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RESEARCH PAPER

The Effect of Human IL-17F on the Growth of Human Hepatocarcinoma Xenograft Tumors in Nude Mice

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Abstract: The human interleukin-17F (hIL-17F) gene was amplified by RT-PCR from PHA-activated human peripheral blood mononuclear cells (PBMCs). It was then subcloned into the retrovirus vector pSIV-1. The pSIV-1/hIL-17F together with its two-helper virus vectors pHIT456 and pHIT60 cotransfected into the package cell 293T by lipofectin to produce mature recombinant retrovirus, which was then used to infect SMMC-7721 hepatocarcinoma cells (HCCs), and the cells were selected in the presence of G418. The integration, transcription, and expression of hIL-17F gene in SMMC-7721 cells were identified by PCR, RT-PCR, and Western blot, respectively. MTT and FCM showed that hIL-17F could not alter the proliferation and cell cycle of SMMC-7721 cells, but ELISA showed that it could down-regulate IL-6, IL-8, and VEGF expressions. The effect of rhIL-17F supernatant on the growth suppression of ECV304 cells was observed by MTT. The experiment on human hepatocarcinoma xenografts tumor in nude mice showed that the formation and growth rates of hIL-17F-transgenic SMMC-7721 HCCs showed an obvious decline, and VEGF and CD34 expressions and angiogenesis of the transgenic neoplasms were also evidently reduced. hIL-17F can inhibit markedly the growth of human hepatocarcinoma xenograft tumors in nude mice by antiangiogenesis. This study provided an experimental evidence for further conducting the tumor gene therapy by targeting vascularity and exploiting antiangiogenic novel medicine related to hIL-17F.

Key Words: human interleukin-17F; retrovirus; hepatocarcinoma; antitumor effect

Angiogenesis-inhibiting factors have potential application in the therapies against diseases of vascular proliferation such as tumors[1,2] because tumors' growth and metastasis depends on angiogenesis. Recently, it has become a novel strategy to research and manufacture angiogenesis inhibitors to control tumors' growth and metastasis[3]. The human IL-17F (hIL-17F) was first identified and isolated from $CD4^+$T cells and monocytes by Starnes and colleagues; it can markedly inhibit angiogenesis, manifested by endothelial capillary tubule formation assays and has certain application prospects in cancer immunotherapies by inhibiting the generation of tumor vascular supply[4]. Although hIL-17F has marked anti-angiogenic activities, its mechanisms are seldom reported in China and abroad, and its antineoplaston studies stay in vacuity even today. The hIL-17F gene from activated peripheral blood mononuclear cells (PBMCs) using RT-PCR was successfully cloned, the recombinant retroviral vectors of hIL-17F were constructed, and the transgenic cells of SMMC-7721, a kind of HCCs, were established. Its effects on biological characteristics of HCCs were observed, and subsequently the models of nude mice bearing human hepatocarcinoma xenograft tumors were also established to further evaluate the antiangiogenic activities and antitumor effects of hIL-17F *in vivo*.

1 Materials and methods

1.1 Materials

1.1.1 Plasmids, cells, and strains: pUCm-T vector was purchased from Sangon Company, China; retroviral vector

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pSIV-1, two-helper virus vectors pHIT456 and pHIT60, and package cells 293T were provided by Dr Chen Yong-Jing of Soochow University; *E. coli* DH5α, human hepatocarcinoma cells (HCCs) SMMC-7721, and human umbilical vein endothelial cells ECV304 were all kept by our department.

1.1.2 Reagents: Trizol RNA Extraction Kit (Sangon) and various kinds of enzymes (TaKaRa); Rapid Plasmid DNA Daily Mini-prep Kit, DNA Gel Extraction Kit, PCR Product Purification Kit (V-gene); Lipofectamin™ (Invitrogen); ELISA Kits for hIL-6, hIL-8, and VEGF (Jingmei); goat antihuman polyclonal antibody against IL-17F[IL-17F (C-13): *sc*-20599], rabbit antigoat IgG-AP, rabbit antihuman polyclonal antibody against VEGF, mouse antimouse monoclonal antibody against CD34 (Santa Cruz); UltraSensitive™ SP Kit (Maixin).

