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Critical role of IL-32 invasion and metastasis of colonic cancer through upregulation of matrix metalloproteinase (MMP)-2

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Abstract

This study explored the effects of IL-32 on matrix metalloproteinase (MMP)-2 upregulation and its roles in the invasion and metastasis of colonic cancer. Overexpression of IL-32 in human colonic cancer tissues was involved in both the depth of invasion and lymphatic permeation. As an inducer in the expression of MMP-2, IL-32 directly induced cell invasion of LoVo cells in vitro through Matrigel-coated Transwell chambers with both gelatin zymography and Western blot analysis, and also promoted liver metastasis of LoVo cells in an animal model. In contrast, no apoptosis occurred in LoVo cells upregulated by IL-32. Furthermore, overexpression of IL-32 in colonic cancer cells led to an increased proportion of vinculin-positive cells, which have their focal contacts at the cell periphery and are involved in the early phase of cellular spreading. In conclusion, IL-32 may play important roles in the depth of invasion and metastasis of colonic cancer through upregulation of MMP-2, which is independent of apoptosis. One of the important characteristics of malignant tumors is the significant capacity of cells to metastasize to different tissues and organs. Metastasis is a complex process that includes multiple steps: detachment from the primary tumor, invasion of the surrounding stroma, survival in the circulatory system, extravasation, and growth at distant sites. Patients die of metastasis rather than the primary tumor. To cure this insidious disease, elucidation of the details of the mechanism by which malignant tumors metastasize will be necessary. Cytokines and chemokines which are produced by malignant tumor cells as well as host defense/inflammatory cells at the primary and metastatic sites play a crucial role in regulating metastasis, particularly MMP-2.

Keywords: IL-32; Colonic cancer; PCR; Matrix metalloproteinase (MMP)-2

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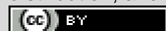

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second in women across the world, and its incidence is rapidly increasing in economically transitioning countries. Over the 5 years after surgery, the BMI was 95% in patients with stage I, 80% in those with stage II, 65% in those with stage III, and 30% in those with stage IV. Tumor prognosis and behavior are highly dependent on the combination of three biological features: the cancer cell's ability to spread to distant organs via lymphovascular invasion, the activity of purging the digestive system, or stromal invasion. Tumor-related sequence has been reported to mediate the progression of colon cancer through the

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acquisition of EMT and the subsequent invasion and metastasis of cancer cells by playing an essential role, and in order to determine whether sequence expression causes colon cancer to progress, six colon cancer patients (age range 47–71 years) used genuine and non-genuine examining the cell line system in which extracted side-by-side type cancer derived cell lines.

Tissue inhibitors in the matrix allow the regulation of MMPs and tumor metastasis in CRC cell lines and through the phosphorylation of RNA sandwiches. When connecting to the receptor of these signal molecules, the signal of matrix can take place that can express MMPs, interact with TNF- or LPS. However, silencing of RNA did not affect the expression of MMP in IL-32-treated cells in recent reports. We have processed to demonstrate the role of sequence in IL-32-mediated colon cancer invasion and metastasis.

The invasion and metastasis resulting from the progression of a localized neoplasm to advanced carcinoma are pivotal in determining clinical outcomes of cancer. We previously reported that IL-32 overexpression was significantly correlated with locoregional progression and peritoneal carcinomatosis of colonic cancer in the immunohistochemistry study solely. But the critical physiological roles of IL-32 in the invasion and metastasis of colonic cancer have not been elucidated from in vitro studies and demonstrated previously.

Accumulating evidence revealed that the IL-32 expression is frequently increased in a variety of malignant diseases, including lymph node metastasis, extrahepatic metastasis, and vascular invasion resulting in distant organ metastasis, such as progressive lung cancer and advanced hepatocellular carcinoma. Next, we will examine whether IL-32 may influence the metastatic behavior and invasion of colonic cancer and elucidate the possible role of IL-32 on cancer invasion and metastasis through the upregulation of matrix metalloproteinases (MMPs) which causes an enhancement of extracellular matrix degradation by in vitro and ex vivo experimental methods on signaling pathways. In this study, for the first time, it will be demonstrated that the overexpression and functioning of IL-32 changes the invasive and metastatic phenotype of colonic cancer as well as lymph node metastasis.

Objective of the Study

Objective of the study. IL-32 is a recently described proinflammatory cytokine mainly produced by monocytes or epithelial cells and activated by Mycobacterium tuberculosis. Although this is a well-known function of IL-32, the role of IL-32 in the development of carcinomas is still accompanied by controversies. This study investigated a potential role of a specific type of IL-32 in cancer invasion in colon cancer.

