. 2002. EVH1 domains: structure, function and interactions. *Febs Lett* **513**: 45–52. doi:10.1016/S0014-5793(01)03291-4
- Basson MA, Akbulut S, Watson-Johnson J, Simon R, Carroll TJ, Shakya R, Gross I, Martin GR, Lufkin T, McMahon AP, et al. 2005. Sprouty1 is a critical regulator of GDNF/RET-mediated kidney induction. *Dev Cell* **8**: 229–239. doi:10.1016/j.devcel.2004.12.004
- Basson MA, Watson-Johnson J, Shakya R, Akbulut S, Hyink D, Costantini FD, Wilson PD, Mason IJ, Licht JD. 2006. Branching morphogenesis of the ureteric epithelium during kidney development is coordinated by the opposing functions of GDNF and Sprouty1. *Dev Biol* **299**: 466–477. doi:10.1016/j.ydbio.2006.08.051
- Bastiani M, Parton RG. 2010. Caveolae at a glance. *J Cell Sci* **123**: 3831–3836. doi:10.1242/jcs.070102
- Boran T, Peterkova R, Lesot H, Lyons DB, Peterka M, Klein OD. 2009. Temporal analysis of ectopic enamel production in incisors from sprouty mutant mice. *J Exp Zoology Part B Mol Dev Evol* **312B**: 473–485. doi:10.1002/jez.b.21254
- Bosc DG, Goueli BS, Janknecht R. 2001. HER2/Neu-mediated activation of the ETS transcription factor ER81 and its target gene MMP-1. *Oncogene* **20**: 6215–6224. doi:10.1038/sj.onc.1204820
- Brems H, Legius E. 2013. Legius syndrome, an update. molecular pathology of mutations in SPRED1. *Keio J Medicine* **62**: 107–112. doi:10.2302/kjm.2013-0002-RE
- Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R, Somers R, Messiaen L, Schepper SD, Fryns J-P, et al. 2007. Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. *Nat Genet* **39**: 1120–1126. doi:10.1038/ng2113
- Buday L, Downward J. 1993. Epidermal growth factor regulates p21^{ras} through the formation of a complex of receptor, Grb2 adapter protein, and SOS nucleotide exchange factor. *Cell* **73**: 611–620. doi:10.1016/0092-8674(93)90146-H
- Bundschu K, Knobloch K-P, Ullrich M, Schinke T, Amling M, Engelhardt CM, Renné T, Walter U, Schuh K. 2005. Gene disruption of *spred-2* causes dwarfism. *J Biol Chem* **280**: 28572–28580. doi:10.1074/jbc.M503640200
- Butland SL, Sanders SS, Schmidt ME, Riechers S-P, Lin DTS, Martin DDO, Vaid K, Graham RK, Singaraja RR, Wanker EE, et al. 2014. The palmitoyl acyltransferase HIP14 shares a high proportion of interactors with huntingtin: implications for a role in the pathogenesis of Huntington's disease. *Hum Mol Genet* **23**: 4142–4160. doi:10.1093/hmg/ddu137
- Casici T, Vinós J, Freeman M. 1999. Sprouty, an intracellular inhibitor of Ras signaling. *Cell* **96**: 655–665. doi:10.1016/S0092-8674(00)80576-0
- Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, et al. 2012. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* **2**: 401–404. doi:10.1158/2159-8290.CD-12-0095
- Collisson EA, Campbell JD, Brooks AN, Berger AH, Lee W, Chmielecki J, Beer DG, Cope L, Creighton CJ, Danilova L, et al. 2014. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* **511**: 543–550. doi:10.1038/nature13385
- Colvin JS, Bohne BA, Harding GW, McEwen DG, Ornitz DM. 1996. Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. *Nat Genet* **12**: 390–397. doi:10.1038/ng0496-390
