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Interaction of IL-22/IL-22R1 promotes cell proliferation and suppresses apoptosis of colorectal cancer via phosphorylation of STAT3

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Abstract: Interleukin-22 (IL-22) is a member of IL-10 cytokine family which is expressed in activated T cells predominantly and in activated natural killer cells at lower levels. Previous studies have demonstrated the link between elevated levels of IL-22 and disease severity of psoriasis, Crohn's disease, rheumatoid arthritis and interstitial lung diseases. However, the function of IL-22 in the development and progression of colorectal cancer (CRC) remains elusive. In this study, we first evaluated the IL-22/IL-22R1 level in CRC patients, and found that tumor tissues have more active expression of IL-22 and IL-22R1 than normal tissues, presenting correlation with the degree of differentiation of tumor tissues. Subsequently, Caspase and cell viability assays were performed on SW-480 cell line which expresses high level of IL-22R1 to examine if the supplementation of IL-22 has an impact on apoptosis and proliferation. In comparison with treatment of 5-FU, supplementation of IL-22 promoted cell proliferation and ameliorated apoptosis. To unveil signal transduction upon activation of IL-22R, we examined the phosphorylation of STAT3 in SW-480 cell line following supplementation of IL-22. The treatment of IL-22 also increased the level of p-Akt, an essential component in PI3K/Akt pathway. Although the link between STAT3 phosphorylation and PI3K/Akt activation remains to be explored, our study revealed the mechanism underlying the effects of IL-22R activation on apoptosis as well as tumor differentiation, indicating the prognostic value of IL-22/IL-22R.

Introduction

In recent years, colorectal cancer (CRC) has an increasing age-standardized incidence and becomes one of the top 3 most common cancers in the world, which accounts for more than one million new diagnosis and over 5 hundred thousand deaths in 2012, compounding on the heavy load on the healthcare systems. Although the therapeutic regimes have been improved substantially, the 5-year survival rate remained far from expectation, which was partially due to a lack of effective early diagnosis of CRC. The transformation from normal colonic mucosa tissue to invasive cancer can be triggered and promoted by the accumulation of genetic and epigenetic changes. Other risk factors include: age beyond 50 years, inherited genetic mutation, incidence of adenomatous polyps, history of colorectal neoplasia and/or inflammatory bowel disease (IBD), smoking, and alcohol consumption, etc. It was reported that approximately 70% adenomatous polyps developed CRCs (Benson, 2007), which can be driven by microbe-instigated focal inflammation involving IL-10-expressing T cells (Dennis *et al.*, 2013).

Most risk factors could evolve to be colorectal cancer through systemic inflammation. For example, immune-mediated mechanisms suggest strong link between IBD and colitis-associated cancer (CAC) which is refractory to treatments and suffers from high mortality (Greten *et al.*, 2004; Terzić *et al.*, 2010). Although there are a few common features between CAC and CRC manifesting no sign of overt inflammatory disease, elevated expression level of proinflammatory cytokines and inflammatory infiltration were extensively observed in CRC that displayed no association with clinically detectable IBD (Atreya and Neurath, 2008; Atreya *et al.*, 2008; Waldner and Neurath, 2008). During the past decade, a bunch of cytokines have been characterized as key effectors in continuous activation of immune system during pathogenesis of IBD and CRCs(Waldner and Neurath, 2008), such as tumor necrosis factor-α (van Horssen, 2006), interleukin-6 (Galizia *et al.*, 2002; Esfandi, Mohammadzadeh *et al.*, 2006), and members of the interleukin-12 family (Peluso *et al.*, 2006), etc. Recently, the link between clinical relevance and IL-22-IL-22R, a novel cytokine-cytokine receptor system, was established.

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IL-22 was originally described as a member of IL-10 cytokine family (Dumoutier *et al.*, 2000; Dumoutier *et al.*, 2000; Xie *et al.*, 2000), and was expressed in activated T cells abundantly and in activated natural killer cells at lower levels (Mandal and Viswanathan, 2015). Of note, $CD4^+$ T_H cells have predominant abundance of IL-22, while in macrophages, dendritic cells, or nonimmune cells, no expression of IL-22 was discovered (Dumoutier *et al.*, 2011), suggesting that the biological function of IL-22 might be implicated in T lymphocyte-mediated diseases. Indeed, previous studies have demonstrated the link between elevated levels of IL-22 and disease severity of psoriasis, Crohn's disease, rheumatoid arthritis and interstitial lung diseases (Ikeuchi *et al.*, 2005; Wolk *et al.*, 2007; Boskabady *et al.*, 2011). Notably, IL-22 resides in regulatory upstream of a subset of molecules in epithelial cells of both digestive and respiratory tracts (Wolk *et al.*, 2006; Aujla *et al.*, 2008; Zheng *et al.*, 2008). In colonic epithelial cells, expression of MUC1, MUC3, MUC10 and MUC13 were stimulated by IL-22 and thereby improves colitis-associated mucus layer destruction and restitution of goblet cell mucus, which attenuates intestinal inflammation in ulcerative colitis mouse model (Sugimoto *et al.*, 2008). However, the mechanism of IL-22 underlying its role in the development of CRC remains elusive, neither does the underlying mechanism.

