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Original Article

IL-33 influenced the development of colorectal cancer via regulating Fra-1

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Abstract: Colorectal cancer is a common digestive tract malignant tumor with the leading morbidity and mortality among all digestive system tumors. It is only second to gastric cancer and esophageal cancer. It was found that Fra-1 was overexpressed in colorectal cancer tissue, especially in the adjacent tissue with inflammatory cells infiltration. At present, the mechanism of Fra-1 in colorectal cancer has not been fully clarified. Interleukin-33 (IL-33) is a type of Th2 cytokine with anti-tumor activity. Exogenous IL-33 can promote tumor specific killer T cell response, thus to enhance antitumor effect. There is still lack of report about the impact of IL-33 in inducing Fra-1 expression on colorectal cancer metastasis. This study intends to investigate the role of Fra-1 in IL-33 mediated colorectal cancer. IL-33 eukaryotic expression vector was constructed and transfected to colorectal cancer cell HCT-116. After transfected for 24 h, the cells were tested by Western blot to determine Fra-1 and IL-33 expression. Wound healing assay and Transwell assay were applied to detect HCT-116 cells migration and invasion. Fra-1 protein obviously declined after IL-33 transfection compared with control ($P < 0.05$). Cell invasion and migration significantly reduced compared with control after transfection ($P < 0.05$). IL-33 plays an inhibitory role on colorectal cancer HCT-116 cell migration and invasion through suppressing Fra-1 expression. It provides theoretical basis for colorectal cancer metastasis related biomarker selection and targeted therapy.

Keywords: Colorectal cancer, Fra-1, IL-33, invasion, migration

Introduction

Colorectal cancer is a common digestive tract malignant tumor that only second to gastric cancer and esophageal cancer. It mainly occurs in rectum or the border between rectum and sigmoid colon, which accounts for about 60% of the disease. In recent years, the morbidity and mortality of colorectal cancer gradually increased in our country. Generally, its onset age is at 60-70 years old. Its incidence in young people is low, which is often accompanied by familial colorectal cancer [1, 2]. Several studies confirmed that the process of colorectal cancer occurrence and development was regulated by multiple genes and factors. Following in-depth investigation, scholars got more understanding on the mechanism of colorectal cancer occurrence [3, 4]. The occurrence of colorectal cancer is associated with cell proliferation, apoptosis, and cell cycle, which are regulated by oncogenes, tumor-suppressor genes, and repair genes.

Fra-1 is a proto-oncogene belonging to the nuclear transcription factor AP-1 family. It is usually overexpressed in malignant tumor. It is found that Fra-1 gene can promote osteocyte and osteoclast differentiation to participate in osteogenesis in the process of embryonic development [5, 6]. In addition, Fra-1 plays an important role in tumor cell migration and invasion. Fra-1 expression obviously increased in the inflammatory lesions around the tumor location, suggesting that Fra-1 might be regulated by some inflammatory cytokines. However, its role and related mechanism in regulating tumor cell occurrence and development has not been fully elucidated [7, 8]. It has been clarified that inflammation is associated with colorectal cancer, of which ulcerative colitis has been considered as a risk factor for colorectal cancer diagnosis. The severity and duration of inflammation is related to the risk of cancer. However, although a variety of cytokines participate in tumor immune response, the regulatory role of each factor in tumor cells is not entirely

