Effect of Progranulin on Migration and Invasion of Human Colon Cancer Cells

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ABSTRACT
Objective: To investigate the effects of progranulin on migration and invasion of human colon cancer cells.
Study Design: An experimental study.
Place and Duration of Study: Department of Histology and Embryology, College of Basic Medical Sciences, Jilin University, Changchun, China, from October 2015 to July 2017.
Methodology: Cell transfection was used to obtain progranulin knocked down/overexpressing cells. MTT and soft agar assay were applied to investigate the cell growth. Migration and invasion were detected using transwell assay.
Results: Tumor growth, migration and invasion decreased in progranulin knocked down cells and increased in progranulin overexpressing cells. The decrease was recovered when exogenous progranulin was added. Progranulin-induced migration and invasion were inhibited by neamine.
Conclusion: Progranulin has a direct effect on tumor growth, migration, and invasion of human colon cancer cells. It may serve as a potential therapeutic target for human colon cancer.


INTRODUCTION
Colon cancer is the most common malignant neoplasms in humans. Despite extensive experimental studies throughout the past decades, the genetic and molecular mechanisms responsible for the development and progression of colon cancer remain primarily unknown. Progranulin is a 88 kDa glycoprotein originally isolated from the highly tumorigenic mouse teratoma PC cells.¹ It is an autocrine growth factor and is the largest member of the granulin/epithelin family of cystein-rich polypeptide growth modulators.² Its expression is up-regulated in many types of cancers including teratoma, breast cancer, renal cell carcinoma, ovarian cancer, multiple myeloma, and others.¹-³ It has been indicated that progranulin was highly expressed in invasive ductal carcinomas in correlation with parameters of poor prognosis, and the expression of progranulin was higher in invasive epithelial ovarian tumors than that in low malignant potential tumors.⁴,⁵ Progranulin has been shown to play a role in tumorigenesis. Progranulin stimulated proliferation of human breast cancer cells and when progranulin expression was inhibited, tumor formation was reduced in vitro and in vivo.⁶ It has been reported that in ovarian cancer, progranulin downregulated cyclin D and CDK 4 to decrease cell proliferation and invasion, and activation of MMP-2 was also involved in the process.⁷ In addition, in normal tissues, progranulin plays a role in wound healing and inflammation.⁸ Yet the mechanisms by which progranulin mediate human colon cancer biological behaviors are still indeterminate.

The present study was conducted to examine the effect of progranulin on tumor cell growth, migration, and invasion in human colon cancer cells.

METHODOLOGY
The experimental study was conducted at Department of Histology and Embryology, College of Basic Medical Sciences, Jilin University, Changchun, China, from October 2015 to July 2017. Human colon cancer cell lines SW480 and SW620 were acquired from the Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences. Cells were cultured in Dulbecco’s modified Eagle’s medium plus 10% (v/v) fetal bovine serum (FBS) at 37°C with 5% CO₂. Progranulin-siRNA (PGRN-siRNA) was obtained from Genechem (Shanghai, China). The pcDNA3.1-Prog (+) was constructed by Sangon Biotech (Shanghai, China). Cell transfections were done by means of Lipofectamine (GIBCO, Invitrogen Corporation, Grand Island, New York, USA).

Semi-quantitative reverse transcriptase polymerase chain reaction assay (RT-PCR) was performed. TRI
reagent (Invitrogen Corporation, Grand Island, NY, USA) was used for total RNA extraction. Primer sequences: 5'-AATGTGACATGGAGGTGAGC-3' (forward primer), 5'-AGCAGGTCTGGTTATCATGG-3' (reverse primer) for progranulin,7 and 5'-ACACTGTGCCCATCTACG-3' (forward primer), 5'-CTCGTCATACTCCTGTTG-3' (reverse primer) for β-actin.9

Enzyme-linked immunosorbent assay (ELISA) was performed to detect progranulin secretion levels. Briefly, plates were coated with 10 μg/ml anti-human progranulin monoclonal antibody (R&D Systems) and blocked with 5mg/ml BSA. After incubated at 4°C overnight, incubated with anti-progranulin polyclonal antibody (R&D Systems) and HRP-conjugated anti-rabbit IgG successively and then TMB solution was added. A multi-scanner autoreader (Multiskan MK 3, Labsystems Dragon, Finland) was used to read the absorbance at 450 nm.

