

MINIREVIEW

Intermolecular Interactions of Sprouty Proteins and Their Implications in Development and Disease

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ABSTRACT

Receptor tyrosine kinase (RTK) signaling is spatially and temporally regulated by a number of positive and negative regulatory mechanisms. These regulatory mechanisms control the amplitude and duration of the signals initiated at the cell surface to have a normal or aberrant biological outcome in development and disease, respectively. In the past decade, the Sprouty (Spry) family of proteins has been identified as modulators of RTK signaling in normal development and disease. This review

summarizes recent advances concerning the biological activities modulated by Spry family proteins, their interactions with signaling proteins, and their involvement in cardiovascular diseases and cancer. The diversity of mechanisms in the regulation of Spry expression and activity in cell systems emphasizes the crucial role of Spry proteins in development and growth across the animal kingdom.

The Sprouty (Spry) protein was first described by Hacohen et al. (1998) as an inhibitor of fibroblast growth factor (FGF)-stimulated tracheal branching during *Drosophila melanogaster* development. Subsequent work established *D. melanogaster* Spry (dSpry) as a widespread inhibitor of receptor-tyrosine kinase (RTK) signaling during organogenesis. For example, *spry*-null flies or flies harboring loss of function mutations on *spry* exhibit eye and wing phenotypes indicative of uncontrolled epidermal growth factor receptor (EGFR) signaling (Minowada et al., 1999).

Four mammalian *spry* genes have been defined based on sequence similarity with *dSpry*. Three homologs of *dSpry* were first identified in a search of the human expressed

sequence tag database (<http://www.ncbi.nlm.nih.gov/dbEST/>) (Hacohen et al., 1998). The fourth mammalian *spry* homolog was originally discovered in mice (de Maximy et al., 1999). Although shorter than dSpry, all of the human homologs of Spry have a C-terminal cysteine-rich domain that is similar to the cognate domain within dSpry (Hacohen et al., 1998). However, similarity in their N termini is limited. The four human Spry proteins are products of different genes located on chromosomes 4q28.1 (*spry1*), 13q31.1 (*spry2*), Xq28/Yq12 (*spry3*), and 5q31.3 (*spry4*). Expression patterns for mammalian Spry isoforms were shown to be highly localized in embryos, some appearing near centers of FGF signaling (de Maximy et al., 1999; Minowada et al., 1999; Tefft et al., 1999; Chambers and Mason, 2000; Chambers et al., 2000; Zhang et al., 2001). These initial studies provided the first clues that growth factors may regulate the expression of Spry proteins.

Concerning the expression of Spry proteins, Su et al. (2002) performed large-scale gene analysis of both human and

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ABBREVIATIONS: Spry, Sprouty; RTK, receptor tyrosine kinase; FGF, fibroblast growth factor; FGFR, FGF receptor; EGF, epidermal growth factor; EGFR, EGF receptor; VEGF, vascular endothelial growth factor; ERK1/2, extracellular signal-regulated kinases 1/2; Cbl, Casitas b-lineage lymphoma proto-oncogene; PtdIns(4,5)P₂, phosphatidylinositol 4,5-bisphosphate; PTP1B, protein tyrosine phosphatase 1B; PTEN, phosphatase and tensin homolog; Grb2, growth factor receptor-bound protein 2; MAPK, mitogen-activated protein kinase; Mnk1, MAPK interacting kinase 1; DYRK1A, dual-specificity tyrosine-phosphorylated and -regulated kinase 1A; TESK1, testicular protein kinase 1; PP2A, protein phosphatase 2A; SHP2, SH2-domain containing tyrosine phosphatase 2; TKB, tyrosine kinase binding; SIAH2, seven in absentia homolog 2; CIN85, Cbl interacting protein of 85 kDa; NSCLC, non-small-cell lung cancer; aa, amino acid(s); TKB, tyrosine kinase binding; xPAPC, *Xenopus laevis* paraxial protocadherin.

mouse samples to create a profile of the normal physiological state of human and mouse transcriptomes (Su et al., 2002). Although *Spry2* appears to be ubiquitously expressed, the expression of the other isoforms is more limited to certain organs and tissues. The expression of human and mouse *Spry* isoforms in fetal tissues, adult tissues, and certain cell lines can be obtained from the following web sites: <http://biogps.gnf.org>, and <http://www.ncbi.nlm.nih.gov/sites/entrez?db=geo>.

Like d*Spry*, mammalian *Spry* proteins were shown to antagonize FGF-stimulated organogenesis (Minowada et al., 1999; Tefft et al., 1999). Since these initial findings, the number of pathways and biological processes regulated by *Spry* proteins has continued to expand. To understand the mechanism(s) involved in the biological actions of *Spry* proteins, significant emphasis has been placed on identifying the proteins that interact with them, the post-translational modifications that may alter the function of *Spry* proteins, and mechanisms that alter cellular levels of these regulators of RTK signaling. In this review, we discuss some of the more recent discoveries pertaining to *Spry* interactions with various proteins, how these interactions allow *Spry* to regulate different signaling pathways and biological processes, as well as how *Spry* expression is affected by these proteins at both the transcriptional and protein levels. Finally, the role of *Spry* proteins in several diseases is briefly reviewed.

Regulation of Biological Processes by *Spry* Proteins

Morphogenesis, Organogenesis, and Development. Tubular networks, which comprise the vascular system, lungs, and kidneys, require tight control to develop and maintain a hierarchical structure (Horowitz and Simons, 2008). Similar to the findings in *D. melanogaster*, mammalian *Spry* proteins have also been shown to play a critical role in tubular morphogenesis associated with tracheal/lung development, uteretic budding, and angiogenesis. The function of individual *Spry* proteins in these processes are elegantly reviewed elsewhere (Cabrita and Christofori, 2008; Horowitz and Simons, 2008; Warburton et al., 2008). FGF signaling, in particular, is crucial to initiating/regulating tubular morphogenesis and is known to up-regulate *Spry* expression in *D. melanogaster* (Hacohen et al., 1998), mice, chicks (Minowada et al., 1999), and zebrafish (Fürthauer et al., 2001). In addition, a recent report of FGF signaling in anthozoan cnidarians (*Nematostella vectensis*) revealed the expression of three FGF ligands, two FGF receptors, and two orthologs of vertebrate *spry* genes, highlighting the importance of the conservation of FGF/antagonist signaling loops among species (Matus et al., 2007). When an intraspecies comparative genomic analysis of the human *spry* genes was performed, investigators were able to show the linkage of *spry4* and *spry1* genes to the *fgf1* and *fgf2* genes, respectively (Kato and Kato, 2006). Except for the nematodes (which, interestingly, contain no *spry* genes), a conservation of function for FGF signaling implies a crucial role for *Spry* in development and growth across the animal kingdom.

Besides the role of *Spry* proteins in tubular morphogenesis (Hacohen et al., 1998), limb development (Minowada et al., 1999), patterning of the midbrain, and anterior hindbrain (Lin et al., 2005), recent reports have provided additional evidence for *Spry* protein involvement in craniofacial and trunk development. Because the functions of *Spry* proteins in embryonic development have been reviewed by others

(Cabrita and Christofori, 2008; Horowitz and Simons, 2008; Warburton et al., 2008), we have focused mainly on the role of *Spry* proteins in craniofacial features. As early as 2001, a hint of *Spry*'s role in maintaining epithelial-mesenchymal interactions for craniofacial and trunk development in vertebrates became apparent after examining the expression profiles of *Spry1*, -2, and -4 during mouse embryogenesis (Zhang et al., 2001). Although *spry4* knockout mice exhibited growth retardation and sustained FGF-mediated extracellular signal regulated kinase (ERK) activation (Taniguchi et al., 2007), mice deficient in *spry2* exhibited clefting of the palate, excessive cell proliferation, and aberrant expression of downstream target genes of FGF receptor signaling (Welsh et al., 2007). Moreover, *Spry2*-BAC transgenic mice were able to rescue palate defects of mice with a deletion of *spry2* in a dosage-dependent manner (Welsh et al., 2007). On the other hand, overexpression of *Spry2* did not disrupt FGF signaling during facial development of avian embryos, and craniofacial defects such as cleft palate were still observed, suggesting that overexpression of *Spry2* may mimic the actions of *Spry* deficiency (Goodnough et al., 2007). A role for *Spry2* in facial development is also suggested by a report identifying cleft palate candidate genes in which D20A and K68N point mutations in *Spry2* were revealed (Vieira et al., 2005). So far, however, no studies suggest that the D20A or K68N substitutions in *Spry2* alter its ability to regulate growth factor signaling. It is noteworthy that *spry2/4* double-knockout mice were embryonic lethal with severe craniofacial, limb, and lung abnormalities (Taniguchi et al., 2007), suggesting that *Spry2* and *Spry4* may each compensate to some extent for the other's functions.

The pleiotropic effects of *Spry* proteins in mouse development also include a role for *Spry2* during inner ear development (Shim et al., 2005), lens morphogenesis (*Spry1* and -2) (Boros et al., 2006), tooth elongation (*Spry4* together with *Spry1* or -2) (Klein et al., 2008), and tooth development (for review, see Tummers and Thesleff, 2009). In the case of inner ear development, both *Spry2* and the FGF receptor 3 (FGFR3) are required for normal hearing in the mouse (Shim et al., 2005). *spry2*-null mice exhibit impaired hearing with an increase in the numbers of outer hair cells and supporting cells in the organ of Corti. Whereas decreasing *fgf8* gene dosage was able to rescue hearing in these mice, decreasing *fgf8* gene dosage in the *spry2*(-/-) mice did not alter the extra numbers of sensory and nonsensory cells (Shim et al., 2005). Although no definitive studies of *Spry* protein participation in lens development have been performed, expression profiles of *Spry2* in the developing lens suggests a role for *Spry* proteins in this process (Boros et al., 2006). Other developmental processes involving *Spry* proteins, not reviewed in this section, are delineated in Table 1.

Cellular Proliferation and Migration. Because of their ability to regulate RTK signaling, the role of *Spry* proteins in regulating cellular proliferation and migration has been the focus of several studies. A number of papers were published in 2001 concerning *Spry*'s effect on cell proliferation and migration. Yigzaw et al. (2001) showed that *Spry2* inhibited the proliferation and migration of HeLa cells in response to a number of growth factors including EGF, FGF, VEGF, and platelet-derived growth factor. This study also showed that the microtubule association and membrane translocation domains described by Lim et al. (2000) are necessary for the antiproliferative and antimigratory actions of *Spry2*. This

report was followed by the demonstration that overexpression of mammalian Spry1 and -2 inhibited FGF-, VEGF-, and EGF-induced proliferation and migration in human umbilical vein endothelial cells (Impagnatiello et al., 2001). Likewise, Spry1 and -2 overexpression inhibited proliferation and differentiation of NIH3T3 cells by modulation of Ras activation (Gross et al., 2001). In subsequent years, Spry2 was shown to inhibit proliferation and migration of rat intestinal epithelial (IEC-6) cells (Poppleton et al., 2004), human umbilical vein endothelial cells (Lee et al., 2004), vascular smooth muscle cells, and inhibition of neointimal hyperplasia in blood vessels after injury (Zhang et al., 2005).

Spry2 translocates to the plasma membrane upon activation of cells with growth factors (Lim et al., 2000; Yigzaw et al. 2001). Although, the mechanisms governing this translocation are not clearly understood, three scenarios have been postulated. First, because the Cys-rich C-terminal region of Spry2 that contains the membrane translocation domain has been shown to bind phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂] (Lim et al., 2000), the binding of Spry2 to PtdIns(4,5)P₂ may be responsible for the translocation of Spry2 to membrane ruffles (Lim et al., 2000). Second, palmitoylation in the cysteine-rich C terminus of Spry2 may facilitate its anchoring to the membrane; previously, palmitoylation of Spry1 and -2 was shown to occur in endothelial cells (Impagnatiello et al., 2001). However, whether palmitoylation of Spry proteins is a dynamic process regulated by stimulation of cells with growth factors remains to be determined. Finally, Spry proteins may translocate to plasma membranes via interactions through their C-terminal regions with caveolin-1 after serine phosphorylation in response to growth factor stimulation (Impagnatiello et al., 2001; Cabrita et al., 2006). Whatever the mechanism, it is clear that translocation of Spry1 and Spry2 is necessary for inhibition of growth factor-stimulated cell migration, proliferation, and differentiation by Spry proteins (Yigzaw et al., 2001).