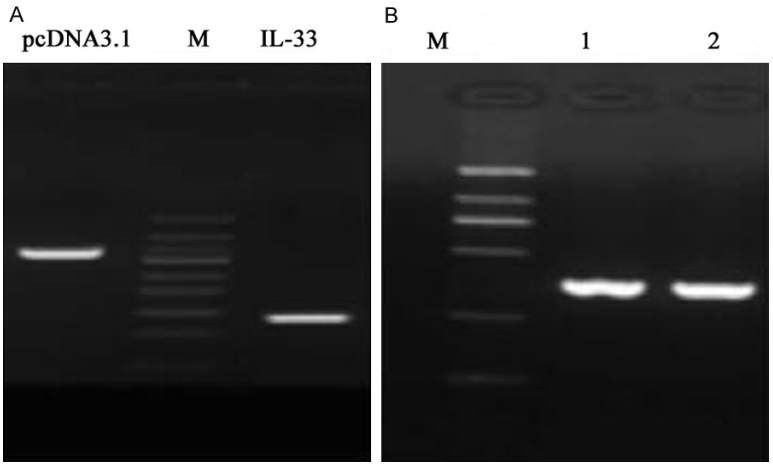

Figure 1. Recombinant human IL-33 expression vector pcDNA-IL33 construction. A: pcDNA3.1 and IL-33 double digestion. B: PCR amplification of pcDNA3.1-IL33 plasmid. 1, sample 1. 2, sample 2. M, DNA marker ladder.

consistent [9]. IL-33 is a newly discovered inflammatory gene with a wide range of gene regulation function. It can activate lymphocytes and eosinophils to produce Th2 cytokines, thus plays a critical role in a variety of pathological processes, such as inflammation, bacterial infection, and autoimmune deficiency disease, etc. At present, though the specific mechanism is still controversy, IL-33 has become the hot spot in the study of tumor [10].

This study combined IL-33 and Fra-1 to investigate their functions in colorectal cancer occurrence and development, aiming to clarify the role of IL-33 induced Fra-1 in colorectal cancer. Colorectal cancer HCT-116 cells were transfected with recombinant human IL-33 cytokine to test Fra-1 expression and cell metastasis. It intends to provide theoretical basis for colorectal cancer metastasis related biomarker selection and targeted therapy.

Materials and methods

General information

HCT-116 cell line was got from Ai Yan Shanghai Biological Technology co., LTD. Medium and FBS were from Gibco. Penicillin-streptomycin was from Beyotime. PBS and enzyme were from Hyclone. pcDNA3.1 vector was from Biofeng. Petri dish was from Corning. Transwell boyden chamber and Matrigel were from BD. Recombinant human IL-33 cytokine was from ACRO Biosystems. β-actin, IL-33, and Fra-1 primary antibodies were from Santa Cruz. RNA extraction kit was from Bioteke. RNA reverse transcription kit was from Takara. Agarose gel DNA extraction kit was from Promega. T4DNA ligase was bought from Takara. EcoRI and NotI restriction enzymes were from Promega. BSA was from Solarbio. Western blot related products were bought from Beyotime. Primers synthetization and gene sequencing were performed by Beijing Genomics Institute.

Recombinant human IL-33 vector construction

The mRNA sequence of human IL-33 in NCBI (NM_001014336) was treated as a template. The primers used were designed by Primer 5.0 software as follows: forward, ATGGCCTCTCACTCAGGCCCCTC, reverse, TTAAGTGGTGGCCTGTTGGGC. Two restriction enzyme loci for EcoRI and NotI were inserted. Peripheral blood RNA was extracted according to the manual and reverse transcribed to cDNA. The system contained 1 μl RNA, 1 μl oligo dT, and 8 μl RNase free water. The reaction was performed at 70°C for 10 min, and then added with 4 μl reverse transcriptase buffer, 2 μl DTT, and 1 μl dNTPs. After incubated at 42°C for 5 min, the system was added with reverse transcriptase and incubated at 42°C for 1 h. At last, the reaction was stopped at 65°C. The obtained cDNA was used as template to amplify IL-33 (510 bp at length) by PCR. The PCR reaction was performed at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 35 s, and 72°C for 1 min, and finally 72°C elongation for 10 min. PCR product was examined by 1.5% agarose gel electrophoresis and recycled. The IL-33 and pcDNA3.1 vector were applied to construct pcDNA-IL33 expression vector through EcoRI and NotI double enzyme digestion, recycling, and directional connection. The vector was used for the following experiments after sequencing.