Epidemiology and Incidence

Colorectal cancer (CRC) is the third most frequently diagnosed malignancy and the cause of an increasing number of deaths among men and women in economically developed countries. Regarding colon cancer, the incidence rate is known to be different according to ethnicity, dietary pattern, lifestyle, and genetic predisposition. The highest incidence is observed in Australia and New Zealand (age-standardized rates, 61.4 for males and 43.3 for females per 100,000), the lowest ones in WB (2.8) and in AS (3.9) in males and in Eastern Africa (3.4) and in AS (3.2) in females. Age is another important

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factor that is inversely related to survival of patients and is differently distributed among countries and ethnicities.

The typical epidemiological characteristics observed in CRC also underline the impact of IL-32 and MMPs in the progression of the disease. A close link between the onset of cancer and various cellular processes leads to the formation of the hallmark of cancer, i.e., uncontrolled cell growth. This is facilitated by evasion of growth suppressors, resistance to programmed cell death, limitless replicative potential, sustained angiogenesis, insensitivity to antigrowth signals, generation of invasion and metastasis, and activation of invasion of other parts of the body. A number of these processes are favored by the expression of specific cytokines and their receptors, enzymes, and other proteins that are related to or part of the extracellular matrix (ECM). Matrix metalloproteinases (MMPs) are among these proteinases which have been associated with cancer progression. Real-time PCR and western blots were used to evaluate expression of two MMPs: MMP-2 and MMP-9, targeting IL-32 isoforms in Caco-2 and HT-29 colonic cells.

Pathophysiology and Staging

Colonic cancer arises via an accumulation of multiple genetic and epigenetic events, ultimately leading to the deregulated cycle ratio of the colonocytes. During the early phase, mutations occurring in APC/β-catenin or K-RAS may be the main factors for developing early phase cancer, which is a heterogeneous subgroup of cancer. An imbalance between cell growth and apoptosis regulation leads to a progressive accumulation of genetic and epigenetic events, resulting in a benign, then malignant cancer. Finally, the transformed cell acquires an invasive phenotype, leading to local disease persistence and spread to distant organs of the body - metastatic disease.

The staging of colonic cancer is important for the future treatment of cancer. Initial colonic cancer evaluation, using preoperative colonoscopy or radiological evaluation, shows that approximately 20-25% of colon cancer is localized to the colon wall, predominantly with a primary tumor node metastasis (TNM) stage of:

- T2: Involves the muscularis propria
- T3: Extends into the subserosa, or into non-peritonealized pericolic or perirectal tissues
- T4: Penetrates the serosa and into the visceral peritoneum, or is directly invading or adherent to other organs or structures.

Invasion and metastasis of colonic cancer to the distal lymph nodes, blood, and organs contribute to a worse prognosis. Involvement of lymph nodes ultimately reduces the prognosis by about 35% at 5 years and 1.3-6% at ten years.

Interleukin-32 (IL-32)

Interleukin 32 (IL-32), a proinflammatory cytokine induced by tumor necrosis factor (TNF)-α, IL-1β, and lipopolysaccharide, strongly induces other apoptosis-related cytokines and chemokines in peripheral blood mononuclear cells. It has been suggested that IL-32 expression differs according to tumor type and function. There are no data concerning IL-32 expression in colonic cancer. The purpose of the present study was to determine whether overexpression of IL-32 is involved in the

invasion and metastasis of colonic cancer, and whether there is a correlation between IL-32 and MMP-2 expression.

Interleukin-32 (IL-32) is a newly described cytokine that has been found to be involved in inflammatory diseases, including Crohn's disease and rheumatoid arthritis. Recently, IL-32 has been reported to be upregulated in the tumor cells of colon cancer. It was reported that IL-32 up-regulated the endothelial cell activity and solidified the colonic cancer survival signal. Furthermore, the expression of IL-32 was related to the level of Dukes' staging classification. However, little information is available concerning the roles in the invasion, angiogenesis, and metastasis of colonic cancer. Matrix metalloproteinases (MMPs) play a major role in the degradation of extracellular matrix, including type IV collagen, which is essential for basement membranes and mesangial matrix. Based on the close interconnection between these studies, this investigation was undertaken to ascertain the potential role of IL-32 in invasion, angiogenesis, and lymphangiogenesis of cancer cells. And through this study, we found for the first time that IL-32 upregulated the expression of matrix metalloproteinase (MMP)-2 in colonic cancer.