Cell Survival. Because of the antiproliferative actions of Spry proteins, the ability of Spry proteins to regulate cell survival or apoptosis has also been an area of interest. A recent study showed that siRNA-mediated silencing of endogenous human Spry2 in adrenal cortex adenocarcinoma cells mitigated the antiapoptotic actions of serum, and these effects involved interactions of Spry2 with c-Cbl [discussed under *Ubiquitin Ligases (cCbl and SIAH2)*] (Edwin and Patel, 2008). Silencing of Spry2 also reduced phosphorylation of

the pro-apoptotic factor BAD through inhibition of the Erk1/2-p90 ribosomal S6 kinase cascade activation (Edwin and Patel, 2008). Likewise, Spry2 was shown to inhibit UV-induced apoptosis in oncogenic HRas(Val12)-transformed human fibroblasts cells (Lito et al., 2009). Using short hairpin RNA specific for Spry2, Lito et al. (2009) showed that knockdown of Spry2 resulted in decreased phosphorylation of Akt, diminished stabilization of HDM2, and increased levels of p53—all major players in the apoptotic cascade induced by DNA damage (Stiewe, 2007). It is noteworthy that in Swiss 3T3 cells, tumor necrosis factor- α decreased Spry2 amounts in a p38 MAPK-dependent manner leading to decreased cell survival. Heterologous expression of Spry2 prevented the apoptotic actions of tumor necrosis factor- α on these cells, which is consistent with a pro-survival role for Spry2 (Ding and Warburton, 2008).

Overall, Spry proteins can inhibit cell migration and proliferation and also seem to be essential for promoting cellular survival. Because all three of these key processes play an important role in normal development as well as pathological conditions such as cancer or vascular restenosis (discussed later under *Sprouty Proteins in Pathological States*), a number of studies have focused on elucidating the fundamental mechanisms by which Spry proteins regulate these processes. Some of these mechanisms have been elucidated by identifying the interacting partners of the Spry proteins.

Post-Translational Modification and Interacting Partners of Spry Proteins. Post-translational modifications and protein-protein interactions are common mechanisms involved in regulating the functional activity of proteins. The following sections will review a number of adaptor proteins, kinases, phosphatases, and ligases that interact with the Spry proteins to fine-tune their biological actions. Figure 1 schematically represents most of these interactions and their functional consequences.

Adaptor Proteins and Raf1. RTK signaling is initiated by binding of adapter proteins to the activated receptors (Lowenstein et al., 1992; Pawson, 1995). Because Spry proteins modulate the actions of RTKs, several studies have examined the effects of Spry proteins on activation of Erk1/2 by different growth factors. It is noteworthy that the effect of the different Spry isoforms on the activation of Erk1/2 varies depending on the growth factor used (Table 2). However, Spry1, Spry2, and Spry4 inhibit FGF-elicited Erk1/2 activation. Because of the involvement of adapter proteins in Erk1/2 activation, investigations have focused on interac-

TABLE 1
Role of sprouty proteins in organogenesis and development

Recent reports of Spry role in organogenesis and development are listed, including relevant growth factor, ligand, or signaling pathway. Additional information describing the role of Spry proteins in morphogenesis and development can be found in the following reviews: Warburton and Bellusci, 2004; Mason et al., 2006; Horowitz and Simons, 2008.

Biological Event	Experimental System	Sprouty Isoform(s)	Growth Factor/Ligand	References
Placental villi sprouting	Placenta	hSpry1,-2,-3	FGF4, FGF10	Anteby et al., 2005
Placental villi sprouting	Placenta	hSpry2	FGF10	Natanson-Yaron et al., 2007
Kidney development	Embryonic kidney explants	mSpry1		Gross et al., 2003
Ureteric branching	<i>spry</i> -null mice	mSpry1	Gdnf/Ret/Wnt11	Basson et al., 2005, 2006
Ureteric branching	Kidney transgenic mice	hSpry2	Wnt11/FGF 7/Gdnf	Chi et al., 2004
Kidney development	Human renal mesenchymal cells	hSpry4	LIF/FGF2/TGF α	Price et al., 2007
Male sex organogenesis	Testes	mSpry2	FGF9	Chi et al., 2006
Neuronal differentiation	Immature neurons	mSpry2	BDNF	Gross et al., 2007
Muscle regeneration	Soleus muscle	hSpry1,-2,-4	FGF6	Laziz et al., 2007
Pancreas development	Pancreatic beta cells	mSpry4		Jäggi et al., 2008

LIF, leukemia inhibitory factor; BDNF, brain-derived neurotrophic factor.

tions between Spry proteins and the adapter proteins that bind activated RTKs. Further rationale to pursue such investigations was provided by studies in *D. melanogaster* S2 cells that demonstrated that Spry acts downstream of FGF recep-

tor and either at or above Ras and Raf1 (Casici et al., 1999). *D. melanogaster* Spry was found to interact with Drk, an SH2-SH3 domain containing adaptor protein homologous to mammalian Grb2 and Gap1, a Ras GTPase-activating protein

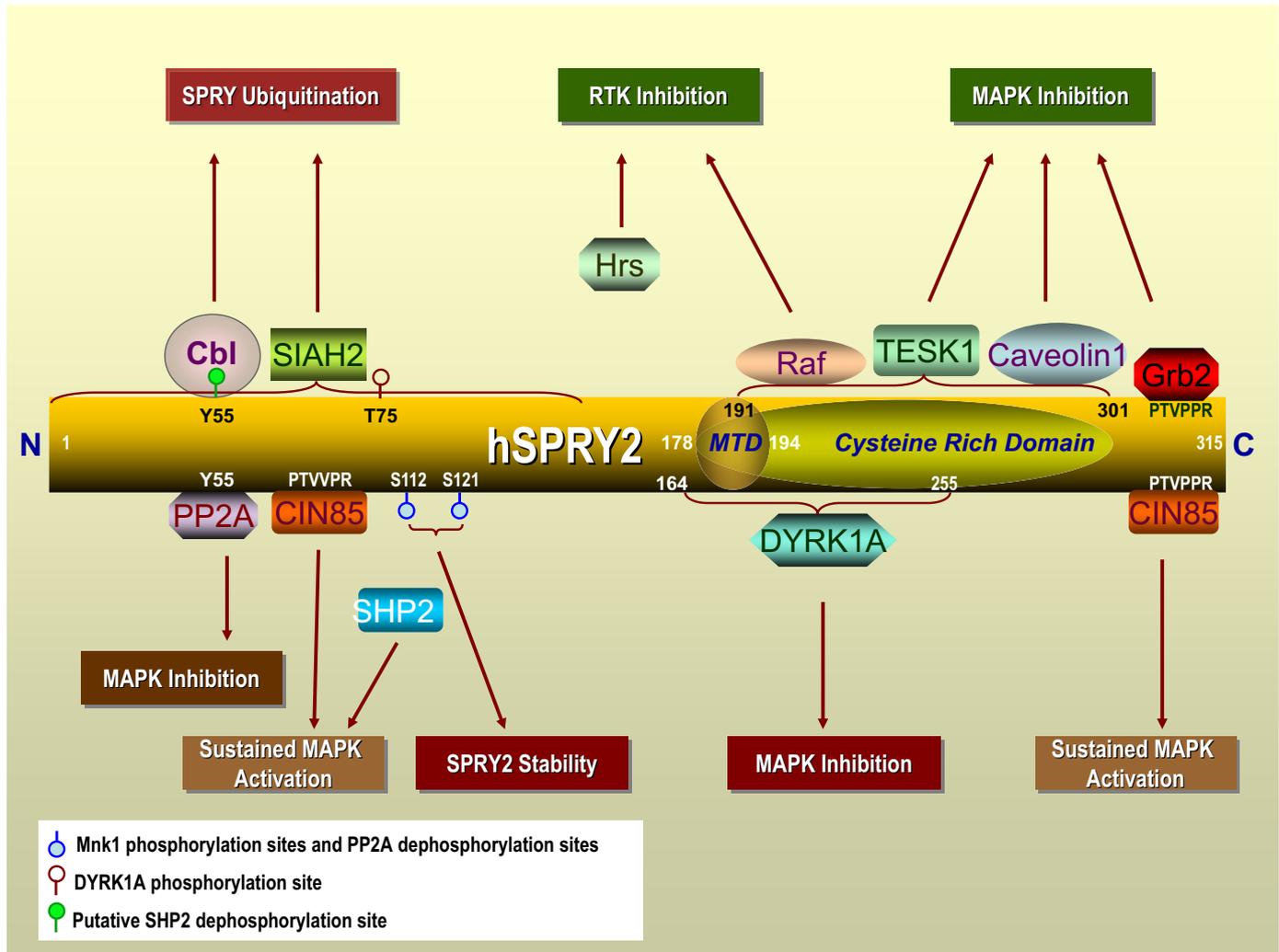


Fig. 1. Schematic representation of the proteins that interact with human Spry2. The functional consequences of each interaction are represented as colored rectangular boxes and indicated by solid arrows. Some of the known phosphorylation sites (Tyr55, Ser112/Ser121, Thr75) are shown, and the kinases or phosphatases that modify these sites are provided in the key box. The adaptor protein CIN85 binds to more than one PXXXPR motif on Spry2. The interaction sites of proteins such as Hrs and SHP2 are not clear; therefore, these proteins are not placed next to Spry2. Proteins that are known to interact with the N-terminal or C-terminal regions of Spry2 without knowledge of the precise sites are located on the brackets that demark the N and C termini. Proteins such as PTP1B and PTEN have been shown to be involved in Spry2 function. However, their physical interaction with Spry2 has not been observed. MTD, membrane translocation domain.

TABLE 2
Effect of overexpressed Sprouty Isoforms on ERK1/2 activation by different growth factors

	EGF	FGF	PDGF	VEGF
Sprouty1	↑↔ Egan et al., 2002; Ozaki et al., 2005	↓↔ Hanafusa et al., 2002; Yusoff et al., 2002; Ozaki et al., 2005	?	↓ Impagnatiello et al., 2001; Huebert et al., 2004
Sprouty2	↑↔ Fürthauer et al., 2001; Egan et al., 2002; Wong et al., 2002; Ozaki et al., 2005	↓ Fürthauer et al., 2001; Impagnatiello et al., 2001; Yusoff et al., 2002; Ozaki et al., 2005	↓ Kajita et al., 2007	↓ Impagnatiello et al., 2001
Sprouty3	?	?	?	?
Sprouty4	↔ Fürthauer et al., 2001; Sasaki et al., 2003; Ozaki et al., 2005	↓↔ Fürthauer et al., 2001; Lee et al., 2001; Yusoff et al., 2002; Ozaki et al., 2005	?	↓ Lee et al., 2001; Sasaki et al., 2003

PDGF, platelet-derived growth factor; ↑, overexpression of sprouty-activated ERK activation; ↓, overexpression of sprouty-inhibited ERK activation; ↔, overexpression of sprouty did not affect ERK activation; ?, no current reports.