Colorectal cancer cell culture and transfection

Colorectal cancer HCT-116 cells were seeded in six-well plate at 1×10^5 cells/well and changed

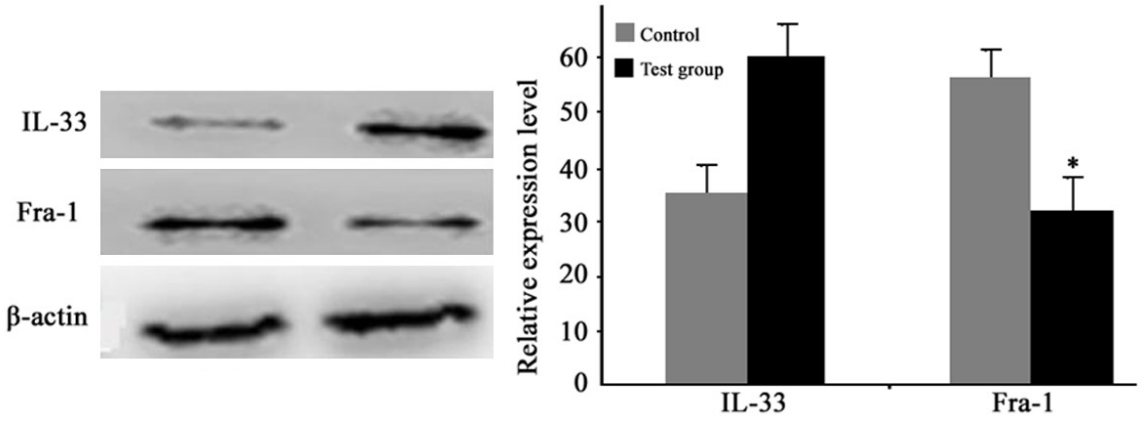

Figure 2. IL-33 and Fra-1 protein expression after pcDNA-IL33 transfection. *P < 0.05, compared with control.

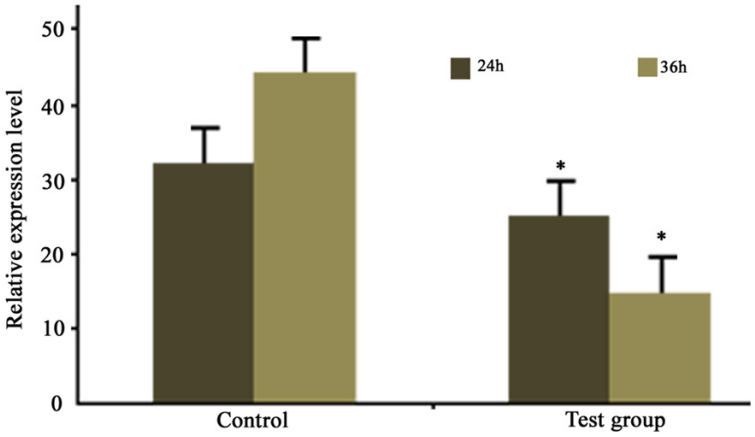

Figure 3. Transwell boyden chamber detection of IL-33 impact on cell invasion. *P < 0.05, compared with control.

to medium without antibiotics before transfection. A total of 50 μl opti-MEM medium was used to dilute 1 μg pcDNA-IL33 expression vector, while 50 μl opti-MEM was adopted to dilute 2 μl lipofectamine. After standed at RT for 5 min, the abovementioned liquids were mixed at RT for 20 min. A total of 200 μl mixture was added to each well for 4 h, and then the cells were further cultured in complete medium.

Western blot

Total protein was extracted from cells at 24 h after transfection and stored at -80°C. The protein was separated by SDS-PAGE and incubated at primary antibody (1:100) at 4°C overnight. After washed by TBST for three times, the membrane was incubated in secondary antibody (1:500) at RT for 4 h. At last, the membrane was developed by ECL and detected by BioRad auto detector. β-actin was selected as internal reference.

