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CONSTRUCTION AND IDENTIFICATION OF RETROVIRAL VECTOR EXPRESSING HUMAN INTERLEUKIN-17 GENE*

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Human interleukin 17(IL-17) is a newly found cytokine secreted by activated T lymphocytes. Many of its characteristics remain unknown. To provide a way for understanding its role in immunology and hematology further, we constructed a recombinant retroviral vector pL17SN containing the human IL-17 gene about 1.3kb with the coding region. The constructed retroviral vector packaged in CRIP cells could infect human primary fibroblasts, and could stimulate the primary fibroblasts secreting human GM-CSF and IL-6. The retroviral vector containing the human IL-17 gene we constructed may present an efficient way to understand the biological functions of human IL-17 and to investigate applications of IL-17 in cancer gene therapy.

Key words: Interleukin-17, Retroviral vector, Gene expression, GM-CSF, Interleukin-6.

Human interleukin 17(IL-17) was found recently and is one of cytokines. Its special bridge role between T lymphocytes and hematology evokes attention out of occasionally. It was found that the major source of human IL-17 was the activated T lymphocytes. In the presence of IL-17, the fibroblasts was able to sustain $CD34^+$ hematopoietic progenitor cells and direct their maturation towards neutrophils.[1] The exact pathway of its known biological activities and many of the characteristics are still in merge. The construction of a retroviral vector containing this cytokine may present an effective way to study its biological function and its application in gene therapy.

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MATERIAL AND METHODS

Reagents

The endonuclease, T4 DNA ligase and Taq DNA polymerase were purchased from Promega. The Lipofectin, recombinant human IL-6 and G418 were the products of Gibco. The human GM-CSF ELISA kit was from R&D. The retroviral vector pLXSN was kindly provided by Dr. Thomas Blankenstein from MDC for Molecular Medicine in Berlin, Germany.

Cell Culture

The retroviral vector packaging cell line CRIP and NIH3T3 cell lines were cultured in DMEM complete media containing 10% FCS. The IL-6 dependent cell line B9.9 was cultured in RPMI-1640 complete media supplemented with 10% FCS and 10ng/ml recombinant human IL-6. All the cells were incubated at 37 ℃ in 5% CO_2.

Cloning of the Human IL-17 Gene Fragment

The PCR primer for cloning human IL-17 gene fragment was designed by the aid of computer. The forward is: TGAATTCGGCACGAGCATCCATCCC-CAGTTGATTG; the backward is: AGCTCGA-GTTTTTTTTTTTTTTTTTTCTTTTGAAACTTTAT

TATTAC. The template is the reversely transcripted single strand DNA from mRNA of PHA stimulated human PBMC. PCR condition: 95 ℃, 20second; 55 ℃, 30 second; 72 ℃, 1minute; total 30cycles. The amplified gene fragment was determined by Hind III digestion. The amplified PCR fragment was inserted into the sequencing vector pBLUEscriptSK after EcoR I and Xho I double digestion, and the sequence was determined by ABI373 DNA sequencing.

Construction of Retroviral Vector Containing the Human IL-17 Gene

The retroviral vector pLXSN and the amplified PCR fragment were digested with EcoR I and Xho I. The two digested fragments were ligated with T4 DNA ligase at 16 ℃ overnight. The ligated products were transferred into the DH5a competent cells and the positive recombinants were selected by EcoR I and Xho I double digestion.

Packaging of the Recombinant Retroviral Vector

The constructed retroviral vector containing the human IL-17 gene pL17SN was transferred into the retroviral vector packaging cell line CRIP by Lipofectin transfection. The transferred CRIP cells were selected in 800mg/ml G418 for at least 2 weeks. The survived CRIP cells were terminated CRIP-L17SN. The titer of packaged recombinant retroviral particles was determined with NIH3T3 cells.

Assay of Biological Activity

The biological activity of IL-17 was assayed indirectly based on the characteristic that IL-17 could stimulate fibroblasts to secret IL-6 and GM-CSF. The culture supernatants of human skin primary fibroblast were collected after infected with packaged recombinant retroviral particles for 48 hours. IL-6 in the supernatants was assayed by MTT methods with B9.9 cells. GM-CSF was determined by ELISA.

RESULTS

Cloning and Sequencing of the Human IL-17 Gene

The primer was designed based on the known human IL-17 cDNA sequence. Electrophresis of the PCR amplified products represents a single special amplified band about 1.3kb. The amplified band was cut into two shorter fragments with Hind III and could be considered to accord with the known sequence preliminary. The amplified sequence was inserted into the sequencing vector pBLUEscriptSK after it was digested with EcoR I and Xho I. The positive recombinant was sequenced as followed:

