Review

Molecular Mechanisms for Defining Tectum Territory in Chick Embryogenesis

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Optic tectum derives from the midbrain alar plate in early chick development. To know the molecular mechanism defining tectum territory along the anteroposterior (AP) and dorsoventral (DV) axis in neural development, we have carried out the gain-of-function experiments using a newly developed gene transfer system, *in ovo* electroporation.

Misexpression of *Otx2/Gbx2* and *Shh* changed the expression of tectum-related genes, resulting in the fate change of the tectum structure along the AP axis and the DV axis, respectively. We conclude that these genes are involved in the establishment of tectum territory in embryogenesis.

Key words: Tectum, Midbrain, *Otx2*, *Gbx2*, *Shh*, Electroporation

I. Introduction

Optic tectum derives from the midbrain alar plate in chick embryogenesis. It has been shown that the genes expressed in the midbrain and the midbrain-hindbrain boundary (isthmus) including *En1/2*, *Pax2/5*, and *Fgf8* are involved in inducing tectum structure and in defining tectum territory along the anteroposterior (AP) axis [3–5, 10, 11]. In early chick development, *Otx2* is expressed in the prosencephalon and mesencephalon, complementary to the expression of *Gbx2* in the rhombencephalon (Fig. 1a–d). Loss-of-function studies of these genes in mice have revealed that *Otx2* is indispensable for the development of the anterior brain segment [1, 2, 8], and that *Gbx2* is required for the development of the isthmus [14]. These observations suggest that both genes may contribute to tectum compartment formation. To know their roles in tectum formation, we carried out gain-of-function experiments of these genes in chick embryos with a newly developed gene transfer system, *in ovo* electroporation [5].

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II. Midbrain Patterning along AP Axis

When *Otx2* was ectopically expressed caudally beyond the midbrain-hindbrain boundary, a large swelling emerged in the metencephalic region at E10 (Fig. 2a). In normal embryos the right and left rhombic lips are fused at the midline to form a single cerebellum by this stage. On the other hand, in the transfected embryos the right and left hemispheres were separated at the metencephalon. A transversal section at the level of nucleus laminalis showed that the right hemisphere has a typical laminated structure of the optic tectum (Fig. 2b). In contrast to the ten layers in the normal embryos (Fig. 2c), it contained nine layers without the outermost layer x which is composed of the optic fibers (Fig. 2d). These results suggest that the alar plate of the metencephalon differentiated into the optic tectum instead of differentiating into the cerebellum by *Otx2* misexpression.

When *Gbx2* was ectopically expressed at the mesencephalon, the transfected tectum became smaller (Fig. 3a) and the caudal limit of the tectum shifted rostrally (Fig. 3b). Consistent with this morphological change, the caudal limit of *Otx2* expression shifted rostrally (Fig. 3d), which corresponds to the rostral shift of *Gbx2* expression in the isthmus (Fig. 3c).

We found that ectopic *Otx2* and *Gbx2* interacted to repress each other's expression. Moreover, ectopic *Otx2* and *Gbx2* repressed endogenous expression of *Fgf8* at the

Fig. 1. Normal expression of *Otx2* (purple) and *Gbx2* (red) in early chick embryogenesis. (a) stage 5 (b) stage 7 (c) stage 10 (d) stage 17. *Fgf8* expression at stage 9 is shown on panel (e). *Otx2* and *Gbx2* are expressed complementarily during early stages. Scale bars = 200 μ m.

Fig. 2. Morphological effects of *Otx2* misexpression in the metencephalon. (a) Dorsal view of E10 transfected brain. (b) Transversal section of the brain at the level indicated as b on panel a. (c) Higher magnification of the control tectum at E10, composed of ten layers. (d) Higher magnification of the ectopic tectum indicated as d on panel b, composed of 9 layers. *Otx2* misexpression induced a large separated hemisphere on the transfected side (right), instead of a single cerebellum. It has a typical laminated structure of the tectum. Scale bars = 500 μ m (a); 200 μ m (b); 100 μ m (c). cel, cerebellum; cer, cereberum; di, diencephalon; fp, floor plate; La, nucleus laminaris; ne, neuroepithelial layer; te, tectum; te-ect, ectopically differentiated tectum; roman numerals indicate the layer of the tectum.

