Expression of Wnt5a and β-Catenin in Chinese Oral Squamous Cell Carcinoma of Tongue

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Abstract: Wnt family regulates cell growth, differentiation and proliferation during embryonic development and also plays important roles in tumorigenesis. By the method of immunohistochemistry, a paralleled stydy was conducted to check Wnt5a and β-Catenin expression in OSCC formalin fixed-paraffin embedded tissues.The results demonstrated (1) Wnt5a and β-Catenin cytoplasmic expression appeared in most OSCC.(2) Statistical results showed positive correlation between the level of Wnt5a expression and OSCC differentiation ($P<0.05$) and inverse correlation between the level of β-Catenin cytoplasmic expression and OSCC differentiation ($P<0.05$). Cytoplasmic accumulation of β-Catenin protein also associated with lymph node metastasis ($P<0.05$), and loss of β-Catenin protein on cell membrane was correlative differentiation level of OSCC inversely ($P<0.05$).These data indicate Wnt/β-Catenin pathway associates with tumorigenesis, metastasis and prognosis of OSCC,and Wnt5a also associates with histological grade of OSCC.

Key words: Oral Squamous Cell Carcinoma (OSCC), Wnt5a, β-Catenin, Immunohistochemistry

Introduction

Wnt family regulates cell growth, differentiation and proliferation during embryonic development and also plays important roles in tumorigenesis. As the key regulation factor of Wnt/ β-Catenin canonical pathway, β-Catenin activity depends on the cytoplasmic accumulation and nuclear translocation in the tumor cells. Whenever any up-stream component in canonical pathway has mutation, it will lead to cytoplasmic accumulation and nuclear translocation of β-Catenin, which induces the transcription of its target genes .Therefore, most members of Wnt family are considered to be oncogene[1]. Wnt5a, which is a unique member of Wnt family, stimulates the non-canonical Wnt/ Ca2+ pathway and also effects on tumorigenesis, invasion and metastasis[2]. Newest knowledge of Wnt signaling pathway indicates Wnt5a may inhibit the canonical Wnt pathway by promoting β-Catenin degradation. Therefore, Wnt5a can be considered as a candidate of tumor suppressor in tumorigenesis and progression. Our study is to investigate the distribution and level of Wnt5a and β-Catenin proteins, as well as their correlation with OSCC histological differentiation and clinical progression. Another aim is to find the relationship between Wnt5a and canonical pathway of Wnt/ β-Catenin in OSCC,such would be of clinical significance.

Materials and Methods

The surgical speciments of 41 OSCC cases selected from Chinese Medical University according to certain standards were staged from T1 to T4, N0 to N3. The histological differentiation of tumor was classified as well (23 cases) , moderate and poor (18 cases).

The paraffin-embedded OSCC tissue were constructed and sectioned in 5 um thickness and used for immunohistochemical profiling of wnt expression and the patterns of β-Catenin distribution under the same experimental conditions. Goat anti-human Wnt5a polyclonal antibody (dilution 1:50, R&D Systems Inc., the U.S.A.) and mouse anti-human β-Catenin monoclonal antibody (dilution 1:40, MAIXIN Inc. FUJIAN) are used as the primary antibodies. Three independed observers evaluated the staining results as below: -:0-5%, +: 5-30%, ++: 30-70% and +++: ±70%. Relationships between immunohistochemical results with various clinico-pathological features were analyzed by Kruskal-Wallis Test and Spearman Correlation Test.

Results and Discussion

(1) Expression of Wnt5a and β-Catenin proteins

In normal oral epithelium positive staining for Wnt5a was moderately localized in the cytoplasm of basal cells, and stronger staining of β-Catenin was observed to localize on the membrane of basal cell and acanthocyte. In OSCC, immunohistochemical staining of Wnt5a was localized in the cytoplasm of tumor cells. Stronger staining of Wnt5a was observed in acanthocyte, while weaker expression was detected in the peripheral cells of tumor nests; in some cases staining of Wnt5a also disappeared in the center of tumor nests. Expression of β-Catenin in Membrane and cytoplasm was shown unequally, partly disappeared in membrane of peripheral cells of some well differentiation tumor nests. Furthermore, significant reduced level of membrane β-Catenin was detected at invasive portion of OSCC cell nests. Postive staining of Wnt5a and β-Catenin were also shown in endoepithelial cells of capillary, fibroblasts and part of inflammation cells in the tumor stroma. (Fig.1)

(2)Correlation of Wnt5a and β-Catenin expression in Table 1

Positive staining of Wnt5a was detected from 40 out of 41 cases OSCC (97.6%) and positive staining of β-Catenin located in cytoplasm was detected from 39 out of 41 cases of OSCC (95.1%).No correlation was found between Wnt5a and cytoplasmic expression of β-Catenin ($P>0.05$), and also no correlation between Wnt5a and membrane of expression β-Catenin ($P>0.05$).