Biological Functions

In addition to the IL-18 induction, IL-32 can interact and potentiate the activation of other interleukins such as IL-1β, which is an inflammatory cytokine that plays a key role in the immune and inflammatory systems. IL-32 is also able to regulate the activity of various transcription factors including nuclear factor-kB (NF-kB). Surprisingly, although MMP-2 has been reported to be induced by the proinflammatory cytokines, whether MMP-2 can be upregulated by NF-kB remains unexplained in previous reports. On the regulation of NF-kB on MMP-2, no direct evidence is available regarding the NF-kB regulation of MMP-2 expression. This study was designed to investigate the detailed function of IL-32 in invasion, metastasis, and death of patients with colonic cancer, primarily concentrating on IL-32-induced MMP-2 expression. Our study included an investigation of the signaling pathways involved in IL-32-induced MMP-2, especially focusing on the relationship between IL-32 and nuclear factors, Signal transducer and activator of transcription 3 (STAT3, which is also important in the survival of cells). Finally, we addressed the exertion of IL-32 during the occurrence and development of colonic cancer.

Expression in Colonic Cancer

IL-32 has been shown to be overexpressed in many cancer cells and tissues, including ovarian serous adenocarcinoma, colorectal carcinoma, pulmonary large carcinoma, gastric cancer, pancreas ductal adenocarcinoma, colon carcinoma, cervix carcinoma, and mesothelioma. The expression of IL-32 may be associated with characteristics such as invasion and metastasis. However, the role of IL-32 in the characteristics of colonic cancer is not known. The pattern and distribution of IL-32 expression and its family are shown in colonic cancer and adjacent normal colon.

Genetically, IL-32 mRNA was significantly increased in Caco2, SW480, HT-29, and HCT-116 cells. The results were confirmed by RT-PCR, and membrane-bound IL-32 was detected only in cancer cell lines.

Matrix Metalloproteinase (MMP)-2

Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, actively participate in many biological processes. MMPs regulate vital physiological functions, such as growth, differentiation, and repair of cells and tissues, by physical hydrolysis of extracellular matrix (ECM) proteins and proteolytic processing of certain cytokines, chemokines, or growth factors. In the tissues of colorectal cancer, the degradation balance of MMP to the ECM is significantly disrupted. Specifically, both increased and decreased expression of certain MMPs in colorectal cancer/DDs organ tissues may contribute to organ destruction by favoring proliferation, resistance to apoptosis, invasion, and ultimately metastasis, but recurrence, and poor prognosis. Matrix metalloproteinase (MMP)-2 functions as a key molecule at "initiation" and "invasion" levels in high invasive and low secretory cells, respectively. MMP-2 appears in the ECM as secreted MMP-2 (active or zymogen MMP-2) and pro-MMP-2 (inactive or latent MMP-2). The C-terminal hemopexin domain of pro-MMP-2 contains 3 fibronectin type II-like collagen-binding repeats, which allows pro-MMP2 to strongly bind/connect to the ECM at the local tumor invasion front to maintain a high MMP-2 local concentration. Pro-MMP-2 is maximally activated by dual metalloproteinases (densin/ADAM) (ADAMOTS and MT-MMPs) with cysteine support. MMP-2 has a 1.5-fold stronger catalytic activity towards denatured collagen than MMP-9 and it cannot degrade interstitial collagen-based fibrotic fibers.

In this study, immunohistochemical analysis was used to investigate the correlation between (IL)-32 expression and clinicopathological features of colonic cancers and the relationship between IL-32 and tumor invasion. IL-32 was highly expressed in colonic tumor cells. IL-32 was significantly expressed in the colorectal cancer stroma, the end of cancer cells of various sizes, on the resection surface of the cancer tissue, and in the cytoplasm locally. According to statistical analysis of the expression of MMP-2 in IL-32 (+) and IL-32 (-) colorectal tissue, it was observed that MMP-2 was significantly secreted into the stroma of IL-32 (+) tissues, especially to the ECM and directly around the IL-32 (+) cancer nests. In this study, we deduced that IL-32 was released from colonic cancer cells, incorporated into colon stromal fibroblasts, and secreted as a protein. In an in vitro experiment, when cancer cells with a relatively higher potential for metastasis possessed a large volume of energy storage incorporation-associated lipid droplets, elasticity was higher if co-cultured with stromal cells that secrete IL-32. In other words, the generation of a stronger "local hard environment" was detected, and the tumor invasion response increased.