In this study, we first evaluated the IL-22/IL-22R1 level in CRC patients, performed histochemistry assay of IL-22/IL-22R1 on tumor tissue and normal adjacent tissues of them, and found that tumor tissues have higher expression level of IL-22 and IL-22R1 than normal tissues, and the expression level presents correlation with the degree of differentiation of tumor tissues. Subsequently, Caspase and cell viability assay was performed on SW-480 cell line which expresses high level of IL-22R1 to examine if the supplementation of IL-22 has an impact on apoptosis and proliferation. In comparison with treatment of 5-FU, supplementation of IL-22 promoted cell proliferation and ameliorated apoptosis. To unveil the signal transduction upon activation of IL-22R, we examined the phosphorylation of STAT3 in SW-480 cell line following supplementation of IL-22. The treatment of IL-22 also increased the level of p-Akt, an essential component in PI3K/Akt pathway. Although the link between STAT3 phosphorylation and PI3K/Akt activation remains to be explored, our study revealed the mechanism underlying the effects of IL-22R activation on apoptosis and tumor differentiation, indicating the prognostic value of IL-22/IL-22R cells.

Material and Methods

Patients and tissues

A total of 30 patients who received elective colorectal resection of tumor tissues at the second affiliated hospital of Hebei Medical University between March 2015 and September 2016 were recruited. Patients who manifest acute presentation, were in pregnancy or breast-feeding were excluded from this study. Demographic and clinical information including tumor characteristics, preoperative risk prediction, comorbidities and operative characteristics were recorded. Biochemical and hematological parameters for every piece of tissue were determined. Lymph nodes from the CRC patients were collected during resection surgery, which were stained with hematoxylin and eosin. Those lymph nodes were grouped as MLNs or normal lymph nodes depending on the hematoxylin and eosin staining results. All samples were frozen in liquid nitrogen until further analysis. This study was approved by the ethics committee of the second affiliated hospital of Hebei Medical University. Written informed consents for all recruited patients were obtained.

Immunohistochemistry assay

Thirty pairs of primary CRC samples and adjacent normal samples were analyzed by anti-IL-22 and IL-22R1 immunostaining. Anti-IL-22 and anti-IL-22R1 monoclonal antibody (R&D Systems, Minneapolis, MN) were used to stain the formalin-fixed and paraffin-embedded (FFPE) sections. Briefly, rehydration was performed, followed by washing with phosphate buffered saline (PBS). As a routine procedure to block endogenous peroxidase activity, 3% hydrogen peroxide (3%) was used to treat those sections for 30 min at room temperature. Retrieval of antigen was performed by adding in 0.01M citrate buffer at pH 6.0, and the mixture was kept in a water-bath at 97°C for 15 min, followed by incubation with 10% goat serum for 30 min at room temperature to avoid nonspecific binding. The excess serum was then disposed, and primary antibodies against IL-22 and IL-22R1 monoclonal antibodies were added to the sections and incubated overnight at 4°C. After incubation with biotinylated goat anti-rabbit IgG at 37°C for half an hour, peroxidase-labelled streptavidin was soon applied, followed by staining with 3,3‘-diaminobenzidine substrate and counterstaining with haematoxylin for 3 min, respectively. After staining, the sections were then dehydrated, and cover slipped. Tumor differentiation grades and stage classification were determined according to the histological classification of the World Health Organization and the 6^{th} edition of tumor-node-metastasis (TNM) classification of the International Union Against Cancer, respectively.

Quantitative realtime-PCR

SV total RNA isolation kit was used to extract total RNA from CRC and adjacent non-tumor liver tissues according to vendor's manual, which was then reverse transcribed to cDNA using Primescript RT reagent kit (Takara Bio. Inc., Tokyo, Japan) at 48°C for 30 min. Amplification of products of reverse transcription was performed by real-time quantitative PCR using SYBR Premix Ex Taq (Takara). The expression of IL-22, IL-22R1, STAT3 and Akt were normalized to β-actin mRNA. Comparative threshold cycle (Ct) method was employed for data analysis, in which fold changes in mRNA abundance of target genes were calculated as equal to $2^{-\Delta\Delta Ct}$.

Cell viability assay

Cells were seeded in 96-well plates (BD Biosciences, USA) at a cell density of 1×10^4 cells/well and left to adhere overnight. For experimental group, cells were seeded into 96-well plates and then transfected with IL-22 siRNA, and mock control was treated with lipofectamine alone. After cellular adhesion, MTT solution (20 μl of 5 mg/ml for each well) was added to cultures and then incubated at 37°C for 4 hours.

removing the culture medium, 150 μL dimethylsulfoxide was added to the supernatant. After 10 min of agitation at 100 rpm, the absorbance at 570 nm were then measured with microplate specctrophotometer (Bio-Rad, US). All experiments were run in triplicate.

