

Expression of Wnt Signaling Pathway Components in RA-induced Cleft Palate Mouse

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Abstract: In order to analyze the potential teratogenic mechanism of RA in secondary palate development, we established RA-induced cleft palate (CP) model by using C57BL/6J mice and performed immunohistochemical staining by using Wnt5a, β -Catenin and Bcl-2 antibodies. Higher level expression of Wnt5a was detected in mesenchymal cells of secondary palate at E13 and the expression decreased from E14 in wild type. In RA-induced CP Wnt5a decreased severely at E15 and disappeared earlier. No relative of β -catenin and Wnt5a was detected in both wide type and RA-induced CP mouse. We also found the expression of Bcl-2 located in MEE cells of RA-induced CP group was restrained in horizontal stage of palate development.

Introduction

Cleft lip and/or cleft palate (CL/P) is one of the most common malformations among live births, occurring with an incidence of 1‰~2‰

in the world and 1.82‰ in China. The palate is sensitive to many teratogenic drugs because the development of palate is very complicated. Retinoic acid (RA) is a kind of drug with distinct teratogenic action. It has been reported that RA can down-regulate the express of Bcl-2 in tumor tissue, which results in cell apoptosis. But little is known about what effect Bcl-2 has on palate development and what effect RA has on Bcl-2 in palate development.

Wnt family is one of the molecular signals regulating the epithelium-mesenchyme interaction. Wnt5a is a unique member in the family since it is the only Wnt expressed in progress zone of growing bone, and is required for extension of the primary embryonic anterior-posterior axis and outgrowth of the limb proximal-distal axis. There is no report about the role of Wnt5a in regulating the development of facial primordium, especially in the secondary palate development.

We established CP model induced by RA, and studied the temporal and spatial distribution of Wnt5a as well as β -catenin the crucial factor of Wnt canonical pathway in order to indicate Wnt5a signaling pathway involving in secondary palate development. and observed the expression level of Bcl-2 in MEE cells.

Materials and Methods

All the pregnant C57BL/6J mice were randomly divided into two groups: control group (n=12) and RA-treated group (n=24). Pregnant mice in RA-treated group received by gavage a single dose of RA at 100mg/kg body weight on GD10. Pregnant mice in control group received 0.2ml corn oil at the same time. 12 Pregnant mice in RA-treated group were killed in GD 16 to observe the malformation situation of the fetuses, and the rest of them were killed on GD13, GD13.5, GD14, GD14.5, GD15 and GD16 for histological and immunohistochemical study followed. 6 Pregnant mice in control group were killed in GD 16 to observe the development situation of the fetuses, and the rest of them were killed on GD13, GD13.5, GD14, GD14.5, GD15 and GD16 for histological and immunohistochemical study followed. In

horizontal stage of palate development, the expression level of Bcl-2 in MEE cells was compared between control group and RA-treated group to make sure what kind of effect RA had on Bcl-2 and to make it clear how RA can result in CP. We detected the expression and distribution of Wnt5a and β -catenin at E13d, E14d, E14.5d, E15d and E16d in control group and RA treated group by immunohistochemistry (Ed: Embryonic day).

Results and Discussion

Of all the 79 fetuses (77 living and 2 dead) in RA-treated group 47 were found to get CP, the CP rate is 59.5%, and no fetus with CP was observed in control group (38 fetuses all living) (Table1). RA can induce CP in C57BL/6J mice, and the 100mg/kg body weight dose is safe and effective (Table2).

The expression of Bcl-2 in MEE cells in RA-treated group was lower than in control group in horizontal stage of palate development (Fig.1 A&B). In horizontal stage of palate development RA can down-regulate the expression of Bcl-2 in MEE cells, which induces MEE cells apoptosis.

