Sonic hedgehog

H S Heussler, M Suri

Implications for human development

The hedgehog (hh) gene is one of the segment polarity genes that regulate segmental and imaginal disc patterning in the fruit fly, *Drosophila melanogaster*. Unlike drosophila and other invertebrates, which only have a single hh gene, vertebrates have a family of genes that are homologous to the hh gene. Mammals have three genes with homology to the hh gene. These comprise Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh).[1] All hedgehog genes encode signalling molecules that are involved in short and long range patterning processes during embryogenesis.

Like all hedgehog proteins Shh protein also undergoes molecular processing in the endoplasmic reticulum. This involves cleavage of its signal peptide, followed by autocatalytic cleavage of the hedgehog protein precursor into a 19 kDa N-terminal domain (Shh-N) and a 25 kDa C-terminal domain (Shh-C). The signalling activity of hedgehog proteins resides in Shh-N.[2] Shh-C has intramolecular cholesterol transferase activity and is responsible for covalently attaching a cholesterol molecule to the C-terminal end of Shh-N.[3] The addition of cholesterol plays an important role in spatially restricting the zone of activity of Shh-N by anchoring it to the cell membrane and restricting its diffusion from the site of secretion.[4] It is believed that inborn errors of cholesterol synthesis such as Smith–Lemli–Opitz syndrome (microcephaly, growth and mental retardation, facial dysmorphism, syndactyly of the second and third toes, congenital heart disease, hypotonia, and genital abnormalities in males) can interfere with SHH signalling by interfering with its molecular processing, in particular with the cholesterol modification of Shh-N.[5]

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There is evidence to suggest that Shh-N binds to the 12 transmembrane receptor Patched.[6] Patched normally inhibits downstream signalling through a seven transmembrane receptor called Smoothened. When Shh-N binds to Patched, Smoothened is released from repression and signalling proceeds via the GLI gene family and HRK4 (human Kruppel related gene 4).[7 8] The GLI gene family and HRK4 are transcription factors. How Smoothened signals via the GLI gene family and HRK4 in humans is not known.

EXPRESSION OF SONIC HEDGEHOG

The expression pattern of Sonic Hedgehog has been studied in several species. Studies in rodents have shown that Shh is essential for dorso–ventral neural tube patterning, foregut development, several aspects of gut development, and antero–posterior patterning of the limb buds.[9] Malformations of several organ systems have been described in Shh knockout mice. Mice homozygous for a disrupted Shh gene showed absence of distal limb structures, cyclopia, and absence of the spinal column and most of the ribs.[10] Homozygous Shh null mutant mice were also shown to have foregut defects, such as oesophageal atresia/stenosis, tracheo–oesophageal fistula, and hypoplastic lungs with a single lobe,[11] and gastrointestinal defects including gut malrotation, reduced smooth muscle, intestinal transformation of stomach, annular pancreas, duodenal stenosis, and imperforate anus.[12]

Shh has been shown to play a crucial role in patterning in a wide range of tissues. Its expression in the mesenchyme of the posterior limb bud is important for the development of the antero–posterior axis and its secretion in the notochord and floor plate is essential for the dorso–ventral patterning of the neural plate. In the mouse mutant, there is an additional anterior digit as a result of the ectopic expression of Shh. Several polydactyly syndromes are thought to result from mutations in GLI3. Protein kinase A dependent processing of vertebrate Gli3 in developing limb generates a potent repressor, as demonstrated by Wang *et al*.[13] This is antagonised by apparent long range signalling from posteriorly localised Sonic hedgehog protein.[13] These authors concluded that the resulting anterior–posterior GLI3 repressor gradient can be perturbed by mutations of the GLI3 gene in human genetic syndromes or by misregulation of Gli3 processing in the chicken mutant talpid-2, producing a range of limb patterning malformations. This is involved in the transduction of the Shh signal.

In the mouse embryo, pancreatic endoderm does not express Shh or Ihh, suggesting that an absence of Shh expression permits mesodermal differentiation and development of the dorsal pancreas. Ectopic expression of Shh in the pancreatic endoderm in transgenic mice results in a dysmorphic pancreas, with endocrine pancreatic cells producing insulin, but with pronounced disorganisation of the architecture. Thus, hedgehog signalling is important in early pancreatic development and also continues to have an effect on the regulation of insulin production.[14]

Transgenic mice overexpressing Shh in the skin develop many features of the naevoid basal cell carcinoma syndrome, thereby demonstrating that Shh can induce basal cell carcinomas in mice.[15]

Shh is involved in the initiation of rightward looping of the cardiac tube in early embryogenesis. Smoothened and SHh/Ihh compound mutant embryonic mice demonstrate failure to develop left to right situs, with a small linear heart, open gut, and cyclopia. Before looping, Shh is expressed on the left side only and induces a nodal related morphogen. This morphogen is thought to be crucial for left to right differentiation in cardiac looping. On the right, Shh expression is suppressed and thereby creates left to right asymmetry. The evidence for this includes the random control of looping seen when Shh is expressed on the right.[16] There is a requirement for hedgehog signalling in cardiac morphogenesis.[17]

Murine Shh cDNA transferred via an adenoviral vector to the skin of postnatal mice has demonstrated increased mRNA expression of Shh, Shh receptor patched, and Gli1 (a transcription factor). Treated mice showed an increased acceleration into anagen (growth) with increased hair growth subsequently.[18]

In human embryos, SHH is known to be expressed in the notochord, floor plate of the neural tube, brain, and in the zone of polarising activity in the developing limbs and gut. Multiple actions of Shh during central nervous system development have been noted, and these include dorso–ventral patterning, specification of oligodendrocytes, proliferation of neural precursors, and control of axon growth.