Cell growth was analysed by MTT assay. Cells were seeded into 96-well plates, after incubation, 5 mg/ml MTT solution was added. The reaction was stopped by 100μl of solubilisation buffer (10% sodium dodecyl sulfate in 0.1% HCl). The absorbance was readed at 540 nm.

Soft agar assay was performed to determine anchorage-independent cell proliferation. Cells were seeded in 0.35% agar with a density of 4×103 cells per 35 mm dish and cultured for 14 days at 37°C. Colonies were counted in the entire dish, the diameters of colonies in 10 microscope fields were measured.

Cell migration and invasion were determined by transwell assay. Cells were added to the top chambers of 24-well transwell plates (invitrogen) in medium containing 1% bovine serum albumin. For invasion assay, the top chambers were coated with 1 mg/ml matrigel. Medium in the bottom chambers contained 10% fetal bovine serum. After incubated for 48 hours, cells were fixed and stained with 0.2% crystal violet.

Data analysis was performed using the Statistical Package for the Social Sciences, version 22 (IBM Corp. Armonk, NY, USA). Experimental results were expressed as means ± SD of at least three independent experiments. For statistical analysis, Student’s t-test was used. A value of p <0.05 was considered significant.

RESULTS

Progranulin increased the cell growth of human colon cancer cells. RT-PCR and enzyme-linked immunosorbent assay was performed to detect the expression of progranulin in human colon cancer cells and the results showed that human colon cancer SW620 cells with high metastatic potentiality expressed high level of progranulin, while the low metastatic potential human colon cancer SW480 cells expressed a lower level of progranulin (Figure 1). SW620 cells were transfected with PGRN-siRNA to knock down the expression of progranulin, and SW480 cells were transfected with pcDNA3.1-Prog (+) and the progranulin overexpressing cells were obtained. As shown in Figure 1, progranulin was decreased in PGRN-siRNA transfectants (Prog-) of SW620 cells and increased in pcDNA3.1-Prog (+) transfectants (Prog+) of SW480 cells.

Figure 1: Expression of progranulin in transfectants. (A) The mRNA expression of progranulin in transfectants was detected by reverse transcriptase polymerase chain reaction (RT-PCR). Beta-actin mRNA expression was used as an internal control. (B) Secreted progranulin was determined by enzyme-linked immunosorbent assay(ELISA). The data shown were means ±SD of four independent experiments(using cells from different preparations). *p<0.001 compared with control. (exact p-value = 0.000)
MTT assay showed that the SW480 cell growth was increased when progranulin overexpressed and SW620 cell growth reduced when progranulin was knocked down compared with controls (Figure 2A). The growth of SW620 (Prog-) cells decreased by 16.83% (n=6, p<0.001), and the SW480 (Prog+) cell growth increased by 62.26% (n=6, p<0.001) at 48 hours. These results indicate a specific role for progranulin in cell growth of human colon cancer cells.

The anchorage-independent cell proliferation was analysed by a colony formation assay in soft agar (Figure 2B). In the SW480 (Prog+) cells colony number increased by 57.75%, from 258 ±18 to 407 ±25 (n=6, p<0.001), the colony size increased by 63.64% from 88 ±16 to 144 ±21 (n=6, p<0.001), and in SW620 (Prog-) cells, colony number decreased by 62.56%, from 406 ±29 to 152 ±13 (n=6, p<0.001), colony size decreased by 65.71% from 140 ±11 to 48 ±9 (n=6, p<0.001).

