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Transcription factor Runx2 knockdown regulates colon cancer transplantation tumor growth in vitro: an experimental study

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ABSTRACT

Objective: To study the effect of transcription factor Runx2 knockdown on colon cancer transplantation tumor growth in vitro. **Methods:** Colon cancer cell lines HT29 were cultured and transfected with negative control (NC) - shRNA plasmids and Runx2-shRNA plasmids respectively, the colon cancer cells transfected with shRNA were subcutaneously injected into C57 nude mice, and they were included in NC group and Runx2 knockdown group respectively. 1 week, 2 weeks and 3 weeks after model establishment, serum was collected to determine the contents of tumor markers, and tumor lesions were collected to determine proliferation and apoptosis gene expression. **Results:** CCSA-2, CEA and CA19-9 levels in serum as well as Rac1, Wnt3a, PLD2 and FAM96B protein expression in transplantation tumor lesions of Runx2 knockdown group were significantly lower than those of NC group while MS4A12, ASPP2 and Fas protein expression in transplantation tumor lesions of Runx2 knockdown group were significantly higher than those of NC group. **Conclusion:** Transcription factor Runx2 knockdown could inhibit the colon cancer transplantation tumor growth in vitro.

1. Introduction

Colon cancer is the malignant digestive tract tumor with rising incidence in recent years[1], but the exact pathogenesis is still not clear. Runt-related transcription factor 2 (Runx2) is a member of Runx transcription factor family, and it adjusts the expression of a variety of downstream target genes to influence cellular biological behavior. In recent years, abnormal Runx2 expression is confirmed to be closely related to the occurrence of a variety of malignant tumors[2,3], knocking down Runx2 expression can effectively restrain the colon cancer cell proliferation and invasion, but it is unclear whether Runx2 knockdown can affect the growth of colon cancer lesions. In the following study, the Runx2 shRNA was designed and transfected into the colon cancer cells to establish Runx2 knockdown cell lines, and then colon cancer transplantation tumor mouse model was established to analyze the influence of Runx2 on colon cancer transplantation tumor growth in vitro.

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2. Experimental animals, reagents and instruments

2.1 Experimental animals

Experimental animals were a total of 48 male C57 nude mice that were 4-6 weeks old and with body weight 18-22 g, they were purchased and bred by Vital River Laboratory Animal Center, the breeding conditions were SPF grade, 24 h circadian rhythm and free eating and drinking, and the animal license was SYXK (Beijing), 2011-0028. Animal experiments were reviewed by the hospital ethics committee, and the animal experiments and animal processing after death were performed according to regulations.

2.2 Experimental reagents and instruments

Colon cancer cell lines HT29 were bought in Shanghai Jian Blunt Biological Technology Co., LTD., Runx2 shRNA and corresponding

plasmid were synthesized by Shanghai Genepharma Company, reagents for cell culture, amplification and sub-culture were bought from Gibco Company, protein extract RIPA was bought from Shanghai Beyotime Company, and enzyme-linked immunosorbent assay kits were purchased from Nanjing SenBeiJia Biological Technology Company.

2.3 Cell culture and Runx2 knockdown methods

HT29 cells were cultured in RPMI1640 culture medium containing 10% fetal bovine serum and digested and sub-cultured with trypsin after the cell density reached around 80%, Lipofectamine2000 transfection reagent was used after sub-culture amplification to transfect the negative control - shRNA plasmids and Runx2-shRNA plasmid into cells, 5 μg/mL puromycin was used after 96 h of transfection to screen for stably expressed cells, digest the cells and then adjust the cell density to 1×10^7/mL.

2.4 Transplantation tumor mouse model establishment methods

1×10^7/mL HT29 cell suspension was taken for subcutaneous injection in the dose of 0.3 mL/mouse, the injection site was the right fore, 8 mice from each group were executed 1 week, 2 weeks and 3 weeks, and the peripheral blood was collected and centrifuged to separate serum and store it in a -80 ℃ low-temperature refrigerator; the transplantation tumor lesions were anatomized, washed with normal saline and then stored in a -80 ℃ low-temperature refrigerator.

2.5 Serum index and gene expression detection methods

Serum specimens were collected, and enzyme-linked immunosorbent assay kits were used to determine the content of CCSA-2, CEA and CA19-9; transplantation tumor lesions were taken and added in protein extract RIPA to separate the protein within the tissue, and enzyme-linked immunosorbent assay kits were used to detect the content of Rac1, Wnt3a, PLD2, FAM96B, MS4A12, ASPP2 and Fas.

2.6 Statistical methods

SPSS 20.0 software was used to input serum index and gene expression data, analysis of above data between two groups was by *t* test and $P<0.05$ indicated the statistical significance in differences.

