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Effect of hGC-MSCs from human gastric cancer tissue on cell proliferation, invasion and epithelial-mesenchymal transition in tumor tissue of gastric cancer tumor-bearing mice

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ABSTRACT

Objective: To study the effect of hGC-MSCs from human gastric cancer tissue on cell proliferation, invasion and epithelial-mesenchymal transition in tumor tissue of gastric cancer tumor-bearing mice.

Methods: BABL/c nude mice were selected as experimental animals and gastric cancer tumor-bearing mice model were established by subcutaneous injection of gastric cancer cells, randomly divided into different intervention groups. hGC-MSCs group were given different amounts of gastric cancer cells for subcutaneous injection, PBS group was given equal volume of PBS for subcutaneous injection. Then tumor tissue volume were determined, tumor-bearing mice were killed and tumor tissues were collected, mRNA expression of proliferation, invasion, EMT-related molecules were determined.

Results: 4, 8, 12, 16, 20 d after intervention, tumor tissue volume of hGC-MSCs group were significantly higher than those of PBS group and the more the number of hGC-MSCs, the higher the tumor tissue volume; mRNA contents of Ki-67, PCNA, Bcl-2, MMP-2, MMP-7, MMP-9, MMP-14, N-cadherin, vimentin, Snail and Twist in tumor tissue of hGC-MSCs group were higher than those of PBS group, and mRNA contents of Bax, TIMP1, TIMP2 and E-cadherin were lower than those of PBS group.

Conclusion: hGC-MSCs from human gastric cancer tissue can promote the tumor growth in gastric cancer tumor-bearing mice, and the molecular mechanism includes promoting cell proliferation, invasion and epithelial-mesenchymal transition.

1. Introduction

Mesenchymal stem cells (MSCs) are the stem cells from mesoderm of early embryo development that have the features such as highly self-renewal ability, easily transferring into exogenous genes and oncolytic virus, and weak immunogenicity [1,2]. Studies in recent years have confirmed that stem cells have good affinity to solid tumors and participate in the occurrence and development of a variety of malignant tumors. MSCs in vitro isolation, culture and amplification are relatively easy, and they are the ideal carrier for the verification of malignant tumor pathogenesis and the targeted treatment of malignant tumors [3–5]. However, the relationship between MSCs and the occurrence and development of gastric cancer is not yet clear at present, and in the following study, hGC-MSCs from human gastric cancer tissue were isolated and cultured, and gastric cancer tumor-bearing mice model were established, aiming at verifying the effect of hGC-MSCs from human gastric cancer tissue on tumor growth of gastric cancer tumor-bearing mice through animal experiments.

2. Materials and methods

2.1. Materials

Human gastric cancer cell lines SGC-7901 were bought from the cell bank of Chinese academy of sciences, BABL/c nude mice were purchased by the university animal center [license: SCXK (Su) 2011-0003], 1640 culture medium, fetal bovine serum and trypsin for cell culture were bought from Gibco Company, and the RNA extraction and PCR detection kits were bought from Beijing Tiangen Biotech Company.

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2.2. Tumor-bearing mouse model establishment

SGC-7901 cell lines were recovered, then cultured with 1640 culture medium containing 10% fetal bovine serum, amplified and then digested with trypsin, cell density was adjusted to 5×10^7/mL, 200 μL cell suspension was collected and subcutaneously inoculated in the back of BABL/c nude mice, the volume of tumor tissue was measured after 8 d, and those with volume more than 0.1 cm^3 were collected for subsequent research.

2.3. Separate culture and injection of hGC-MSCs

Fresh gastric carcinoma tissue was collected, saved under aseptic conditions, sent to the super clean bench, cut up to tissue fragments of 1 mm^3, then inoculated in a Petri dish for 2 h of adherence, culture medium was added for continuous incubation for 24 h, half the medium was changed, un-adhered tissue mass and cells were discarded, medium was changed every 3 d, cells were digested and sub-cultured after 80%–90% of fusion, and hGC-MSCs were obtained for treatment. HGC-MSCs group received injection of 0.5×10^6, 1.0×10^6 and 2.0×10^6 hGC-MSCs respectively around the transplantation tumor, and PBS group received injection the same volume of PBS.