Transwell boyden assay

The Matrigel was rewarmed according to the manual and added to the upper chamber of Transwell boyden at 100 μl. A total of 200 μl cell suspension at 1×10^5 cells/ml was added to the upper chamber, while 1 ml complete medium was added to the lower chamber for 24 h and 36 h, respectively. The membrane was stained by 0.1% crystal violet and observed under inverted microscope (100×). Three vision fields were randomly selected to calculate the cell number.

Wound healing assay

The six-well plate was drew a line at the width of 1 cm on the back using a marker. A total of 5×10^5 cells were seeded in each well and cultured for 24 h. The cells were scratched using a 100 μl tip according to the line and washed by PBS. After 24 h incubation, the cells were imaged and analyzed by Image J software.

Results

Recombinant human IL-33 expression vector construction

pcDNA3.1 vector and the PCR product of IL-33 were double digested by EcoRI and NotI and

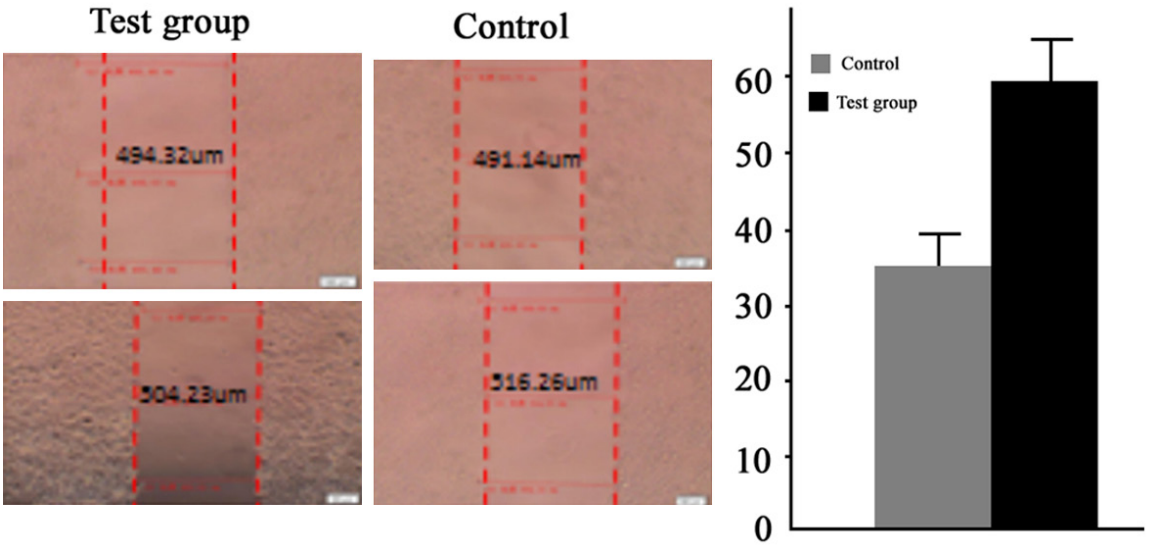

Figure 4. Wound healing assay detection of cell migration after IL-33 transfection. *P < 0.05, compared with control.

tested by agarose gel electrophoresis (**Figure 1A**). After connection, PCR was applied to determine whether IL-33 was reconstructed to pcDNA3.1. **Figure 1B** showed that pcDNA-IL33 expression vector was successfully established. Two plasmids received sequencing and no mutation or deletion occurred, suggesting that our constructed pcDNA-IL33 could be used for transfection. Plasmid 1 was selected for the following experiments.

IL-33 and Fra-1 expression after transfection

Western blot was applied to detect IL-33 and Fra-1 protein expression in HCT-116 cells after transfection for 24 h. As shown in **Figure 2**, IL-33 expression significantly increased in recombinant pcDNA-IL33 plasmid transfection group compared with control (P < 0.05). Fra-1 level was obviously declined after transfection compared with control (P < 0.05), suggesting that IL-33 showed negative correlation with Fra-1 in colorectal cancer cells.

