Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60080-8

Effect of WNT5A on epithelial-mesenchymal transition and its correlation with tumor invasion and metastasis in nasopharyngeal carcinoma

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ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 15 January 2014
Accepted 15 March 2014
Available online 20 June 2014

Keywords:
WNT5A
Epithelial-mesenchymal transition
Matrix metalloproteinase-2
E-cadherin
Nasopharyngeal cancer
Metastasis

ABSTRACT

Objective: To investigate the correlation between nasopharyngeal carcinoma cell WNT5A and epithelial-mesenchymal transition (emt)/metastasis, and investigate its possible mechanisms. **Methods:** RT-PCR and gene transfection were used to detect the expression of nasopharyngeal carcinoma cell strains WNT5A and EMT related factor 5-8F. Transient transfection of NPC cell line 5-8F was determined by liposome of plasmid with WNT5A gene. The differential expressions of WNT5A and EMT-related factors in cells before and after transfection were detected by RT-PCR. Cell scratch assay and Transwell assay were used to detect the motility abilities of cells before and after 5-8F transfection. **Results:** The expressions of WNT5A and EMT related factors matrix metalloproteinase-2 of the WNT5A transferred group in the nasopharyngeal carcinoma cell line 5-8F were higher than the blank control group and the empty vector transferred group, and the transfer ability of the WNT5A transferred group was higher than that in the blank control group and the empty vector transferred group, while the expressions of EMT related factors E-cadherin were lower than that in the blank control group and the empty vector transferred group, and the transfer ability of the WNT5A transferred group was higher than that in the blank control group and the empty vector transferred group. **Conclusions:** In nasopharyngeal carcinoma cells, WNT5A can regulate the epithelial-mesenchymal transition and affect the ability of tumor invasion and metastasis of nasopharyngeal carcinoma.

1. Introduction

Nasopharyngeal carcinoma is one of the common head and neck cancer which had high malignant degree; over 95% of this disease are poorly differentiated carcinoma. The patients had a tendency of cervical lymph nodes metastasis in their early stage, then the disease systemic spread and the prognosis is poor[1]. Currently because of its sensitivity to radiotherapy, radiotherapy should be considered as a standard strategy, but the 5-year survival rates are still low, so understanding the pathogenesis of nasopharyngeal carcinoma and the metastasis and invasion of NPC has become a research hotspot. WNT5A as an important Wnt family members play an extremely important role in many physiological and pathological processes[2,3], it also plays an important role in the epithelial-mesenchymal transition of tumors growth and development[4-6]. This study used RT-PCR and gene transfection to investigate the effect of WNT5A on the process of nasopharyngeal carcinoma cell proliferation, differentiation and invasion and metastasis, and investigate its possible mechanisms.

2. Materials and methods

2.1. Materials and reagents

Human nasopharyngeal carcinoma cell line 5-8F was purchased from the Cell Center of XX University.

Agarose, ethidium bromide (EB), chloroform, isoamyl

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alcohol, ethanol, isopropanol were provided by the laboratory, Trizol, DEPC purchased from PBL Biotechnology; RT-PCR kit was purchased from TaKaRa Biotechnology (Dalian) Co., Ltd.; WNT5A, E-cadherin, MMP-2 and GAPDH mRNA PCR primers were purchased from Wuhan Boster biotechnology company, Transwell chambers were purchased from Sigma-Aldrich Company, plasmid pSUPER RetroWNT5A-RNAi was purchased from BD Biosciences. PCR amplification icycler type (invitrogen; United States), DC2000 gel imaging analyzer (Qiagen, Germany).

2.2. Cell culture

After isolated human nasopharyngeal carcinoma cell line 5-8F, the cells were placed in DMEM culture medium with 10% fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin, 2 mmol/L glutamine, then placed in 5% CO_2 37 ℃ constant temperature incubator. The medium was changed every other dayand wea divided into three groups 1 d before transfection, WNT5A transferred group, the blank control group and the empty vector transferred group,

2.3. WNT5A-RNAi transfection

WNT5A full-length cloning was used as a template to amplify the coding frame sequence in GenBank. The sense strand: CAT CGA AGG TGG AAC TGC A, antisense strand: GCA AGT TGG TAC AGG TCA ACA. The digestion site of BglII and HindIII were arranged at 5′ and 3′ ends of the oligonucleotide chain respectively. The WNT5A targeting shRNA sequences were cloned into the vector plasmid and mixed. The mixture was added to the cells and incubated at room temperature.