Caspase-3 assay

The caspase-3 activity kit (Beyotime, shanghai, China) was used to evaluate the activity of caspase-3. Cells were centrifuged at 10,000 rpm, 4°C for 10 min and washed with PBS, after which the supernatants were removed. Supernatants were transferred to a new Eppendorf tubes, mixing with 50 μL reaction buffer, 50 μL sample and 5μL DEVD-pNA substrate, and incubated at 37°C, 5% CO_2 for 2 hours. Absorbance was measured under 405 nm wavelength using an xMark™ Microplate Absorbance Spectrophotometer (Bio-Rad Laboratories, Inc. California, United States). pNA concentration in the experiments was determined by the pNA concentration *vs.* absorbance plot.

Apoptosis assessment by flow cytometry

The degree of apoptosis of cells was analyzed by Annexin V-FITC (Becton Dickinson, NJ, USA) and propidium iodide (PI) flow cytometry. SW-480 cells were seeded at a density of 5×10^4 cell/cm^2 and treated with a range of concentrations of evodiamine. After 48 hours of treatment with IL-22 siRNA lipofectamine, 100 μL cells were suspended in 1 × Annexin-binding buffer and then incubated with 5μL fluorescein isothiocyanate (FITC) annexin V and 5μL PI (Invitrogen, US) for 15min at room temperature in darkness. The viability and apoptosis were analyzed by flow cytometry under 530nm and > 575nm, respectively (BD Bioscience), where viable cells are negative for Annexin V and PI, cells under early stage apoptotic process were negative for PI and positive for Annexin V, and cells at late apoptotic phase were positive for both PI and Annexin V.

siRNA transfection

siRNA of STAT3 and negative control siRNAs was purchased from Invitrogen (Thermo Fisher, MA, USA). Cells are seeded and cultured to 90% confluence before transfection. siRNA transfection was induced via Lipofectamine 2000 (Thermo Fisher, MA, USA). In brief, Lipofectamine 2000 was gently mixed, from which 0.25 μL was diluted in 25 μL serum-free Opti-MEM Medium, incubated at room temperature for 5 min. Dilute 15 pmol STAT3 siRNA or control siRNA in 25 μL serum-free Opti-MEM Medium and then mix with Lipofectamine 2000 prepared previously. The mixture was incubated at room temperature for 15 min. Add 100 μL complete growth medium without antibiotics with 2×10^4 cells to each well containing siRNA-Lipofectamine 2000 complexes.

Western blot

Cells were gathered by centrifugation, washed twice in ice-cold PBS, and lysed in RIPA buffer at 4°C for 30 min. Protease inhibitor was supplemented to prevent the degradation of proteins. The supernatant was subject to protein denaturation with buffer containing 3% sodium dodecyl sulfate (SDS; Sigma). The total protein was quantified by Bio-Rad protein DC assay kit (Bio-Rad, Hercules, CA) against the standard curve derived from bovine serum albumin of known content. Proteins were then separated using 8% SDS gel electrophoresis (SDS-PAGE) and transferred to a 0.45 mm PVDF membrane. Monoclonal antibody against STAT3, p-STAT3, IL-22 and IL-22R1 were used to probe the resultant blots at 4°C overnight. After twice washes with PBS, the gel was incubated with horseradish peroxidase-conjugated Ig secondary antibodies (1:5000, Sigma Aldrich, St. Louis, MO, USA). Following washes with PBS, the staining was read by an enhanced chemiluminescence detection system (GE Healthcare, Chicago, Il, USA).

Statistical analysis

All numerical data were expressed as mean ± SE, and categorical data were presented as percentage. Significance of differences were analyzed by Student's *t*-tests or one-way ANOVA (analysis of variance) using Microsoft Excel (Microsoft Campus, Redmond, WA, USA). $p < 0.05$ was considered statistical significance.

Results

Expressions of IL-22 and IL-22R1 are correlated with tumor stage, differentiation and metastasis

The oncogenic role of IL-22 in regulation of cell growth and death has been characterized in glioblastoma, while its function in colorectal cancer remains elusive and controversial. To investigate the roles of IL-22 in CRC, the levels of IL-22 and IL-22R1 of CRC tumor tissues were measured in 30 CRC patients. All recruited patients had undergone surgical resection with histologically negative resection margins. qRT-PCR was performed to determine the transcriptional level of IL-22 and IL-22R1. The positiveness of IL-22 and IL-22R1 were analyzed together with clinicopathological parameters of these patients. Tumor stages were in positive correlation with IL-22/IL-22R1 (Tab. 1, $p < 0.05$), while the degree of tumor differentiation was negatively correlated with the level of IL-22 and IL-22R1 ($p < 0.05$). In addition, IL-22- and IL-22R1-positive patients were enriched in the patients with lymphatic metastasis ($p < 0.05$). Later, we performed immunohistochemistry assay to validate the correlation between IL-22/IL-22R1 levels and tumor status. In normal adjacent tissues, IL-22 staining was rarely seen, while in cancer tissues, the highly differentiated tissues carried lower level of IL-22/IL-22R1 staining, and the poorly differentiated tissues have stronger IL-22/IL-22R1 stains (Figs. 1, 2). These results suggest that IL-22/IL-22R1 is critical for tumor progression.

