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LRP2, AN AUXILIARY RECEPTOR THAT CONTROLS SONIC HEDGEHOG SIGNALING IN DEVELOPMENT AND DISEASE

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Abstract

To fulfill their multiple roles in organ development and adult tissue homeostasis Hedgehog morphogens (HH) act through their receptor Patched (PTCH) on target cells. However, HH actions also require HH binding proteins, auxiliary cell surface receptors that agonize or antagonize morphogen signaling in a context-dependent manner. Here, we discuss recent findings on the LDL receptor-related protein 2 (LRP2), an exemplary HH binding protein that modulates sonic hedgehog activities in stem and progenitor cell niches in embryonic and adult tissues. LRP2 functions are crucial for developmental processes in a number of tissues, including the brain, the eye, and the heart, and defects in this receptor pathway are the cause of devastating congenital diseases in humans.

Introduction

Hedgehogs (HH) are morphogens that govern central patterning processes in the embryo but that are also essential for homeostasis of adult tissues. In mammals, HH encompass three related proteins, called sonic hedgehog (SHH), desert hedgehog (DHH), and indian hedgehog (IHH) (Echelard et al., 1993). Their mode of action is prototypical for morphogens, being released from a local source and establishing a concentration gradient to pattern a target field. In recent years, much has been learned about the mechanisms that determine HH gradient formation in tissues involving active transport through cell-cell contacts as well as passive diffusion processes (Torroja et al., 2005; Dessaud et al., 2008; Roy et al., 2011; Sanders et al., 2013). Also, the mechanism of signaling at the primary cilium, the organelle that transduces HH cues into target cells, has been defined in molecular detail (see text box 1). Still, the complexity of HH activities in embryonic and adult tissues remains puzzling, in some instances promoting while in others impeding cell fate decisions.

An intriguing twist in understanding the context-dependent modes of HH actions came with the identification of HH binding proteins, cell surface proteins that act as auxiliary receptors in HH binding and cellular signal reception. The structural organization of HH binding proteins is diverse, including glycosylphosphatidylinositol (GPI)-anchored molecules, immunoglobulin domain containing proteins, as well as endocytic receptors. Jointly with the HH receptors Patched (PTCH) 1 and 2, these HH binding proteins are referred to as the HH receptorsome.

The structural distinctions but also the unique spatial and temporal expression patterns of HH binding proteins argue for specific functions in modulation of HH signaling. In recent years, findings from animal models as well as from studies

in patients have substantiated this notion, identifying important roles for the HH receptorsome in balancing morphogen activities. In this review, we will provide an overview of the molecular mechanisms of HH co-receptor functions and their implications for mammalian development and disease. Specifically, we will focus on the LDL receptor-related protein 2 (LRP2), a member of the LDL receptor gene family that exemplifies the context-dependent actions of auxiliary HH receptors.

The HH receptorsome

In mammals, HH proteins control key processes during embryonic development including patterning of the limb buds and neural tube (by SHH) (Ericson et al., 1995; Zuniga et al., 1999), formation of bone and cartilage (by IHH) (Vortkamp et al., 1996; St-Jacques et al., 1999), and development of germ cells (by DHH) (Bitgood et al., 1996). Consequently, the absence of HH activities results in various congenital defects in patients and animal models. This fact is highlighted by the loss of SHH signaling resulting in a failure to ventralize the midline of the neural tube and in midline formation defects (Chiang et al., 1996). Affected individuals suffer from severe craniofacial malformations and from a fusion of the forebrain hemispheres, a syndrome known as holoprosencephaly (HPE) (Roessler et al., 1996). Mutations in several components of the SHH signaling cascade cause HPE, including defects in the genes encoding SHH and PTCH1, or its downstream targets GLI2, SIX3, and ZIC2 (reviewed in (Roessler and Muenke, 2010)).

The signaling properties of HH proteins are explained by the interaction with their primary receptor PTCH1 on targeted cells (see text box 1). Thus, it came as a surprise when additional cell surface proteins were identified that bound HH proteins and affected morphogen activities (Fig. 1). The first HH binding

protein to be uncovered was the HH interacting protein (HHIP). HHIP was identified by expression cloning approaches aimed to characterize novel components of the HH signaling machinery in the mouse limb bud. HHIP is an 80-kDa protein anchored to the cell surface by a single membrane-spanning domain. The protein is unique to vertebrates and binds all three mammalian HH variants (Chuang and McMahon, 1999). Loss as well as overexpression of HHIP in mice impact bone and cartilage formation, suggesting a major role for HHIP in control of IHH function (Chuang and McMahon, 1999; Chuang et al., 2003). HHIP is an antagonist of the HH pathway, likely attenuating signaling by sequestration of the morphogen. Thus, its activity partially overlaps with that of unliganded PTCH1 (Jeong and McMahon, 2005; Holtz et al., 2013).

Whereas HHIP acts as an inhibitor of HH signaling, three additional HH binding proteins were identified subsequently because of their ability to promote SHH signaling. Growth arrest specific protein 1 (GAS1) is a 45-kDa polypeptide tethered to the cell surface via a GPI anchor (Stebel et al., 2000). It was retrieved by expression cloning of novel SHH binding proteins from cultured cells (Lee et al., 2001a), and later shown to impair craniofacial and eye development when inactivated in mice (Lee et al., 2001b; Martinelli and Fan, 2007). The relevance of GAS1 for SHH-dependent forebrain patterning was substantiated by the identification of missense mutations in *GAS1* in HPE patients. These mutations reduce the affinity of the receptor for SHH (Pineda-Alvarez et al., 2012). Additional HH binding proteins, that facilitate SHH signaling, are two related receptors of the immunoglobulin super family, termed CDON (cell adhesion molecule-related/down regulated by oncogenes) and BOC (brother of CDON). In contrast to the vertebrate-specific GAS1, CDON and BOC homologues are also found in invertebrates (Lum et al., 2003; Yao et al., 2006;