- Costa RM, Federov NB, Kogan JH, Murphy GG, Stern J, Ohno M, Kuchelapati R, Jacks T, Silva AJ. 2002. Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. *Nature* **415**: 526–530. doi:10.1038/nature711
- Cui Y, Costa RM, Murphy GG, Elgersma Y, Zhu Y, Gutmann DH, Parada LF, Mody I, Silva AJ. 2008. Neurofibromin regulation of ERK signaling modulates GABA release and learning. *Cell* **135**: 549–560. doi:10.1016/j.cell.2008.09.060
- Demille MMC, Kimmel BE, Rubin GM. 1996. A *Drosophila* gene regulated by rough and glass shows similarity to *ena* and *VASP*. *Gene* **183**: 103–108. doi:10.1016/S0378-1119(96)00506-9
- Denayer E, Ahmed T, Brems H, Woerden GV, Borgesius NZ, Callaerts-Vegh Z, Yoshimura A, Hartmann D, Elgersma Y, D'Hooze R, et al. 2008. *Spred1* is required for synaptic plasticity and hippocampus-dependent learning. *J Neurosci* **28**: 14443–14449. doi:10.1523/JNEUROSCI.4698-08.2008
- Dry JR, Pavey S, Pratilas CA, Harbron C, Runswick S, Hodgson D, Chresta C, McCormack R, Byrne N, Cockerill M, et al. 2010. Transcriptional pathway signatures predict MEK addiction and response to selumetinib (AZD6244). *Cancer Res* **70**: 2264–2273. doi:10.1158/0008-5472.CAN-09-1577
- Dunzendorfer-Matt T, Mercado EL, Maly K, McCormick F, Scheffzek K. 2016. The neurofibromin recruitment factor *Spred1* binds to the GAP related domain without affecting Ras inactivation. *Proc Natl Acad Sci* **113**: 7497–7502. doi:10.1073/pnas.1607298113
- Economou AD, Ohazama A, Porntaveetus T, Sharpe PT, Kondo S, Basson MA, Gritli-Linde A, Cobourne MT, Green JBA. 2012. Periodic stripe formation by a Turing mechanism operating at growth zones in the mammalian palate. *Nat Genet* **44**: 348–351. doi:10.1038/ng.1090
- Engelhardt CM, Bundschu K, Messerschmitt M, Renné T, Walter U, Reinhard M, Schuh K. 2004. Expression and subcellular localization of *spred* proteins in mouse and human tissues. *Histochem Cell Biol* **122**: 527–538. doi:10.1007/s00418-004-0725-6
- Ethridge LE, White SP, Mosconi MW, Wang J, Byerly MJ, Sweeney JA. 2016. Reduced habituation of auditory evoked potentials indicate cortical hyper-excitability in fragile X syndrome. *Transl Psychiat* **6**: e787. doi:10.1038/tp.2016.48
- Fenckova M, Blok LER, Asztalos L, Goodman DP, Cizek P, Singgih EL, Glennon JC, Int'Hout J, Zweier C, Eichler EE, et al. 2019. Habituation learning is a widely affected mechanism in *Drosophila* models of intellectual disability and autism spectrum disorders. *Biol Psychiat* **86**: 294–305. doi:10.1016/j.biopsych.2019.04.029
- Führer S, Ahammer L, Ausserbichler A, Scheffzek K, Dunzendorfer-Matt T, Tollinger M. 2017. NMR resonance assignments of the EVH1 domain of neurofibromin's recruitment factor *Spred1*. *Biomol Nmr Assignm* **11**: 305–308. doi:10.1007/s12104-017-9768-1
- Hacohen N, Kramer S, Sutherland D, Hiromi Y, Krasnow MA. 1998. Sprouty encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airways. *Cell* **92**: 253–263. doi:10.1016/S0092-8674(00)80919-8
- Hall AB, Jura N, DaSilva J, Jang YJ, Gong D, Bar-Sagi D. 2003. *Hspry2* is targeted to the ubiquitin-dependent proteasome pathway by c-Cbl. *Curr Biol* **13**: 308–314. doi:10.1016/S0960-9822(03)00086-1