(Casci et al., 1999). Because Drk (Grb2) and Gap1 are important components of RTK signaling pathways, Spry, by binding these proteins, may negatively affect Ras activation. Further support for this hypothesis was provided in mammalian system by studies showing that Spry1, Spry2, and Spry4 inhibit the Ras/Raf/MAPK pathway by preventing Ras activation (Gross et al., 2001; Leeksa et al., 2002). Although two earlier studies suggested that Spry2 and Spry4 bind Raf1 (Tefft et al., 2002; Sasaki et al., 2003), these findings have not been consistently reproduced by others (Gross et al., 2001); therefore, the role of Raf1 association with Spry proteins in regulation of downstream activation of Erk1/2 is debatable. On the other hand, among proteins upstream of Ras, the interactions of Spry2 with Grb2 have been more consistently observed, and these interactions are enhanced by growth factors that augment the tyrosine phosphorylation of Spry proteins (Gross et al., 2001; Tefft et al., 2002; Lao et al., 2006; Martínez et al., 2007). Because one of the critical tyrosine residues phosphorylated on Spry proteins is the residue corresponding to Tyr55 on Spry2, studies have investigated the role of this tyrosine phosphorylation in the interactions between Spry2 and Grb2. Using the Y55F mutant of Spry2 and a tyrosine phosphorylated octapeptide corresponding to the residues around Tyr55 on Spry2, one study concluded that phospho-Tyr55 and surrounding residues form a site for the SH2 domain of Grb2 (Hanafusa et al., 2002). This study also suggested that Spry1, which is phosphorylated on a cognate site (Tyr53), and also has surrounding residues that are similar to those around Tyr55 on Spry2, binds Grb2. However, direct binding of SH2 domain of Grb2 to the residues surrounding Tyr55 on Spry2 or Tyr53 on Spry1 was not shown in this study. Moreover, others have not observed an interaction between Spry1 and Grb2 (Gross et al., 2001; Lao et al., 2006). Thus, the residues surrounding Tyr55 seem not to be the Grb2 binding site. More likely, as suggested by Lao et al. (2006), growth factor-mediated phosphorylation of Tyr55 on Spry2 alters its conformation to reveal a cryptic PXXPXR motif (aa 304–309) in the extreme C terminus of Spry2 that serves to bind the SH3 domain of Grb2. Spry2 binding to Grb2 sequesters Grb2 away from Sos, thereby inhibiting the Erk1/2 pathway (Lao et al., 2006). It is noteworthy that the PXXPXR motif in the C terminus of Spry2 is not present in the other Spry isoforms, and therefore may explain why Spry2 is more effective at inhibiting FGFR signaling as well as the findings concerning the lack of interactions between Spry1 and Grb2 (Gross et al., 2001; Lao et al., 2006).

Kinases (Mnk1, DYRK1A, and TESK1). Spry2 migrates as multiple bands on SDS-polyacrylamide gels. Although palmitoylated Spry2 may account for one of these bands, the initial evidence that Spry1 and Spry2 are phosphorylated was derived from the elegant studies of Impagnatiello et al. (2001) who showed that in vitro treatment of cell lysates with protein phosphatase caused the disappearance of the slower migrating Spry2 bands. One of the first growth factor-mediated phosphorylation events that became evident was the phosphorylation of Spry2 on Tyr55 by Src family of protein kinases (Hanafusa et al., 2002; Rubin et al., 2003; Mason et al., 2004). Tyr55 on Spry2 is also conserved on other isoforms of Spry. The phosphorylation of Spry on the conserved Tyr is necessary for its activity as an inhibitor of RTKs in response to FGF (Sasaki et al., 2001; Hanafusa et al., 2002). It is

noteworthy that the Y55F mutant of Spry2 also migrates as multiple bands, demonstrating that phosphorylation on Tyr55 is not responsible for the slower migrating species of Spry2. The phosphorylation of the Tyr corresponding to Tyr55 of Spry2 in other Spry isoforms is also essential for some of their interactions with other proteins such as c-Cbl and PP2A and is, therefore, referred to as the critical Tyr residue in some sections of this review.

It is now clear that the more slowly migrating Spry2 bands that disappeared with phosphatase treatment (Impagnatiello et al., 2001) were due to Ser/Thr phosphorylation. In this context, a report investigating the stability of Spry2 by ubiquitination and proteosomal degradation (DaSilva et al., 2006) showed that Ser112 and Ser121 on Spry2 are phosphorylated by Mnk1 kinase, and these phosphorylations regulate phosphorylation of Tyr55. Thus, substitution of Ser112 and Ser121 with Ala or inhibition of Mnk1 activity increases the rate of ligand induced degradation of Spry2 by augmenting phosphorylation of Tyr55, thereby enhancing the binding and ubiquitylation of Spry2 by c-Cbl; the S112/121A mutant of Spry2 also migrated faster on SDS-polyacrylamide gel electrophoresis, indicating that these phosphorylation sites account for the slower migrating bands (DaSilva et al., 2006). Using site-directed mutagenesis, Lao et al. (2007) also showed that a number of Ser or Thr residues in a Ser/Thr-rich region (aa 108–132) on Spry2 contributed to Spry2 bands that migrated more slowly than was observed in response to growth factor stimulation (Lao et al., 2007). Although several of the Ser/Thr residues in this region on Spry2 form a consensus site for casein kinase 2, no evidence showing phosphorylation of Spry by casein kinase 2 has been forthcoming. However, at least two of the phosphorylated Ser residues (Ser112 and Ser115) are dephosphorylated by protein phosphatase 2A (PP2A) (Lao et al., 2007) [discussed further under *Phosphatases (PTP1B, PP2A, SHP2, and PTEN)*].

Besides the phosphorylation of Ser112 and Ser121, two reports suggest that other phosphorylation events may also acutely modulate Spry2 function. DYRK1A (dual-specificity tyrosine-phosphorylated and -regulated kinase 1A) has been shown to interact with and phosphorylate Spry2 on Thr75 (Aranda et al., 2008). This interaction and Thr75 phosphorylation of Spry2 by DYRK1A seem to negatively affect the function of Spry2 as an antagonist of RTK signaling, because substitution of Thr75 with Ala increased the ability of Spry2 to inhibit Erk1/2 activation by FGF (Aranda et al., 2008). However, the role of Thr75 phosphorylation has to be further substantiated by other approaches including silencing or knockout studies of DYRK1A. Likewise, although the phosphorylation of Tyr227 by FGF, but not by EGF, has been suggested to augment the ability of Spry2 to inhibit the FGF-activated Erk1/2 cascade (Rubin et al., 2005b), this sole report needs to be explored further to determine the mechanisms, including structural changes, by which phosphorylation of Tyr227 may augment the inhibitory actions of Spry2.

In addition to being regulated by kinases, Spry interactions with kinases may also regulate their functional activity. In this respect, testicular protein kinase 1 (TESK1) was identified from a human fetal liver cDNA library as a binding partner of hSpry4 (Leeksa et al., 2002). TESK1 is a serine/threonine kinase that phosphorylates cofilin and plays a critical role in integrin-mediated actin cytoskeletal reorganization and cell spreading. The Spry4-TESK1 interaction increases upon growth factor stimulation, and the two proteins

colocalize in apparent cytoplasmic vesicles (Leeksa et al., 2002). Spry4, by binding through the C-terminal cysteine-rich region, inhibits the kinase activity of TESK1 and suppresses integrin- and TESK1-mediated cofilin phosphorylation during the spreading of cells on laminin (Tsumura et al., 2005). More recently, TESK1 has been shown to interact with Spry2 and localize the protein to vesicular compartments including endosomes (Chandramouli et al., 2008). TESK1, independent of its kinase activity, attenuates the ability of Spry2 to inhibit growth factor actions, primarily by interfering with Spry2/Grb2 interactions and dephosphorylation of Ser residues by PP2A (Chandramouli et al., 2008).

Using electron-capture dissociation mass spectrometry, a recent study has identified 15 Ser/Thr/Tyr phosphorylation sites on Spry2 (Sweet et al., 2008). Besides Tyr55, three of these Ser/Thr phosphorylation sites were previously identified as phosphorylation sites (DaSilva et al., 2006; Lao et al., 2007). However, the other 11 sites have not previously been documented as phosphorylation sites on Spry2. It is noteworthy that some of the sites on Spry2, including Thr75, Tyr227, Ser112, and Ser121 discussed above in this section were not identified in this study (Sweet et al., 2008). This may be because none of the phosphorylation studies were exhaustive enough to include different cell types or different experimental conditions that lead to phosphorylation of the various sites. Nonetheless, there seem to be at least 19 phosphorylation sites on Spry2, and the role of phosphorylation of the majority of these sites, as well as the identity of the kinases that phosphorylate them, will be an area of active investigation. Besides Tyr55, some of the residues that are phosphorylated on Spry2 are also conserved in other Spry isoforms. However, whether these additional sites on other isoforms of Spry are also phosphorylated, and whether their phosphorylation modulates the functions of other Spry isoforms, remains to be determined.

Phosphatases (PTP1B, PP2A, SHP2, and PTEN). Just as some kinases have been shown to phosphorylate Spry proteins, evidence that the function of Spry proteins is either mediated or regulated by phosphatases has also been forthcoming. Yigzaw et al. (2003) showed that Spry2 increases the amount of soluble protein tyrosine phosphatase 1B (PTP1B) without changing the total amount of cellular PTP1B. The increase in soluble PTP1B decreases tyrosine phosphorylation of proteins such as p130Cas involved in focal adhesion formation and migration (Liu et al., 1998). Indeed, the expression of a “dominant-negative” PTP1B attenuated the ability of Spry2 to inhibit cell migration, but not its ability to inhibit cell proliferation (Yigzaw et al., 2003). The role of PTP1B in mediating the functions of Spry proteins has been further corroborated by recent findings that the transgenic expression of Spry4 in pancreatic β -cells increases soluble PTP1B content and inhibits development of islets of Langerhans in mice (Jäggi et al., 2008). However, interactions between PTP1B and Spry2 (D. Chaturvedi and T. B. Patel, unpublished observations) or Spry4 (Jäggi et al., 2008) have not been observed, and the mechanism(s) by which Spry2 or Spry4 increases soluble amounts of PTP1B remain elusive. Because elevations in soluble PTP1B activity by Spry2 decrease tyrosine phosphorylation of p130Cas, the Spry2-mediated decrease in Rac1 activation (Poppleton et al., 2004) has been suggested to be due to a decrease in formation of an

active Rac1 GTP/GDP exchange factor composed of p130Cas, CrkII, ELMO, and DOCK180.

Unlike PTP1B, an association of Spry2 with the catalytic and regulatory subunits of PP2A has been documented (Lao et al., 2007). This association of PP2A with Spry2 is enhanced by activated growth factor receptors, after which PP2A dephosphorylates Ser112 and Ser115 on Spry2. This dephosphorylation of Spry2 could be an activation process because phosphorylation of Ser residues in this region has also been shown to modulate phosphorylation of Tyr55, the c-Cbl binding site [discussed under *Ubiquitin Ligases (cCbl and SIAH2)*]. It is noteworthy that the N-terminal region (aa 50–60) around Tyr55 is also the region on Spry2 that binds PP2A, and PP2A competes with c-Cbl for binding to this region when Tyr55 is phosphorylated (Lao et al., 2007). The studies of Lao et al. (2007) also suggest that distinct pools of Spry in the cells interact with PP2A and c-Cbl. Moreover, as mentioned under *Kinases (Mnk1, DYRK1A, and TESK1)*, interactions of Spry2 with TESK1 inhibit PP2A-mediated dephosphorylation of Ser residues on Spry2 (Chandramouli et al., 2008).

As suggested for PP2A, in forced-expression studies, the Src homology-2 containing phosphotyrosine phosphatase (SHP2) has also been implicated in regulating the biological activity of Spry proteins. Hanafusa et al. (2004) showed that expression of constitutively active SHP2 resulted in dephosphorylation of Spry1 and Spry2 on Tyr53 and Tyr55, respectively. Decreased phosphorylation of Tyr55 was suggested to decrease binding of Grb2 and, therefore, to decrease the inhibitory actions of Spry2 on receptor tyrosine kinase signaling. Although one study has shown that the association between SHP2 and Spry1 is enhanced by activated growth factor receptors (Jarvis et al., 2006), a previous report showed that Spry2 association with SHP2 was decreased by FGF activation (Tefft et al., 2002). The reports suggesting that dephosphorylation of Spry1 on Tyr53 and Spry2 on Tyr55 decrease their functional activity and relieve the inhibitory effect on RTK signaling (Hanafusa et al., 2004; Jarvis et al., 2006) may provide a tenable mechanism for SHP2-mediated augmentation of growth factor signaling (Noguchi et al., 1994; Xiao et al., 1994; Tang et al., 1995; Yamauchi et al., 1995; Bennett et al., 1996; Deb et al., 1998; Wu et al., 2006). However, the discrepancies between whether growth factor activation increases or decreases interactions between SHP2 and Spry proteins (Tefft et al., 2002; Jarvis et al., 2006), as well as the lack of any data concerning the role of endogenous SHP2 in regulating the ability of Spry proteins to modulate growth factor signaling, call into question the physiological significance of SHP2 in modulating the function of Spry proteins. Moreover, as discussed by Chan et al. (2008), *spry1*- and *spry2*-null mice do not show the phenotype of the active SHP2 transgenic mice.