Higher level expression of Wnt5a was detected in mesenchymal cells of secondary palate at E13d when palatal shelves were in vertically position, and the expression decreased from E14d when palatal shelves went up to the horizontal position. The expression of Wnt5a was detectable evenly in the developing MEE cells until the palatal shelves fused following apoptosis happened in the MEE cells. In RA-induced cleft palate, the expression of Wnt5a decreased faster, and was lower at E15d also disappeared earlier than control group. No significant difference of Wnt5a expression was observed in mesenchymal cells of secondary palate between the control group and RA treated group. Wnt5a was expressed both in epithelial and mesenchymal components during palate development, the data proved that Wnt5a signaling molecule may involve in the growth and development of secondary palate. Higher level expression of Wnt5a expressed at rapid proliferating stage of E13d and reduced level at the following stage of epithelial differentiation suggested that Wnt5a take part in the proliferation and rarely join the differentiation. RA possibly disabled MEE cells to proliferate and differentiate at the pivotal stage when palate shelves were to contact and fuse, MEE cells were dysplastic, and then both side of palatal shelves could not contact and fuse. The decreased expression of Wnt5a at this pivotal stage proved that

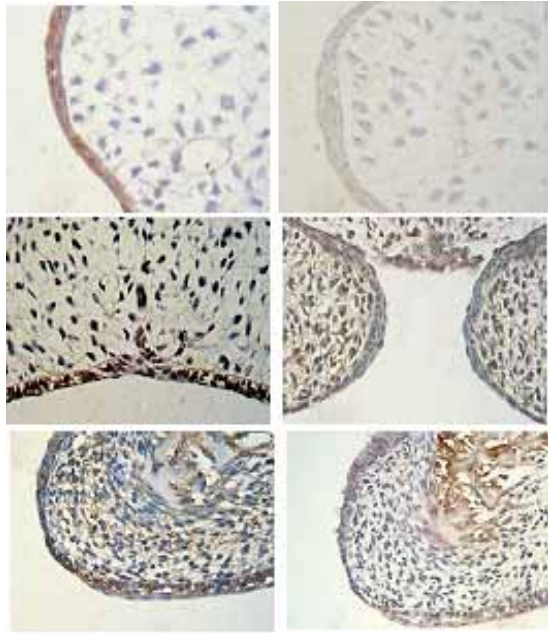


Fig.1. Expression of Bcl-2, Wnt5a and β -catenin in RA-induced Cleft Palate. Positive staining of Bcl-2 showed in Control group (A) and negative in RA -treated group (B). Positive staining of Wnt5a was also showed in Control group (C) and negative in RA -treated group (D), but positive staining of β -catenin was showed in both Control group (E) and negative in RA -treated group (F).

Table2. Relationship between CP Rate and Fetus Number of Each Litter

embryos in each litter	pregnant mice number	total embryo number	Embryo with CP number	rate of CP
>6	7	54	23	42.6%
-6	5	25	21	84.0%

RA may influence the proliferation and differentiation of MEE cells and then induce cleft palate by regulating Wnt5a and other signaling molecules (Fig.1 C&D).

β -catenin was detectable both in control group and RA induced cleft palate. There was no significant change in the expression and distribution of β -catenin, and also no relative of β -catenin and Wnt5a distribution pattern and level was observed during palate development. Further detection of β -catenin protein in the control group and RA-induced cleft palate did not show any significant changes. Wnt5a may not function through the canonical Wnt/ β -catenin pathway in regulating the development of palate (Fig.1 E&F).

References

- 1) Yamaguchi TP, Bradley A, McMahon AP, et al.: A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Dev*, 126: 1211-1223, 1999,.
- 2) Li C, Xiao J, Hormi K, Borok Z and Minoo P: Wnt5a Partipates in Distal Lung Morphogenesis. *Dev Biol*, 248: 68-81, 2002.
- 3) Brown NL, Knott L, Sandy JR, et al: Microarray analysis of murine palatogenesis: Temporal expression of genes during normal palate development. *Dev Growth Differ*, 45(2): 153-165, 2003.
- 4) Veeman MT, Axelrod JD, Moon RT. A second canon. function and mechanisms of beta-catenin-independent wnt signaling. *Dev Cell*, 5(3): 367-377, 2003.

Table1. Poisonous Effect on Fetus Mouse by RA

	Pregnant mice number	Living embryo number	dead embryo number	absorpe dembryo number
RA-treated group	12	77	2	5
Control group	6	38	0	3