Camp et al., 2010; Zheng et al., 2010). Involvement of CDON in SHH signaling was documented by loss of function phenotypes in mice causing holoprosencephalic syndrome (Cole and Krauss, 2003; Zhang et al., 2006). Also, mutations in CDON, disrupting its ability to interact with PTCH1, have been identified in HPE patients (Bae et al., 2011). Inactivation of *Boc* in mice results in misguidance of commissural axons in the spinal cord, a defect in line with the role of SHH as midline derived chemoattractant (Okada et al., 2006). Although absence of BOC does not impact forebrain development per se, it aggravates the spectrum of forebrain malformation when combined with *Gas1* (Izzi et al., 2011) or *Cdon* (Zhang et al., 2011) defects in mice.

According to current concepts, GAS1, CDON, and BOC directly interact with PTCH1 to form high affinity receptor complexes that facilitate signal reception under limiting SHH concentrations (as in the developing neural tube). Agonistic actions are unique to each of the receptors as judged from the distinct phenotypes seen in *Cdon*, *Gas1,* and *Boc* single mutant mice, and by the progressive worsening of SHH-related phenotypes when combining individual receptor gene defects (Allen et al., 2007; Izzi et al., 2011; Zhang et al., 2011). In fact, the full spectrum of phenotypes reminiscent of *Shh* mutant mice requires elimination of all three HH binding proteins (Allen et al., 2011).

The latest addition to the HH receptorsome is a 600-kDa cell surface protein called LRP2 (also known as megalin). LRP2 is a type-1 transmembrane receptor of the LDL receptor gene family, a group of structurally related endocytic receptors (see text box 2). LRP2 is expressed in vertebrate and invertebrate species, but its role in HH signaling has so far only been confirmed in mammals. As with other members of the HH receptorsome, involvement of LRP2 in SHH action has

been suggested by HPE-related phenotypes seen in *Lrp2* gene-targeted mice (Willnow et al., 1996) and in humans with Donnai-Barrow syndrome (DBS), an autosomal recessive defect in *LRP2* (Kantarci et al., 2007; Kantarci et al., 2008). In the ventral neural tube, LRP2 interacts with PTCH1 to promote SHH sequestration and signaling during early neurulation (Christ et al., 2012).

The identification of several HH binding proteins, seemingly performing related functions in promoting SHH signaling during forebrain formation, remains puzzling. Yet, recent studies have uncovered unique context-dependent functions for GAS1, CDON, BOC, and LRP2 in HH signaling that extend their documented roles in forebrain patterning to other organ systems in the embryonic and the adult organism, and even encompass functions as agonist or antagonist of the HH pathway. In the main part of this article, we will focus on LRP2 as an example of an auxiliary HH receptor detailing how it promotes SHH action during forebrain development and why familial *LRP2* mutations cause forebrain malformation in humans. We will discuss possible roles for LRP2 in control of stem cell niches in the adult brain and retina, and how receptor dysfunction in these niches alters cell fate decisions and may cause cognitive dysfunction and eye disease. We will review new findings implicating LRP2 in SHH signaling during heart development, and we will present genetic data implicating this receptor in SHH-dependent tumor formation in pancreas and prostate.

LRP2, an endocytic receptor directing ligands into different cellular sorting pathways

LRP2 is the phylogenetically most ancient member of the LDL receptor gene family conserved from nematodes to man (reviewed in (Christensen and Birn, 2002)). In

mammals, the receptor is expressed in specialized absorptive epithelia including kidney, lung, reproductive organs, and brain. LRP2 has best been characterized as an endocytic receptor in the renal proximal tubules responsible for clearance of low-molecular weight plasma proteins from the glomerular filtrate. Ligands for LRP2 are plasma carriers for vitamins and steroid hormones, such as the vitamin D binding protein (DBP) and the retinol binding protein (RBP) (Christensen et al., 1999; Nykjaer et al., 1999). Retrieval of carrier proteins in the proximal tubules prevents filtration loss of vitamins and hormones bound to these carriers and conserves plasma levels of these essential metabolites. Absence of LRP2 in humans and mouse models results in loss of plasma proteins into the urine (low-molecular weight proteinuria) and in hypovitaminosis (Leheste et al., 1999; Storm et al., 2013). A similar function for LRP2 in cellular uptake of androgens and estrogens bound to their carrier, the sex hormone binding protein, has been documented in reproductive organs (Hammes et al., 2005).

A role for LRP2 as clearance receptor for multiple ligands has been documented in several epithelia. Remarkably, the fate of ligands internalized by LRP2 in various tissues is distinct, dependent on the cellular context and the physiological needs (Fig. 2). In most instances, ligands internalized from the apical cell surface are directed to late endosomal/lysosomal compartments for catabolism (as with DBP and RBP). However, some ligands resist lysosomal degradation and move with the receptor through the recycling compartments back to the apical cell surface for re-secretion (e.g., SHH) (Morales et al., 2006). A third route involves ligands trafficked by LRP2 from the apical to the basolateral plasma membrane, enabling transcytosis through polarized epithelia (e.g., thyroglobin, the carrier for thyroid hormones) (Marino et al., 2003). The molecular mechanisms governing different fates for LRP2

ligands are poorly understood but likely involve the interaction of the receptor tail with cytoplasmic adaptors that sort the receptor into different cellular pathways (Fig. 2). As detailed below, the ability of LRP2 to direct ligands into different intracellular fates may also provide explanatory models for the context-dependent functions of this receptor in SHH signaling.