EcoR

GAATTCGGCACGAGCATCCATCC CCAGTTGATT GGAAGAAACA
ACGATGACTCCTGGGAAGACCTCATTGGTG TCACTGCTAC TGCTGCTGAG
CCTGGAGGCCATAGTGAAGGCAGGAATCACAATCCCACGAAATCCAGGAT
GCCCAAATTCTGAGGACAAGAACTTCCCCCGGACTGTGATGGTCAACCTG
AACATCCATAACCGGAATACCAATACCAATCCCAAAAGGTCCTCAGATTA
CTACAACCGATCCACCTCACCTTGGAATCTCCACCGCAATGAGGACCCTG
AGAGATATCCCTCTGTGATCTGGGAGGCAAAGTGCCGCCACTTGGGCTGC
ATCAACGCTGATGGGAACGTGGACTACCACATGAACTCTGTCCCCATCCA
GCAAGAGATCCTGGTCCTGCGCAGGGAGCCTCCACACTGCCCCAACTCCT
TCCGGCTGGAGAAGATACTGGTGTCCGTGGGCTGCACCTG TGTCACCCCG
ATTGTCCACCATGTGGCCTAAGAGCTCTGGGGAGCCCACACTCCCCAAAG
CAGTTAGACTATGGAGAGCCGACCCAGCCCCTCAGGAACCCTCATCCTTC
AAAGACAGCCTCATTTCGGACTAAACTCATTAGAGTTCTTAAGGCAGTTT
GTCCAATTAAAGCTTCAGAGGTAACACTTGGCCAAGATATGAGATCTGAA
TTACCTTTCCCTCTTTCCAAGAAGGAAGGTTTGACTGAGTACCAATTTGCT
TCTTGTTTACTTTTTTAAGGGCTTTAAGTTATTTATGTATTTAATATGCCCT
GAGATAACTTTGGGGTATAAGATTCCATTTTAATGAATTACCTACTTTATT
TTGTTTGTCTTTTTAAAGAAGATAAGATTCTGGGCTTGGGAATTTTATTAT

TTAAAAGGTAAAACCTGTATTTATTTGAGCTATTTAAGGATCTATTTATG
TTTAAGTATTTAGAAAAAGGTGAAAAAGCACTATTATCAGTTCTGCCTAG
GTAAATGTAAGATAGAATTAAATGGCAGTGCAAAATTTCTGAGTCTTTAC
AACATACGGATATAGTATTTCCTCCTCTTTGTTTTTAAAAGTTATAACATG
GCTGAAAAGAAAGATTAAACCTACTTTCATATGTATTAATTTAAATTTTG
CAATTTGTTGAGGTTTTACAAGAGATACAGCAAGTCTAACTCTCTGTTCC
ATTAAACCCTTATAATAAAATCCTTCTGTAATAATAAAGTTTCAAAAGAA
AAGAAAAAAAAAAAAAAAAAACTCGAG
Xho I

The sequence is coincidence with the published sequence. The cloned fragment includes the complete reading frame and part of the 3' sequences.

Construction of the Recombinant Retroviral Vector

The human IL-17 fragment was cut from the pBLUEscriptSK-hIL17 with EcoR I and Xho I and inserted into the pLXSN large fragment digested with the same endonuclease. The constructed vector was terminated pL17SN and its brief structure is represented in Figure 1.

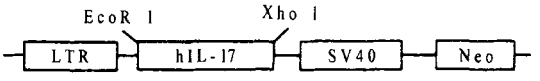

Fig. 1. The illustration of pL17SN.

The recombinants were determined by EcoR I and Xho I double digestion. The clone reproducing a 1.3kb long fragment was positive (Figure 2).

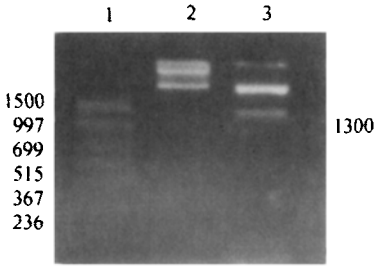

Fig. 2. The determination of pL17SN by endonuclease digestion. (From left to right: lane 1, DNA marker; lane 2, the undigested pL17SN ; lane 3, the pL17SN digested with EcoR I and Xho I).

One recombinants was selected to transfect the CRIP cells. After two weeks' selective culture in G418, some positive clones were picked and tested for their viral titers. The target cells was NIH3T3 cells. The comparatively high titer of packaged retroviral particles was $4\sim5\times10^4$CFU/ml.

Biological Activity of pL17SN

There is no way to determine the activity of IL-17 directly update. But it has been demonstrated that IL-17 could induce fibroblasts producing IL-6, IL-8 and G-CSF,[1,2] so the biological activity of IL-17 can be assayed by testing those cytokines secreted by fibroblasts cultured in the presence of IL-17. The levels of IL-6 and GM-CSF in the supernatant of human skin primary fibroblasts were determined to be 40ng/ml and 10pg/ml, respectively, after infected with packaged recombinant retroviral particles.

DISCUSSION

For nearly ten years, unordinary effort has been devoted to identify T cell-derived molecules that are unique to the activation state of a T cell or specific to T cell function.[3-6] An overall understanding of the processes involved in and the consequences of T cell activation has been contributed with the discovery of such molecules, coupled with their sequence comparisons to known gene families and super-families. IL-17 was considered to be one of these molecules. The most exciting data in the limited studies may be that IL-17 might be a major vehicle by which T cells communicate with hematopoietic system. In particular, fibroblasts, when cultured in the presence of IL-17, are able to sustain $CD34^+$ hematopoietic progenitor cells and direct their maturation towards neutrophils.[2] Many of functions and characteristics of IL-17 remain unknown, no more than the exact way of its function. The purpose of our

constructing a retroviral vector containing IL-17 gene is to provide a simple tool to study the functions and characteristics of IL-17. From the results, the pL17SN we constructed could be packaged by CRIP cells and produce infectious recombinant retroviral particles. It was also determined indirectly that human primary fibroblasts infected with pL17SN could produce IL-17. So the constructed pL17SN would be useful in the understanding of the roles of IL-17.

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