The Hedgehog Signaling Pathway in Vertebrates

The hedgehog signaling pathway is a mechanism that regulates cell growth and differentiation during embryonic development, called embryogenesis, in animals. The hedgehog signaling pathway works both between cells and within individual cells. The hedgehog gene (hh) was observed in fruit flies (Drosophila melanogaster) in 1980, and later in vertebrates in 1993. Unlike flies, which have one hh gene, vertebrates have several hh genes. The hedgehog signaling pathway controls a wide range of developmental processes in the vertebrate embryo, and researchers found that dysfunction in the hedgehog signaling pathway leads to birth defects including extra digits, cyclopia with one eye and no forebrain, and cancers in adults and juveniles.

In 1980, Christiane Nüsslein-Volhard and Eric F. Wieschaus in Germany researched how genes control fruit fly development, and they named the hedgehog gene. They named the gene hedgehog because mutant fruit fly larva demonstrated an abnormal pattern of spiky projections, called denticles, on their exoskeletons that resembled the spines of a hedgehog. Until the early 1990s, researchers studied the hh gene (the abbreviation for the hedgehog gene) in the Drosophila hedgehog pathway. They determined that the hh gene encodes a family of hh proteins, which mediate both cell-to-cell interactions and has long-range effects in developing Drosophila embryos.

In 1992, three research teams published the sequence of the hh gene from Drosophila: Jym Mohler and Kodela Vani at Barnard College in New York, New York; Phillip A. Beachy's group at Johns Hopkins University School of Medicine in Baltimore, Maryland; and Thomas B. Kornberg's group at the University of California San Francisco in San Francisco, California. Analyses of the hh DNA sequence data led researchers to discover gene homologs, or genetic sequences similar to those in fruit flies but in vertebrates, a result that revealed a high degree of genetic conservation between species.

In 1993, Clifford Tabin and Andrew P. McMahon in the US and Philip W. Ingham in England published the DNA sequences of related genes, or homologs, to the Drosophila hh gene in several vertebrate families. Unlike the fly, in which there is only one hh gene, the researchers identified different hh genes in vertebrates. There are three classes of vertebrate hh genes: Sonic hedgehog, Indian hedgehog, and Desert hedgehog, with most vertebrate species possessing one member from each gene family. Mammals, including humans, and birds have one gene from each family: Sonic hh (Shh), Indian hh (Ihh), and Desert hh (Dhh). Zebrafish have at least five hh genes: two Sonic class genes, Shh and Tiggy-Winkle hh (Twhh); two Indian class genes, qiqihar hedgehog (Qhh) and echidna hedgehog (Ehh); and one Desert class gene, Dhh.

In their 1993 experiment, Tabin's group at the Harvard Medical School in Boston, Massachusetts, isolated a vertebrate Sonic hh gene related to the Drosophila hh gene in the developing limbs, or limb buds, of chicks (Gallus gallus). The group demonstrated that Shh gene expression occurs in the area of the margin of the developing limb bud, called the zone of polarizing activity (ZPA), and in other regions of the chick embryo. Their research demonstrated that the Shh gene controls front to back or anterior to posterior pattering in the chick limb, and prompts limbs to develop. When researchers removed the ZPAs from limb buds and attached them to other areas of the chicks, new limb buds develop in the presence of Shh.

McMahon's group at the Harvard Medical School in Boston, Massachusetts, identified three members of mammalian hh gene family in mice (Mus musculus) related to the Drosophila hh gene: Shh, Dhh, and Ihh. They then compared, across species, the sequences of the genes and the proteins made from the genes to determine the evolutionary relatedness of each hedgehog gene. The protein sequence of the Dhh protein in mice is the most closely related to the hh protein in Drosophila,

indicating that it is the oldest and most conserved between species. Ihh and Shh DNA sequences indicated that those genes more closely related to each other than to the Dhh gene, and therefore resulted from a more recent evolutionary duplication event.

Ingham's group at the Molecular Embryology Laboratory in Oxford, England, identified three members of the hh gene family in five hh genes found in zebrafish (Brachydanio rerio). In the zebrafish embryos, Ingham's group identified a Dhh gene and the Shh gene as active in the notochord, a structure in chordate embryos, in the floor plate, a structure that in vertebrate embryos develops into the nervous system. They also found that the genes functioned in the posterior fin mesoderm, the tissues associated with polarizing activities. Shh and Twhh genes are expressed in fin bud development, but only Shh is required for proper development in zebrafish. The pattern of Shh expression in zebrafish mutants affects axial structures consistent with the role for the Shh gene in floor plate induction.

Ihh proteins function in developing bones, and they regulate the proliferation and differentiation of cartilage cells, called chondrocytes. Dhh proteins also function in the development of sperm cells during a process called spermatogenesis. Dhh proteins are necessary for the development of the glia cells that insulate the peripheral nerves, called Schwann cells after Theodor Schwann who obseved them in nineteenth century Germany. Shh proteins function in the development of the central nervous system, and they establish lateral asymmetry, and function to establish the front-to-back (anterior-posterior) limb axis.

The Shh gene became one of the most studied of the hh genes. Researchers experimented with vertebrate sonic hedgehog proteins (N-Shh), and their results indicated that those proteins traveled outside of cells. Once in the extracellular environment, and with help from other molecules, N-Shh proteins can move at least twelve cell diameters to form a distribution gradient to target cells. For all hh signaling between cells, small differences in the concentration of the proteins can alter cellular functions and processes.

A flexible rod-shaped structure found in vertebrate embryos, called the notochord, defines the primary axis of the embryo. The notochord, located under the neural tube of the developing vertebrate's central nervous system, secretes the N-Shh protein to form a gradient. The gradient of N-Shh protein specifies five distinct types of neuron cells, called V0, V1, V2, and V3 interneurons, or motor neurons. In addition, the cells of the floor plate, which develop into the nervous system, respond to the highest concentration of N-Shh secreted by the notochord and become non-neuron supporting cells of the nervous system, called glial cells. The floor plate glia cells begin to secrete N-Shh protein themselves. The remaining neural tube cells develop into various types of neural cells, specified by different concentrations of N-Shh proteins. Notochord cells develop into V3 cells in response to the second highest concentration of N-Shh protein after the floor plate. The third and fourth highest concentrations yield the motor neurons, V2, V1, and V0 neurons respond to the lowest concentration of N-Shh protein.

The hh family of proteins regulates multiple developmental processes in the vertebrate embryo, and can cause cancer or birth defects. Hedgehog family proteins are involved in cancers, for example, a human skin cancer called basal cell carcinoma, and a childhood brain cancer called medulloblastoma. Mutations and improper expression of Shh proteins lead to various embryonic defects and birth defects, especially those affecting the brain, head, and limbs. Shh protein functions for multiple events during embryogenesis, including craniofacial development. Mice lacking the Shh gene (Shh null) are born without their forebrains or mid-faces, a disorder called holoprosencephaly, and they have only one eye, a phenomenon called cyclopia. If the Shh gene fails to produce Shh proteins in vertebrates, birth defects result, especially in heads and faces.

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