2.4. RT-PCR

Conventional method trizol reagent was applied to collect cells in each group, 2 μg RNA volume was calculated according to the concentration, and reverse transcription was carried out according to the process of reverse transcription kit. Primers were as follow: WNT5A (273 bp) upstream primer sequence: 5′-CTTCGCCCAGGTTGTAATTGAAGC-3′, downstream: 5′-CTGCCAAAAACAGAGGTGTTATCC-3′, WNT5A reaction conditions: 94 ℃ 1 min, 94 ℃ 30 s → 58 ℃ 30 s → 72 ℃ 45 s, 35 cycles of amplification. E-cad (497 bp) upstream: 5′-GGGTCTTGCTATGTTGCC-3′, downstream: 5′-GTTCCGCTCTGTCTTTGC-3′, reaction conditions: after denaturation at 95 ℃ for 5 min, 95 ℃ denaturation 30 s → anneal at 60 ℃ for 30 s → extended at 72 ℃ for 40 s, 35 cycles of amplification.MMP-2 (225 bp) upstream: 5′-AGATCTTCTTCTTCAAGGACCGGTT-3′, downstream: 5′-GGCTGGTCAGTGGCTTGGGGTA-3′, reaction conditions: after denaturation at 95 ℃ for 5 min, 95 ℃ denaturation 30 s → anneal at 63 ℃ for 30 s → extended at 72 ℃ for 30 s, 35 cycles of amplification. Internal reference sequence was GADPH (452 bp) upstream: 5′-ACCACAGTCCATGCCATCAC-3′, downstream: 5′-TCCACCACCCTGTTGCTGTA-3′, synthesized by Shanghai Biological Engineering Co., Ltd. It was amplified by the PCR instrument, the optical density of each bands was analyzed. Internal reference GADPH optical density was used to standardize the absorbance values of WNT5A, E-cad and MMP-2 mRNA, the relative content of WNT5A, E-cad, MMP-2 mRNA expression was obtained.

2.5. Scratch adhesion test

Cells were seeded at logarithmic growth phase in 24-well plate, 5×10^5 each well. When the cells grew to 100% confluence, 1-2 straight lines were drawn for the cells in each well with 10 μL pipette tip. Cells were cultured in each group, photographed at 0, 12, 24, 48 h and the difference speed of cell damage repair was observed.

2.6. Transwell experiment

Human nasopharyngeal carcinoma cells were collected in logarithmic growth phase and washed with PBS. The single cell suspension was obtained. Cell density was adjusted to 1×10^5/mL, 200 μL was taken respectively then added to each Transwell chamber. The Transwell plate was cultured in 5% CO_2 37 ℃ for 10-14 days. After 3 days they were replaced with fresh culture medium, the cells at the surface of upper chamber were wiped off, fixed with alcohol and HE stained. 10 fields of vision were selected randomly from each slice (×200), the number of cells at lower chamber were counted and the result was expressed as mean value.

2.7. Statistical analysis

The data were analyzed by SPSS 16.0 statistics software and the measurement data were expressed as mean±SD values. *t*-test was used and One-Way ANOVA was applied in the comparison between groups, $P<0.05$ has statistical significance.

3. Results

3.1 Detection of WNT5A transfection

Retrovirus infection method can successfully construct the NPC cells which can transfect WNT5A plasmid, the transfection efficiency were 80% to 90%. RT-PCR results showed that the WNT5A expression of nasopharyngeal carcinoma cells which with WNT5A gene plasmid was significantly increased.

3.2. WNT5A, E-cad, MMP-2-related factor expression in the cells of each group

MRNA was extracted according to previously methods and Semi-quantitative assay of mRNA was used. The average values of WNT5A, E-cad, MMP-2 expression levels by semi-quantitative analysis in the WNT5A transferred group, the blank control group and the empty vector transferred group were shown in Table 1. The WNT5A and MMP-2 expressions of the WNT5A transferred group were significantly higher than the blank control group and the empty vector transferred group, and the differences had statistical significance ($P<0.05$). The E-cad expression levels of the WNT5A transferred group were significantly lower than the blank control group and the empty vector transferred group, and the differences had statistical significance ($P<0.05$). The WNT5A, E-cad, MMP-2 expression levels of the blank control group and the empty vector transferred group showed no significant difference ($P> 0.05$) (Figure 1).

Table 1
WNT5A, E-cad, MMP-2 mRNA expression in the cells of each group.

Group	WNT5A	E-cad	MMP-2
Blank control group	0.42±0.11	0.55±0.07	0.38±0.12
Empty vector transferred group	0.40±0.10	0.56±0.08	0.40±0.10
WNT5A transferred group	1.78±0.24*△	0.32±0.08*△	1.78±0.24*△

Note: *compared with blank control group, $P<0.05$; △ compared with empty vector transferred group, $P<0.05$.

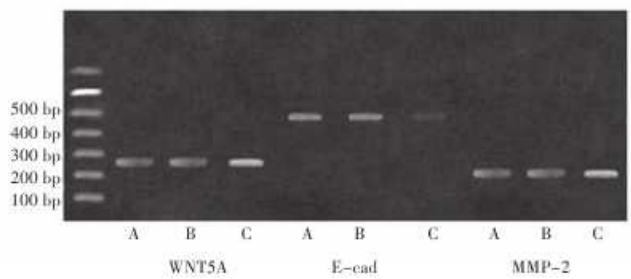

Figure 1. WNT5A, E-cad, MMP-2 mRNA expression in the cells of each group.
A: the blank control group; B: the empty vector transferred group; C: the WNT5A transferred group.