It is noteworthy that by sequestering c-Cbl, Spry proteins can also augment RTK signaling (Egan et al., 2002; Wong et al., 2002; Fong et al., 2003; Guy et al., 2003; Hall et al., 2003; Rubin et al., 2003; Mason et al., 2004; Haglund et al., 2005). Because Tyr55 on Spry2 has to be phosphorylated to bind c-Cbl, SHP2, by dephosphorylating this tyrosine, would be expected to decrease the ability of Spry2 to sequester c-Cbl. This would then diminish the protection of RTKs from down-regulation by c-Cbl. This scenario predicts that by decreasing Spry2 function, SHP2 would also diminish cell surface growth factor receptors and signaling capacity if these receptors were c-Cbl substrates. However, to our knowledge, no

experimental evidence supports such a scenario, perhaps because SHP2/Spry2 interactions may be transitory and may not affect long-term trafficking of the EGFR.

The interactions of tumor suppressor phosphatase and tensin homolog (PTEN), another phosphatase, with Spry proteins have been difficult to show, but, like PTP1B, PTEN activity is necessary for the biological function of Spry proteins. In HeLa cells, overexpression of Spry2 increases the total amount of cellular PTEN (Edwin et al., 2006). Overexpression of Spry2 also decreased the amount of PTEN that is phosphorylated (Edwin et al., 2006). Because dephospho-PTEN is more enzymatically active than phospho-PTEN, Spry2 expression increases PTEN activity by both increasing its amount as well as decreasing its phosphorylation. The resultant increase in PTEN activity decreases activation of AKT and downstream signaling (Edwin et al., 2006). Most importantly, by silencing PTEN or by performing experiments in PTEN-null mouse embryonic fibroblasts, PTEN was found to be necessary for Spry2-mediated inhibition of cell proliferation (Edwin et al., 2006).

Ubiquitin Ligases (cCbl and SIAH2). A prominent binding partner of Spry is c-Cbl. Cbl proteins are ubiquitin ligases, which, through mono- and polyubiquitination of the RTKs, initiate their endocytosis and/or lysosomal/proteosomal degradation (Levkowitz et al., 1998; Miyake et al., 1998). c-Cbl and b-Cbl interact through their SH2-like tyrosine kinase binding (TKB) domain with the N-terminal region of Spry proteins that encompass the critical tyrosine residue equivalent to Tyr55 on Spry2 (Wong et al., 2001; Fong et al., 2003; Rubin et al., 2003). This interaction requires phosphorylation of the tyrosine equivalent to Tyr55 on Spry2 (Fong et al., 2003; Hall et al., 2003; Mason et al., 2004).

In a detailed structural study using Cbl TKB domain and peptides of Cbl substrates, Ng et al. (2008) showed that the region encompassing Tyr55 on Spry2 has the highest affinity for Cbl TKB. The cognate region on Spry4 binds weakly to Cbl TKB, perhaps because of the lack of a conserved Thr residue adjacent to the conserved tyrosine residue in Spry4 that is phosphorylated (Ng et al., 2008).

Because of its high affinity for Cbl TKB, Spry2 effectively sequesters Cbl and protects receptors such as the EGFR from ubiquitination, endocytosis, and degradation. Stabilized cell surface EGFR are then able to sustain EGF-induced Erk signaling that culminates in the differentiation of PC12 cells (Wong et al., 2001; Egan et al., 2002; Rubin et al., 2003). The competitive interplay between Cbl, Spry, and EGFR seems to occur in the endosomal compartments, and it regulates the amplitude and longevity of intracellular signals. A more direct study on this aspect showed that hSpry2 interferes with the trafficking of activated EGFR specifically at the step of progression from early to late endosomes (Kim et al., 2007). This effect seems to be mediated by the binding of Spry2 to the endocytic regulatory protein hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) (Kim et al., 2007). Thus, Spry proteins can augment RTK signaling and are therefore referred to as modulators of RTKs rather than inhibitors (for reviews, see Guy et al., 2003; Li et al., 2003; Kim and Bar-Sagi, 2004; Rubin et al., 2005a; Cabrita and Christofori, 2008). By binding Cbl and protecting growth factor receptors from down-regulation, Spry2 also enhances the antiapoptotic actions of serum (Edwin and Patel, 2008) as well as Erk signaling by the EGF receptor (Wong et al., 2001;

Egan et al., 2002; Rubin et al., 2003). One of the caveats with these studies is that they either used forced expression of Spry2 or were performed with high concentrations (≥ 100 ng/ml) of EGF. As described by Sigismund et al (2005), at low physiological concentrations of EGF, the EGFR is not ubiquitinated and is internalized via a clathrin-mediated pathway, whereas at high EGF concentrations, the EGFR undergoes an ubiquitination and lipid raft-mediated internalization process. At present, whether Spry2 alters the EGFR internalization and degradation at low physiological concentrations of EGF remains unknown.

Despite the presence of Tyr residue equivalent to Tyr55 in Spry2, Spry4 does not interfere with down-regulation of the EGFR (Wong et al., 2002). This is perhaps related to the fact that the Spry4 does not contain the Thr residue immediately next to Tyr55 that is important for Cbl binding. Although Spry4 binds the c-Cbl TKB domain with moderate affinity *in vitro*, its binding to c-Cbl in intact cells is limited because Spry4 is poorly phosphorylated (Ng et al., 2008). Moreover, in the case of Spry2, additional adaptor molecules may be involved to modulate receptor down-regulation. For instance, Cbl recruits endocytic complexes, including the Cbl-interacting protein of 85 kDa (CIN85)/endophilins, to activated RTKs to facilitate endocytosis (Petrelli et al., 2002; Soubeyran et al., 2002; Szymkiewicz et al., 2002). Haglund et al. (2005) identified CIN85 as a new interacting partner of Spry2 that plays an important role in Cbl-mediated down-regulation of growth factor receptors and EGF-induced differentiation of PC12 cells. The CIN85 SH3 domains A and C bind specifically to Pro/Arg-rich motifs present in the N- (aa 59–64, PTVVPR) and C-terminal (aa 304–309, PTVPPR) regions of Spry2, respectively (Haglund et al., 2005); these regions are not present in other Spry isoforms. In addition, the SH3C domain of CIN85 may bind the Pro-rich region encompassed by amino acids 67 to 72 on Spry2 (Haglund et al., 2005). Substitution of Arg309 with Ala markedly reduced the association of Spry2 with CIN85 SH3A domain, whereas R64A substitution modestly decreased interaction with SH3A domain. On the other hand, when Arg309 and Arg64 were simultaneously substituted, the association between CIN85 SH3A domain and Spry2 was abolished, whereas this double mutant did not affect the binding to CIN85-SH3C domain, suggesting a multivalent mode of binding between these proteins (Haglund et al., 2005). Moreover, substitution of R309A on CIN85 abrogated the effects of Spry2 on EGFR down-regulation (Haglund et al., 2005). Thus, in addition to the interaction with Cbl, Spry2/CIN85 association seems necessary to down-regulate the EGFR. The importance of the C-terminal CIN85 binding region on Spry2 in facilitating the antiapoptotic actions of serum was also shown by the findings that wild-type Spry2, but not its mutant with disrupted CIN85 binding site (aa 304–309), could rescue the functions of Spry2 when the endogenous Spry2 was silenced (Edwin and Patel, 2008).

Besides binding Spry, c-Cbl ubiquitinates Spry in a growth factor-dependent manner (Hall et al., 2003; Rubin et al., 2003). The c-Cbl-mediated ubiquitination of Spry2 targets the protein for degradation by the 26S proteasome (Hall et al., 2003; Rubin et al., 2003). This process may also be regulated by the phosphorylation of other residues on Spry2. Hence, phosphorylation of Ser112 and Ser121 on Spry2 may decrease the phosphorylation of Tyr55 and, therefore, bind-

ing of and ubiquitination by c-Cbl (DaSilva et al., 2006). Conversely, when Spry2 is not phosphorylated on Ser112 and Ser121, phosphorylation of Tyr55 is enhanced with a concomitant increase in c-Cbl binding, increasing Spry2 ubiquitination and degradation (DaSilva et al., 2006).

Like c-Cbl, the E3 ligase Siah2 binds to the N-terminal region of Spry2. Siah2 interacts with Spry2 via its ring finger domain but in a tyrosine phosphorylation independent manner (Nadeau et al., 2007). Overexpression of Siah2, but not Siah1, resulted in the ubiquitination and proteosomal degradation of Spry1, Spry2, and, to a lesser extent, Spry4. In keeping with a role for Siah2 in regulating the amount of Spry proteins, a recent study showed that the expression of a dominant-negative Siah2 ring finger mutant increased the amount of Spry2 in SW1 melanoma cells and reduced tumorigenesis and metastasis (Qi et al., 2008).

The published findings with the two ubiquitin ligases, c-Cbl and Siah2, illustrate that different mechanisms regulate Spry2 degradation—one (c-Cbl) that depends on tyrosine phosphorylation of Spry2 and the other (Siah2) that is independent of tyrosine phosphorylation of Spry2.

Phospholipase C and Protein Kinase C. A formal association of Spry proteins with either phospholipase C or protein kinase C has not been demonstrated. However, Spry4 overexpression decreases VEGF-A-mediated hydrolysis of PtdIns(4,5)P₂, thereby decreasing the formation of inositol1,4,5-trisphosphate and diacylglycerol with a resultant decrease in elevation of intracellular Ca²⁺ concentrations and activation of protein kinase C. This action of Spry4 probably results from binding of PtdIns(4,5)P₂ to the C terminus of Spry4, thereby limiting or altering substrate availability to PLC (Ayada et al., 2009). As with all overexpression studies, the physiological significance of these findings remain to be determined.

Paraxial Protocadherin. The intracellular cytosolic domain of *Xenopus laevis* paraxial protocadherin (xPAPC) has been shown to interact with Spry1 and -2 (Wang et al., 2008). This interaction negatively affects the inhibitory function of Spry, consequently enhancing the planar cell polarity signaling and thus gastrulation movements in *X. laevis*. Interestingly, RhoA activation, as well as the membrane localization of Dishevelled and protein kinase C δ , were inhibited upon overexpression of xSpry1 in *X. laevis*; however, expression of xPAPC rescued both RhoA activation and the recruitment of Dishevelled and protein kinase C δ to the membrane. The antagonistic properties of xPAPC and xSpry1 on the planar cell polarity signaling pathway suggests that sprouty/xPAPC interactions may play a role in β -catenin independent Wnt-signaling (Wang et al., 2008).

Hetero-Oligomers of Spry Proteins. In recent years, it has become evident that the different Spry isoforms can interact with each other to form homo- and heterodimers or -oligomers (Ozaki et al., 2005). This interaction among the different isoforms seems to occur via their Cys-rich C-terminal regions. Because the Spry isoforms interact with different signaling proteins and in some cases with the same protein but with various affinities, the finding that Spry proteins form hetero-oligomers has the following significance. First, when studying the interactions of a given isoform of Spry with another protein, it is possible that the interacting protein is associated with a Spry isoform other than the one being investigated. Second, by binding different

proteins in a pathway, two Spry isoforms may have a greater effect than a single isoform by itself. Indeed, Ozaki et al. (2005) showed that the coexpression of Spry1 and Spry4, which interact with Grb2 and SOS, respectively, inhibited FGF2-elicited activation of Erk1/2 to a greater extent compared with each individual isoform; similar results were also obtained when endogenous Spry isoforms in Swiss 3T3 cells were silenced (Ozaki et al., 2005). A third implication of the hetero-oligomerization of Spry proteins is that the differential expression of the four isoforms in various cell types or tissues may modulate a given pathway to different extents. This could be biologically important in fine-tuning the actions of Spry proteins during development of different organs. Perhaps the different phenotypes observed with *spry2* or *spry4* single knockouts versus double knockout of these genes (reviewed above under *Morphogenesis, Organogenesis, and Development* and in Mason et al., 2006; Cabrita and Christofori, 2008) may be due to oligomerization of these Spry isoforms.