- Hanafusa H, Torii S, Yasunaga T, Nishida E. 2002. Sprouty1 and Sprouty2 provide a control mechanism for the Ras/MAPK signalling pathway. *Nat Cell Biol* **4**: 850–858. doi:10.1038/ncb867
- Hanafusa H, Torii S, Yasunaga T, Matsumoto K, Nishida E. 2004. *Shp2*, an SH2-containing protein-tyrosine phosphatase, positively regulates receptor tyrosine kinase signaling by dephosphorylating and inactivating the inhibitor Sprouty. *J Biol Chem* **279**: 22992–22995. doi:10.1074/jbc.M312498200

- Harmer NJ, Sivak JM, Amaya E, Blundell TL. 2005. 1.15 Å crystal structure of the *X. tropicalis* Spred1 EVH1 domain suggests a fourth distinct peptide-binding mechanism within the EVH1 family. *Febs Lett* **579**: 1161–1166. doi:10.1016/j.febslet.2004.11.114
- Hirata Y, Brems H, Suzuki M, Kanamori M, Okada M, Morita R, Llano-Rivas I, Ose T, Messiaen L, Legius E, et al. 2016. Interaction between a domain of the negative regulator of the Ras-ERK pathway, SPRED1 protein, and the GTPase-activating protein-related domain of neurofibromin Is implicated in Legius syndrome and neurofibromatosis type 1. *J Biol Chem* **291**: 3124–3134. doi:10.1074/jbc.M115.703710
- Hyman SL, Shores A, North KN. 2005. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. *Neurology* **65**: 1037–1044. doi:10.1212/01.wnl.0000179303.72345.ce
- Impagnatiello MA, Weitzer S, Gannon G, Compagni A, Cotten M, Christofori G. 2001. Mammalian sprouty-1 and -2 are membrane-anchored phosphoprotein inhibitors of growth factor signaling in endothelial cells. *J Cell Biol* **152**: 1087–1098. doi:10.1083/jcb.152.5.1087
- Inoue H, Kato R, Fukuyama S, Nonami A, Taniguchi K, Matsumoto K, Nakano T, Tsuda M, Matsumura M, Kubo M, et al. 2005. Spred-1 negatively regulates allergen-induced airway eosinophilia and hyperresponsiveness. *J Exp Medicine* **201**: 73–82. doi:10.1084/jem.20040616
- Jané-Valbuena J, Widlund HR, Perner S, Johnson LA, Dibner AC, Lin WM, Baker AC, Nazarian RM, Vijayendran KG, Sellers WR, et al. 2010. An oncogenic role for *ETV1* in melanoma. *Cancer Res* **70**: 2075–2084. doi:10.1158/0008-5472.CAN-09-3092
- Janknecht R. 1996. Analysis of the ERK-stimulated ETS transcription factor ER81. *Mol Cell Biol* **16**: 1550–1556.
- Jiang C-F, Shi Z-M, Li D-M, Qian Y-C, Ren Y, Bai X-M, Xie Y-X, Wang L, Ge X, Liu W-T, et al. 2018. Estrogen-induced miR-196a elevation promotes tumor growth and metastasis via targeting SPRED1 in breast cancer. *Mol Cancer* **17**: 83. doi:10.1186/s12943-018-0830-0
- Kachroo N, Valencia T, Warren AY, Gnanapragasam VJ. 2013. Evidence for downregulation of the negative regulator SPRED2 in clinical prostate cancer. *Brit J Cancer* **108**: 597–601. doi:10.1038/bjc.2012.507
- Kato R, Nonami A, Taketomi T, Wakioka T, Kuroiwa A, Matsuda Y, Yoshimura A. 2003. Molecular cloning of mammalian Spred-3 which suppresses tyrosine kinase-mediated Erk activation. *Biochem Biophys Res Commun* **302**: 767–772. doi:10.1016/S0006-291X(03)00259-6
- Kawazoe T, Taniguchi K. 2019. The sprouty/spred family as tumor suppressors: coming of age. *Cancer Sci* **110**: 1525–1535. doi:10.1111/cas.13999
- Kimmel HD. 1973. Habituation, habitability, and conditioning. In *Habituation: behavioral studies, volume 1* (ed. Peeke HVS, Herz MJ), pp. 219–238. Academic Press, New York.