TABLE 1

The relationship between clinical characteristics of colorectal cancer patients and expression of IL-22/IL-22R1 in colorectal cancer

Characteristics	Patients (N)	IL-22 expression negative (%)	positive (%)	*P*	IL-22R1 expression negative (%)	positive (%)	*P*
Gender							
Male	16	43.75	56.25	> 0.05	50.00	50.00	> 0.05
Female	14	42.86	57.14		42.86	57.14	
Age							
≤ 50 years	8	37.50	52.50	> 0.05	37.50	52.50	> 0.05
> 50 years	22	40.91	59.09		45.45	54.55	
Tumor stage							
Ⅰ	3	66.67	33.33	< 0.05	66.67	33.33	< 0.05
Ⅱa	7	57.14	42.86		42.86	57.14	
Ⅱb	3	33.33	66.67		33.33	66.67	
Ⅲb	8	37.50	62.50		37.50	62.50	
Ⅲc	9	33.33	66.67		22.22	77.78	
Tumor size							
≤ 3 cm	6	50.00	50.00	> 0.05	50.00	50.00	> 0.05
> 3 cm	24	45.83	54.17		54.17	45.83	
Tumor differentiation							
High	5	80.00	20.00	< 0.05	80.00	20.00	< 0.05
Medium	13	46.15	53.85		38.46	61.54	
Poor	12	25.00	75.00		33.33	66.67	
Lymphatic metastasis							
Yes	17	35.29	64.71	< 0.05	29.41	70.59	< 0.05
No	13	61.54	38.46		53.85	46.15	

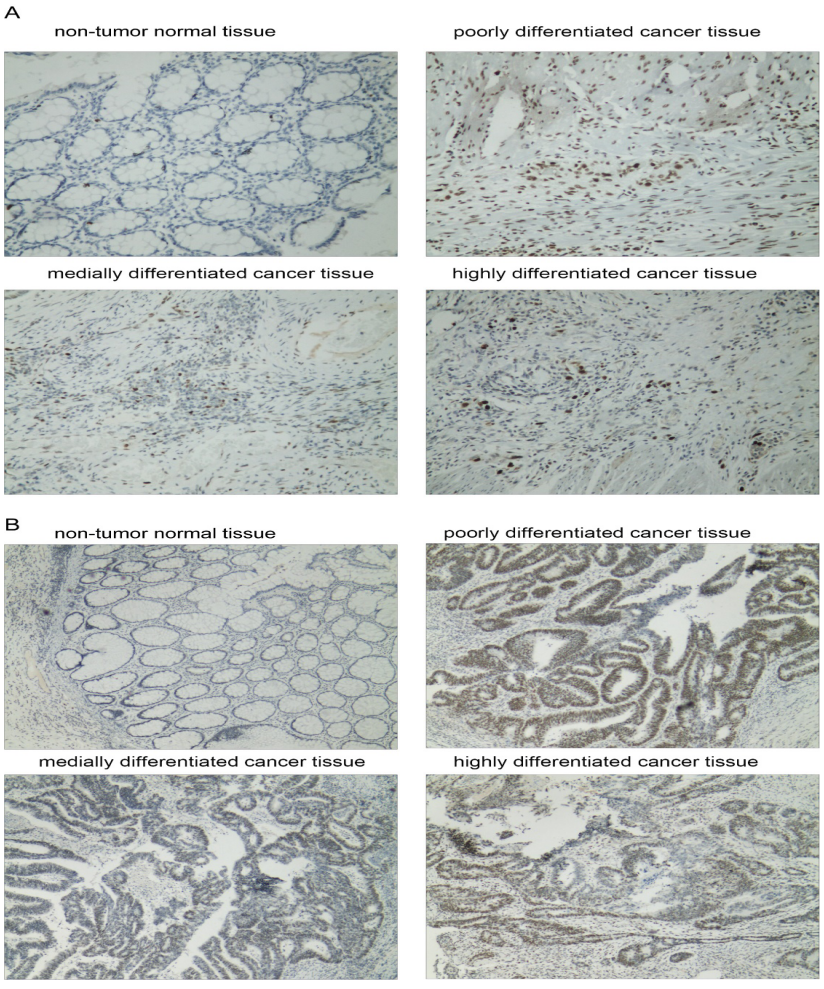

FIGURE 1. Immunohistochemistry stain of IL-22/IL-22R1 in non-tumor normal tissue, poorly differentiated cancer tissue, medially differentiated cancer tissue, and highly differentiated cancer tissue (× 200). A. IL-22 staining; B. IL-22R1 staining.