2.4. Determination of tumor tissue growth

(1) 0, 4, 8, 12, 16 and 20 d after establishment of tumor-bearing mice model, the long diameter and short diameter of tumor were determined, and tumor tissue volume was calculated according to long diameter × short diameter × short diameter/2; (2) on the 21 d, tumor-bearing mice were executed and anatomized to get tumor tissue, RNA extraction kit was used to extract RNA and reverse-transcribe it into cDNA, PCR kit was used for amplification and detection, and mRNA contents of Ki-67, PCNA, Bcl-2, Bax, P53, MMP-2, MMP-7, MMP-9, MMP-14, TIMP1, TIMP2, E-cadherin, N-cadherin, Vimentin, Snail and Twist were calculated.

2.5. Statistical methods

SPSS20.0 software was used for analysis, differences among groups were compared by single factor analysis of variance, the pair-wise comparison was by LSD method and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1. hGC-MSCs promoted tumor tissue growth in tumor-bearing mice

Different doses of hGC-MSCs could all promote the tumor tissue growth in tumor-bearing mice. At each point in time, tumor tissue volume of hGC-MSCs group were significantly higher than those of PBS group, and the larger the dose of hGC-MSCs, the more significant the increase of tumor tissue volume, and it was significantly dose-dependent (see Table 1).

3.2. hGC-MSCs regulated the expression of Ki-67, PCNA, Bcl-2 and Bax in tumor tissue

Different doses of hGC-MSCs could all increase the expression of Ki-67, PCNA and Bcl-2, and inhibit the expression of Bax in tumor tissue. mRNA contents of Ki-67, PCNA and Bcl-2 in tumor tissue of hGC-MSCs group were higher than those of PBS group, and mRNA content of Bax was lower than that of PBS group; the larger the dose of hGC-MSCs, the more significant the change in the mRNA contents of Ki-67, PCNA, Bcl-2 and Bax, and it was significantly dose-dependent (see Table 2).

3.3. hGC-MSCs regulated the expression of MMPs and TIMPs in tumor tissue

Different doses of hGC-MSCs could all increase the expression of MMP-2, MMP-7, MMP-9 and MMP-14, and inhibit the expression of TIMP1 and TIMP2 in tumor tissue. mRNA contents of MMP-2, MMP-7, MMP-9 and MMP-14 in tumor tissue of hGC-MSCs group were higher than those of PBS group, and mRNA contents of TIMP1 and TIMP2 were lower than those of PBS group; the larger the dose of hGC-MSCs, the more significant the change in the mRNA contents of MMP-2, MMP-7, MMP-9, MMP-14, TIMP1 and TIMP2, and it was significantly dose-dependent (see Table 3).

3.4. hGC-MSCs regulated the expression of EMT-related molecules in tumor tissue

Different doses of hGC-MSCs could all increase the expression of N-cadherin, Vimentin, Snail and Twist, and inhibit the expression of E-cadherin in tumor tissue. mRNA contents of

Table 2
mRNA contents of proliferation-related molecules in tumor tissue of every group.

Groups	Proliferation-related molecules			
	Ki-67/β-actin	PCNA/β-actin	Bcl-2/β-actin	Bax/β-actin
2×10^6 hGC-MSCs	2.95 ± 0.32	3.38 ± 0.39	3.14 ± 0.33	0.29 ± 0.28
1×10^6 hGC-MSCs	2.11 ± 0.24	2.28 ± 0.24	2.39 ± 0.25	0.55 ± 0.07
0.5×10^6 hGC-MSCs	1.67 ± 0.18	1.74 ± 0.21	1.49 ± 0.15	0.78 ± 0.09
PBS group	1.00 ± 0.12	1.00 ± 0.11	1.00 ± 0.08	1.00 ± 0.14

Table 1
Tumor tissue volume of all groups (cm^3).

Groups	Action time					
	0 d	4 d	8 d	12 d	16 d	20 d
2×10^6 hGC-MSCs	0.105 ± 0.012	0.528 ± 0.065	1.229 ± 0.128	1.912 ± 0.228	2.585 ± 0.278	3.255 ± 0.339
1×10^6 hGC-MSCs	0.112 ± 0.011	0.394 ± 0.052	0.924 ± 0.104	1.449 ± 0.179	2.003 ± 0.231	2.783 ± 0.315
0.5×10^6 hGC-MSCs	0.101 ± 0.010	0.304 ± 0.036	0.683 ± 0.080	1.214 ± 0.097	1.583 ± 0.178	2.218 ± 0.252
PBS group	0.108 ± 0.009	0.194 ± 0.020	0.489 ± 0.057	0.881 ± 0.094	1.133 ± 0.128	1.331 ± 0.148