- King JAJ, Straffon AFL, D'Abaco GM, Poon CLC, ISTT, Smith CM, Buchert M, Corcoran NM, Hall NE, Callus BA, et al. 2005. Distinct requirements for the Sprouty domain for functional activity of Spred proteins. *Biochem J* **388**: 445–454. doi:10.1042/BJ20041284
- Klein OD, Lyons DB, Balooch G, Marshall GW, Basson MA, Peterka M, Boran T, Peterkova R, Martin GR. 2008. An FGF signaling loop sustains the generation of differentiated progeny from stem cells in mouse incisors. *Development* **135**: 377–385. doi:10.1242/dev.015081
- Knosp WM, Knox SM, Lombaert IMA, Haddox CL, Patel VN, Hoffman MP. 2015. Submandibular parasympathetic gangliogenesis requires Sprouty-dependent Wnt signals from epithelial progenitors. *Dev Cell* **32**: 667–677. doi:10.1016/j.devcel.2015.01.023
- Lim J, Yusoff P, Wong ESM, Chandramouli S, Lao D-H, Fong CW, Guy GR. 2002. The cysteine-rich Sprouty translocation domain targets mitogen-activated protein kinase inhibitory proteins to phosphatidylinositol 4,5-bisphosphate in plasma membranes. *Mol Cell Biol* **22**: 7953–7966. doi:10.1128/MCB.22.22.7953-7966.2002
- Martin GA, Viskochil D, Bollag G, McCabe PC, Crosier WJ, Haurbruck H, Conroy L, Clark R, O'Connell P, Cawthon RM, et al. 1990. The GAP-related domain of the neurofibromatosis type 1 gene product interacts with *ras* p21. *Cell* **63**: 843–849. doi:10.1016/0092-8674(90)90150-D
- Mason JM, Morrison DJ, Bassit B, Dimri M, Band H, Licht JD, Gross I. 2004. Tyrosine phosphorylation of sprouty proteins regulates their ability to inhibit growth factor signaling: a dual feedback loop. *Mol Biol Cell* **15**: 2176–2188. doi:10.1091/mbc.E03-07-0503
- Messiaen L, Yao S, Brems H, Callens T, Sathienkijkanchai A, Denayer E, Spencer E, Arn P, Babovic-Vuksanovic D, Bay C, et al. 2009. Clinical and mutational spectrum of neurofibromatosis type 1-like syndrome. *JAMA* **302**: 2111–2118. doi:10.1001/jama.2009.1663
- Nan X, Tamgüney TM, Collisson EA, Lin L-J, Pitt C, Galeas J, Lewis S, Gray JW, McCormick F, Chu S. 2015. Ras-GTP dimers activate the mitogen-activated protein kinase (MAPK) pathway. *Proc Natl Acad Sci* **112**: 7996–8001. doi:10.1073/pnas.1509123112
- Nobuhisa I, Kato R, Inoue H, Takizawa M, Okita K, Yoshimura A, Taga T. 2004. Spred-2 suppresses aorta-gonad-mesonephros hematopoiesis by inhibiting MAP kinase activation. *J Exp Medicine* **199**: 737–742. doi:10.1084/jem.20030830
- Nonami A, Taketomi T, Kimura A, Saeki K, Takaki H, Sanada T, Taniguchi K, Harada M, Kato R, Yoshimura A. 2005. The Sprouty-related protein, spred-1, localizes in a lipid raft/caveola and inhibits ERK activation in collaboration with caveolin-1. *Genes Cells* **10**: 887–895. doi:10.1111/j.1365-2443.2005.00886.x
- Oh S, Shin S, Lightfoot SA, Janknecht R. 2013. 14-3-3 proteins modulate the ETS transcription factor ETV1 in prostate cancer. *Cancer Res* **73**: 5110–5119. doi:10.1158/0008-5472.CAN-13-0578