Regulation of Spry Proteins. As modulators of RTK signaling, the cellular content of Spry proteins would be expected to regulate the extent of their actions. As with all proteins, the cellular content of the Spry proteins is determined by the balance between its degradation and synthesis. Concerning degradation, the regulation of cellular amounts of Spry proteins by ubiquitination-mediated proteosomal degradation has been most extensively studied for Spry2 and, so far, involves the ubiquitin ligases c-Cbl and Siah2. The regulation of Spry proteins by transcriptional control is reviewed in the following section.

Transcriptional Regulation. Transcriptional regulation of Spry in response to growth factor stimulation was one of the first mechanisms revealed to alter sprouty expression (Hacohen et al., 1998; Minowada et al., 1999). In *D. melanogaster*, Spry's ability to act as a negative feedback inhibitor is due in part to its up-regulation in response to growth factor stimulation (Hacohen et al., 1998). This phenomenon was also observed in vertebrates (discussed in the Introduction) with studies by Minowada et al. (1999) showing the regulation of Spry expression in both mice and chicks through FGF signaling in various tissues. Besides growth factors, Ozaki et al. (2001) found that activation of protein kinase C by phorbol 12-myristate-13-acetate and active Raf kinase were also able to induce *spry* gene expression. Although MAPK signaling has been shown to effectively stimulate Spry induction by several mechanisms, Abe and Naski (2004) showed that Spry expression could be regulated by other pathways, such as through calcium-dependent and PLC γ signals.

Several transcription factor-binding sites have been identified in the *spry2* (Ding et al., 2003) and *spry4* promoters (Ding et al., 2004); however, little work has centered on identifying regulatory sequences in the promoters of *spry* genes. Although DNA methylation was shown to down-regulate Spry4 (Wang et al., 2006) and Spry2 in prostate cancer (McKie et al., 2005), no such preferred methylation status was identified in the *Spry2* promoter of breast cancer samples to account for the reduced Spry2 levels observed in both a cancer profiling array study and reverse transcription-polymerase chain reaction study of breast cancer samples (Lo et al., 2004). Likewise, although Spry2 levels are decreased in prostate cancer, no hypermethylation of the *Spry2* promoter was observed (Fritzsche et al., 2006). Is it possible that

other sites in the promoter of *Spry* genes participate in their regulation in normal cell development and disease progression? Recent papers suggest that this is possible. For example, a report by Lagha et al. (2008) revealed that Pax3, a transcription factor crucial for myogenesis and progenitor cell survival (Buckingham and Relaix, 2007), may target Spry1 in progenitor cells. In their study, transcripts corresponding to Spry1 were detected in Pax3-positive progenitor cells within the dermomyotome and myotome. *Pax3(-/-)* somite cells, however, displayed reduced Spry1 and increased phosphorylation of Erk, indicating the loss of Spry1 function (Lagha et al., 2008). In addition, another study (Katoh and Katoh, 2006) identified two Wnt-mediated-TCF/LEF- β -catenin sites in the 5' promoter of *Spry4* in the human, chimpanzee, rat, and mouse sequences. This is interesting because non-small-cell lung cancer (NSCLC) samples exhibit decreased Wnt-7a expression (Winn et al., 2005). In fact, overexpression of Wnt-7a reverses cell transformation, decreases anchorage-independent growth, and in conjunction with Fzd-9 (Wnt receptor), induces Spry4 expression (Winn et al., 2005). This suggests that the tumor suppressor function of Wnt7a/Fzd-9 signaling in NSCLC may be reduced as a result of low levels of Wnt-7a mRNA (Winn et al., 2005), which in turn may affect Spry expression and function.

Spry Proteins in Pathological States

Cancer. Evidence that the expression of Spry proteins is dysregulated in a number of cancers has been accumulating over the past few years (Table 3). These reports have suggested the potential of using cellular Spry content as tumor markers. A recent review (Lo et al., 2006) has comprehensively described past progress in this area; therefore, the discussion here is limited to the most recent findings pertinent to Spry proteins in cancer biology.

Consistent with a role for Spry2 in inhibiting both Erk1/2 signaling as well as proliferation, Spry2 amounts are decreased in hepatocellular carcinomas (Fong et al., 2006) and lung carcinomas (Sutterlüty et al., 2007). In mice, inhibition of the biological activity of Spry2 by hydrodynamic injection of Spry2 Y55F accelerated activated β -catenin-induced Erk1/2 activation and neoplastic phenotype in the liver (Lee et al., 2008), suggesting that loss of Spry2 function contributes to hepatic tumorigenesis. Likewise, in patients with NSCLC, lowered Spry2 expression was reported, and ectopi-

cally expressed Spry2 not only significantly reduced the proliferation of cell lines derived from these tumors but also blocked tumor formation from these cell lines in mice (Sutterlüty et al., 2007). In this study, Spry2 inhibited proliferation of mutant K-Ras-transformed NSCLC cells without inhibiting Erk1/2 activation, suggesting that Spry2 inhibits tumor formation by modulating other pathways (Sutterlüty et al., 2007). Further support for a role of Spry2 in modulating lung tumors is derived from the observations that specific overexpression of Spry2 in mouse lung epithelium significantly inhibited urethane-induced lung tumorigenesis (Minowada and Miller, 2009). Fewer tumors with a smaller diameter were observed in Spry2-overexpressing mice exposed to urethane (Minowada and Miller, 2009). The tumor-suppressing effect of Spry2 is apparently exerted at a level below K-Ras mutation, because Spry2 overexpression did not alter the frequency of occurrence of K-Ras mutations in urethane-induced tumors (Minowada and Miller, 2009). Through an as-yet-unknown mechanism, the loss of Spry2 function increases the number and size of lung tumors of mice harboring the K-RasG12D germline mutation (Shaw et al., 2007).

As mentioned under *Ubiquitin Ligases (cCbl and SIAH2)*, by sequestering c-Cbl, Spry2 can protect certain RTKs such as the EGFR from down-regulation and degradation and sustain their signaling. In theory, this mode of regulation should have a positive effect on tumor formation. Indeed, in H-Ras(V12)-transformed fibroblasts, the expression of EGF receptors and Spry2 was found to be elevated (Lito et al., 2008). The EGF receptor was necessary for oncogenic H-Ras transformed cells to grow in the absence of growth factors and form large anchorage independent colonies (Lito et al., 2008). Silencing of Spry2 in these H-Ras(V12)-transformed fibroblasts markedly attenuated their ability to form large colonies in agarose or form tumors in athymic mice (Lito et al., 2008). The effects of Spry2 were found to be specific for oncogenic Ras transformed cells because in control, nononcogenic H-Ras-transformed cells, Spry2 inhibited H-Ras and Erk1/2 activation (Lito et al., 2008). These data suggest that in oncogenic H-Ras-transformed fibroblasts, Spry2 may act as a tumor promoter by protecting EGF receptor down-regulation, whereas in control fibroblasts, it acts upstream of Ras to inhibit the Erk1/2 cascade. Another situation in which up-regulation of Spry2 has been observed is in melanoma cell

TABLE 3

Sprouty proteins in the etiology of several diseases and disorders

Reports of down- and up-regulated Spry levels in different diseases are listed, including type of disease and Spry isoform(s) along with appropriate references.

Disease	Isoforms	Reference
Down-regulated spry levels		
Hepatocellular carcinoma	Spry2	Fong et al., 2006; Lee et al., 2008
Breast cancer	Spry1, -2	Lo et al., 2004
Prostate cancer	Spry1	Kwabi-Addo et al., 2004
	Spry2	McKie et al., 2005
	Spry4	Wang et al., 2006
	Spry1, -2	Fritzsche et al., 2006
Non-small-cell lung cancer	Spry2	Sutterlüty et al., 2007
	Spry4	
Schizophrenia/bipolar disorder	Spry2	Pillai, 2008
Cardiovascular disease	Spry1	Thum et al., 2008
	Spry2	Sayed et al., 2008
Up-regulated spry levels		
Melanoma (B-Raf and N-Ras mutated cell lines)	Spry2	Bloethner et al., 2005
TDII thanatophoric dysplasia II	Spry2	Guo et al., 2008

lines with mutant B-Raf and N-Ras (Bloethner et al., 2005). However, there were several other genes also up-regulated in these cells; whether Spry2 acts as a tumor promoter in these cells is not entirely clear. Nevertheless, given our understanding of how Spry proteins both positively and negatively regulate signaling and biological actions of growth factor receptors, the cellular context and repertoire of changes in signaling elements in a given type of cancer may determine whether Spry proteins may act as tumor suppressors or tumor promoters.

Cardiovascular Diseases. Evidence linking Spry proteins to the regulation of cardiovascular diseases was derived from the studies of Zhang et al. (2005), which showed that Spry2 by inhibiting vascular smooth muscle cell proliferation and migration also diminished neointimal hyperplasia after balloon angioplasty of rat carotid artery. This study also showed that the expression of Spry2 in the carotid artery was markedly decreased immediately after balloon angioplasty and was then elevated 1 to 2 weeks after injury. Moreover, Huebert et al. (2004) showed that human heart samples obtained from patients with left ventricular assist device implants had increased levels of Spry1 mRNA and protein levels. Increased Spry1 levels were shown in the microvasculature and appeared to be responsible for a decrease in VEGF-induced proliferation of the endothelial cells (Huebert et al., 2004), confirming the findings that Spry proteins inhibit the proliferation of cultured endothelial cells (Impagnatiello et al., 2001). However, the mechanism(s) involved in the up-regulation of Spry proteins by left ventricular assist device remain unidentified.

More recently, studies have linked the expression of Spry proteins to changes in microRNAs that are up-regulated during cardiac hypertrophy and heart failure. MicroRNAs, which are non-protein-coding RNAs, participate in gene regulation in mammalian cells by repressing protein translation (for review, see Cannell et al., 2008). MicroRNA21 (miR21) is of particular interest because it is one of the microRNAs commonly up-regulated in different forms of cancer (Volinia et al., 2006; Meng et al., 2007) and in cardiac hypertrophy (Tatsuguchi et al., 2007; Sayed et al., 2008). In effect, Spry2 was identified as a potential target of miR21 in cardiac myocytes and colon cancer (SW 480) cells (Sayed et al., 2008). Overexpression of miR21 repressed Spry2 expression and induced the formation of branch-like outgrowths in cardiomyocytes that formed gap junctions between cells (Sayed et al., 2008). These morphological changes in cultured cardiomyocytes may represent the increased connectivity and conductance velocity observed in early stages of hypertrophy (Cooklin et al., 1998). A similar morphological change was also observed in cardiac myocytes after silencing of Spry2. Furthermore, β -adrenergic receptor activation, by increasing miR21 expression and decreasing Spry levels, also led to the branching of cardiomyocytes to form intracellular gap junctions (Sayed et al., 2008). This effect of β -adrenergic receptor activation was abrogated by the expression of “eraser miR21” that suppressed miR21 levels and resulted in increased expression of Spry2 (Sayed et al., 2008). Likewise, in colon cancer (SW480) cells, suppression of miR21 amounts increased cellular Spry2 content and inhibited cell migration. Given these findings, it is tempting to speculate that the down-regulation of Spry proteins in certain cancers (re-

viewed above under *Cancer* and in Table 3) may be secondary to the overexpression of miR21.

