Focus on Molecules: Six3 — Master or Apprentice?

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1. Structure

Six3 (also known as six homeobox-3), a vertebrate homolog of the Drosophila 'sine oculis' (so) gene, is a member of the evolutionarily conserved SIX family. The members of SIX family are found in diverse organisms including flatworms, fruit fly, medaka fish, chickens, frogs, zebrafish, mice and humans (Oliver et al., 1995).

Members of the SIX gene family encode transcription factors characterized by the presence of evolutionarily conserved DNA-binding homeodomain and an upstream SIX domain, which may be involved both in determining DNA-binding specificity and in mediating protein–protein interactions (Fig. 1A). DNA-binding activity of so is mediated through the Ppd class of homeobox nucleic acid recognition domain (HD). SIX proteins contain lysine residue at position fifty (K50), which assign SIX3 to an Orthodenticle sub-group within the Ppd class. The site bound by SIX3 contains the traditional ATTAT homeodomain core recognition sequence. Even though there are individual differences, the consensus binding sites for SIX proteins in flies and worms are TGATA and GGTTGTA. The SIX domain which is the second interaction domain in the six3 protein, is 146 amino acids long, and lies just 5’ and directly adjacent to the HD. This domain is involved in mediating protein–protein interactions.

2. Function

In Drosophila, So is expressed in the eye imaginal disc (a monolayer epithelium that develops into the adult eye of fly) in nuclei of both differentiating retinal photoreceptors as well as retinal precursor cells (Fig. 1B). The SD domain of So physically interacts with transcriptional coactivator Eyes absent (Eya) to promote eye development in Drosophila. This interaction is conserved across the animal kingdom.

The evolution of six 1–6 class of genes in the vertebrate lineage involves a genome duplication event (Fig. 1C).

Genes in the six family are shown to be expressed in head, retina, lens, ear, nose, brain, kidney, muscle and gonads, and play major role in vertebrate and insect development or maintenance of the differentiated state of tissues. SIX3 and SIX6 are the major SIX proteins in the retina. SIX3 is a member of complex network of eye field transcription factors that regulate retina and lens development. SIX3 is expressed at multiple points during the development of the vertebrate eye. It is first localized to the optic vesicle and optic stalk but then expands to include the neural retina and developing lens placode (Oliver et al., 1995). In humans, northern blot analysis revealed that the SIX3 gene was expressed only in the eye. In human embryos, expression of SIX3 was detected as early as 5–7 weeks of gestation and found to be maintained in the eye throughout the entire period of fetal development. At 20 weeks of gestation, expression of SIX3 in the human retina was detected in the ganglion cells and in cells of the inner nuclear layer. In vertebrates the nuclear protein SIX3 recruits EYA4, a transcriptional coactivator, one of the four orthologs of Drosophila gene eyes absent.

Injection of six3 RNA into medaka fish embryos caused ectopic pax6 and Rx2 expression in midbrain and cerebellum, resulting in the formation of ectopic retinal primordia. Misexpression of six3 in chick eye using retrovirus disrupts the integrity of corneal endothelium and affects corneal transparency. Gain-of-function studies suggested that six3 plays a role in eye anterior segment morphogenesis. Injected mouse six3 mRNA initiated ectopic expression of endogenous medaka six3, uncovering a feedback control of six3 expression. Initiation of ectopic retina formation demonstrated a pivotal role for six3 in vertebrate retina development and hinted at a conserved regulatory network underlying vertebrate and invertebrate eye development. SIX3 plays a role in regulation of anterior eye segment development in vertebrates.

Six3 is involved in the proliferation of retinal precursor cells. Many cell-cycle regulators are regulated in cell-cycle dependent manner. SIX3 is a partner of DNA replication-inhibitor Geminin (Gem). Gem inhibits cell-cycle progression by sequestering Cdt1, the key component for the assembly of the pre-replication complex. SIX3 competes with Cdt1 directly to bind to Gem. SIX3 and Gem act antagonistically in controlling proliferation and differentiation. During early stages of development, high levels of SIX3 inhibit Gem activity, which allows proliferation and prevents premature neuronal differentiation. Later, Gem promote cell-cycle exit, a necessary prerequisite for the initiation of neuronal differentiation. At this stage Gem does not overlap with SIX3 and is maintained in the differentiating central neural retina. Recently, it has been seen that Drosophila cdc25 homolog, string, and mammalian cyc A are direct transcriptional targets of So and SIX1.

Six3 is a major player in lens development. SIX3 normally exerts its effect by directly activating pax6, a gene considered to be the “master regulator of eye development.” In the absence of six3, pax6 and Sox2 are...
Six3 has not been directly involved with eye diseases. This could be explained by its role in the pax6 circuit as discussed above. However, it has been associated with holoprosencephaly (HPE) (Wallis et al., 1999). Six3 is one of the earliest genes to be expressed in the anterior forebrain. SIX3 regulates Sonic hedgehog, Wnt, BMP and Nodal signaling in forebrain and is thought to enable these cells to adopt anterior cell fate. Interestingly, the map position of human SIX3 overlaps the locations of dominant disorder holoprosencephaly type 2 with ocular phenotypes. HPE exhibit signs like single central incisor, hypotelorism, microcephaly, or other craniofacial findings that can be present with or without associated brain malformations. Several evidences from studies indicated that misregulation of SIX transcription and/or post-translational modification is an underlying cause for a wide range of primary cancers and metastatic lesions. The role of SIX proteins in cell cycle is significant and may be important in tumorigenesis and cancer biology.

4. Future studies

The elucidation of the 3-D structure of six3 will be of paramount interest in delineating how it interacts with other members of the pax6 circuit. Also more elaborate studies of the physical interaction with other important members of the circuit, such aseya and dach will shed light of how the circuit is structured and how regulation is achieved in order to control differentiation and morphogenesis of the eye.

Acknowledgements

A.S. is supported by Ohio Cancer Research Associate grant. P.A.T is supported by NIH grant EY10540. We apologize to authors whose work cannot be cited due to limits for reference citation. We thank J. Kumar for Fig. 1B.

References