LRP2 promotes SHH activity in neurogenic niches of the developing and adult brain

In the early mouse embryo, expression of LRP2 is restricted to the apical surface of the neuroepithelium. Receptor expression in neuroepithelial cells is sustained following neurulation and seen most prominent in the ventral domain of the neural tube. The relevance of LRP2 for proper specification of the CNS is documented by loss of function phenotypes in mouse models with ENU-induced or with targeted disruptions of *Lrp2* resulting in aberrant dorso-ventral patterning of the neural tube and in forebrain formation defects reminiscent of HPE (Willnow et al., 1996; Zarbalis et al., 2004; Spoelgen et al., 2005). The identification of *LRP2* mutations as cause of the autosomal recessive disorder DBS confirmed the relevance of LRP2 for the development of forebrain and facial structures in humans (Kantarci et al., 2007; Kantarci et al., 2008; Rosenfeld et al., 2010). DBS is a facio-oculo-acoustico-renal syndrome that also includes microforms of HPE (Khalifa et al., 2015). All known *LRP2* mutations affect the extracellular domain of the receptor polypeptide, in most instances causing expression of a truncated soluble receptor ectodomain. Genotype-phenotype correlations are not apparent in these cases. Because of the holoprosencephalic features of LRP2- deficient individuals and because of the ability of LRP2 to bind SHH in cultured cells (McCarthy and Argraves, 2003), a role for this

receptor in SHH action during forebrain development was proposed. This hypothesis was substantiated in mouse models of LRP2 deficiency (Christ et al., 2012). In these studies, LRP2 was shown to facilitate SHH signaling in the rostral diencephalon ventral midline (RDVM), a major forebrain organizer region (Fig. 3A). During neurulation, SHH is produced by the prechordal plate (PrCP), a rostral extension of the notochord. PrCP-derived SHH moves to the overlying RDVM to pattern this target field. In the RDVM, LRP2 forms a co-receptor complex with PTCH1 facilitating SHH binding and internalization of SHH/PTCH1 complexes, a prerequisite for pathway activation (Fig. 3B). The predominant localization of LRP2 in the ciliary pocket, a cell surface compartment of high endocytic activity, supports a role for this receptor in endocytosis of SHH/PTCH1 complexes from the apical cell surface (Christ et al., 2012). The necessity of LRP2 for SHH-dependent endocytosis of PTCH1 has been extensively documented in cell lines and whole embryo cultures (Christ et al., 2012). In contrast, the fate of the receptor ligand remains less clear. Because SHH co-localizes with LRP2 to the recycling compartment in neuroepithelial cells, SHH molecules internalized by this receptor may be subjected to re-secretion, promoting accumulation of the morphogen in its target field (Christ et al., 2012). Whether LRP2 may also provide the pathway for initial trafficking of SHH molecules from the PrCP to the apical surface of the RDVM remains highly speculative, but the ability of LRP2 to mediate epithelial trafficking of SHH has been suggested by studies in the epididymis (Morales et al., 2006).

Although LRP2 is expressed throughout the entire neural tube, its activity in SHH signaling is most relevant for the anterior part of the developing CNS (i.e., the forebrain). By contrast, the receptor does not seem to play a crucial role in SHH action in the caudal neural tube, as no major patterning defects are seen in the

spinal cord of receptor-deficient mice (Wicher and Aldskogius, 2008). Zebrafish mutants lacking *lrp2* suffer from resorption deficiency of the pronephros but show normal forebrain development, arguing that a role of LRP2 in forebrain formation may be specific to mammals (Anzenberger et al., 2006; Kur et al., 2011).

While the role of LRP2 in HH signaling in other phyla still awaits clarification, its relevance for SHH signaling in the mammalian brain likely goes beyond forebrain formation. Thus, in the adult brain, expression of LRP2 persists in the ependyma, the epithelial cell layer lining the ventricular system. In the lateral ventricles, expression of LRP2 is restricted to the lateral wall but not seen in the medial wall of the ependyma. This observation is remarkable as ependymal cells of the lateral wall face the subventricular zone (SVZ), a neurogenic niche of the adult mammalian brain (Merkle and Alvarez-Buylla, 2006; Ninkovic and Gotz, 2007). Loss of LRP2 expression in the ependyma results in depletion of neural stem cells and in decreased neurogenesis in the SVZ of adult mice (Gajera et al., 2010). Several morphogen pathways are implicated in adult neurogenesis in the SVZ, including SHH (Ahn and Joyner, 2005; Ihrie and Alvarez-Buylla, 2011). Distinct SHH-responsive domains exist in the ventral and in the dorsal SVZ, specifying the formation of different subtypes of olfactory bulb interneurons and oligodendroglial cells (Ihrie et al., 2011; Tong et al., 2015). Although ependymal cells are not a part of the neural stem cell population, they play a vital role in maintaining the microenvironment of the SVZ to enable adult neurogenesis (Lim et al., 2000; Colak et al., 2008; Ihrie and Alvarez-Buylla, 2011). Conceivably, LRP2 on ependymal cells may regulate SHH levels in the SVZ enabling SHH-dependent neurogenesis to proceed in this stem cell niche.

LRP2 has also been genetically associated with autism spectrum disorders in several patient cohorts (Ionita-Laza et al., 2012). Although hypothetical at present, this activity may also involve a role in control of SHH signaling as defects in this morphogen pathway have been implicated in developmental disturbances and in impaired neuroprotective mechanisms in autism (Lee and Tierney, 2011; Al-Ayadhi, 2012).