Another recent report has also shown that miR21 levels are increased in fibroblasts derived from failing hearts (Thum et al., 2008). In this study, miR21 levels were not altered in the cardiomyocytes of failing hearts. Although at first sight this seems to be at odds with the findings of Sayed et al. (2008), the findings from the two laboratories may not be inconsistent. Although Sayed et al. (2008) observed an increase in miR21 expression in cardiac myocytes of hypertrophied hearts, the expression of miR21 was decreased during the decompensation stage (i.e., in the failing heart). Like its effect in cardiac myocytes, the increase in miR21 in cardiac fibroblasts decreases the expression of Spry1 and thereby up-regulates the activation of the Erk1/2 cascade (Thum et al., 2008). This increase in the Erk1/2 signaling in the failing heart contributes to fibrosis and remodeling of the heart. Overall, the two studies show the involvement of miR21 in the regulation of Spry2 and Spry1 in cardiomyocytes and cardiac fibroblasts of the hypertrophied and failing heart, respectively, suggesting that there are coordinated temporal changes in miR21 expression in different cell types in the hypertrophic and failing heart. Thus, during early stages of cardiac hypertrophy, miR21 is increased in cardiac myocytes, and as the heart progresses toward decompensation and failure, miR21 levels in the cardiomyocytes are no longer elevated but instead become elevated in cardiac fibroblasts. The increase in miR21 in cardiac myocytes, by decreasing Spry2, may augment the activation of the Erk1/2 pathway that is known to play a critical role in induction of cardiac hypertrophy. The increase in miR21 in the later stages (i.e., decompensation) may contribute to the fibrosis and remodeling of the failing heart.

Other Pathological States or Correlates. By regulating the strength and duration of growth factor signaling, Spry proteins may also play a critical role in the etiology or progression of other diseases. In this context, Spry2 levels are elevated in chondrocytes of mice and humans with thanatophoric dysplasia type II (Guo et al., 2008). Thanatophoric dysplasia type II is a lethal form of chondrodysplasia. Chondrodysplasias result from inherited activating mutations in FGFR3, which negatively regulates proliferation and terminal differentiation of chondrocytes in growth plates, leading to abnormal linear bone growth. It has been suggested that the increase in Spry2 content in chondrocytes of thanatophoric dysplasia type II, by sequestering c-Cbl and protecting FGFR3 from down-regulation, may exacerbate the progression of the disease (Guo et al., 2008).

A decrease in the amount of Spry2 in prefrontal cortex of schizophrenic and bipolar patients has been correlated with a decrease in brain-derived neurotrophic factor (Pillai, 2008). However, whether these changes contribute to the etiology or progression of the disease is not known. Because Spry proteins modulate the proliferation of cells, one study has examined Spry2 expression after electroconvulsive therapy (Ongür et al., 2007). The findings from these studies show that electroconvulsive therapy decreases Spry2 expression, augments glial cell proliferation and thereby, increases neuronal plasticity (Ongür et al., 2007).

Concluding Remarks

As evident from the literature reviewed here, remarkable progress has been made in understanding the functional role of a group of proteins that was discovered just a decade ago. The Spry proteins are clearly involved in the pathogenesis of a number of cancers and in cardiovascular and other diseases. Although significant progress has been made toward understanding some of the molecular mechanism(s) by which Spry proteins fine-tune the biological actions of RTKs, future studies will have to consider the following: 1) that the different Spry family members associate with different signaling molecules; 2) that the Spry isoforms themselves may form homo- or heterodimers/oligomers, depending on which isoforms are expressed in a given cell type or tissue; and 3) that some of the Spry interacting partners also associate with key regulators of other pathways, permitting Spry proteins to affect more than one signaling pathway. Hence, the modulation of a given biological function or pathway by a specific Spry isoform will depend on the repertoire of its interacting partners. Elucidating the precise composition of the Spry complexes (Spry signalosomes) to regulate a given biological outcome will be fruitful ground for future investigations.

Moreover, it should be noted that the Spry proteins also are similar to the Spred family of proteins in the conserved cysteine rich domain (for review, see Bundschu et al., 2006, 2007). Whether the coexpression of the Spred family of proteins also modulates the function of Spry proteins is not yet clear. Therefore, future experimental systems will have to be more sophisticated to better understand how the Spry proteins, sometimes referred to as “master modulators of receptor tyrosine kinases,” mediate their functions in different tissues and cell types. Although Spry proteins have been implicated in different diseases, presently no “small molecules” alter their functions. Hence, future efforts may have to be directed toward development of drugs that alter Spry function to treat pathological conditions associated with aberrant Spry expression.

References

Abe M and Naski MC (2004) Regulation of sprouty expression by PLCgamma and calcium-dependent signals. *Biochem Biophys Res Commun* **323**:1040–1047.

Anteby EY, Natanson-Yaron S, Greenfield C, Goldman-Wohl D, Haimov-Kochman R, Holzer H, and Yagel S (2005) Human placental Hofbauer cells express sprouty proteins: a possible modulating mechanism of villous branching. *Placenta* **26**:476–483.

Aranda S, Alvarez M, Turró S, Laguna A, and de la Luna S (2008) Sprouty2-mediated inhibition of fibroblast growth factor signaling is modulated by the protein kinase DYRK1A. *Mol Cell Biol* **28**:5899–5911.

Ayada T, Taniguchi K, Okamoto F, Kato R, Komune S, Takaesu G, and Yoshimura A (2009) Sprouty4 negatively regulates protein kinase C activation by inhibiting phosphatidylinositol 4,5-bisphosphate hydrolysis. *Oncogene* **28**:1076–1088.

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.

Basson MA, Watson-Johnson J, Shakya R, Akbulut S, Hyink D, Costantini FD, Wilson PD, Mason IJ, and 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.

Bennett AM, Hausdorff SF, O'Reilly AM, Freeman RM, and Neel BG (1996) Multiple requirements for SHPTP2 in epidermal growth factor-mediated cell cycle progression. *Mol Cell Biol* **16**:1189–1202.

Bloethner S, Chen B, Hemminki K, Müller-Berghaus J, Ugurel S, Schadendorf D, and Kumar R (2005) Effect of common B-RAF and N-RAS mutations on global gene expression in melanoma cell lines. *Carcinogenesis* **26**:1224–1232.

Boros J, Newitt P, Wang Q, McAvoy JW, and Lovicu FJ (2006) Sef and Sprouty expression in the developing ocular lens: implications for regulating lens cell proliferation and differentiation. *Semin Cell Dev Biol* **17**:741–752.

Buckingham M and Relaix F (2007) The role of Pax genes in the development of tissues and organs: Pax3 and Pax7 regulate muscle progenitor cell functions. *Annu Rev Cell Dev Biol* **23**:645–673.

Bundschu K, Walter U, and Schuh K (2006) The VASP-Spred-Sprouty domain puzzle. *J Biol Chem* **281**:36477–36481.

Bundschu K, Walter U, and Schuh K (2007) Getting a first clue about SPRED functions. *Bioessays* **29**:897–907.

Cabrita MA and Christofori G (2008) Sprouty proteins, masterminds of receptor tyrosine kinase signaling. *Angiogenesis* **11**:53–62.

Cabrita MA, Jäggi F, Widjaja SP, and Christofori G (2006) A functional interaction between sprouty proteins and caveolin-1. *J Biol Chem* **281**:29201–29212.

Cannell IG, Kong YW, and Bushell M (2008) How do microRNAs regulate gene expression? *Biochem Soc Trans* **36**:1224–1231.

Casci T, Vinós J, and Freeman M (1999) Sprouty, an intracellular inhibitor of Ras signaling. *Cell* **96**:655–665.

Chambers D and Mason I (2000) Expression of sprouty2 during early development of the chick embryo is coincident with known sites of FGF signalling. *Mech Dev* **91**:361–364.

Chambers D, Medhurst AD, Walsh FS, Price J, and Mason I (2000) Differential display of genes expressed at the midbrain-hindbrain junction identifies sprouty2: a FGF8-inducible member of a family of intracellular FGF antagonists. *Mol Cell Neurosci* **15**:22–35.

Chan G, Kalaitzidis D, and Neel BG (2008) The tyrosine phosphatase Shp2 (PTPN11) in cancer. *Cancer Metastasis Rev* **27**:179–192.

Chandramouli S, Yu CY, Yusoff P, Lao DH, Leong HF, Mizuno K, and Guy GR (2008) Tesk1 interacts with Spry2 to abrogate its inhibition of ERK phosphorylation downstream of receptor tyrosine kinase signaling. *J Biol Chem* **283**:1679–1691.

Chi L, Itäranta P, Zhang S, and Vainio S (2006) Sprouty2 is involved in male sex organogenesis by controlling fibroblast growth factor 9-induced mesonephric cell migration to the developing testis. *Endocrinology* **147**:3777–3788.

Chi L, Zhang S, Lin Y, Prunskaitė-Hyryläinen R, Vuolteenaho R, Itäranta P, and Vainio S (2004) Sprouty proteins regulate ureteric branching by coordinating reciprocal epithelial Wnt11, mesenchymal Gdnf and stromal Fgf7 signalling during kidney development. *Development* **131**:3345–3356.

Cooklin M, Wallis WR, Sheridan DJ, and Fry CH (1998) Conduction velocity and gap junction resistance in hypertrophied, hypoxic guinea-pig left ventricular myocardium. *Exp Physiol* **83**:763–770.

DaSilva J, Xu L, Kim HJ, Miller WT, and Bar-Sagi D (2006) Regulation of sprouty stability by Mnk1-dependent phosphorylation. *Mol Cell Biol* **26**:1898–1907.

de Maximy AA, Nakatake Y, Moncada S, Itoh N, Thiery JP, and Bellusci S (1999) Cloning and expression pattern of a mouse homologue of *Drosophila* sprouty in the mouse embryo. *Mech Dev* **81**:213–216.

Deb TB, Wong L, Salomon DS, Zhou G, Dixon JE, Gutkind JS, Thompson SA, and Johnson GR (1998) A common requirement for the catalytic activity and both SH2 domains of SHP-2 in mitogen-activated protein (MAP) kinase activation by the ErbB family of receptors. A specific role for SHP-2 in map, but not c-Jun amino-terminal kinase activation. *J Biol Chem* **273**:16643–16646.

Ding W and Warburton D (2008) Down-regulation of Sprouty2 via p38 MAPK plays a key role in the induction of cellular apoptosis by tumor necrosis factor- α . *Biochem Biophys Res Commun* **375**:460–464.

Ding W, Bellusci S, Shi W, and Warburton D (2003) Functional analysis of the human Sprouty2 gene promoter. *Gene* **322**:175–185.

Ding W, Bellusci S, Shi W, and Warburton D (2004) Genomic structure and promoter characterization of the human Sprouty4 gene, a novel regulator of lung morphogenesis. *Am J Physiol Lung Cell Mol Physiol* **287**:L52–L59.

Edwin F and Patel TB (2008) A novel role of Sprouty 2 in regulating cellular apoptosis. *J Biol Chem* **283**:3181–3190.

Edwin F, Singh R, Endersby R, Baker SJ, and Patel TB (2006) The tumor suppressor PTEN is necessary for human Sprouty 2-mediated inhibition of cell proliferation. *J Biol Chem* **281**:4816–4822.

Egan JE, Hall AB, Yatsula BA, and Bar-Sagi D (2002) The bimodal regulation of epidermal growth factor signaling by human Sprouty proteins. *Proc Natl Acad Sci U S A* **99**:6041–6046.

Fong CW, Chua MS, McKie AB, Ling SH, Mason V, Li R, Yusoff P, Lo TL, Leung HY, So SK, et al. (2006) Sprouty 2, an inhibitor of mitogen-activated protein kinase signaling, is down-regulated in hepatocellular carcinoma. *Cancer Res* **66**:2048–2058.

Fong CW, Leong HF, Wong ES, Lim J, Yusoff P, and Guy GR (2003) Tyrosine phosphorylation of Sprouty2 enhances its interaction with c-Cbl and is crucial for its function. *J Biol Chem* **278**:33456–33464.

Fritzsche S, Kenzelmann M, Hoffmann MJ, Müller M, Engers R, Gröne HJ, and Schulz WA (2006) Concomitant down-regulation of SPRY1 and SPRY2 in prostate carcinoma. *Endocr Relat Cancer* **13**:839–849.

Fürthauer M, Reifers F, Brand M, Thisse B, and Thisse C (2001) sprouty4 acts in vivo as a feedback-induced antagonist of FGF signaling in zebrafish. *Development* **128**:2175–2186.

Goodnough LH, Brugmann SA, Hu D, and Helms JA (2007) Stage-dependent craniofacial defects resulting from Sprouty2 overexpression. *Dev Dyn* **236**:1918–1928.

Gross I, Armant O, Benosman S, de Aguiar JL, Freund JN, Keding M, Licht JD, Gaidon C, and Loeffler JP (2007) Sprouty2 inhibits BDNF-induced signaling and modulates neuronal differentiation and survival. *Cell Death Differ* **14**:1802–1812.