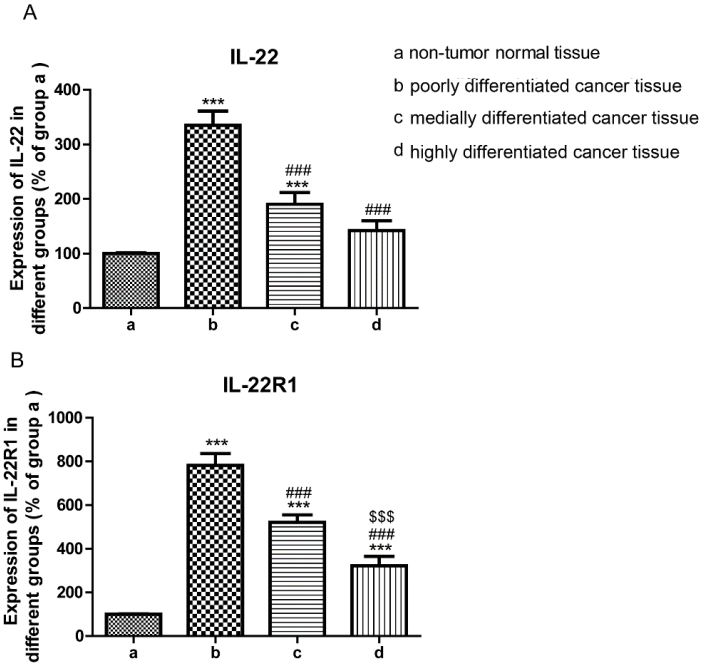

FIGURE 2. Quantification of IL-22 and IL-22R1 in non-tumor normal tissue, poorly differentiated cancer tissue, medially differentiated cancer tissue, and highly differentiated cancer tissue. A. Expression of IL-22 in the four types of tissues; B. Expression of IL-22R1 in the four types of tissues. (significance $p < 0.05$; ***: significantly different than non-tumor normal tissue; ###: significantly different than poorly differentiated tissue; $$$: significantly different than medially differentiated tissue).

IL-22 supplementation promotes cell viability and suppresses cell apoptosis

The fact that positiveness of IL-22/IL-22R1 was correlated with tumor status, we next sought to investigate if the interaction between IL-22 and IL-22R1 promotes tumor progression. Firstly, the IL-22R1 level of FHC, SW-480, HCT-116 and COLO-205 were measured and SW-480 expresses the highest level of IL-22R1 (Fig. 3(A)), and thus was selected for further experiments. IL-22 was supplemented to SW-480 cells to investigate the effect of IL-22-IL-22R1 interaction on cell viability and apoptosis. Compared with control, supplementation of IL-22 significantly decreased caspase-3 and caspase-9 activity (Fig. 3(B)), indicating that the apoptosis was suppressed. Moreover, IL-22 supplementation also rescued SW-480 cells treated with 5-FU (Fig. 3(C)). On the contrary, the IL-22 supplementation elevated the cell viability of SW-480 and counteracted the inhibition of 5-FU on cell viability on SW-480 (Fig. 3(D)).

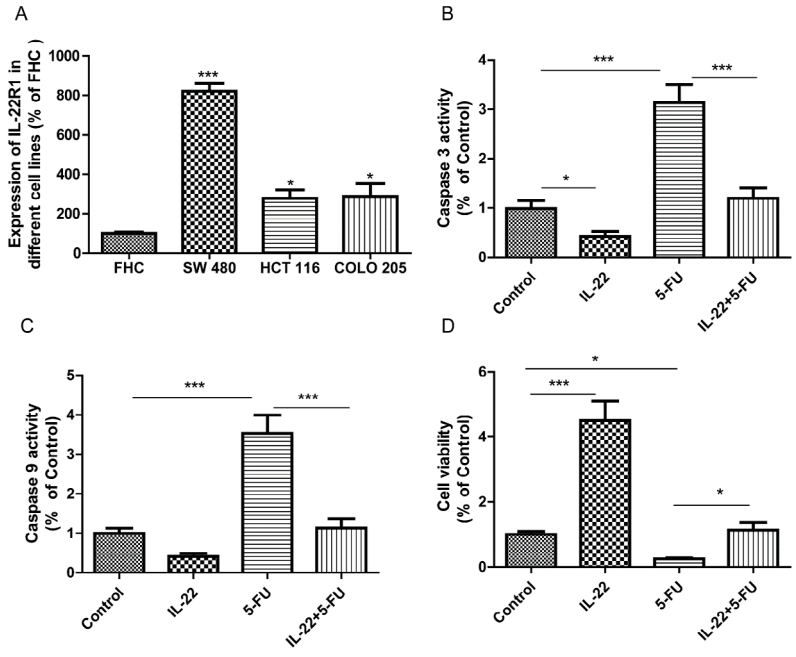

FIGURE 3. The effect of IL-22 supplementation to high IL-22R1 expressing cell line SW-480. A. Expression of IL-22R1 in FHC, SW-480, HCT-116 and COLO-205 cells. B. Caspase-3 activity of SW-480 cells with/without IL-22 supplementation and/or 5-FU. C. Caspase-9 activity of SW-480 cells with/without IL-22 supplementation and/or 5-FU. D. Cell viability SW-480 cells with/without IL-22 supplementation and/or 5-FU. (*: $p < 0.05$, **: $p < 0.01$).