LRP2 regulates distinct steps of SHH function during eye development

DBS patients exhibit multiple pathologies including enlarged eyes (buphthalmos) and extreme nearsightedness (high myopia). Massive overgrowth of the eye globe is also seen in mice (Cases et al., 2015) and zebrafish (Veth et al., 2011) lacking this receptor. At first, implicating the loss of LRP2 as an auxiliary SHH receptor in these phenotypes seemed counterintuitive as holoprosencephalic phenotypes are typically not associated with enlarged and exophthalmic eyes. A possible resolution of this mystery came with a new study that substantiated the role of LRP2 as SHH binding protein in the retina. However, in this tissue, the receptor acts as an antagonist rather than an agonist of the morphogen pathway (Christ et al., 2015).

In the developing mammalian eye, SHH is produced by retinal ganglion cells. It provides inductive signals to retinal progenitor cells, resulting in patterning of the retina in a central to peripheral direction (Wang et al., 2002) (Fig. 3C). Intriguingly, SHH activity is absent from the distal margin of the developing retina, the ciliary marginal zone (CMZ), resulting in quiescence of progenitor cells in this tissue (Zhao et al., 2002; Cho and Cepko, 2006). By contrast, in non-mammalian species, such as amphibians and fish, the retinal margin constitutes a stem cell niche exhibiting proliferative capacity throughout adult life (Lamba et al., 2008). What

mechanism causes absence of SHH from the CMZ and why this tissue has to stay quiescent in the mammalian eye remains enigmatic. Recent studies now document that LRP2 is specifically expressed in the CMZ of the mammalian retina where it may act as a clearance receptor for SHH. In this model, LRP2-mediated uptake and lysosomal catabolism prevents spread of SHH activity from the central retina into the retinal margin and protects progenitor cells in this niche from adverse mitogenic stimuli (Fig. 3D). Loss of this "morphogen sink" in the LRP2 mutant retina increases the active concentration of SHH in the CMZ, resulting in aberrant expansion of the retinal progenitor cell pool and in hyperproliferation of the retinal margin. Enhanced retinal progenitor cell proliferation as a consequence of increased SHH signaling is also seen in mice heterozygous for the *Ptch1* gene defect (Moshiri and Reh, 2004) or in animals lacking SUFU, an inhibitor of the SHH pathway (Cwinn et al., 2011), supporting this concept. Although not proven formally yet, hyperproliferation of the embryonic CMZ possibly contributes to the increase in postnatal eye size in *Lrp2*$^{-/-}$ mice (and DBS patients), as mice with conditional *Lrp2* inactivation in the adult lack such eye pathologies (Storm et al., 2014). Of note, the ability to act as an inhibitor of HH signaling during eye development has also been shown for CDON in zebrafish (Cardozo et al., 2014). Clearly, the exact mode of LRP2 action in the retinal margin still awaits further experimental consolidation (Fig. 3D). For example, what mechanism may prevent LRP2-mediated endocytosis of PTCH1 in the CMZ (but not in the RDVM) in the presence of SHH remains unclear. Conceptually, the cell-type specific interaction of LRP2 and PTCH1 (or the lack thereof) may be determined by the existence of additional interactors that facilitate or prevent co-receptor interaction. Such a mechanism is operable in the WNT pathway with transmembrane proteins Kremen 1 and 2 blocking LRP5/6 interaction with Frizzled (Mao et al., 2002).

The ability to sort SHH into recycling versus degradation fates potentially determines the action of LRP2 as agonist or antagonist of the SHH pathway. A third possibility how LRP2 may control SHH action entails sorting of the morphogen into vesicles destined for transcytosis. During eye formation, SHH also acts as a chemoattractant guiding the migration of oligodendrocyte precursor cells from the preoptic area to the optic nerve where they differentiate into myelin-producing oligodendrocytes (Fig. 3E). LRP2 contributes to the formation of such SHH guidance cues because the receptor, expressed on optic nerve astrocytes, captures locally produced SHH and presents it to oligodendrocyte precursor cells (Fig. 3F). In line with this activity, blockade of LRP2 activity by inhibitory antibodies partially abolishes chemoattraction of oligodendrocyte precursor cells in optic nerve explants (Ortega et al., 2012). An effect on SHH-dependent guidance processes during eye development is not unique to LRP2 as BOC has been shown to restrict the number of retinal ganglion cells that project their axons across the optic chiasm (contralateral projections). This activity of BOC encompasses its ability to drive the formation of ipsilateral projecting retinal ganglion cells (Sanchez-Arrones et al., 2013) and to cause SHH-induced repulsion of axonal projection across the optic chiasm (Fabre et al., 2010).

Emerging functions for LRP2 in SHH action

During mammalian heart development, two specific tissues, the anterior heart field and the cardiac neural crest, contribute to the formation of the septum that divides the cardiac outflow tract into pulmonary artery and aorta. In approximately 2% of congenital heart conditions in humans, this truncus arteriosus fails to separate properly. As a consequence, a common arterial trunk (CAT) arises from the left and

right ventricles and a mixture of oxygenated and deoxygenated blood enters the circulatory system. CAT patients suffer from cyanosis (poor oxygen saturation) and possible heart failure. SHH is crucial for proper outflow tract formation as *Shh*$^{-/-}$ mice exhibit a single outflow tract (Washington Smoak et al., 2005). Specifically, SHH determines the ability of cardiac neural crest cells to populate the outflow tract cushions, and it enables myocardial cells to complete septation following cushion formation (Goddeeris et al., 2007). In a recessive genetic screen, mutations in *Lrp2* were identified as a novel cause of outflow tract septation defects in mice, implicating this receptor in SHH function in the embryonic heart (Li et al., 2015). Mice having combined defects in *Gas1* and *Shh* (Martinelli and Fan, 2007), or in *Gas1*, *Cdon*, and *Boc* (Allen et al., 2011) suffer from heart looping defects, a phenotype also seen in *Smo* deficient animals (Zhang et al., 2001). Jointly, the above findings underscore the relevance of members of the HH receptorsome for multiple steps of heart morphogenesis.