Structure and Function

Matrix metalloproteinases (MMPs) are a group of zinc-binding proteinases that serve several major enzymatic activities: hydrolysis of peptide bonds in the ECM, degrading members of the ECM. MMPs could be implicated in driving disease progression and recent ground-reversal in evaluating the function of matrix metalloproteinase has helped us gain a more comprehensive view of the "reciprocal regulation" of tissue formation and related diseases. Thus, MMP-2 and MMP-9 are thought to be more

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likely involved in the regulation of tumor progression, neurological diseases, microbial infection, and even the development of "collagen disease" that has been postulated for 117-135 years.

Experimental Methods

HCT-116 cells were provided by Cancer Research Institute, Seoul National University. RPMI-1640 medium supplemented with 5% heat-inactivated fetal bovine serum and 2 mM L-glutamine was used as a standard growth medium. All cells were maintained in a humidified 5% CO2 atmosphere at 37 °C. Cells (5 × 105) were treated with recombinant human IL-32b or IL-32s for 72 h at the indicated concentration, and the proteolytic activation of MMP-9 was determined in a gelatin zymographic assay. IL-32 levels were determined by quantitative PCR. The following primers were used: IL-32 (reverse 5′-CTCATCTTCACGTGGACGAAGTCC-3′; forward 5′-GAGGAAGAAGACCACCGTCAGGCT-3′). The housekeeping gene GAPDH was used as a loading control. HCT-116 cells (5 × 105) were seeded in 6-well plates and cultured overnight in RPMI + 5% FBS. The following day, cells were treated with human recombinant IL-32b or IL-32s for a further 72 h. Cell culture supernatant was collected and centrifuged to remove cells, and 250 μL of cold methanol was added to the cell culture media and incubated at -20 °C to precipitate proteins. Samples were spun at 14,000× g to precipitate proteins, media from the cell cultures was then removed, and the cells washed and resuspended in PBS. Protein content was determined in cell pellets using BCA protein assay kit. MMP-2 activity was analyzed by gelatin zymographic assay. Supernatant collected from HCT-116 cells (5 × 105) pretreated as above and activated for 48 h was used to determine MMP-2 activity. 5× cell lysate was loaded onto 10% zymography gel (which contains 0.1% gelatin) and electrophoresed at 100 V for 135 min. The gel was then incubated in 1% Triton X-100 for 2 × 15 min at room temperature to remove SDS and then incubated at 37 °C in developing buffer. Subsequently, the gel was stained with Coomassie Brilliant Blue G-250 in a 45% methanol/10% acetic acid solution and then destained with a 20% methanol/10% acetic acid solution. The level of gelatinolytic activity was visualized and assessed by the appearance of white bands against the dark blue background. MMP activity was determined using a densitometer. All incubation mixtures contained aprotinin (10 μg/mL), phenylmethylsulphonyl fluoride (1 mM), and sodium orthovanadate (1 mM) to inhibit serine, cysteine (autolysis), and tyrosine (gelatinase activation) proteases, respectively. Insets show the results of quantification by volume densitometry.

Cell Culture and Maintenance

The human colonic cancer HT29, HCT116, and SW480 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; CaCl2 1.5 mM, glucose 4.5 g/L, HCO3 3.7 g/L, NaHCO3 3.3 g/L, pyruvate 67 mg/L, phenol red 10 mg/L) containing 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 units/mL of penicillin G, and 100 μg/mL of streptomycin. Cells were placed in a 5% CO2 fully humidified incubator in an atmosphere of 95% air at 37°C. At confluence, the growing cells were subcultured using a 0.25% trypsin-EDTA solution. For the mRNA and protein isolation, HT29, HCT116, and SW480 cells were grown to confluence in six-well tissue culture plates. The medium was removed, and the plates were washed. To study whether there was an association between IL-

32 and MMP-2, we stimulated the cells to express IL-32 and MMP-2. The serum concentration was decreased to 1% (final concentration) to sensitize the cells, as previously described.

To stimulate IL-32 expression, we used 10 ng/mL tumor-necrosis factor (TNF)-a. To stimulate MMP-2 expression, we used 10 ng/mL IL-22. For the negative control, the cells receiving no stimulation were exposed to the growth medium containing 1% FBS for the duration of the experiment. After the stimulation period, cells were harvested for mRNA/protein isolation (see below). An in vitro cell-substrate adhesion assay was carried out on IL-32 to investigate its cell invasive potential. After being isolated, the mRNA samples were treated with DNase I Amplification Grade, following the procedure of the manufacturer. A fraction of each mRNA sample was used in a test reaction to ensure that the mRNA was DNA free.