The impact of IL-33 transfection on cell invasion

Transwell boyden chamber was adopted to determine cell invasion after IL-33 transfection. After stained by 0.1% crystal violet, the membrane was observed under the inverted microscope. Three vision fields were randomly selected to calculate the cell number. It was revealed that transmembrane cell number in transfection group was markedly lower than that of control (P < 0.05) (**Figure 3**).

The effect of IL-33 transfection on cell migration

Wound healing assay was performed to evaluate cell migration after IL-33 transfection. As shown in **Figure 4**, the migration distance in transfection group was 10.32 ± 3.13 μm, which was significantly lower than that in control as 24 ± 6.85 μm (P < 0.05), indicating that IL-33 induced Fra-1 reduction can inhibit colorectal cancer cell migration.

Discussion

Colorectal cancer is a common digestive tract malignant tumor. Most colorectal cancer patients have appeared metastasis when diagnosed. Following in-depth basic research, scholars' understanding of the pathogenesis of colorectal cancer obviously improves. However, it is still controversy about the regulatory mechanism of cancer cell metastasis and invasion. Inflammation is the response to external stimulation, thus playing a critical role in maintaining homeostasis [11, 12]. It was confirmed that inflammatory cells infiltration was often accompanied to cancer lesions. Inflammatory factors abnormal expression may further lead to tumor suppressor gene mutation and tumor signaling pathways abnormal activation, thus to promote cancer cell migra-

tion and invasion [13-16]. Therefore, investigating the regulatory role of inflammatory factors to colorectal cancer cells can further clarify the mechanism of colorectal cancer cell migration and invasion.

Currently, it was found that IL-6 cytokine induction can affect colorectal cancer cell migration, proliferation, and invasion through STAT3-Fra-1 signaling pathway [17-20]. It was further confirmed that Fra-1 played an important role in regulating tumor occurrence and development. However, because of various types of cytokines in inflammation and complicated interaction mechanism, single factor cannot fully reflect the whole regulatory network. Therefore, investigation of the regulatory mechanism of each important factor is of great significance. As mentioned before, inflammatory cytokine IL-33 can activate lymphocytes and eosinophils to produce Th2 cytokines, which plays an important role in a variety of disease. Fra-1 is a proto-oncogene that overexpressed in some multiple malignant tumors. It can promote tumor cell migration and invasion. Study found that Fra-1 obviously overexpressed in the inflammatory lesions around the tumor location, suggesting that Fra-1 may be regulated by some kind of inflammation factors. However, its role and related regulatory mechanism in tumor development is still unclear.

As a type of proto-oncogene, Fra-1 is associated with various types of tumors. It was found that Fra-1 can regulate breast cancer cell migration and metastasis directly. Fra-1 overexpression was also negatively correlated with chemotherapy resistance. In vitro study showed that downregulating Fra-1 in breast cancer can help resistant to adriamycin and cyclophosphamide, whereas enhancing Fra-1 level may sensitize cancer cells to these drugs.

This study combined IL-33 and Fra-1 to explore their roles in colorectal cancer occurrence and development, aiming to clarify the role of IL-33 induced Fra-1 in colorectal cancer. Our results showed that IL-33 overexpression downregulated Fra-1 level in colorectal cancer cells, indicating that Fra-1 was regulated by IL-33, which was in accordance with the regulatory role of IL-6 on Fra-1. Moreover, Fra-1 expression presented positive correlation with colorectal cancer cell migration and invasion, suggesting that Fra-1 played a critical role in colorectal cancer occurrence and development.

IL-3 suppressed colorectal cancer HCT-116 cell migration and invasion by restraining Fra-1 expression.

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Disclosure of conflict of interest

None.

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