As well as in embryonic development, recent studies also implicate HH binding proteins in pathway activities in the adult organism, most notably in tumor formation. Aberrant SHH signaling is causally involved in tumorigenesis in a number of tissues, foremost in cerebellum and skin, but also in prostate, bladder, and pancreas (reviewed in (Ruiz i Altaba et al., 2002; Rubin and de Sauvage, 2006)). Thus, pharmacological intervention with this pathway has proven successful in the therapy of basal cell carcinoma of the skin (Von Hoff et al., 2009). The ability of SHH to maintain the proliferative capacity of progenitor cell populations infers its tumorigenic potential in case of constitute activation of the pathway in adult tissues. For example, constitutive pathway activation is seen with activating mutations in *SMO* (Xie et al., 1998) or inactivation mutations in *PTCH1* (Hahn et al., 1996;

Johnson et al., 1996) in patients with basal cell carcinoma. Also, inappropriate GLI1 target gene expression causes the uncontrolled proliferation of granule neuron precursor cells in medulloblastoma, a malignant tumor of the cerebellum (Lee et al., 2010). Concerning involvement of HH binding proteins in tumor formation, increased levels of BOC are detected in medulloblastoma tissue in patients and mouse models, and inactivation of the encoding gene in mice reduces tumor burden (Mille et al., 2014). Along the same lines, low expression levels of GAS1 correlate with poor prognosis in colorectal cancer (Jiang et al., 2011), while inactivation of *Cdon* promotes tumor formation in an experimental model of intestinal cancer in mice (Delloye-Bourgeois et al., 2013). At present, the involvement of LRP2 in SHH-dependent tumorigenesis is speculative, but *LRP2* polymorphisms are associated with the risk and poor prognosis of cancers of prostate and pancreas (Holt et al., 2008; Jones et al., 2008).

Outlook

Much has been learned about LRP2 as an auxiliary receptor to PTCH1 in SHH signal trasnduction, a function that parallels the established role of the related receptors LRP5 and LRP6 as co-receptors to Frizzled in WNT signaling (reviewed in (Niehrs and Shen, 2010)). It is noteworthy that LRP2 has also been shown to bind and mediate the endocytic clearance of bone morphogenetic proteins (Spoelgen et al., 2005). Thus, an exciting topic to be address in the future may be the potential of LRP2 to engage different types of morphogens and to act as convergence point for the integration of multiple morphogen pathways in target cells. Also, while a role of LRP2 in SHH signaling in the mammalian organism unfolds, the relevance of this receptor for HH signaling in non-mammalian species remains an unanswered

question. Thus, LRP2 is expressed in organisms as primitive as *C. elegans* (where the receptor is called LRP-1) (Yochem and Greenwald, 1993). Although the existence of the HH signaling pathway in nematodes is still a matter of debate, inactivation of the hedgehog-related gene *qua-1* (Hao et al., 2006) and of *lrp-1* (Yochem et al., 1999) causes similar larval molting defects. Disruption of *mgl*, the gene encoding LRP2 in *Drosophila,* results in lethality of fly larvae (Riedel et al., 2011). The underlying mode of receptor action in the fruit fly remains enigmatic. However, disruption of *lrp2* in the zebrafish mutant *bugeye* causes massive enlargement of the eye globe, a phenotype that might be influenced by altered HH signaling in the mutant fish eye (Veth et al., 2011). Elucidating the molecular mechanisms of LRP2 action in various species will be important to dissect conserved versus newly acquired functions for this receptor in the mammalian organism.

For reasons of focus, this review has concentrated on the role of LRP2 in SHH signaling. Obviously, a large body of exciting work, not covered here, has identified key contributions of BOC, CDON, and GAS1 to morphogen action in vertebrate and non-vertebrate species. Intriguingly, these various HH co-receptors do not act autonomously but functionally interact in control of morphogen signaling. Dependent on the physiological context, these interactions may be redundant, synergistic, or even opposing. This notion is exemplified by the progressive worsening of craniofacial malformations in mice with combined receptor deficiencies (Allen et al., 2007; Izzi et al., 2011; Zhang et al., 2011), or by the observation that loss of *Gas1* and *Boc* promotes whereas the combined loss of *Gas1*, *Boc*, and *Cdon* abrogates pancreatic cancer progression (Mathew et al., 2014). In this respect, clarifying the genetic interaction of *Lrp2* with other members of the HH

receptorsome, as in new mouse models with combined receptor gene defects is clearly warranted.

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Text box 1: Cellular SHH signal transduction

Common to all phyla, the HH pathway in cells is repressed through a cascade of inhibitory interactions that are relieved in the presence of the morphogen. In brief, binding of HH to the 12-pass transmembrane protein Patched 1 (PTCH1) on the surface of target cells alleviates its inhibitory effect on the 7-pass transmembrane protein Smoothened (SMO). Activated SMO triggers the conversion of the full-length form of the glioma-associated (GLI) family of transcription factors into their active form, resulting in induction of target gene transcription (reviewed in (Goetz and Anderson, 2010; Briscoe and Therond, 2013)). Unique to vertebrates, the activity of the HH pathway is intimately linked to a cellular organelle, called the primary cilium (Huangfu et al., 2003). Primary cilia are solitary antenna-like structures consisting of a basal body, a transition zone, and a filamentous axoneme (see figure). The basal body is composed of nine microtubuli triplets that, at the level of the transition zone, turn into the doublet microtubuli structure of the axoneme. The transition zone (containing transition fibers, Y-linkers, and septins) acts as a selective barrier between the cytoplasm and the microenvironment of the axoneme. In contrast to motile cilia, arranged in nine microtubuli doublets with an inner microtubule pair (9+2), the axoneme of a primary cilium lacks the inner microtubule pair (9+0) and shows no motility. The plasma membrane at the base of the primary cilium forms the ciliary pocket, a membrane invagination with high endocytic activity and enriched in PTCH1 molecules. Components of the SHH pathway including PTCH1, SMO, and GLI proteins display dynamic movements along the basal body and the ciliary axoneme with their specific localization depending on the state of activation of the canonical SHH pathway (Corbit et al., 2005; Rohatgi et al., 2007). In the absence of SHH (repressed state), PTCH1 localizes to the ciliary base and the axoneme, preventing