1.1.3 Primers for PCR: The following primers shown in Table 1 were all synthesized by Sangon Company

Table 1 Primer sequences used for the PCR amplification

Number	Primer sequences
(1)	5′-CAGCGCAACATGACAGTGAAGAC-3′
(2)	5′-CACCTCTTACTGCACATGGTGGAT-3′
(3)	5′-GCAGAATTCATGGTCAAGTACTTGCTG-3′
(4)	5′-CTCGGATCCTTACTGCACATGGTGGATG-3′
(5)	5′-CTCCATGAACTCCGTTCCCATCCAGCAAG-3′
(6)	5′-GGAACGGAGTTCATGGAGATGTCTTCCT-3′

1.2 Methods

1.2.1 Cloning of hIL-17F gene: PBMCs were isolated and cultivated for 24 h with stimulator PHA (10 μg/mL), and their total RNA were extracted and reversely transcribed into the primary chain of cDNA. Subsequently, hIL-17F genes were amplified by PCR with P1 and P2 as primers, and the products of RT-PCR were subcloned into pUCm-T vectors, and finally the recombinant cloning vectors pUCm-T/hIL-17F were identified by PCR and sequencing.

1.2.2 Construction of recombinant retroviral vector pSIV-1/hIL-17F: Plasmids pUCm-T/hIL-17F were amplified by PCR, using P3, P6 as primers and P4, P5 as primers, respectively, and the products were, respectively, PCR1 and PCR2; after purification, the products PCR1 and PCR2 served as templates, and they were amplified with P3 and P4 as primers also by PCR, and the products were PCR3 (i.e., the restriction site of *Eco*R I 'GAATTC' was synonymously mutated into 'GAACTC' in the 462 bp exon of hIL-17F containing the signal peptide sequence of 20 amino acids). PCR3 was then subcloned into the retroviral vector pSIV-1, and the new recombinant vector was identified by PCR, restriction endonuclease digestion, and sequencing.

1.2.3 Package and maturity of recombinant retrovirus: The mixture of pSIV-1/hIL-17F, two-helper virus vectors pHIT456 and pHIT60 in proportion of 2:1:1 were cotransfected into the package cells 293T, which were about 50 %–60 % confluent, to pack and release the mature and infectious recombinant retrovirus (RV-hIL-17F). Meanwhile, the mature blank retrovirus (RV) was also prepared, and both the RV and the normal 293T cells without vectors were kept as negative control groups. The cytopathic effect (CPE) of 293T cells were observed under a microscope, and the supernatant of virus RV-hIL-17F, RV, and the normal 293T cells were collected and kept as templates for PCR identification with P3 and P4 as primers.

1.2.4 Infection of SMMC-7721 cells and screening for positive clones: One day before transfection to the experimental group (EG), 2 mL of SMMC-7721 cells were planted into a 6-well plate at the density of 1×10^5/mL, and when the cells were 50 %–60 % confluent, the medium was replaced with 1 mL of the above-mentioned supernatant of virus RV-hIL-17F containing 8 ng/mL polybrene. After the plate was kept at 37 °C for 6 h, RPMI 1640 medium containing 10 % calf serum was added to each well of the plate until a concentration of 2.5 mL/well was attained. This process was then repeated daily for another twice. And the supernatants of RV and normal 293T cells were treated in the same manner and were kept as negative control groups (CG). Seventy-two hours after the first infection, SMMC-7721 cells of each group were, respectively, collected, diluted six times, and cultivated in selective medium containing 500 μg/mL of G418 for 3 weeks to screen and amplify the positive clones.

1.2.5 Identification of the transgenic HCCs: The positive clones of transgenic HCCs, SMMC-7721/RV-hIL-17F, and their cultivation supernatant were collected separately, and the genomic DNA and total RNA of SMMC-7721/RV-hIL-17F were extracted; the PCR process was then carried out with the genomic DNA as templates, P3 and P4 as primers to identify the integration of hIL-17F gene into the genome; RT-PCR assay was carried out with the total RNA as templates and P3 and P4 as primers to identify the transcription of hIL-17F gene in the transgenic HCCs; meanwhile, the hIL-17F protein expressed by the transgenic HCCs was identified by Western blot. As well, both cells of SMMC-7721, SMMC-7721/RV and their cultivation supernatants were subjected to the same treatment and were kept as negative CGs.

1.2.6 Detection of proliferation vitality and cell cycle of the transgenic HCCs: The density of SMMC-7721/RV-hIL-17F in logarithmic phase in cell suspensions were regulated at the density of 1×10^5/mL, and 100 μL of such suspensions were plated into each well of EG in a 96-well plate. SMMC-7721 and SMMC-7721/RV were also treated in the same manner to be kept as cell CG and blank vector CG, respectively. The experiment was performed in four replicates; meanwhile a blank well was set up. All these cells were cultivated under the same conditions, and after 1, 2, and 3 days, MTT methods were used to detect the cellular proliferation vitality in each group, and also about 1×10^6 cells in each group were collected and fixed by 70 % ethanol for the detection of cell cycle