3.3. Scratch adhesion test to detect the transfer ability of cells in each group

The migration distance changed with different period of time of cells in each group. After 48 h, the migration distance of the WNT5A transferred group was significantly higher than the blank control group and the empty vector transferred group, scratch wounds have covered 80% to 90%. Scratch wounds of the blank control group and the empty vector transferred group were only 40% to 60%, and the differences had statistical significance ($P<0.05$) (Table 2).

3.4. Transwell invasion assay

Under the 200 times microscope, compared the number of cells that entered the lower chamber of Transwell between any two groups, the number of NPC in the WNT5A transferred group was the most (85.58±9.86), and the invasion and metastases ability was the highest, which were significantly higher than that in the blank control group and the empty vector transferred group, and the differences had statistical significance ($P<0.05$). The blank control group (55.47±6.72) and the empty vector transferred group (52.36±6.54) showed no significant difference ($P>0.05$).

4. Discussion

WNT5A gene is located on human chromosome 3p14-p21, which plays its role in many physiological and pathological processes by autocrine or paracrine, especially in the development of normal structure and tumor. Recent studies have found that WNT5A have different effect on different types of tumors, and its mechanism remains unclear in NPC formation. Epithelial-mesenchymal transition is an important pathogenesis of tumor invasion and metastasis[7]. EMT was originally used to describe the evolution process of interstitial cells during the developmental stages of embryo, which has been demonstrated to play a decisive role in tumor invasion and metastasis[8]. But there are less reports on whether EMT is involved in the occurrence and development of NPC and whether WNT5A is involved in the regulation of EMT. E-cad, MMP-2 play a more important role in the regulation of EMT.E-cad is a class of cadherin with deeply-research. This is because it is closely related with tumor invasion and metastasis, which can suppresse

Table 2
Transfer ability of cells in each group.

Group	Migrating cells accounted for scratch wound area (%)			
	0 h	12 h	24 h	48 h
Blank control group	0	6.34±1.59	17.84±3.62	48.67±7.62
Empty vector transferred group	0	6.23±1.44	18.35±4.23	49.39±6.85
WNT5A transferred group	0	10.46±2.76*△	29.58±5.38*△	87.45±12.54*△

Note: *compared with the blank control group, $P<0.05$; △compared with the empty vector transferred group, $P<0.05$.

the tumor invasion and metastasis in many steps[9].

MMP-2 gene as one of the most important MMPs can degrade a variety of ECM components, which play an important role in the invasion and metastasis of tumor cells, including the degradation of extracellular matrix membrane effective components, regulating cell adhesion and tissue remodeling and wound healing. There is an inhibition or antagonism[10,11] between the two. In this study, RT-PCR and gene transfection methods were used to detect the WNT5A, E-cad MMP-2 mRNA expression in the nasopharyngeal tissues or cells, the results showed that the epithelial marker E-cad expression was decreased in nasopharyngeal carcinoma, while WNT5A and MMP-2 expression were increased in the nasopharyngeal carcinoma.WNT5A transfection can lead to E-cad, MMP-2 corresponding changes, that suggested WNT5A may be involved in the development and progression of NPC. Because E-cad decreased, the tightness between cells will reduced and the cells easy to escape or metastasis and the cell polarity disappears. The MMP-2 is increased, which further lead to the disintegration between cells and cytoskeletal rearrangement, cell motility enhanced[12].These results illustrate that WNT5A involved in the invasion and metastasis of the EMT, which is consistent with the reports of Polakis P, thereby contributing to the results of NPC metastasis[13].

EMT occurrence involved in multiple signaling pathways, the specific mechanism is still unclear.

In this study, the scratch test and Transwell invasion assay confirmed nasopharyngeal carcinoma cells have strong athletic ability in tumor pathogenesis. The invasion progress of cell lines with WNT5A transfection increased with the increased expression of WNT5A and the extension of time. It is considered that WNT5A and EMT-related gene expression E-cad, MMP-2 is closely related and there is an interaction, they play an important role in the regulation of cell movement. Some scholars believe that WNT5A leading to EMT change by adjusting the molecular signaling pathway[14,15].

In summary, WNT5A involved in the invasion and metastasis of nasopharyngeal carcinoma EMT regulation. WNT5A can regulate the expression of E-cad and MMP-2 by targeting and promote the transfer of EMT to nasopharyngeal carcinoma .The specific mechanism remains unclear. The regulation of various signaling pathways and changes of cytokine levels between each other still need to further explore. It can provide experimental data for the study of WNT5A inhibitor and the molecular mechanisms of nasopharyngeal carcinoma metastasis treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

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