entry of SMO into the cilium. Because of the absence of activated SMO, full-length GLI (GLIFL) fails to accumulate inside the cilium. Although low amounts are still found within the axoneme, most GLIFL molecules are subject to phosphorylation by protein kinase A (PKA) at the ciliary base (Tuson et al., 2011). Phosphorylated GLIFL is targeted for proteolytic processing by the proteasome, generating the repressor form of the transcription factors (GLIR) (Wang et al., 2000). The most prominent repressor form is GLI3R that translocates to the nucleus and inhibits target gene transcription. Repression of the SHH pathway is facilitated by two additional factors, SUFU (Suppressor of fused) and KIF7. Binding of SUFU prevents conversion of GLIFL into the transcriptional activator form (GLIA), while the kinesin-4 family protein KIF7 promotes GLI3R formation. Binding of SHH to PTCH1 results in de-repression of the pathway (activated), induced by internalization and lysosomal degradation of PTCH1/SHH complexes (Incardona et al., 2000). Depletion of PTCH1 from the cell surface enables SMO to enter the cilium, either via lateral transport from the plasma membrane or from an intracellular vesicle pool (Milenkovic et al., 2009; Wang et al., 2009). SMO binds to the ciliary proteins EVC (Ellis-van Creveld syndrome protein) and EVC2 in a region of the cilium just above the transition zone, inducing conformational changes to SMO that cause inhibition of phosphorylation of GLIFL by PKA (Dorn et al., 2012). Also upon pathway activation, KIF7 translocates into the cilium to promote GLIFL accumulation in the tip of the cilium. In addition, KIF7 inhibits SUFU and enables activated GLIA (mainly GLI2A) to move to the nucleus where it activates target gene transcription. Anterograde transport of HH pathway components to the ciliary tip is performed by the intraflagellar transport (IFT)-B complex and the kinesin-2 motor, while their retrograde transport is arranged

by dynein motor proteins and the IFT-A complex (Haycraft et al., 2005; Tran et al., 2008).

Text box 2: The LDL receptor gene family

The LDL receptor gene family encompasses a group of type-1 transmembrane proteins that share structural similarity with the LDL receptor, the main receptor for uptake of lipoproteins in vertebrate cell types (reviewed in (Dieckmann et al., 2010; Willnow et al., 2012)). The figure depicts the mammalian members of this gene family. Their extracellular domains contain clusters of complement-type repeats (the sites for ligand binding) as well as β-propellers (for pH-dependent release of such ligands in endosomes). Similar to the LDL receptor, most LDL receptor-related proteins (LRPs) act as clearance receptors internalizing a multitude of ligands, from lipoproteins to vitamin carriers to signaling molecules. The LDL receptor is dispensable for embryogenesis as judged by the normal development of humans or animal models lacking this receptor (Goldstein et al., 2001). By contrast, most LRPs are required for embryogenesis and are the cause of congenital defects in humans when being mutated. Commonly, LRPs act as co-receptors to signaling proteins, modulating ligand binding and/or cellular signal transduction (reviewed in (Willnow et al., 2012)). This concept has best been elucidated for LRP5 and LRP6, co-receptors to Frizzled in WNT signaling. *Lrp6* mutations in mice result in axial truncation and abnormal head and limb structures (Pinson et al., 2000), whereas *LRP5* mutations cause bone formation defects in humans and mouse models (Gong et al., 2001; Kato et al., 2002; Little et al., 2002). The role of LRP2 as a co-receptor to PTCH1 in SHH signaling is detailed in the main text.

FIGURE LEGENDS

Fig. 1: The HH receptorsome

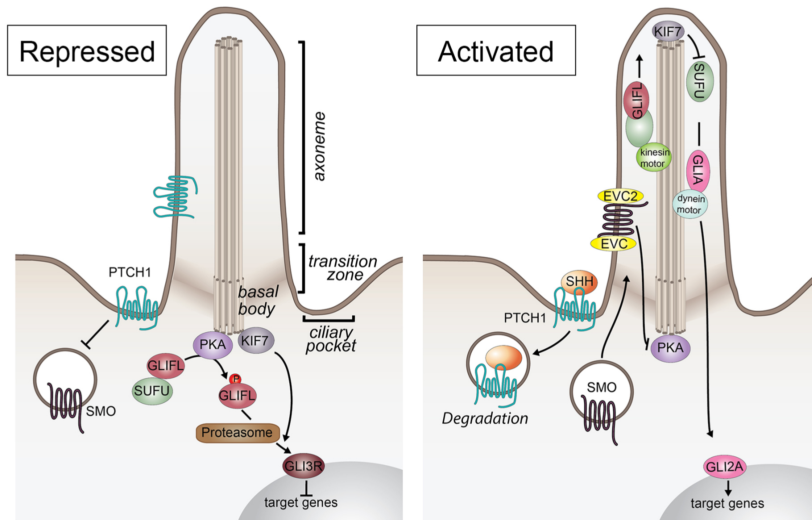
This scheme depicts the structural organization of PTCH1 and PTCH2 and of HH binding proteins identified in mammals. Known interaction sites for HH (asterisk) and PTCH1 (star) in the receptors are indicated.