Gross I, Bassit B, Benezra M, and Licht JD (2001) Mammalian sprouty proteins inhibit cell growth and differentiation by preventing ras activation. *J Biol Chem* **276**:46460–46468.

Gross I, Morrison DJ, Hyink DP, Georgas K, English MA, Mericskay M, Hosono S, Sassoon D, Wilson PD, Little M, et al. (2003) The receptor tyrosine kinase regulator Sprouty1 is a target of the tumor suppressor WT1 and important for kidney development. *J Biol Chem* **278**:41420–41430.

Guo C, Degnin CR, Laederich MB, Lunstrum GP, Holden P, Bihlmaier J, Krakow D, Cho YJ, and Horton WA (2008) Sprouty 2 disturbs FGFR3 degradation in thanatophoric dysplasia type II: a severe form of human achondroplasia. *Cell Signal* **20**:1471–1477.

Guy GR, Wong ES, Yusoff P, Chandramouli S, Lo TL, Lim J, and Fong CW (2003) Sprouty: how does the branch manager work? *J Cell Sci* **116**:3061–3068.

Hacohen N, Kramer S, Sutherland D, Hiromi Y, and Krasnow MA (1998) sprouty

- encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airways. *Cell* **92**:253–263.
- Haglund K, Schmidt MH, Wong ES, Guy GR, and Dikic I (2005) Sprout2 acts at the Cbl/CIN85 interface to inhibit epidermal growth factor receptor downregulation. *EMBO Rep* **6**:635–641.
- Hall AB, Jura N, DaSilva J, Jang YJ, Gong D, and Bar-Sagi D (2003) hSpry2 is targeted to the ubiquitin-dependent proteasome pathway by c-Cbl. *Curr Biol* **13**:308–314.
- Hanafusa H, Torii S, Yasunaga T, and Nishida E (2002) Sprout1 and Sprout2 provide a control mechanism for the Ras/MAPK signalling pathway. *Nat Cell Biol* **4**:850–858.
- Hanafusa H, Torii S, Yasunaga T, Matsumoto K, and 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.
- Horowitz A and Simons M (2008) Branching morphogenesis. *Circ Res* **103**:784–795.
- Huebner RC, Li Q, Adhikari N, Charles NJ, Han X, Ezzat MK, Grindle S, Park S, Ormazza S, Fermin D, et al. (2004) Identification and regulation of Sprouty1, a negative inhibitor of the ERK cascade, in the human heart. *Physiol Genomics* **18**:284–289.
- Impagnatiello MA, Weitzer S, Gannon G, Compagni A, Cotten M, and 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.
- Jäggi F, Cabrera MA, Perl AK, and Christofori G (2008) Modulation of endocrine pancreas development but not beta-cell carcinogenesis by Sprouty4. *Mol Cancer Res* **6**:468–482.
- Jarvis LA, Toering SJ, Simon MA, Krasnow MA, and Smith-Bolton RK (2006) Sprouty proteins are in vivo targets of Corkscrew/SHP-2 tyrosine phosphatases. *Development* **133**:1133–1142.
- Kajita M, Ikeda W, Tamaru Y, and Takai Y (2007) Regulation of platelet-derived growth factor-induced Ras signaling by poliovirus receptor Necl-5 and negative growth regulator Sprouty2. *Genes Cells* **12**:345–357.
- Katoh Y and Katoh M (2006) FGF signaling inhibitor, SPRY4, is evolutionarily conserved target of WNT signaling pathway in progenitor cells. *Int J Mol Med* **17**:529–532.
- Kim HJ and Bar-Sagi D (2004) Modulation of signalling by Sprouty: a developing story. *Nat Rev Mol Cell Biol* **5**:441–450.
- Kim HJ, Taylor LJ, and Bar-Sagi D (2007) Spatial regulation of EGFR signaling by Sprouty2. *Curr Biol* **17**:455–461.
- Klein OD, Lyons DB, Baloch G, Marshall GW, Basson MA, Peterka M, Boran T, Peterkova R, and Martin GR (2008) An FGF signaling loop sustains the generation of differentiated progeny from stem cells in mouse incisors. *Development* **135**:377–385.
- Kwabi-Addo B, Wang J, Erdem H, Vaid A, Castro P, Ayala G, and Ittmann M (2004) The expression of Sprouty1, an inhibitor of fibroblast growth factor signal transduction, is decreased in human prostate cancer. *Cancer Res* **64**:4728–4735.
- Lagha M, Kormish JD, Rocancourt D, Manceau M, Epstein JA, Zaret KS, Relaix F, and Buckingham ME (2008) Pax3 regulation of FGF signaling affects the progression of embryonic progenitor cells into the myogenic program. *Genes Dev* **22**:1828–1837.
- Lao DH, Chandramouli S, Yusoff P, Fong CW, Saw TY, Tai LP, Yu CY, Leong HF, and Guy GR (2006) A Src homology 3-binding sequence on the C terminus of Sprouty2 is necessary for inhibition of the Ras/ERK pathway downstream of fibroblast growth factor receptor stimulation. *J Biol Chem* **281**:29993–30000.
- Lao DH, Yusoff P, Chandramouli S, Philip RJ, Fong CW, Jackson RA, Saw TY, Yu CY, and Guy GR (2007) Direct binding of PP2A to Sprouty2 and phosphorylation changes are a prerequisite for ERK inhibition downstream of fibroblast growth factor receptor stimulation. *J Biol Chem* **282**:9117–9126.
- Laziz I, Armand AS, Pariset C, Lecolle S, Della Gaspera B, Charbonnier F, and Chanoine C (2007) Sprouty gene expression is regulated by nerve and FGF6 during regeneration of mouse muscles. *Growth Factors* **25**:151–159.
- Lee CC, Putnam AJ, Miranti CK, Gustafson M, Wang LM, Vande Woude GF, and Gao CF (2004) Overexpression of sprouty 2 inhibits HGF/SF-mediated cell growth, invasion, migration, and cytokinesis. *Oncogene* **23**:5193–5202.
- Lee SA, Ho C, Roy R, Kosinski C, Patil MA, Tward AD, Fridlyand J, and Chen X (2008) Integration of genomic analysis and in vivo transfection to identify sprouty 2 as a candidate tumor suppressor in liver cancer. *Hepatology* **47**:1200–1210.
- Lee SH, Schloss DJ, Jarvis L, Krasnow MA, and Swain JL (2001) Inhibition of angiogenesis by a mouse sprouty protein. *J Biol Chem* **276**:4128–4133.
- Leeksma OC, Van Achterberg TA, Tsumura Y, Toshima J, Eldering E, Kroes WG, Mellink C, Spaargaren M, Mizuno K, Pannekoek H, et al. (2002) Human sprouty 4, a new ras antagonist on 5q31, interacts with the dual specificity kinase TESK1. *Eur J Biochem* **269**:2546–2556.
- Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, Beguinot L, Geiger B, and Yarden Y (1998) c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev* **12**:3663–3674.
- Li X, Wheldon L, and Heath JK (2003) Sprouty: a controversial role in receptor tyrosine kinase signalling pathways. *Biochem Soc Trans* **31**:1445–1446.
- Lim J, Wong ES, Ong SH, Yusoff P, Low BC, and Guy GR (2000) Sprouty proteins are targeted to membrane ruffles upon growth factor receptor tyrosine kinase activation. Identification of a novel translocation domain. *J Biol Chem* **275**:32837–32845.
- Lin W, Jing N, Basson MA, Dierich A, Licht J, and Ang SL (2005) Synergistic activity of Sef and Sprouty proteins in regulating the expression of Gbx2 in the mid-hindbrain region. *Genesis* **41**:110–115.
- Lito P, Mets BD, Appledorn DM, Maher VM, and McCormick JJ (2009) Sprouty 2 regulates DNA damage-induced apoptosis in Ras-transformed human fibroblasts. *J Biol Chem* **284**:848–854.
- Lito P, Mets BD, Kleff S, O'Reilly S, Maher VM, and McCormick JJ (2008) Evidence that sprouty 2 is necessary for sarcoma formation by H-Ras oncogene-transformed human fibroblasts. *J Biol Chem* **283**:2002–2009.
- Liu F, Sells MA, and Chernoff J (1998) Protein tyrosine phosphatase 1B negatively regulates integrin signaling. *Curr Biol* **8**:173–176.
- Lo TL, Fong CW, Yusoff P, McKie AB, Chua MS, Leung HY, and Guy GR (2006) Sprouty and cancer: the first terms report. *Cancer Lett* **242**:141–150.
- Lo TL, Yusoff P, Fong CW, Guo K, McCaw BJ, Phillips WA, Yang H, Wong ES, Leong HF, Zeng Q, et al. (2004) The ras/mitogen-activated protein kinase pathway inhibitor and likely tumor suppressor proteins, sprouty 1 and sprouty 2 are deregulated in breast cancer. *Cancer Res* **64**:6127–6136.
- Lowenstein EJ, Daly RJ, Batzer AG, Li W, Margolis B, Lammers R, Ullrich A, Skolnik EY, Bar-Sagi D, and Schlessinger J (1992) The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* **70**:431–442.
- Martínez N, García-Domínguez CA, Domingo B, Oliva JL, Zarich N, Sánchez A, Gutiérrez-Eisman S, Llopis J, and Rojas JM (2007) Sprouty2 binds Grb2 at two different proline-rich regions, and the mechanism of ERK inhibition is independent of this interaction. *Cell Signal* **19**:2277–2285.
- Mason JM, Morrison DJ, Bassit B, Dimri M, Band H, Licht JD, and 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.
- Mason JM, Morrison DJ, Basson MA, and Licht JD (2006) Sprouty proteins: multifaceted negative-feedback regulators of receptor tyrosine kinase signaling. *Trends Cell Biol* **16**:45–54.
- Matus DQ, Thomsen GH, and Martindale MQ (2007) FGF signaling in gastrulation and neural development in *Nematostella vectensis*, an anthozoan cnidarian. *Dev Genes Evol* **217**:137–148.
- McKie AB, Douglas DA, Olijslagers S, Graham J, Omar MM, Heer R, Gnanaprasagam VJ, Robson CN, and Leung HY (2005) Epigenetic inactivation of the human sprouty2 (hSPRY2) homologue in prostate cancer. *Oncogene* **24**:2166–2174.
- Meng F, Henson R, Wehbe-Jane K, Ghoshal K, Jacob ST, and Patel T (2007) MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* **133**:647–658.
- Minowada G and Miller YE (2009) Overexpression of Sprouty 2 in mouse lung epithelium inhibits urethane-induced tumorigenesis. *Am J Respir Cell Mol Biol* **40**:31–37.
- Minowada G, Jarvis LA, Chi CL, Neubüser A, Sun X, Hachohen N, Krasnow MA, and Martin GR (1999) Vertebrate Sprouty genes are induced by FGF signaling and can cause chondrodysplasia when overexpressed. *Development* **126**:4465–4475.
- Miyake S, Lupher ML, Jr., Druker B, and Band H (1998) The tyrosine kinase regulator Cbl enhances the ubiquitination and degradation of the platelet-derived growth factor receptor alpha. *Proc Natl Acad Sci U S A* **95**:7927–7932.
- Nadeau RJ, Toher JL, Yang X, Kovalenko D, and Friesel R (2007) Regulation of Sprouty2 stability by mammalian Seven-in-Absentia homolog 2. *J Cell Biochem* **100**:151–160.
- Natanson-Yaron S, Anteby EY, Greenfield C, Goldman-Wohl D, Hamani Y, Hochner-Celnikier D, and Yagel S (2007) FGF 10 and Sprouty 2 modulate trophoblast invasion and branching morphogenesis. *Mol Hum Reprod* **13**:511–519.
- Ng C, Jackson RA, Buschdorf JP, Sun Q, Guy GR, and Sivaraman J (2008) Structural basis for a novel intrapeptide H-bond and reverse binding of c-Cbl-TKB domain substrates. *EMBO J* **27**:804–816.
- Noguchi T, Matozaki T, Horita K, Fujioka Y, and Kasuga M (1994) Role of SH-PTP2, a protein-tyrosine phosphatase with Src homology 2 domains, in insulin-stimulated Ras activation. *Mol Cell Biol* **14**:6674–6682.
- Ongür D, Pohlman J, Dow AL, Eisch AJ, Edwin F, Heckers S, Cohen BM, Patel TB, and Carlezon WA Jr. (2007) Electroconvulsive seizures stimulate glial proliferation and reduce expression of Sprouty2 within the prefrontal cortex of rats. *Biol Psychiatry* **62**:505–512.
- Ozaki K, Kadomoto R, Asato K, Tanimura S, Itoh N, and Kohno M (2001) ERK pathway positively regulates the expression of Sprouty genes. *Biochem Biophys Res Commun* **285**:1084–1088.
- Ozaki K, Miyazaki S, Tanimura S, and Kohno M (2005) Efficient suppression of FGF-2-induced ERK activation by the cooperative interaction among mammalian Sprouty isoforms. *J Cell Sci* **118**:5861–5871.
- Pawson T (1995) Protein modules and signalling networks. *Nature* **373**:573–580.
- Petrelli A, Gilestro GF, Lanzardo S, Comoglio PM, Migone N, and Giordano S (2002) The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature* **416**:187–190.
- Pillai A (2008) Decreased expression of Sprouty2 in the dorsolateral prefrontal cortex in schizophrenia and bipolar disorder: a correlation with BDNF expression. *PLoS ONE* **3**:e1784.
- Poppleton HM, Edwin F, Jaggar L, Ray R, Johnson LR, and Patel TB (2004) Sprouty regulates cell migration by inhibiting the activation of Rac1 GTPase. *Biochem Biophys Res Commun* **323**:98–103.
- Price KL, Long DA, Jina N, Liapis H, Hubank M, Woolf AS, and Winyard PJ (2007) Microarray interrogation of human mesenchymal cells highlights potentially important molecules in vivo. *Physiol Genomics* **28**:193–202.
- Qi J, Nakayama K, Gaitonde S, Goydos JS, Krajewski S, Eroshkin A, Bar-Sagi D, Bowtell D, and Ronai Z (2008) The ubiquitin ligase Siah2 regulates tumorigenesis and metastasis by HIF-dependent and -independent pathways. *Proc Natl Acad Sci U S A* **105**:16713–16718.
- Rubin C, Gur G, and Yarden Y (2005a) Negative regulation of receptor tyrosine kinases: unexpected links to c-Cbl and receptor ubiquitylation. *Cell Res* **15**:66–71.
- Rubin C, Litvak V, Medvedovsky H, Zwang Y, Lev S, and Yarden Y (2003) Sprouty fine-tunes EGF signaling through interlinked positive and negative feedback loops. *Curr Biol* **13**:297–307.
- Rubin C, Zwang Y, Vaisman N, Ron D, and Yarden Y (2005b) Phosphorylation of carboxyl-terminal tyrosines modulates the specificity of Sprouty-2 inhibition of different signaling pathways. *J Biol Chem* **280**:9735–9744.
- Sasaki A, Taketomi T, Kato R, Saeki K, Nonami A, Sasaki M, Kuriyama M, Saito N, Shibuya M, and Yoshimura A (2003) Mammalian Sprouty4 suppresses Ras-independent ERK activation by binding to Raf1. *Nat Cell Biol* **5**:427–432.