Fig. 3. Effects of *Gbx2* misexpression. (a) Dorsal view of the E7.5 transfected brain. (b) Horizontal section. (c-e) Expression of *Otx2* (d, e) and *Gbx2* (c) of E3.5 transfected brain on the transfected side (c, d) and the control side (e). *Gbx2* misexpression resulted in the anterior shift of *Otx2*-*Gbx2* expression boundary (asterisks on panels c, d) and the caudal limit of the optic tectum (arrows on panel a). Scale bars = 500 μ m (a); 200 μ m (b-e). di, diencephalon; ms, mesencephalon; mt, metencephalon; rl, rhombic lip; te, tectum.

isthmus (Fig. 1e) but induced *Fgf8* expression at the interface between *Otx2* and *Gbx2* expression. *Fgf8* carries the isthmus organizing activity and can induce ectopic tectum in the diencephalon [4, 7]. Thus, it is suggested that interaction between *Otx2* and *Gbx2* determines the site of *Fgf8* expression and the posterior limit of the tectum to control the tectum territory along the AP axis [6].

III. Midbrain Patterning along DV Axis

While the alar plate differentiates into the optic tectum, the basal plate gives rise to the tegmentum during the midbrain development. It is largely unknown how the differences between these two structures are molecularly controlled. The secreted protein Sonic hedgehog (Shh) produced in the notochord and floor plate has the activity to induce differentiation of ventral cell types of the central nervous system [12]. To evaluate the role of Shh for the establishment of dorsoventral polarity in the developing midbrain, we have ectopically expressed the *Shh* unilaterally in the midbrain of E1.5 chick embryos *in ovo*.

Besides the endogenous expression in the floor plate (Fig. 4a), ectopic *Shh* expression was detected in the unilateral side of the midbrain at E3.5 (Fig. 4b). We found that normal growth of the tectum was suppressed in the *Shh*-transfected region, producing dorsally enlarged tegmentum (Fig. 5a, b). In later stages, the tectum territory of the *Shh*-transfected side was significantly reduced and was fused with that of the untransfected

Fig. 4. Development of the *Shh*-transfected midbrain at E3.5. (a, b) Expression of the *Shh* in normal (a) and the transfected (b) brain. The *Shh*-transfected tectum was not developed properly. (c, d) Ectopic nerves were detected with anti-neurofilament antibody 3A10 in the transfected side (d), which did not exist in the control side (c). (e, f) Transversal section at the level of oculomotor nerves. Motor neuron marker SC1 was positive (f), in the *Shh*-transfected domain (e). Scale bars=500 μ m (b, d); 200 μ m (f). di, diencephalon; hind, hindbrain; mid, midbrain; hind, hindbrain; tel, telencephalon.

side, resulting in the formation of a single optic lobe at the midline (Fig. 5c, d).

Ectopic Shh suppressed the expression of several genes in the midbrain and isthmus such as *En2*, *Pax7*,

Pax2/5 and *Fgf8*, which are crucial for the tectum formation [3–5, 7, 9, 10]. Markers for the midbrain roof plate were inhibited, indicating that the roof plate was not fully generated. Moreover, the Shh induced a considerable amount of SC1 positive ectopic nerves (Fig. 4c, d), indicating they are motor neurons [13]. Dopaminergic and serotonergic neurons were also generated in the dorsally extended region by the *Shh* misexpression. These changes indicate that ectopic Shh changed the fate of the mesencephalic alar plate for the basal plate derivatives. Taken together our results suggest that Shh signaling restricts the tectum territory by controlling the molecular cascade for the tectum formation along the DV axis (Fig. 6) [15].

Fig. 5. Development of the normal (a, c) and the *Shh*-transfected (b, d) midbrain. At E5.5 (a, b) tectum territory is significantly reduced and the dorsal midline dividing right-left tectum is unclear (arrows). At E18.5 (c, d), a single fused tectum was generated at the dorsal position. Scale bar=2 mm. Cel, cerebellum; Cer, cerebrum; Di, diencephalon; Tc, tectum; Tel, telencephalon.

Fig. 6. Role of the Shh in midbrain development. In the basal plate the Shh inhibits the molecular cascade for the tectum formation, which results in the promotion of neural differentiation to form the tegmentum. On the other hand, the alar plate does not receive the Shh signaling so that it continues massive cell proliferation to form an enlarging tectum.

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V. References

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