Fig. 2: Cellular fate of ligands internalized by LRP2

Endocytosis starts with an extracellular ligand binding to LRP2 at the apical cell surface of epithelial cells. Receptor/cargo complexes are internalized from the plasma membrane and delivered to early endosomes. A drop in luminal pH in early endosomes disrupts binding of most ligands to LRP2, enabling the unliganded receptor to recycle back to the cell surface while the ligand moves through the late endosomal compartments to lysosomes for catabolism. However, some ligand/LRP2 complexes resist the low pH in early endosomes and recycle back to the cell surface, causing re-secretion of the ligand. A third route involves trafficking of receptor cargo from early endosomes to the basolateral cell membrane, resulting in transcytosis of such ligands. Cytosolic adaptors binding to the cytoplasmic domain of LRP2 to promote (+) or inhibit (-) distinct steps in receptor sorting are indicated. AP2, adaptor related protein complex 2; ARH, autosomal recessive hypercholesterolemia; DAB2, Disabled homolog 2; GIPC, PDZ domain containing family member 1.

Fig. 3: Proposed mechanisms for LRP2-mediated control of SHH trafficking and signaling

(**A-B**) LRP2 promotes SHH signaling during forebrain patterning. (A) At neurulation, SHH, produced by the prechordal plate (PrCP) acts on the overlying neuroepithelium to pattern the rostral diencephalon ventral midline (RDVM), a major forebrain organizer region. LRP2 is expressed in the apical surface of the RDVM (green line). (B) On the apical surface of neuroepithelial cells, SHH is sequestered by a co-receptor complex of LRP2 and PTCH1. SHH binding induces uptake of receptor-ligand complexes, lifting PTCH1-dependent suppression of SMO activity. Internalized PTCH1 is subject to lysosomal catabolism while SHH is potentially delivered to the cellular recycling pathway to increase local morphogen concentration in the RDVM. (**C-D**) LRP2 antagonizes SHH signaling in the developing retina. (C) SHH, produced by retinal ganglion cells patterns the developing retina in a central to peripheral direction. SHH activity is absent from the ciliary marginal zone (CMZ). LRP2 expression in the retina is restricted to non-pigmented ciliary epithelial cells in the CMZ (green line). (D) Binding of SHH to LRP2 on non-pigmented ciliary epithelial cells results in cellular uptake and lysosomal degradation of the morphogen. As a consequence of absent SHH ligand, PTCH1 inhibits SMO activity in the CMZ. (**E-F**) LRP2 facilitates SHH action in the developing optic nerve. (E) During optic nerve formation, SHH, produced by retinal ganglion cells in the retina, acts as a chemoattractant for oligodendrocyte precursor cells (OPC) that migrate from the preoptic area to the optic nerve and differentiate into myelin-forming oligodendrocytes. LRP2 is expressed on astrocytes in the optic nerve (green line). (F) In astrocytes, LRP2 possibly mediates transcytosis of SHH from the apical to the basolateral site of the cells, delivering SHH molecules to OPC that respond to the morphogen signal with SMO activation.



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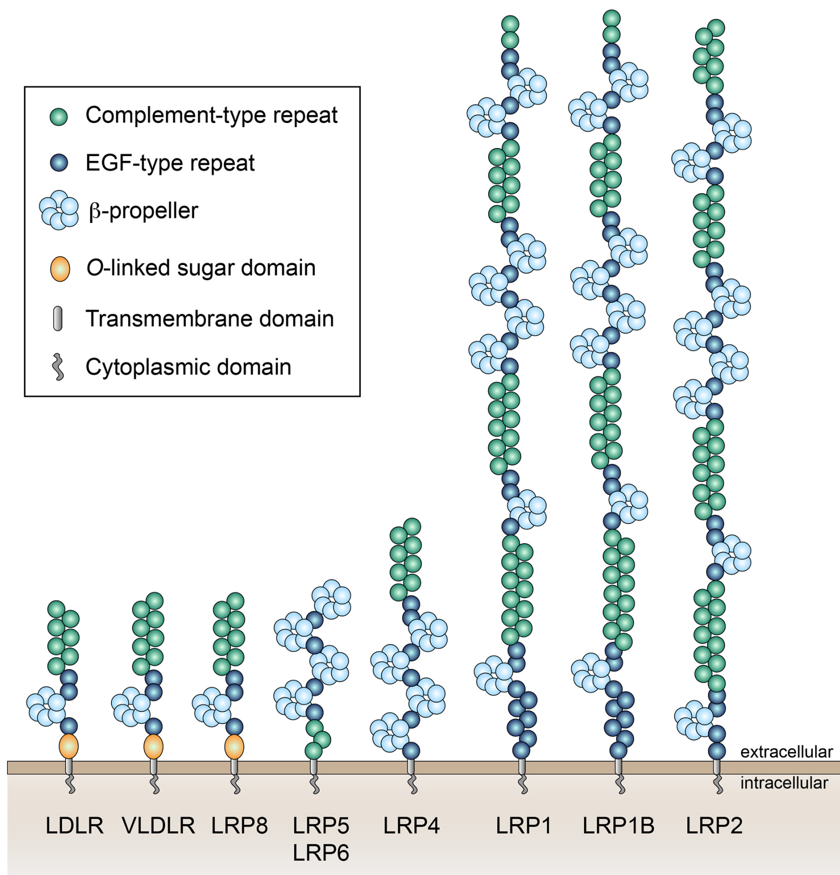