Shh is an important signalling pathway in the development of the human gut both in the anterior–posterior and the radial postioning of the gut. A recent study showed that high amounts of Shh mRNA are expressed in fundic glands of the stomach in adults and in a few cells in the base of the crypts of the small intestine and colon. In areas of intestinal

metaplasia of the fundus of the stomach SHH expression is lost. In early development, lack of SHH results in intestinal transformation of the stomach. SHH is also expressed in fundic gland heterotopia in Meckel's diverticulae. It also has a role in the conversion of squamous cells in the oesophagus to gastric fundic cells (Barrett's oesophagus).[19]

Sonic hedgehog has also been characterised in human fetal prostate with the ductal budding of the prostatic urothelium. It is also present in the female urogenital sinus. However, ductal budding appears contemporaneously with testosterone production.[20]

HUMAN PHENOTYPES

In humans, SHH is located on the distal long arm of chromosome 7 (7q36). Heterozygous mutations of SHH result in one form of autosomal dominant holoprosencephaly (HPE3).[21] There is wide clinical variability, with family members presenting with holoprosencephaly, facial abnormalities (cyclopia, premaxillary agenesis, mild hypotelorism, or a single central maxillary incisor) to mild microcephaly alone. A heterozygous mutation of this gene has also been identified in a large three generation family with solitary median maxillary incisor.[22] This abnormality is considered to be a "microform" of holoprosencephaly and it is usually associated with normal cognition and neuroimaging.

It has been postulated that preaxial polydactyly in humans could result from ectopic expression of Shh in the anterior limb bud. Mutations in GLI3, a downstream target of SHH, can be seen in Grieg syndrome (craniofacial anomalies with postaxial polydactyly of hands and preaxial polydactyly and syndactyly of toes), Pallister–Hall syndrome (hypothalamic hamartoblastoma, hypopituitarism, craniofacial anomalies, postaxial polydactyly, renal and cardiac anomalies, and imperforate anus), and autosomal dominant postaxial polydactyly type A/B and preaxial polydactyly type IV.[23 24]

The Sonic hedgehog–Patched–Smoothened pathway is also rapidly emerging as one of the most important regulators of oncogenic transformation. Mutations in the PTCH gene, which encodes the patched receptor, are responsible for the naevoid basal cell carcinoma syndrome (Gorlin syndrome). Activating mutations in SHH and Smoothened lead to similar phenotypes to those seen in loss of function mutations in PTCH.[25] Studies using cyclopamine (an Shh pathway antagonist) in preclinical models of medulloblastoma demonstrated blocking of murine medulloblastoma cell proliferation in vitro. They also demonstrated induction of rapid cell death in freshly resected human medulloblastoma, but not in other brain tumours, thus establishing a role in the pathogenesis of medulloblastoma.[26]

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It has been postulated that the VATER association (vertebral anomalies, anal atresia, tracheo–oesophageal fistula with or without oesophageal atresia, renal, and radial anomalies) may be related to abnormal SHH signalling. Observation has been made of similar developmental anomalies in Gli mutant mice.[27]

FUTURE PROSPECTS

Future studies will help to clarify the precise role of Shh and its downstream targets in the embryogenesis of several mammalian systems, including humans. A more precise understanding of the role of SHH in the development of the human nervous system may shed new light on several syndromes/disorders and the basis of the teratogenic effects of many agents such as ethanol and retinoic acid. Both these agents have long been recognised to cause central nervous system and craniofacial malformations. Recent work suggests that these effects may be mediated through the Sonic hedgehog pathway.[28]

The study accompanying this editorial demonstrates that this pathway has a role in gastric fundic gland heterotopia and loss of function regulates intestinal metaplasia, suggesting potential roles and possible treatments in these disorders.[19]

The role of the Shh pathway in the regulation of oncogenic transformation is a new and exciting field. The work by Berman *et al* suggests that these pathways may well offer the potential for new treatments for medulloblastoma.[26] In the longer term, better understanding of the regulatory role of this pathway may offer new targets for therapeutic manipulation.

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Authors' affiliations
H S Heussler, Division of Child Health, School of Human Development, University of Nottingham NG7 1EN, UK
M Suri, Division of Clinical Genetics, City Hospital, Hucknall Road, Nottingham NG5 1PB, UK

Correspondence to: Dr H S Heussler, Division of Child Health, School of Human Development, University of Nottingham NG7 1EN, UK; honey.Heussler@nottingham.ac.uk

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