SI-1

Tbx5 and the retinotectum projection.

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Accumulating data indicate that Tbx5 gene plays a key role during both morphogenesis and organogenesis. In developing limb buds, Tbx5 and Tbx4 genes determine the wing/leg (forelimb/hindlimb) identity (*Nature* 398, 810-814, 1999). Tbx5 expressed exclusively in the left ventricle controls the left/right asymmetry of heart. These lines of evidence strongly suggest that Tbx5 gene, which is expressed in the dorsal side of eye cup, also regulates both eye morphogenesis and the retinotectum projection along the dorsal-ventral axis of eye.

To explore this possibility, we used a novel *in ovo* electroporation technique to misexpress Tbx5 gene in the ventral side of eye cup. This method allows rapid and temporary site-directed expression of foreign genes. Misexpression of Tbx5 gene in the ventral side of eye cup induces complete re-organization of several dorsal and ventral markers; EphrinB1 and EphrinB2 genes, which are normally expressed in the dorsal side, was induced in the ventral portion whereas the ventral markers EphB2, EphB3, Pax2 and Vax genes were repressed in the ventral side of eye cup. Consequently, misexpression of Tbx5 gene induces the double dorsal appearance of eye morphology.

In addition, we analyzed the projection patterns of retinal ganglion cell axons between the retina and the tectum. Normally, ganglion cell axons from the dorsal retina project to the ventral tectum and the ventral axons to the dorsal tectum. When Tbx5 gene was misexpressed in the ventral side by the conventional retrovirus method, ventral ganglion cell axons entered the dorsal side of tectum but did not make a tight focus. Instead, axons extended to the ventral side where normally dorsal axons innervate. Abnormal arborization and synaptic varicosities were also observed. This indicates that misexpression of Tbx5 in the ventral ganglion cells dramatically changes the projection properties of their axons and that Tbx5 gene acts as a topographic determinant of the visual projection between retina and tectum (*Science* in press).

Taken together, our data indicate that Tbx5 gene plays a critical role during morphogenesis and organogenesis of multiple organs, highlighting the functional significance of this gene in eye development.

SI-2

Retinal transplantation of the neural stem cells

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Beginning in 1990's, neural stem or progenitor cells have been isolated from the central nervous system (CNS). Adult rat hippocampus-derived neural stem cells (AHSCs) isolated by Palmer et al. are one of the few cell lines which have been proven to have multipotency and self-renewability by clonal analysis. In vitro, they continue to proliferate in serum-free medium containing bFGF. When bFGF is withdrawn and retinoic acid is added, they begin to differentiate into neurons, astrocytes and oligodendrocytes. It is also reported that they demonstrated site-specific neuronal differentiation once they were grafted into adult hippocampus and olfactory bulb. To investigate the plasticity of clonal AHSCs *in vivo*, we grafted AHSCs heterotopically into the rat eye.

A clone chosen for this work carried marker genes encoding cytoplasmic β-galactosidase (β-gal). Ten to thirty thousand of these cells were injected into the vitreous space of the adult rat eye or the newborn rat eye. To evaluate the influence of the damage of host retinas, the adult retina was injured with a hooked 30-gauge needle by scratching parallel to the equator under direct observation with a surgical microscope, and then the AHSCs were injected into the vitreous space. Two, 4 or 8 weeks later, rats were sacrificed and eyes were fixed in 4% paraformaldehyde. They were processed for histology.

Four weeks after grafting, the grafted AHSCs incorporated in the developing pups' retinas and injured adult retinas but not in normal adult retinas. Integrated cells showed the successful differentiation into both glia (GFAP 9.9% of the integrated cells) and neuron (MAP2ab 9.7% of the integrated cells) even in adult retinas. Although, the AHSCs failed to differentiate into retina-specific phenotypes as shown by expression of HPC-1, calbindin and rhodopsin, possibly because of their basic inability or an absence of local cues which are essential for differentiation into retinal neurons. In conclusion, this study has yielded basic and important information regarding the transplantation of the neural stem cells into retinas.

The successful integration and differentiation of the neural stem cells even in the adult retina indicates their potential as cell source of retinal regeneration, although there exists limitation of use of the AHSCs for heterotopic retinal transplant.