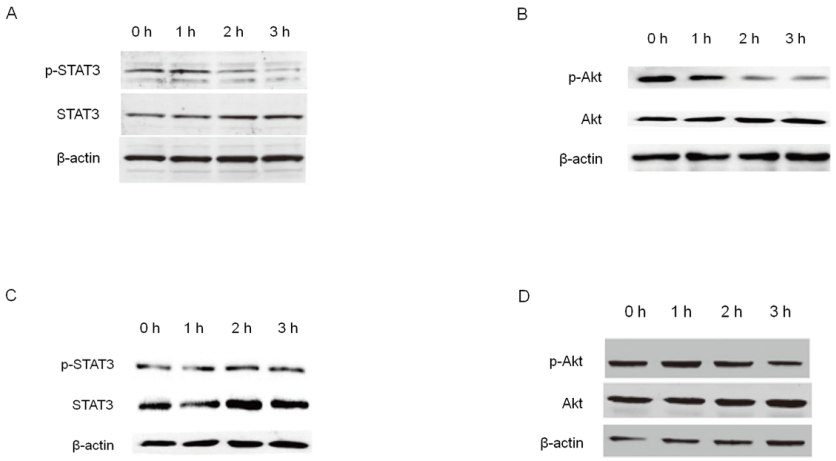

FIGURE 4. IL-22 activates STAT3 signaling pathway in SW-480 cells. A. The protein expression of p-STAT3 in SW-480 cells stimulated by IL-22. B. The protein expression of p-Akt in SW-480 cells stimulated by IL-22. C. The protein expression of p-STAT3 in HCT-116 cells stimulated by IL-22. D. The protein expression of p-Akt in HCT-116 cells stimulated by IL-22.

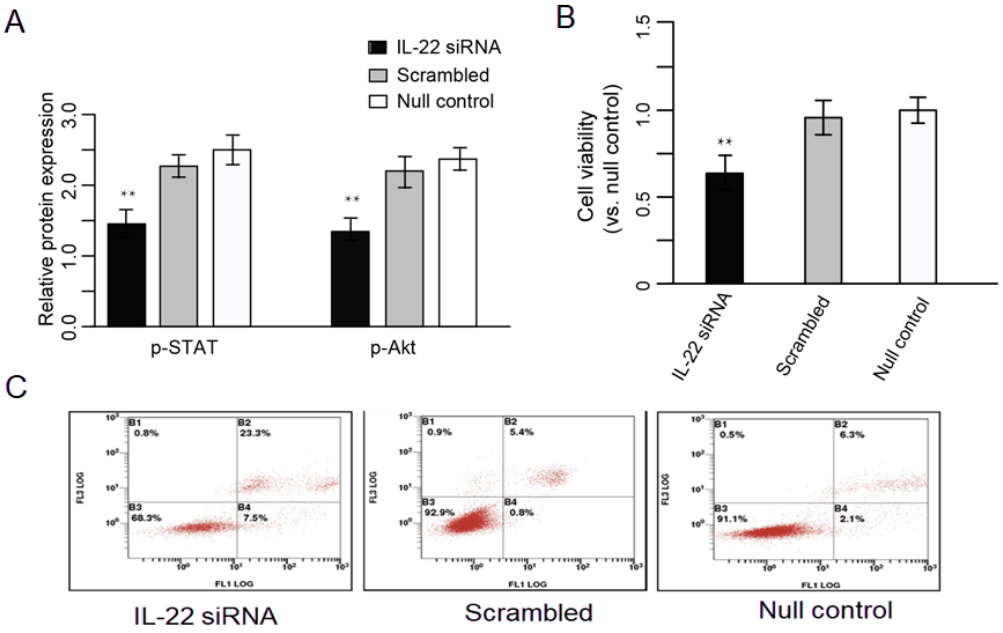

FIGURE 5. The cell viability and apoptosis assay of SW-480 cells with IL-22 knocked down. A. Relative protein expression of p-STAT3 and p-Akt in SW-480 cells with IL-22 knocked down. B. Cell viability assay of SW-480 cells with IL-22 knocked down. C. Annexin V flow cytometry analysis of SW-480 cells with/without IL-22 knocked down. y-axis indicates propidium iodide (PI) staining and x-axis shows Annexin V-FITC staining. *: $p < 0.05$, **: $p < 0.01$.

IL-22 induces phosphorylation of STAT3 in SW-480 cells

To determine downstream signal transduction pathway of IL-22-IL-22R1 interaction, we detected the degree of STAT3 phosphorylation in SW-480 cells after supplementing IL-22. The p-STAT3 reached its maximum level at 15 min following IL-22 stimulation, which diminished with time and the residual p-STAT3 was detectable at 3 hours (Fig. 4(A)). The Akt phosphorylation demonstrated similar trend after IL-22 supplementation (Fig. 4(B)). While in HCT-116, treatment of IL-22 did not significantly increase p-STAT3 or p-Akt levels (Figs. 4(C) and 4(D)). These results suggest that IL-22R1 stimulated by IL-22 could triggered phosphorylation of STAT3 and Akt.

Inhibition of IL-22 reduces p-STAT3 and p-Akt and impairs cell viability of SW-480

We then knocked down IL-22 in SW-480 cells to examine the effect of IL-22. Consistently, p-STAT3 and p-Akt were both reduced in SW-480 cells transfected with IL-22 siRNA (Fig. 5(A)). The cell viability of SW-480 cells transfected with IL-22 siRNA was inhibited (Fig. 5(B)), while the proportion of apoptotic cells was significantly elevated (Fig. 5(C)).