- Ozaki K, Kadomoto R, Asato K, Tanimura S, Itoh N, Kohno M. 2001. ERK pathway positively regulates the expression of sprouty genes. *Biochem Biophys Res Commun* **285**: 1084–1088. doi:10.1006/bbrc.2001.5295
- Packer LM, East P, Reis-Filho JS, Marais R. 2009. Identification of direct transcriptional targets of ^{V600E}BRAF/MEK signalling in melanoma. *Pigm Cell Melanoma R* **22**: 785–798. doi:10.1111/j.1755-148X.2009.00618.x
- Pasmant E, Ballerini P, Lapillonne H, Perot C, Vidaud D, Leverger G, Landman-Parker J. 2009. SPRED1 disorder and predisposition to leukemia in children. *Blood* **114**: 1131. doi:10.1182/blood-2009-04-21850
- Pasmant E, Gilbert-Dussardier B, Petit A, de Laval B, Luscan A, Gruber A, Lapillonne H, Deswarte C, Goussard P, Laurendeau I, et al. 2015. SPRED1, a RAS MAPK pathway inhibitor that causes Legius syndrome, is a tumour suppressor downregulated in paediatric acute myeloblastic leukaemia. *Oncogene* **34**: 631–638. doi:10.1038/onc.2013.587
- Peterson FC, Volkman BF. 2014. Diversity of polyproline recognition by EVH1 domains. *Front Biosci* **14**: 833–846.
- Pratils CA, Taylor BS, Ye Q, Viale A, Sander C, Solit DB, Rosen N. 2009. V600EBRAF is associated with disabled feedback inhibition of RAF-MEK signaling and elevated transcriptional

- output of the pathway. *Proc Natl Acad Sci* **106**: 4519–4524. doi:10.1073/pnas.0900780106
- Ran L, Sirota I, Cao Z, Murphy D, Chen Y, Shukla S, Xie Y, Kaufmann MC, Gao D, Zhu S, et al. 2015. Combined inhibition of MAP kinase and KIT signaling synergistically destabilizes ETV1 and suppresses GIST tumor growth. *Cancer Discov* **5**: 304–315. doi:10.1158/2159-8290.CD-14-0985
- Riccardi VM. 1992. Piebaldism and neurofibromatosis 1. *Pediatr Dermatol* **10**: 288. doi:10.1016/0045-9380(92)90053-2
- Schepper SD, Maertens O, Callens T, Naeyaert J-M, Lambert J, Messiaen L. 2008. Somatic mutation analysis in NF1 café au lait spots reveals Two NF1 hits in the melanocytes. *J Invest Dermatol* **128**: 1050–1053. doi:10.1038/sj.jid.5701095
- Sevick-Muraca EM, King PD. 2014. Lymphatic vessel abnormalities arising from disorders of Ras signal transduction. *Trends Cardiovasc Med* **24**: 121–127. doi:10.1016/j.tcm.2013.09.004
- Sherekar M, Han S-W, Ghirlando R, Messing S, Drew M, Rabara D, Waybright T, Juneja P, O'Neill H, Stanley CB, et al. 2020. Biochemical and structural analyses reveal that the tumor suppressor neurofibromin (NF1) forms a high-affinity dimer. *J Biol Chem* **295**: 1105–1119. doi:10.1074/jbc.RA119.010934
- Shim K, Minowada G, Coling DE, Martin GR. 2005. Sprouty2, a mouse deafness gene, regulates cell fate decisions in the auditory sensory epithelium by antagonizing FGF signaling. *Dev Cell* **8**: 553–564. doi:10.1016/j.devcel.2005.02.009