IL-32 and MMP-2 Expression Analysis

To evaluate the relationship between the expression levels, International Union Against Cancer (UICC) stages, and disease outcome according to IL-32 and MMP-2 expression, chi-square tests were applied by SPSS software, version 12.0 (SPSS, Inc., Chicago, IL, USA). Results were assumed to be significant at a value of $p<0.05$. Immunohistochemical staining: Slides were deparaffinized and rehydrated through graded alcohol followed by blockade of endogenous peroxidase using 3% hydrogen peroxide for 10 min. The slides were washed with phosphate buffer saline (PBS) for 5 min and then blocked with 5% BSA for 10 min. After being washed with PBS, the tissue sections were separately incubated with mouse monoclonal anti-IL-32 antibody and MMP-2 antibody, that were incubated overnight at 4°C. Slides were rinsed in PBS for three times and then incubated with the biotinylated secondary antibody for 15 min. After three washes with PBS, the sections were incubated with streptavidin-peroxidase for 10 min and then washed again three times. Liquid diaminobenzidine (DAB) was added to visualize the target protein according to the manufacturer's instructions (Maixin, Fuzhou, China). Sections were then filmed with liquid DAB plus substrate-chromogen system (Dako Real Envision Detection System, DAKO, Dako, Kyoto, Japan). Finally, the sections were counterstained slightly with Jenner's stain, coated with coverslips, and left to air-dry at room temperature. Expression of IL-32 and MMP-2 were evaluated on the basis of a semiquantitative score obtained by considering both the intensity and the percentage of positive immunoreactive cancer cells.

Results

As presented in Supplementary Figure S1, the expression of the construct was easily detected comparing HCT15 cells transiently expressing the siRNA-GFP with control siRNA-GFP of Scl27 cells. Therefore, we co-transfected IL-32 siRNA with siRNA-GFP into SW480 to investigate whether IL-32 exerts a critical role in colonic cancer cells. Unexpectedly, the number of metastasized foci on liver was greatly reduced by transient transfection of IL-32 siRNA-GFP when compared to control siRNA-GFP, as presented in Figure 1A. In fact, well formation of the HCT15 cell line required ~2 weeks, because cells were to some extent spindular, which is easy to be observed, and there were very few of well formations of parental cells in Matrigel-free dishes, as compared to HCT15 IL-32 siRNA-GFP cells, as shown in Figure 1A. These indicated that upregulation of IL-32 probably via soluble factor(s),

since no direct cell-to-cell contact occurred, contributed to the invasion and metastasis of the SW480 cells. Blockade of upregulated endogenous IL-32 with IL-32 siRNA-GFP led to a drastic reduction in the ability to invade through extracellular matrix (ECM).

IL-32 Upregulation in Colonic Cancer Cells

In a previous study, we found that expression of an inflammatory cytokine, interleukin-32 (IL-32), was upregulated in colonic cancer patients. In the present study, we investigated the functions of upregulated IL-32 in colonic cancer cells. For the first time, we found the overexpression of tumors in the orthotropic xenograft model of colonic cancer. At the same time, we found a negative correlation between the levels of IL-32 expression and cancer differentiation and a positive correlation with the depth of tumor invasion and lymph node metastasis. A correlation was also seen between postoperative metastasis and the degree of IL-32 expression. These data suggest that as a tissue biomarker, IL-32 may provide valuable predictive information especially for invasion and metastasis.

MMP-2 Expression Induced by IL-32

The effect of IL-32 on invasion and metastasis of cancer cells suggests that invasion might be an early change determined by IL-32 in vitro. We found that IL-32 induced the expression and secretion of matrix metalloproteinase (MMP)-2, but not MMP-9. To investigate the molecular mechanisms underlying the IL-32-induced MMP-2 expression, the mRNA expression was examined by RT-PCR. As shown in Figure 3D and 3E, the expression of MMP-2 mRNA was increased by IL-32. However, IL-32 did not increase MMP-9 mRNA. To evaluate the transcriptional activation of the MMP-2 gene, a construct containing -996/+265 region of the human MMP-2 gene was generated and transfected into SW480 and SW620 cells. As seen in Figure 3F, the cells were transiently transfected with pGL3-bas, pGL3-MMP-2 construct only, or together with IL-32 construct. The luciferase activity in the cells co-transfected with pGL3-MMP-2 construct and IL-32 construct was higher than that of pGL3-MMP-2 alone ($P < 0.05$).