- Sasaki A, Taketomi T, Wakioka T, Kato R, and Yoshimura A (2001) Identification of a dominant negative mutant of Sprouty that potentiates fibroblast growth factor-but not epidermal growth factor-induced ERK activation. *J Biol Chem* **276**:36804–36808.
- Sayed D, Rane S, Lypowy J, He M, Chen IY, Vashistha H, Yan L, Malhotra A, Vatner D, and Abdellatif M (2008) MicroRNA-21 targets Sprouty2 and promotes cellular outgrowths. *Mol Biol Cell* **19**:3272–3282.
- Shaw AT, Meissner A, Dowdle JA, Crowley D, Magendanz M, Ouyang C, Parisi T, Rajagopal J, Blank LJ, Bronson RT, et al. (2007) Sprouty-2 regulates oncogenic K-ras in lung development and tumorigenesis. *Genes Dev* **21**:694–707.
- Shim K, Minowada G, Coling DE, and 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.
- Sigismund S, Woelk T, Puri C, Maspero E, Tacchetti C, Transidico P, Di Fiore PP, and Polo S (2005) Clathrin-independent endocytosis of ubiquitinated cargos. *Proc Natl Acad Sci U S A* **102**:2760–2765.
- Soubeyran P, Kowanetz K, Szymkiewicz I, Langdon WY, and Dikic I (2002) Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature* **416**:183–187.
- Stiewe T (2007) The p53 family in differentiation and tumorigenesis. *Nat Rev Cancer* **7**:165–168.
- Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, Orth AP, Vega RG, Sapinoso LM, Moqrich A, et al. (2002) Large-scale analysis of the human and mouse transcripts. *Proc Natl Acad Sci U S A* **99**:4465–4470.
- Sutterlüthy H, Mayer CE, Setinek U, Attens J, Ovtcharov S, Mikula M, Mikulits W, Micksche M, and Berger W (2007) Down-regulation of Sprouty2 in non-small cell lung cancer contributes to tumor malignancy via extracellular signal-regulated kinase pathway-dependent and -independent mechanisms. *Mol Cancer Res* **5**:509–520.
- Sweet SM, Mardakheh FK, Ryan KJ, Langton AJ, Heath JK, and Cooper HJ (2008) Targeted online liquid chromatography electron capture dissociation mass spectrometry for the localization of sites of in vivo phosphorylation in human Sprouty2. *Anal Chem* **80**:6650–6657.
- Szymkiewicz I, Kowanetz K, Soubeyran P, Dinarina A, Lipkowitz S, and Dikic I (2002) CIN85 participates in Cbl-b-mediated down-regulation of receptor tyrosine kinases. *J Biol Chem* **277**:39666–39672.
- Tang TL, Freeman RM, Jr., O'Reilly AM, Neel BG, and Sokol SY (1995) The SH2-containing protein-tyrosine phosphatase SH-PTP2 is required upstream of MAP kinase for early *Xenopus* development. *Cell* **80**:473–483.
- Taniguchi K, Ayada T, Ichiyama K, Kohno R, Yonemitsu Y, Minami Y, Kikuchi A, Maehara Y, and Yoshimura A (2007) Sprouty2 and Sprouty4 are essential for embryonic morphogenesis and regulation of FGF signaling. *Biochem Biophys Res Commun* **352**:896–902.
- Tatsuguchi M, Seok HY, Callis TE, Thomson JM, Chen JF, Newman M, Rojas M, Hammond SM, and Wang DZ (2007) Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. *J Mol Cell Cardiol* **42**:1137–1141.
- Tefft D, Lee M, Smith S, Crowe DL, Bellusci S, and Warburton D (2002) mSprouty2 inhibits FGF10-activated MAP kinase by differentially binding to upstream target proteins. *Am J Physiol Lung Cell Mol Physiol* **283**:L700–L706.
- Tefft JD, Lee M, Smith S, Leinwand M, Zhao J, Bringas P, Jr., Crowe DL, and Warburton D (1999) Conserved function of mSpry-2, a murine homolog of *Drosophila* sprouty, which negatively modulates respiratory organogenesis. *Curr Biol* **9**:219–222.
- Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, et al. (2008) MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* **456**:980–984.
- Tsumura Y, Toshima J, Leeksa OC, Ohashi K, and Mizuno K (2005) Sprouty-4 negatively regulates cell spreading by inhibiting the kinase activity of testicular protein kinase. *Biochem J* **387**:627–637.
- Tummers M and Thesleff I (2009) The importance of signal pathway modulation in all aspects of tooth development. *J Exp Zool B Mol Dev Evol* **312B**:309–319.
- Vieira AR, Avila JR, Daack-Hirsch S, Dragan E, Félix TM, Rahimov F, Harrington J, Schultz RR, Watanabe Y, Johnson M, et al. (2005) Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. *PLoS Genet* **1**:e64.
- Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, et al. (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A* **103**:2257–2261.
- Wang Y, Janicki P, Köster I, Berger CD, Wenzl C, Grosshans J, and Steinbeisser H (2008) Xenopus Paraxial Protocadherin regulates morphogenesis by antagonizing Sprouty. *Genes Dev* **22**:878–883.
- Wang J, Thompson B, Ren C, Ittmann M, and Kwabi-Addo B (2006) Sprouty4, a suppressor of tumor cell motility, is down regulated by DNA methylation in human prostate cancer. *Prostate* **66**:613–624.
- Warburton D and Bellusci S (2004) The molecular genetics of lung morphogenesis and injury repair. *Paediatr Respir Rev* **5** (Suppl A):S283–S287.
- Warburton D, Perin L, Defilippo R, Bellusci S, Shi W, and Driscoll B (2008) Stem/progenitor cells in lung development, injury repair, and regeneration. *Proc Am Thorac Soc* **5**:703–706.
- Welsh IC, Hagge-Greenberg A, and O'Brien TP (2007) A dosage-dependent role for Spry2 in growth and patterning during palate development. *Mech Dev* **124**:746–761.
- Winn RA, Marek L, Han SY, Rodriguez K, Rodriguez N, Hammond M, Van Scoyk M, Acosta H, Mirus J, Barry N, et al. (2005) Restoration of Wnt-7a expression reverses non-small cell lung cancer cellular transformation through frizzled-9-mediated growth inhibition and promotion of cell differentiation. *J Biol Chem* **280**:19625–19634.
- Wong ES, Fong CW, Lim J, Yusoff P, Low BC, Langdon WY, and Guy GR (2002) Sprouty2 attenuates epidermal growth factor receptor ubiquitylation and endocytosis, and consequently enhances Ras/ERK signalling. *EMBO J* **21**:4796–4808.
- Wong ES, Lim J, Low BC, Chen Q, and Guy GR (2001) Evidence for direct interaction between Sprouty and Cbl. *J Biol Chem* **276**:5866–5875.
- Wu JH, Goswami R, Cai X, Exum ST, Huang X, Zhang L, Brian L, Premont RT, Poppel K, and Freedman NJ (2006) Regulation of the platelet-derived growth factor receptor-beta by G protein-coupled receptor kinase-5 in vascular smooth muscle cells involves the phosphatase Shp2. *J Biol Chem* **281**:37758–37772.
- Xiao S, Rose DW, Sasaoka T, Maegawa H, Burke TR, Jr., Roller PP, Shoelson SE, and Olefsky JM (1994) Syp (SH-PTP2) is a positive mediator of growth factor-stimulated mitogenic signal transduction. *J Biol Chem* **269**:21244–21248.
- Yamauchi K, Milarski KL, Saliel AR, and Pessin JE (1995) Protein-tyrosine-phosphatase SHPTP2 is a required positive effector for insulin downstream signaling. *Proc Natl Acad Sci U S A* **92**:664–668.
- Yizgaw Y, Cartin L, Pierre S, Scholich K, and Patel TB (2001) The C terminus of sprouty is important for modulation of cellular migration and proliferation. *J Biol Chem* **276**:22742–22747.
- Yizgaw Y, Poppleton HM, Sreejayan N, Hassid A, and Patel TB (2003) Protein-tyrosine phosphatase-1B (PTP1B) mediates the anti-migratory actions of Sprouty. *J Biol Chem* **278**:284–288.
- Yusoff P, Lao DH, Ong SH, Wong ES, Lim J, Lo TL, Leong HF, Fong CW, and Guy GR (2002) Sprouty2 inhibits the Ras/MAP kinase pathway by inhibiting the activation of Raf. *J Biol Chem* **277**:3195–3201.
- Zhang C, Chaturvedi D, Jaggar L, Magnuson D, Lee JM, and Patel TB (2005) Regulation of vascular smooth muscle cell proliferation and migration by human sprouty 2. *Arterioscler Thromb Vasc Biol* **25**:533–538.
- Zhang S, Lin Y, Itäranta P, Yagi A, and Vainio S (2001) Expression of Sprouty genes 1, 2 and 4 during mouse organogenesis. *Mech Dev* **109**:367–370.

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