- Siljamäki E, Abankwa D. 2016. SPRED1 interferes with K-ras but Not H-ras membrane anchorage and signaling. *Mol Cell Biol* **36**: 2612–2625. doi:10.1128/MCB.00191-16
- Simanshu DK, Nissley DV, McCormick F. 2017. RAS proteins and their regulators in human disease. *Cell* **170**: 17–33. doi:10.1016/j.cell.2017.06.009
- Stiller C, Chessells J, Fitchett M. 1994. Neurofibromatosis and childhood leukaemia/lymphoma: a population-based UKCCSG study. *Brit J Cancer* **70**: 969–972. doi:10.1038/bjc.1994.431
- Stowe IB, Mercado EL, Stowe TR, Bell EL, Osés-Prieto JA, Hernández H, Burlingame AL, McCormick F. 2012. A shared molecular mechanism underlies the human rasopathies legius syndrome and neurofibromatosis-1. *Gene Dev* **26**: 1421–1426. doi:10.1101/gad.190876.112
- Sumner K, Crockett DK, Muram T, Mallempati K, Best H, Mao R. 2011. The SPRED1 variants repository for Legius syndrome. *G3 Genes Genomes Genetics* **1**: 451–456.
- Swartz JR, Wiggins JL, Carrasco M, Lord C, Monk CS. 2013. Amygdala habituation and prefrontal functional connectivity in youth with autism spectrum disorders. *J Am Acad Child Adolesc Psychiatry* **52**: 84–93. doi:10.1016/j.jaac.2012.10.012
- Tadokoro Y, Hoshii T, Yamazaki S, Eto K, Ema H, Kobayashi M, Ueno M, Ohta K, Arai Y, Hara E, et al. 2018. Spred1 safeguards hematopoietic homeostasis against diet-induced systemic stress. *Cell Stem Cell* **22**: 713–725.e8. doi:10.1016/j.stem.2018.04.002
- Taketomi T, Yoshiga D, Taniguchi K, Kobayashi T, Nonami A, Kato R, Sasaki M, Sasaki A, Ishibashi H, Moriyama M, et al. 2005. Loss of mammalian Sprouty2 leads to enteric neuronal hyperplasia and esophageal achalasia. *Nat Neurosci* **8**: 855–857. doi:10.1038/nn1485
- Taniguchi K, Kohno R, Ayada T, Kato R, Ichiyama K, Morisada T, Oike Y, Yonemitsu Y, Maehara Y, Yoshimura A. 2007a. Spreds Are essential for embryonic lymphangiogenesis by regulating vascular endothelial growth factor receptor 3 signaling. *Mol Cell Biol* **27**: 4541–4550. doi:10.1128/MCB.01600-06
- Taniguchi K, Ayada T, Ichiyama K, Kohno R, Yonemitsu Y, Minami Y, Kikuchi A, Maehara Y, Yoshimura A. 2007b. Sprouty2 and Sprouty4 are essential for embryonic morphogenesis and regulation of FGF signaling. *Biochem Biophys Res Commun* **352**: 896–902. doi:10.1016/j.bbrc.2006.11.107
- Taniguchi K, Sasaki K, Watari K, Yasukawa H, Imaizumi T, Ayada T, Okamoto F, Ishizaki T, Kato R, Kohno R, et al. 2009. Suppression of *Sprouty2* has a therapeutic effect for a mouse model of ischemia by enhancing angiogenesis. *PLoS One* **4**: e5467. doi:10.1371/journal.pone.0005467
- Terrell EM, Durrant DE, Ritt DA, Sealover NE, Sheffels E, Spencer-Smith R, Esposito D, Zhou Y, Hancock JF, Kortum RL, et al. 2019. Distinct binding preferences between Ras and Raf family members and the impact on oncogenic Ras signaling. *Mol Cell* **76**: 872–884.e5. doi:10.1016/j.molcel.2019.09.004
