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IL-33/ST2 pathway contributes to metastasis of human colorectal cancer

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ABSTRACT

Interleukin-33 (IL-33) was recently implicated in cancer pathogenesis. However, the possible effect of IL-33 on tumor progression of colorectal cancer (CRC), which is one of the most commonly diagnosed and lethal cancers worldwide, was still unclear. Here we evaluated the potential role of IL-33/ST2 pathway in metastasis of human CRC. We found an elevated expression of IL-33 and ST2 in tumor tissues of CRC patients. Higher expressions of IL-33 and ST2 were observed in poor-differentiated human CRC cells. Of note, IL-33 stimulation promoted the invasion of human CRC cells in a dose dependent manner. Enhanced IL-33/ST2 signaling promoted CRC metastasis, while attenuated IL-33/ST2 signaling decreased CRC metastasis. In consistent, enforced IL-33 expression in human CRC cells enhanced their growth, metastasis and reduced the survival time in nude mice, while decreased IL-33 expression in human CRC cells inhibited their growth, metastasis and prolonged the survival time in nude mice. Finally, we observed an increased expression of IL-6, CXCR4, MMP2 and MMP9 in response to IL-33/ST2 signaling in human CRC cells, which were crucial for the enhanced metastasis by IL-33 stimulation. Collectively, our findings demonstrated that IL-33/ST2 pathway could contribute to the metastasis of human CRC, which could enlarge the understanding of CRC pathogenesis and provide clues for developing new CRC therapeutics.

1. Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed and lethal cancers worldwide [1]. Currently, surgical resection is a major treatment and highly effective for localized CRC [2]. However, up to 30–40% of patients can develop recurrence even within the first few years after initial surgery [2]. Within CRC patients, about 60% have liver metastasis [3]. The CRC patient's 5-year post-surgical survival chances could fall from 90% to 10% or even less after metastasis [4]. Thus, identification of critical effectors involved in CRC metastasis was essential and might ultimately aid the clinical treatment of CRC patients.

Interleukin-33 (IL-33), a member of the IL-1 family of cytokines, could bind to Toll-interleukin 1 (IL-1) receptor (TIR) domain-containing receptor ST2 and induce NF-kB and MAPK activation [5,6]. Accumulating data showed that IL-33/ST2 signaling was implicated in cancer growth and metastasis [5]. As such, IL-33/ST2 axis could promote breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells [7]. High level of serum IL-33 was reported to be a diagnostic and prognostic marker of non-small cell lung cancer and hepatocellular carcinoma [8,9]. Of interest, IL-33 blockade reduced mucositis and enabled prolonged irinotecan (CPT-11) treatment of ectopic CT26 colon carcinoma in mice, suggesting that IL-33/ST2 pathway was participated in CRC pathogenesis [10]. However, whether IL-33/ST2 pathway was involved in the metastasis of CRC remains undefined.

In this study, we evaluated the potential role of IL-33/ST2 pathway in human CRC metastasis. Our findings demonstrated an important role of IL-33/ST2 interaction in metastasis of human CRC cells, which could enlarge the understanding of CRC pathogenesis and facilitate the development of novel CRC therapeutics.

2. Materials and methods

2.1. Patients

The Ethics Committee of Tongji University approved the study with patients. Fifty-two CRC patients were enrolled and given written informed consent. Pathology reports confirmed the diagnosis and provided the differentiation state of tumor cells. Subjects with autoimmune diseases or infections were excluded.

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2.2. Reagents and cell culture

The human CRC cells were isolated from surgical tumor tissues by the Cancer Cell Isolation Kit (Panomics) according to the manufacturer's instructions. SW620 cells were obtained from ATCC and maintained in our lab. Cells were cultured at 37 °C under 5% CO_2 in complete RPMI 1640 medium (GIBCO) containing 10% heat-inactivated fetal bovine serum supplemented with 2 mM glutamine, 100 IU/ml penicillin and 100 mg/ml streptomycin sulfate. For metastasis, CRC cells (2×10^5/ml) were stimulated with recombinant IL-33 protein (Biologend) for 48 h and detected for their invasion. Human full-length IL-33 and ST2 expression vectors were purchased from Sino Biological Inc. Human IL-33 shRNA vector was purchased from Santa Cruz Biotechnology. Human ST2 shRNA vector was purchased from Invivogen. Neutralizing antibody for human ST2, IL-6 and CXCR4 were purchased from R&D Systems. Transfection of CRC cells was achieved using the Amaxa Nucleofector Kit or according to the manual's instructions. MMP inhibitor GM6001 was purchased from Merck Millipore.

2.3. Real-time PCR

Quantitative Real-time RT-PCR was performed as previously described [11,12]. All the primers and probes were obtained from Applied Biosystems. Total RNA was extracted using TRIzol reagent. cDNA was synthesized with the PrimeScript RT reagent Kit (TaKaRa). Quantitative RT-PCR (qRT-PCR) analyses were carried out in duplicate to detect mRNA expression using SYBR Premix Ex Taq (TaKaRa), and β-actin was used as an internal control.