Discussion

Our data on the role of IL-32 in invasion and metastasis of colonic cancer are important not only because they show the key molecule triggering the progression of colonic cancer, but also because upregulation of MMP-2 expression in IL-32 has not been shown and MMP-2 expression, a marker for lymph node and liver metastasis of colonic cancer, has not been focused. The role of MMP-2 in angiogenesis has already been demonstrated, and the results in this study can support the fact that MMP-2 produced by IL-32 is related to the criteria needed to confirm the production of MMP-2 by IL-32, and data on the interaction in molecules would help us to better understand the insight of the metastasis in colonic cancer or the other cancers. IL-32 has been reported to be markedly higher in human colon cancer compared to normal colon mucosa. These include various activities related to the extracellular environment and are considered to have an important characteristic of more invasiveness and metastasis. To address the function of IL-32, we first tried to find the target gene of IL-32 affecting the invasion and metastasis of colonic cancer. We will focus on MMP-2 because of its

importance in invasion and metastasis of colonic cancer. Then, for their possible interaction, we evaluated the correlation between IL-32 and MMP-2 expression with clinicopathological features of colonic cancer patients. The quantity of IL-32 and MMP-2 are well correlated with the progression and metastasis, and propose that the IL-32 production of MMP-2 may increase the metastasis of the colonic cancer. Consideration of the IL-32 small interfering RNA system and the potential MoAb against IL-32 will show more therapeutic potential, and further investigation value will be necessary in chemotherapy for the patients of high IL-32 quantitation.

Our date shows that the expression of IL-32 in human colon carcinoma tissue is related to invasion and metastasis of colon cancer. Even, our date shows that IL-32 can be used as an independent factor for prediction of 5-year survival rate in colonic carcinoma. Our data indicates a novel role for the IL-32 in the promotion of cancer invasion and metastasis.

Two important points of our studies are presented as follows. MMP-2 has been found, in previous studies, to be involved in surface type of metastasis. The interaction between the tumor cell and matrix are dependent on MMP-2 activities. However, if the MMP-2 activity were blocked, the invasion and metastasis ability of tumor cells or secreted iso-MMP-2s are impaired. Some research also demonstrates that only MMP-2 gene deficient mice, can impede tumor cell infiltration and extension. Li-Ya Liu et al. proved that the silencing the expression of MMP-2 gene by dsRNAi could decrease the implantation and distant metastasis of MDA-MB-231 cell in vivo. Our concomitant investigation that rIL-32 could promote MMP-2 in HT29 cells. These data indicated that IL-32 may participate the surface type of invasion and metastasis of colonic cancer through upregulation expression of MMP-2. In addition, IL-32 can also upregulate COX2, EP1 and Orx1 expression in HT29 cells through binding IL-18R. In our case, human HT29 cells contain packed, increased COX2, EP1 and Orx1 expression, IL-32 may facilitate HT29 cells growth through upregulate these gene expression. Moreover, future studies are necessary to examine potential relationship between MMP-2 and the expressions of COX2, EP1 and Orx1. If IL-32 promoted these gene expression in HT29 cells through independent pathway or binding same receptor, then activated cancer biopathway may promote HT29 cells growth more. Otherwise, the IL-32-activated subsequent signal may activate the LT, COX and Orx signal to promote HT29 cells growth. The data in this paper discussed IL-32 action in colon cancer cells in vitro. However, several points were already established that the tumorigenesis involve a complex biopathway and a solid ECM or increased pressures. Cell-cell communication are induced by this milieu. Although, we lack the information of the development from cell-based conclusions to the animal models and clinic specimens, our data could open an avenue of potential clinical treatment for colon cancer. Inhibition is thought to be hopeful, but an antibody against MMP-2 or MMP-9 has been designed to impair enzyme activity at the site of the metalloproteinase-inhibitor, the stroma, rather than in the tumor, leading to no results. However, we believe that IL-32 might activate HT29 tumor cells, to enhance the production of MMP-2, which might be beneficial for IL-32-targeted inhibition of antibodies, decreasing the production and effect of MMP-2. Taken together, these data expand the understanding of IL-32 in colon cancer cells, and likely in other epithelial carcinomas, including oral and laryngeal cancers.