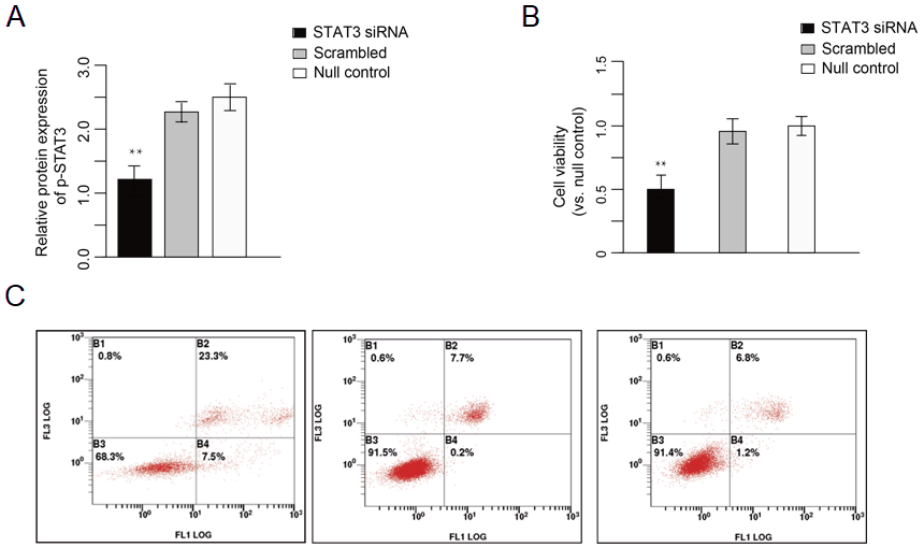

FIGURE 6. The cell viability and apoptosis assay of SW-480 cells with STAT3 knocked down. A. Relative protein expression of p-STAT3 in SW-480 cells with STAT3 knocked down. B. Cell viability assay of SW-480 cells with STAT3 knocked down. C. Annexin V flow cytometry analysis of SW-480 cells with/without STAT3 knocked down. y-axis indicates propidium iodide (PI) staining and x-axis shows Annexin V-FITC staining. *: $p < 0.05$, **: $p < 0.01$.

IL-22 supplementation did not alter the proliferation and apoptosis of STAT3 knocked-down SW-480 cells

To further confirm if STAT3 is a key effector implicated in the function of IL-22 in SW-480, we firstly knocked down STAT3 in SW-480 and supplemented those cells with IL-22. Compared with empty control, the SW-480 transfected with STAT3 siRNAs expressed less STAT3 and p-STAT3 (Fig. 6(A)). Those cells did not show significant increase in cell viability, nor alleviated apoptosis when supplemented with IL-22 (Fig. 6(B)), which was also observed from caspase-3 and caspase-9 assay (Fig. 6(C)).

Discussion

IL-22 exerts critical effects on pancreatic cells, colonic epithelial cells, and hepatocytes, and specific types of fibroblasts and stem cells (Sabat *et al.*, 2014) including T helper 22 (TH22. Tumor promotion involves a consecutive process implicated in the transition of tumor growth from a single premalignant cell into a fully developed tumor, in which cytokines and chemokines promote angiogenesis and suppress immune system to eliminate tumors(Yu *et al.*, 2009; Shalapour and Karin, 2015) signal transducer and activator of transcription (STAT. Previous studies have implicated the function of IL-22 in a variety of cancer types. Human lung cancer cells could secret IL-22 to enhance various biological processes related to cell survival and resistance to chemotherapy, which has been attested in *in vivo* xenograft model with IL-22 silenced (Curd *et al.*, 2012) respiratory tract and skin. Most tumours originating in these sites over-express IL-22R. Interestingly, there is an increase in Th17 frequency within the peripheral blood and tumour microenvironment of advanced cancer patients. Subsequently, IL-17 has been shown to display both pro-tumour and anti-tumour functions. Because many tumours lack expression of the IL-17 receptor, the effects of IL-17 on tumour growth are generated by cells that surround the tumour cells. Like IL-17, high levels of IL-22 have been detected in tumour tissues and the peripheral blood of cancer patients; however, the direct effect of IL-22 on tumour cells has remained largely unknown. In this report, we show that IL-22 stimulated production of vascular endothelial growth factor (VEGF. In liver cancer, IL-22 was expressed abundantly in tumor infiltrated leukocytes, which plays a role in metastasis, tumor growth and tumor stages (Cheng *et al.*, 2015). The transformation of chronic inflammation to CAC can be induced by proinflammatory cytokines secreted therein, leading to mutations in oncogenes and tumor suppressor and genomic instability via various mechanisms(Fearon and Vogelstein, 1990; Terzić *et al.*, 2010) 1958. As a cytokine implicated in inflammatory activities, IL-22 plays a key role in CRCs. For instance, IL-22 not only induced the production of MUC1, MUC3, MUC10 and MUC13 in colonic epithelial cells, but also elevates the expression of BCL-2, BCL-X and MCL1, and antibacterial and regenerative proteins, such as REG3β (Feng *et al.*, 2012; Nelson and Guyer, 2012), we discovered that interleukin-22 (IL-22. High concentrations of IL-22 in subepithelial myofibroblasts could also affect the expression of molecules that participate in cellular mobility, signal transduction and interferon regulatory factors, and metabolisms (Andoh *et al.*, 2005). In this study, we discovered the association between both IL-22 and IL-22R expression level and tumor stage and tumor differentiation in 30 CRC patients, immune histochemistry assay provided further support for this postulation. The highly differentiated cancer tissue expressed high level of IL-22.