3. Experimental results

3.1 Serum indexes of two groups of transplantation tumor mice

1 week, 2 weeks and 3 weeks after transplantation tumor model establishment, analysis of serum tumor markers CCSA-2 (ng/mL), CEA (ng/mL) and CA19-9 (IU/L) contents between two groups of transplantation tumor mice was as follows: 1 week, 2 weeks and 3 weeks after model establishment, serum CCSA-2, CEA and CA19-9 levels of Runx2 knockdown group were significantly lower than those of NC group. Differences in serum CCSA-2, CEA and CA19-9 levels were statistically significant between Runx2 knockdown group and NC group ($P<0.05$).

Table 1.

Comparison of serum tumor markers between two groups of transplantation tumor mice.

Groups	*n*	Time	CCSA-2	CEA	CA19-9
NC group	8	1 week	55.78±8.91	26.72±3.76	46.51±7.52
		2 weeks	67.21±9.25	39.15±6.42	64.21±8.14
		3 weeks	83.36±9.14	55.25±8.21	83.72±10.25
Runx2 knockdown group	8	1 week	42.54±7.47*	20.19±3.12*	34.21±5.26*
		2 weeks	49.26±6.95*	27.64±3.52*	45.33±7.68*
		3 weeks	61.42±8.25*	32.12±4.46*	52.17±9.31*

*: comparison between NC group and Runx2 knockdown group at the same point in time, $P<0.05$.

3.2 Proliferation gene expression in transplantation tumor lesion of two groups of mice

1 week, 2 weeks and 3 weeks after transplantation tumor model establishment, analysis of proliferation genes Rac1, Wnt3a, PLD2 and FAM96B expression in transplantation tumor lesions between two groups of mice was as follows: 1 week, 2 weeks and 3 weeks after model establishment, Rac1, Wnt3a, PLD2 and FAM96B protein expression in transplantation tumor lesions of Runx2 knockdown group were significantly lower than those of NC group. Differences in Rac1, Wnt3a, PLD2 and FAM96B protein expression in transplantation tumor lesions were statistically significant between Runx2 knockdown group and NC group ($P<0.05$).

Table 2.

Comparison of proliferation gene expression in transplantation tumor lesions between two groups of mice (ng/mL).

Groups	*n*	Time	Rac1	Wnt3a	PLD2	PLD2
NC group	8	1 week	3.51±0.64	1.03±0.16	2.35±0.42	0.95±0.12
		2 weeks	6.53±0.89	2.65±0.42	4.56±0.85	2.56±0.42
		3 weeks	10.25±1.42	5.51±0.77	7.75±0.94	6.51±0.89
Runx2 knockdown group	8	1 week	2.26±0.36*	0.67±0.09*	1.67±0.28*	0.56±0.08*
		2 weeks	3.87±0.65*	1.24±0.18*	2.88±0.52*	1.32±0.23*
		3 weeks	5.21±0.78*	1.88±0.26*	4.02±0.67*	2.89±0.41*

*: comparison between NC group and Runx2 knockdown group at the same point in time, $P<0.05$.

3.3 Apoptosis gene expression in transplantation tumor lesion of two groups of mice

1 week, 2 weeks and 3 weeks after transplantation tumor model establishment, analysis of apoptosis genes MS4A12 (ng/mL), ASPP2 (ng/mL) and Fas (μg/mL) expression in transplantation tumor lesions between two groups of mice was as follows: 1 week, 2 weeks and 3 weeks after model establishment, MS4A12, ASPP2 and Fas protein expression in transplantation tumor lesions of Runx2 knockdown group were significantly higher than those of NC group. Differences in MS4A12, ASPP2 and Fas protein expression in transplantation tumor lesions were statistically significant between Runx2 knockdown group and NC group (P<0.05).

Table 3.

Comparison of apoptosis gene expression in transplantation tumor lesions between two groups of mice.

Groups	*n*	Time	MS4A12	ASPP2	Fas
NC group	8	1 week	4.75±0.75	2.15±0.35	0.56±0.07
		2 weeks	4.62±0.81	2.28±0.33	0.51±0.08
		3 weeks	4.48±0.67	2.09±0.31	0.60±0.06
Runx2 knockdown group	8	1 week	6.97±0.93*	4.42±0.67*	0.83±0.11*
		2 weeks	9.21±1.13*	6.79±0.81*	1.21±0.17*
		3 weeks	13.21±1.95*	9.14±1.17*	1.77±0.25*

*: comparison between NC group and Runx2 knockdown group at the same point in time, P<0.05.