2.4. Invasive assay

The invasion assay was performed using BD Biocoat Matrigel Invasion Chamber assay (8 μm, BD Bioscience) as previously described [13]. Briefly, the Matrigel inserts were rehydrated and 5×10^4 of testing cells were resuspended in 0.5 mL of serum-free media and then seeded onto the upper chamber of Matrigel-coated filters. In the lower chambers, 0.75 mL of complete medium was added as a chemoattractant. The whole chamber was placed in one well of a 24-well plate, and cells were cultured in routine conditions. After 24 h, the cells on the upper side of the chamber were scraped, and the ones on the lower side of the chamber were fixed by methanol, stained with hematoxylin, and invaded cells were counted under the microscope. Five predetermined fields were counted for each membrane, and the mean values from three independent experiments in duplicates were used. Data are expressed as the percentage of invasion through the Matrigel Matrix and membrane relative to the migration through the control membrane according to the manufacturer's manual.

2.5. Flow cytometry

The protein expression levels of IL-33 and ST2 in primary CRC cells were analyzed with flow cytometry. Briefly, 5×10^5 cells were stained with FITC-conjugated anti-human IL-33 antibody (Sino Biological Inc.) or PE-conjugated anti-human ST2 antibody (MBL International) after fixation and permeabilization (BD), and analyzed on a FACSCalibur flow cytometer (BD). Appropriate isotype controls were used and all data were analyzed with FlowJo software (Tree Star).

2.6. In vivo experiments

Approval for mouse experiments was obtained from the Institutional Animal Care and Use Committee of Tongji University. BALB/c nude mice of 6–8 weeks old were purchased from Shanghai SLAC laboratory Animal Co. Ltd. and housed under specific pathogen-free conditions. In brief, 3×10^6 SW620 cells were suspended in 0.2 ml PBS and injected subcutaneously into the nude mice. Tumors volumes were measured every 5 days following tumor challenge using vernier calipers and were presented as the means ± SD. Thirty days after challenge, metastasis index to lung was graded as 1–4 and calculated as previously described [13]. Specifically, each metastasis less than 0.5 mm in diameter was graded as 1, between 0.5 and 1 mm as Grade 2, between 1.0 and 2 mm as Grade 3, and >2 mm as Grade 4. All the grade scores were then added to determine the metastatic index for a given animal, and the mean index was then calculated for a given control or experimental group of animals.

2.7. Statistical analyses

Statistical evaluation was performed using *T* tests or two-way analysis of variance (ANOVA) using the program PRISM 6.0 (GraphPad Software Inc., San Diego, CA, USA). $P < 0.05$ was considered as statistical significant.

3. Results

3.1. Elevated expression of IL-33 and ST2 in tumor cells of CRC patients

To evaluate the potential role of IL-33/ST2 pathway in CRC metastasis, we detected the expression of IL-33 and ST2 in tumor tissues and adjacent tissues of CRC patients. As shown in Fig. 1A and B, the expression of IL-33 and ST2 was significantly higher in tumor tissues than that in adjacent tissues ($P < 0.05$). Of note, the expression levels of IL-33 and ST2 in tumor tissues were associated with the clinical stages of CRC patients (Fig. 1C and D, $P < 0.05$). When primary CRC cells were isolated from the surgical tissues and detected for their mRNA expressions of IL-33 and ST2, we found that IL-33 and ST2 mRNA expression levels in poor-differentiated CRC cells were significantly higher than that in medium- and well-differentiated CRC cells (Fig. 1E and F, $P < 0.05$). We then detected the protein levels of IL-33 and ST2 in primary CRC cells and found similar results (Fig. 1G and H, $P < 0.05$). These results suggested an involvement of IL-33/ST2 pathway in CRC metastasis.

3.2. Enhanced IL-33/ST2 pathway promoted the metastasis of human CRC

When human primary CRC cells were treated with an increasing dose of IL-33, a dose dependent manner of enhanced CRC invasion was observed (Fig. 2A, $P < 0.05$). An increased expression of ST2 of primary CRC cells was achieved by the transfection of ST2 expression vector (Fig. 2B, $P < 0.05$). Of important, enforced ST2 expression on primary CRC cells further promoted their invasion induced by IL-33 stimulation (Fig. 2C, $P < 0.05$). In consistent, transfection with IL-33 expression vector also resulted in enhanced invasion of primary CRC cells (Fig. 2D and E, $P < 0.05$). To detect this phenomenon in vivo, nude mice were challenged with SW620 cells that were stably transfected with IL-33 expression vector. We found that enforced IL-33 expression could significantly promote the tumor growth and metastasis of SW620 cells in nude mice (Fig. 2F and G, $P < 0.05$). Further, enhanced IL-33 expression in SW620 cells decreased the survival time of tumor bearing nude mice (Fig. 2H, $P < 0.05$).