Subsequently, we selected CRC cell line SW-480 which expressed high level of IL-22R, and treatment of IL-22 markedly enhanced the survival of SW-480 cells in the presence of 5-FU, indicating that activation of IL-22 and IL-22R could promote the cell proliferation and apoptotic resistance. Consistently, Stefanie *et al.* (de Moura *et al.*, 2009) showed that IL-17 and IL-22 dual positive innate lymph cells were accumulated and enriched in bacteria-induced colon cancer tissues, and mechanistic analysis demonstrated that the proliferation of epithelial cells was promoted by the STAT3 phosphorylation induced by IL-22, highlighting the potential value as a therapeutic target of IL-22 axis in colon

cancer. In this study, we showed that the phosphorylation of Tyr-705 was elevated by supplementation of IL-22, and the PI3K/Akt signaling pathway was activated concordantly.

STAT3, a transcription factor belonging to the STAT family, and has been revealed as an oncogene frequently activated in various cancers (Bromberg and Jr, 2002; Buettner, Mora and Jove, 2002; Mirovni inštitut, 2013) constitutively activated STATs have been detected in a wide variety of human cancer cell lines and primary tumors. STATs are activated by tyrosine phosphorylation, which is normally a transient and tightly regulated process. In tumor cells, constitutive activation of STATs is linked to persistent activity of tyrosine kinases, including Src, epidermal growth factor receptor, Janus kinases, Bcr-Abl, and many others. Such oncogenic tyrosine kinases are often activated as a consequence of permanent ligand/receptor engagement in autocrine or paracrine cytokine and growth factor signaling or represent autonomous constitutively active enzymes as a result of genetic alterations found in tumor but not normal cells. Persistent signaling of specific STATs, in particular Stat3 and Stat5, has been demonstrated to directly contribute to oncogenesis by stimulating cell proliferation and preventing apoptosis. STATs participate in oncogenesis through up-regulation of genes encoding apoptosis inhibitors and cell cycle regulators such as Bcl-x(L. STAT3 could activate downstream effectors such as Bcl-2 and Cyclin D1, thereby initiating pro-apoptotic and proliferation pathways (Bromberg *et al.*, 1999; Bromberg, 2002; Bromberg and Jr, 2002; Real *et al.*, 2002). Activated STAT3 has been reported to mediate oncogenic transformation in culture cells and tumor formation in nude mice (Bromberg *et al.*, 1999). For example, STAT3 was found to be activated by IL-26 in gastric cancer, which upregulated expression of Bcl-2, Bcl-XL and c-Myc, leading to sustained cell proliferation (You *et al.*, 2013). Similar effect of STAT3 signaling was discovered in renal cancer('STAT3 , but not ERKs , mediates the IL-6 – induced proliferation of renal cancer cells , ACHN and 769P', 2002), endometrial cancer(Gao *et al.*, 2009), bladder cancer(Chen *et al.*, 2008) and colon cancers. By contrast, inhibition of STAT3 led to diminished proliferation and active apoptosis in cancer cells (Zhao *et al.*, 2013). Akt plays an essential role in cancer cell proliferation, invasiveness, angiogenesis and metastasis (Populo *et al.*, 2012; Gao *et al.*, 2017; Kim *et al.*, 2017; Sahlberg *et al.*, 2017). Akt signaling cascade can be mediated by caspase family proteins or bcl-2 family members, and affects cell proliferation (Costa *et al.*, 2018). Hussein et al. reported that IL-22 could induced Akt phosphorylation, which contributes to enhanced tumor growth and progression of glioblastoma(Akil *et al.*, 2015). Consistently, our study revealed that inhibition of IL-22 resulted in decrease of phosphorylated STAT3, which was further proved to suppress proliferation and promote apoptosis of SW-480.

Since the pathogenic roles of STAT3 and p-Akt are well characterized in a variety of cancers, IL-22/IL-22R axis has drawn extensive research interest as a driving pathway of cancer progression. Unlike IL-6 and other cytokines that activate STAT3, IL-22 does not elicit response of immune cells, which makes it a promising therapeutic target with less side effect. The present study demonstrated the relation between IL-22 expression and tumor differentiation and validated the effect of IL-22 on apoptosis and cell viability. Alterations in STAT3 phosphorylation and p-Akt level induced by IL-22 supplementation further revealed the mechanism underlying the effects of IL-22R activation on apoptosis as well as tumor differentiation, indicating the prognostic value of IL-22/IL-22R.

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