4. Discussion

Runx2 is an important member of the Runx transcription factor family, and it has a regulatory effect on cell proliferation, invasion and other biological behaviors. Abnormal cell proliferation and invasion is the important link of the occurrence and development of malignant tumor, and the relationship of abnormal Runx2 expression with breast cancer[4], liver cancer[5], pancreatic cancer[6] and other malignant tumors has received more and more attention. The cellular experimental study of the domestic scholars has confirmed that knocking down the expression of Runx2 can effectively inhibit the proliferation and invasion of colon cancer cells[7]. However, it is not yet clear at present about Runx2's influence on the growth of colon cancer lesions. In the growth of malignant tumor lesions, the lesion itself can synthesize a variety of molecules and secrete them into the blood circulation, and detecting the serum levels of corresponding tumor markers can reflect the lesion growth activity. CCSA-2, CEA and CA19-9 are the tumor markers closely related to the growth of colon cancer lesions[8,9], and in order to define the effect of Runx2 knockdown on colon cancer transplantation tumor lesion growth, the contents of the tumor markers in serum were analyzed at first in the study, and the results showed that the rising trend of serum tumor marker levels of Runx2 knockdown group was smoother than that of NC group, and serum CCSA-2, CEA and CA19-9 levels of Runx2 knockdown group after 1 week, 2 weeks and 3 weeks were significantly lower than those of NC group. It means that Runx2 knockdown can effectively inhibit the growth of colon cancer transplanted tumor, and serum levels of tumor markers reflecting the cancer cell growth vigor are lower.

The growth of tumor lesion is closely related to the abnormal cancer cell proliferation, and Runx2 as a transcription factor in cells, can participate in the regulation of the expression of a variety of proliferation-related genes. Rac1, Wnt3a, PLD2, FAM96B and so on are the genes closely associated with colon cancer cell proliferation. Rac1 is a member of the Rho family that can not only promote the actin polymerization and induce invasive cell growth, but can also increase the expression of VEGF and increase the number of new blood vessels[10,11]; Wnt3a is a member of the Wnt family, and it interacts with ALDH1B1 to activate PPARs and thus promote cell growth[12,13]; PLD2 has inhibiting effect on the function of p21, p53 and a variety of other tumor suppressor genes, and it can increase the expression of a variety of cyclin and their kinase so as to accelerate the cell cycle and promote cell proliferation[14]; FAM96B, also known as MIP18 and HSPC118, has inhibiting effect on basic helix - loop - helix protein E2-2, and can weaken the inhibiting effect of E2-2 on biological effects of VEGF so as to promote angiogenesis and blood infiltration of the cancer cells. In the study, analysis of the proliferation gene expression in colon cancer transplantation tumor lesions showed that the rising trend of Rac1, Wnt3a, PLD2 and FAM96B protein expression in transplantation tumor lesions of Runx2 knockdown group was smoother than that of NC group, and Rac1, Wnt3a, PLD2 and FAM96B protein expression in transplantation tumor lesions of Runx2 knockdown group after 1 week, 2 weeks and 3 weeks were significantly lower than those of NC group. It means that Runx2 knockdown can effectively restrain the expression of pro-proliferation genes such as Rac1, Wnt3a, PLD2 and FAM96B so as to inhibit the cancer cell proliferation and lesion growth.

In the growth of colon cancer lesions, the proliferation of cancer cells is not only associated with the high expression of proliferation genes, but also related to the expression deletion of a variety of pro-apoptosis genes. MS4A12, ASPP2 and Fas are the genes closely related to colon cancer cell apoptosis. MS4A12 is an important member in MS4A family, its expression decreases in the G1 phase of cell cycle and increases in S phase, it has hindering effect on the process of cell cycle, and it can make the cell cycle arrest at S phase and inhibit the growth of cells[15,16]; ASPP2 is a pro-apoptosis member of the ASPP family, and it activates p53 through the downstream Ras/MAPK signaling pathways, and then induces apoptosis and inhibits cell proliferation by p53[17,18]; Fas is a molecule in cancer cells mediating death receptor pathway

apoptosis, and it forms apoptotic body and induces cell apoptosis by cascade activation of caspase molecules. In the study, analysis of the proliferation gene expression in colon cancer transplantation tumor lesions showed that the rising trend of MS4A12, ASPP2 and Fas protein expression in transplantation tumor lesions of Runx2 knockdown group was more significant than that of NC group, and MS4A12, ASPP2 and Fas protein expression in transplantation tumor lesions of Runx2 knockdown group after 1 week, 2 weeks and 3 weeks were significantly higher than those of NC group. It means that Runx2 knockdown can effectively increase the expression of pro-apoptosis genes such as MS4A12, ASPP2 and Fas so as to induce cancer cells apoptosis and inhibit the growth of tumor lesions. Based on above animal experiment research, it is believed that knocking down transcription factor Runx2 has inhibiting effect on colon cancer transplantation tumor growth in vitro, and inhibiting pro-proliferation gene expression and increasing pro-apoptosis gene expression are the molecular pathways for Runx2 knockdown to exert carcinostasis.

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