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Conclusion

Our data show that IL-32 induces the invasion and MMP-2 production of colonic cancer cells, and that such induction activity was reduced in the IL-32 siRNA transfected cells. IL-32 induced the invasion and adhesion in a time- and concentration-dependent manner. The MMP-2 production inhibitor reduced the MMP-2 inhibitory activity of cells treated with IL-32. MMP-2 production or invasion ability induced by IL-32 was reduced in the cells that were transiently transfected with MMP-2 siRNA. These data suggest that IL-32 might induce invasion as well as metastasis through MMP-2 upregulation. Based on the results, we suggest the need for further study on treatment of colonic cancer patients with recurrence after treatments including colon resection, hepatic resection, or chemotherapy.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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References

1. Surveillance, Epidemiology, and End Results (SEER) Program. Stat Fact Sheets: Colon and Rectum Cancer. [(accessed on 25 October 2013)]. Available online: http://seer.Cancer.Gov/statfacts/html/colorect.Html/

2. Howlader N, Noone A, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2008. National Cancer Institute; Bethesda, MD, USA: 2011.

3. Hua H, Li M, Luo T, Yin Y, Jiang Y. Matrix metalloproteinases in tumorigenesis: An evolving paradigm. Cell. Mol. Life Sci 2011;68:3853–3868.

https://doi.org/10.1007/s00018-011-0763-x
PMid:21744247

4. Van der Jagt MF, Wobbes T, Strobbe LJ, Sweep FC, Span PN. Metalloproteinases and their regulators in colorectal cancer. J. Surg. Oncol 2010;101:259–269.
PMid:20039272

5. Yousif NG, Zhai Y, Slimani H, Ao L, Fullerton D, Meng X. TLR3-mediated inflammatory response after global myocardial i/r via activation of TLR4-mediated NF-kappa β and TRIF/IRF signaling pathways. SHOCK 2014;41: 34-34.

6. Hu J, van den Steen PE, Sang Q, Opdenakker G. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nat. Rev. Drug Discov. 2007;6:480–498.
https://doi.org/10.1038/nrd2308
PMid:17541420

7. Doyle JL, Haas TL. Differential role of b-catenin in VEGF and histamineinduced MMP-2 production in microvascular endothelial cells. J Cell Biochem 2009;107:272–83.
https://doi.org/10.1002/jcb.22123
PMid:19306293

8. Clements WM, Wang J, Sarnaik A, Kim OJ, MacDonald J, FenoglioPreiser C, et al. b-Catenin mutation is a frequent cause of Wnt pathway activation in gastric cancer. Cancer Res 2002;62:3503–6.
PMid:12067995

9. Yousif NG. Fibronectin promotes migration and invasion of ovarian cancer cells through up-regulation of FAK–PI 3 K/A kt pathway. Cell biology international 2014;38: 85-91.
https://doi.org/10.1002/cbin.10184
PMid:24115647

10. Cho JY, Lim JY, Cheong JH, Park YY, Yoon SL, Kim SM, et al. Gene expression signature-based prognostic risk score in gastric cancer. Clin Cancer Res 2011;17:1850–7.
https://doi.org/10.1158/1078-0432.CCR-10-2180
PMid:21447720 PMCid:PMC3078023

11. Catalano V, Labianca R, Beretta GD, Gatta G, de Braud F, Van Cutsem E. Gastric cancer. Crit Rev Oncol Hematol 2009;71:127–64.
https://doi.org/10.1016/j.critrevonc.2009.01.004
PMid:19230702

12. Naylor TL, Greshock J, Wang Y, Colligon T, Yu QC, Clemmer V, et al. High resolution genomic analysis of sporadic breast cancer using array-based comparative genomic hybridization. Breast Cancer Res 2005;7:R1186–98.
https://doi.org/10.1186/bcr1356
PMid:16457699 PMCid:PMC1410746

13. Shoda H, Fujio K, Yamamoto K. Rheumatoid arthritis and interleukin32. Cell Mol Life Sci 2007;64:2671–9.
https://doi.org/10.1007/s00018-007-7186-8
PMid:17619821

14. Yousif NG, Al-Amran FG, Hadi N, Lee J, Adrienne J. Expression of IL-32 modulates NF-κB and p38 MAP kinase pathways in human esophageal cancer. Cytokine 2013;61: 223-227.
https://doi.org/10.1016/j.cyto.2012.09.022
PMid:23107826

15. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: Trials and

tribulations. Science 2002;295:2387–2392.
https://doi.org/10.1126/science.1067100
PMid:11923519
16. Ogata Y, Matono K, Nakajima M, et al. Efficacy of the mmp inhibitor mmi270 against lung metastasis following removal of orthotopically transplanted human colon cancer in rat. Int. J. Cancer. 2006;118:215–221.
https://doi.org/10.1002/ijc.21285
PMid:16003755
17. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J. Clin. Oncol 2009;27:5287–5297.
https://doi.org/10.1200/JCO.2009.23.5556
PMid:19738110 PMCid:PMC2773480
18. Hasegawa S, Koshikawa N, Momiyama N, et al. Matrilysin-specific antisense oligonucleotide inhibits liver metastasis of human colon cancer cells in a nude mouse model. Int. J. Cancer 1998;76:812–816.
https://doi.org/10.1002/(SICI)1097-0215(19980610)76:6<812::AID-IJC8>3.0.CO;2-0
19. Wu J, Wu G, Lv L, et al. Microrna-34a inhibits migration and invasion of colon cancer cells via targeting to fra-1. Carcinogenesis 2012;33:519–528.
https://doi.org/10.1093/carcin/bgr304
PMid:22198213
20. Novick D, Rubinstein M, Azam T, Rabinkov A, Dinarello CA, Kim SH. Proteinase 3 is an IL-32 binding protein. Proc Natl Acad Sci U S A 2006;103:3316–21.
https://doi.org/10.1073/pnas.0511206103
PMid:16488976 PMCid:PMC1413913
21. Netea MG, Azam T, Ferwerda G, et al. IL-32 synergizes with nucleotide oligomerization domain (NOD) 1 and NOD2 ligands for IL-1b and IL-6 production through a caspase 1- dependent mechanism. Proc Natl Acad Sci U S A 2005;102:16309–14. 18.
22. Japanese Gastric Cancer A. Japanese classification of gastric carcinoma—2nd English edition. Gastric Cancer 1998;1:10–24. https://doi.org/10.1007/s101200050051
https://doi.org/10.1007/PL00011681
23. Yousif NG, Sadiq AM, Yousif MG, Al-Mudhafar RH. Notch1 ligand signaling pathway activated in cervical cancer: poor prognosis with high-level JAG1/Notch1. Archives of gynecology and obstetrics 2015;4: 899-904.
https://doi.org/10.1007/s00404-015-3694-1
PMid:25842263
24. Shih CH, Chen SL, Yen CC, et al. Thyroid hormone receptor-dependent transcriptional regulation of fibrinogen and coagulation proteins. Endocrinology 2004;145:2804–14.
https://doi.org/10.1210/en.2003-1372
PMid:14977860
25. Alhasani S, Yousif NG. Critical role of IL-23 signaling in prostatic cancer. American Journal of BioMedicine 2013;1(1): 4-6.
https://doi.org/10.18081/ajbm/2333-5106-013-11/4-6
26. Wang CL, Wang CI, Liao PC, et al. Discovery of retinoblastoma-associated binding protein 46 as a novel prognostic marker for distant metastasis in nonsmall cell lung cancer by combined analysis of cancer cell secretome and pleural effusion proteome. J Proteome Res 2009;8:4428–40.
https://doi.org/10.1021/pr900160h
PMid:19655816

27. Lee JH, Choi JW, Kim YS. Plasma or serum timp-1 is a predictor of survival outcomes in colorectal cancer: A meta-analysis. J. Gastrointest. Liver Dis 2011;20:287–291.
PMid:21961097
28. Holten-Andersen MN, Christensen IJ, Nielsen HJ, et al. Total levels of tissue inhibitor of metalloproteinases 1 in plasma yield high diagnostic sensitivity and specificity in patients with colon cancer. Clin. Cancer Res 2002;8:156–164.
PMid:11801553
29. Wang DG, Yousif NG, Sadiq AM, et al. Critical role of SEMA5A expression in invasion and metastasis of ovarian cancer cell. American Journal of BioMedicine 2014;2(3): 4-6.
30. Lu KV, Jong KA, Rajasekaran AK, Cloughesy TF, Mischel PS. Upregulation of tissue inhibitor of metalloproteinases (timp)-2 promotes matrix metalloproteinase (mmp)-2 activation and cell invasion in a human glioblastoma cell line. Lab. Investig 2004;84:8–20.
https://doi.org/10.1038/labinvest.3700003
PMid:14631378
31. Park KS, Kim SJ, Kim KH, Kim JC. Clinical characteristics of timp2, mmp2, and mmp9 gene polymorphisms in colorectal cancer. J. Gastroenterol. Hepatol 2011;26:391–397.
https://doi.org/10.1111/j.1440-1746.2010.06504.x
PMid:21261731
32. Zhang M, Liu Y, Feng H, et al. Cd133 affects the invasive ability of hct116 cells by regulating timp-2. Am. J. Pathol 2013;182:565–576.
https://doi.org/10.1016/j.ajpath.2012.10.015
PMid:23195431

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