(3) Correlation between the clinicopathologic characteristics of

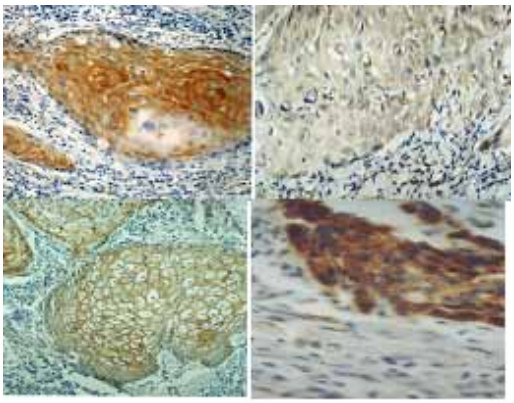

Fig.1 Expression of Wnt5a and b-Catenin in OSCC
Wnt5a was localized in the cytoplasm of tumor cells, over-expression in well-differentiated OSCC (A) and decreased level in poor-differentiated OSCC (B). b-Catenin in Membrane and cytoplasm was shown unequally (C), disappeared in membrane but cytoplasm over-expression in poor-differentiated OSCC (D).

patients with OSCC and the expression of Wnt5a and β-Catenin was summarized in Table 2.

Statistical results showed positive correlation between the level of Wnt5a expression and OSCC differentiation (P<0.05),which indicate that Wnt5a may play its roles as a tumor suppressor in well-differentiated OSCC because of feedback mechanisms, but in poor- differentiated OSCC tumor cells lose the function to secret Wnt5a protein to escape from the inhibition of Wnt5a. There were significant inverse correlation between the level of cytoplasmic expression of β-Catenin, loss of β-Catenin expression on cell membrane and OSCC differentiation (P<0.05). These results suggest that Wnt/β-Catenin signaling pathway regulates the tumorigenesis and progression of OSCC. Furthermore, significant reduced level of β-Catenin protein at the invasive portion of OSCC cell nests indicates that the tumor cells are more aggressive because of the loss of adhesion between the tumor cells. Cytoplasmic accumulation of β-Catenin protein also associated with lymph node metastasis (P<0.05) ,which means Wnt/β-Catenin signaling pathwaymay also play important roles in the OSCC metastasis.Over-expression of Wnt5a protein in endoepithelial cells of capillary and part of inflammation cells in the tumor stroma suggest the Wnt pathway may effects metastasis and prognosis of OSCC by promoting the tumor microenvironment[3]. The underlying mechanisms remain unclear. In conclusion, our results showed that the correlation between the canonical Wnt/ β-Catenin pathway and tumorigenesis, metastasis and prognosis of OSCC, and the expression of Wnt5a which is the key up-stream component of non-canonical Wnt/ Ca^{2+} pathway also associates with histological grade of OSCC . These data suggest the components of Wnt signaling pathway are useful as the marker of clinical and histological diagnosis in OSCC.

References

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Table1. Correlation of Wnt5a and β-Catenin Expression in OSCC

Wnt5a	n	Cytoplasm β-Cat			Membraneβ-Cat		
		+++	++	+/-	+++	++	+/-
+++	25	11	7	7	1	10	14
++	8	6	1	1	0	1	7
+/-	8	2	2	4	1	0	7
Total	41	19	10	12	2	11	28
Spearman P			0.723			0.081	

Table2. Relationship between Expression of Wnt5a and β-Catenin with Clinico-pathologic Features

		Wnt5a			Cytoplasm β-Cat			Membrane β-Cat		
	n	+++	++	+/-	+++	++	+/-	+++	++	+/-
histological differentiation										
good	23	18	2	3	6	8	9	2	10	11
Moderate/poor	18	7	6	5	13	2	3	0	1	17
p		0.024			0.011			0.000		
lymph node metastasis										
yes	25	16	6	3	14	8	3	1	10	14
no	16	9	2	5	5	2	9	1	1	14
p		0.374			0.013			0.061		