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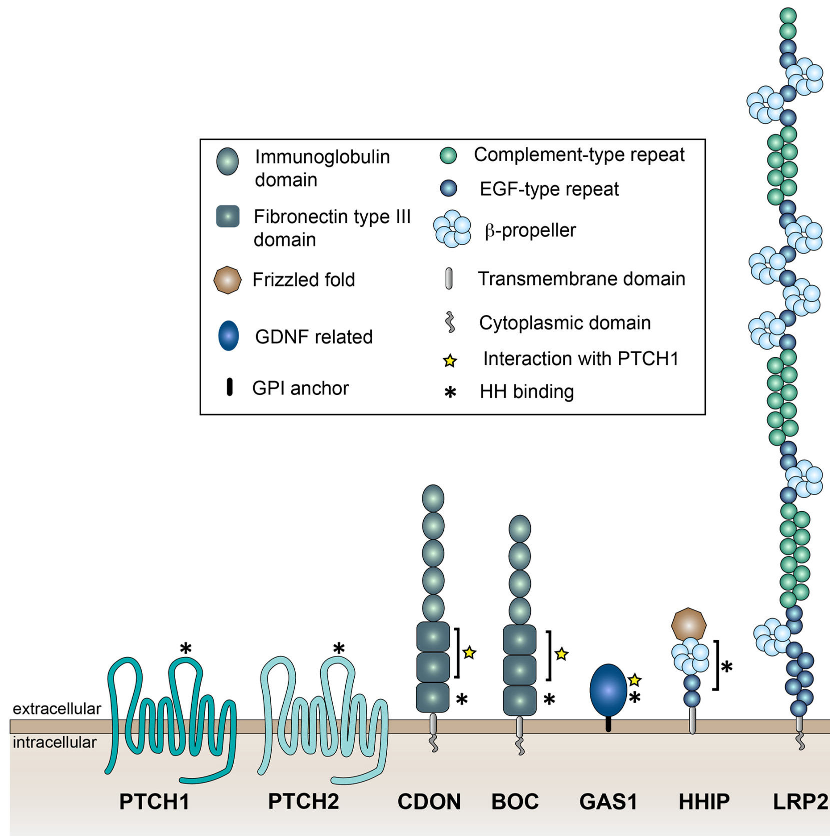

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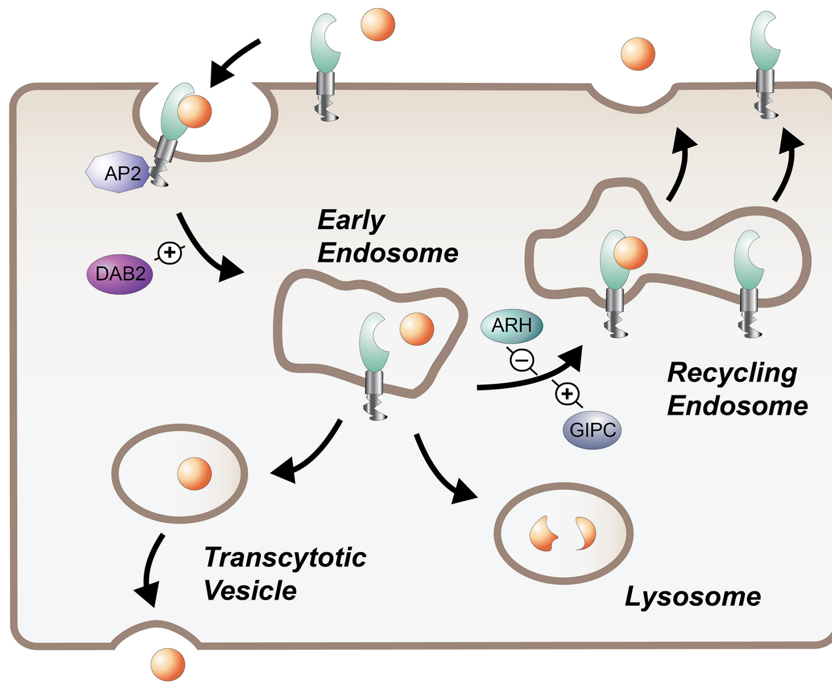
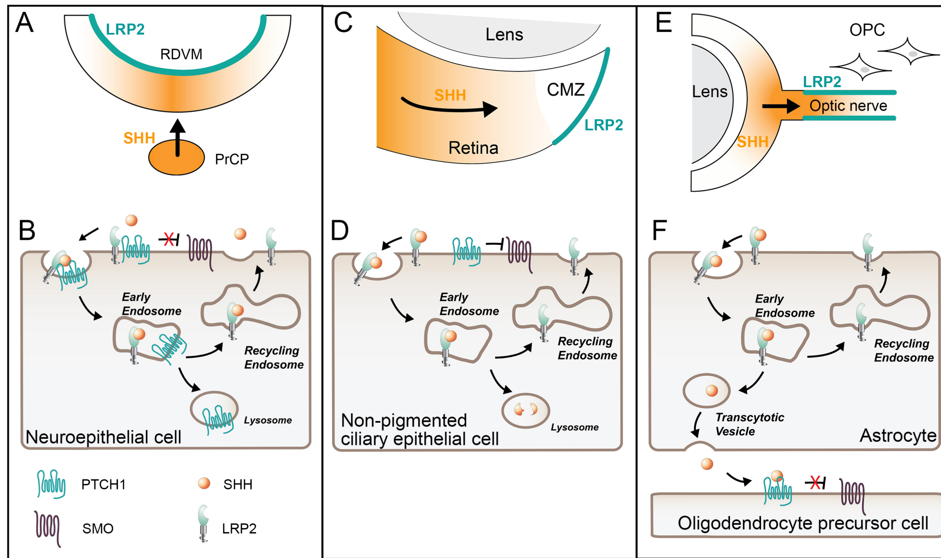


Christ et al., Figure 2



Christ et al., Figure 3