- Tidyman WE, Rauen KA. 2009. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev* **19**: 230–236. doi:10.1016/j.gde.2009.04.001
- Tong J, Elowe S, Nash P, Pawson T. 2003. Manipulation of EphB2 regulatory motifs and SH2 binding sites switches MAPK signaling and biological activity. *J Biol Chem* **278**: 6111–6119. doi:10.1074/jbc.M208972200
- Tuduce IL, Schuh K, Bundschu K. 2010. Spred2 expression during mouse development. *Dev Dynam* **239**: 3072–3085. doi:10.1002/dvdy.22432
- Wakioka T, Sasaki A, Kato R, Shouda T, Matsumoto A, Miyoshi K, Tsuneoka M, Komiya S, Baron R, Yoshimura A. 2001. Spred is a sprouty-related suppressor of Ras signalling. *Nature* **412**: 647–651. doi:10.1038/35088082
- Wang J, Wang W, Li J, Wu L, Song M, Meng Q. 2017. Mir182 activates the Ras–MEK–ERK pathway in human oral cavity squamous cell carcinoma by suppressing RASA1 and SPRED1. *Oncotargets Ther* **Volume 10**: 667–679. doi:10.2147/OTT.S121864
- Whitmarsh AJ. 2007. Regulation of gene transcription by mitogen-activated protein kinase signaling pathways. *Biochimica Et Biophysica Acta Bba-Mol Cell Res* **1773**: 1285–1298. doi:10.1016/j.bbamcr.2006.11.011
- Wright LP, Philips MR. 2006. Thematic review series: lipid post-translational modifications. CAAX modification and membrane targeting of Ras. *J Lipid Res* **47**: 883–891. doi:10.1194/jlr.R600004-JLR200
- Xie Y, Cao Z, Wong EWP, Guan Y, Ma W, Zhang JQ, Walczak EG, Murphy D, Ran L, Sirota I, et al. 2018. COP1–DET1–ETS axis regulates ERK transcriptome and sensitivity to MAPK inhibitors. *J Clin Invest* **128**: 1442–1457. doi:10.1172/JCI94840
- Xu G, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, Dunn D, Stevens J, Gesteland R, White R, et al. 1990. The neurofibromatosis type 1 gene encodes a protein related to GAP. *Cell* **62**: 599–608. doi:10.1016/0092-8674(90)90024-9
- Yan W, Markegard E, Dharmiaiah S, Urisman A, Drew M, Esposito D, Scheffzek K, Nissley DV, McCormick F, Simanshu DK. 2020. Structural insights into the SPRED1–neurofibromin–KRAS complex and disruption of SPRED1–neurofibromin interaction by oncogenic EGFR. *Cell Rep* **32**: 107909. doi:10.1016/j.celrep.2020.107909
- Yoshida T, Hisamoto T, Akiba J, Koga H, Nakamura K, Tokunaga Y, Hanada S, Kumemura H, Maeyama M, Harada M, et al. 2006. Spreds, inhibitors of the Ras/ERK signal transduction, are dysregulated in human hepatocellular carcinoma and linked to the malignant phenotype of tumors. *Oncogene* **25**: 6056–6066. doi:10.1038/sj.onc.1209635
- Yusoff P, Lao D-H, Ong SH, Wong ESM, Lim J, Lo TL, Leong HF, Fong CW, Guy GR. 2002. Sprouty2 inhibits the Ras/MAP kinase pathway by inhibiting the activation of Raf. *J Biol Chem* **277**: 3195–3201. doi:10.1074/jbc.M108368200

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