Progranulin promoted the migration and invasion of human colon cancer cells. Since cancer cell mobility is associated with metastatic potential, the effect of progranulin was next evaluated on cell migration. As shown in Figure 3, SW480 (Prog+) cell migration rates were increased as compared with control cells by about 216.81% (n=3, p<0.001), and the cell migration rates were decreased in SW620 (Prog-) cells by 77.33% (n=3, p<0.001). In addition, the decreased cell migration were completely recovered when exogenous progranulin (400 ng/ml) was added. We further explored the roles of progranulin in cell invasion. SW480 (Prog+) cell invasion abilities were increased as compared with control cells by 616.07% (n=3, p=0.005), and the cell invasion rates were decreased in SW620 (Prog-) cells by 71.92% (n=3, p<0.001). When exogenous progranulin (400 ng/ml) was added, the decreased cell invasions were recovered (Figure 3). To estimate the effect of proliferation rate in similar conditions on migration and invasion assays, cell count was performed after 24 hours of starvation. These results clearly demonstrate that progranulin has an effect on migration and invasion of human colon cancer cells.

Neamine inhibited progranulin-induced migration and invasion. We evaluated the migration and invasion of neamine stimulated cells. The cell migration rates were decreased by 52.12% in SW480 (Prog+) cells stimulated with 300 μmol/L neamine compared with un-stimulated SW480 (Prog+) cells (n=3, p<0.001; Figure 3). The invasion rates of neamine stimulated SW480 (Prog+) cells were decreased by 84.04% compared with un-stimulated SW480 (Prog+) cells (n=3, p <0.001; Figure 3).

DISCUSSION

As a secreted glycoprotein, progranulin was found because of its ability to stimulate its proliferation by an autocrine fashion in highly malignant mouse teratoma-derived cell line PC.1,2 Subsequent studies had mainly focused on how it expressed in different types of cancers, such as breast cancer, glioblastoma and so on.3-6 Colon cancer is the most common malignant neoplasms. In this study, we indicated that the growth factor progranulin might play a significant role in human colon cancer formation and progression by promoting cell growth, migration, and invasion.

The presently reported experiments showed that, progranulin could directly contribute to the cell growth of human colon cancer cells. Cell growth was obviously decreased in progranulin knocked down cells and increased in progranulin overexpressing cells. Most deaths from malignant tumor are due to metastases. The metastatic process involves a sequence of events, and...
the migration and invasion of cancer cells are key steps in this process. The transwell migration and invasion assay showed that the migration and invasion abilities of human colon cancer cells are positively related to the progranulin levels. Migration and invasion rates were increased when progranulin was overexpressed in low metastatic potential SW480 cells and decreased when progranulin was knocked down in high metastatic potential SW620 cells. In addition, exogenous progranulin could recover the decreased migration and invasion completely.

Most cancer cells (such as ovarian cancer cells, breast cancer cells, adrenal tumor cells and prostate cancer cells) originated from epithelial tissue highly express progranulin. The promotion of progranulin on cell proliferation, migration, and invasion is strictly controlled by intracellular and extracellular signals. When cells were stimulated by external factors or physiological function changed, progranulin may play its role through multiple signal transduction pathways. It has been demonstrated that progranulin stimulates proliferation in breast cancer cells, and progranulin was shown to mediate the mitogenic activity of estrogen by stimulating cyclin D1 expression. He has reported that progranulin mediates tissue repair and tumorigenesis and these biological activities are mediated via activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. However, the effects of progranulin on human colon cancer have not been reported before, and the mechanism is not clear. It would be worth examining that whether it is the common mechanism for progranulin in tumor cell growth.

It has been reported that progranulin stimulated migration, invasiveness and up-regulated matrix metalloprotease-9 expression. In addition, progranulin stimulates the cell invasion, increases the expression of matrix metalloproteinase 13 and 17 and protects against anoikis. They also reported that focal adhesion kinase (FAK) signaling pathways was activated by progranulin to promote cell invasion. FAK is known to play a role in a wide variety of biological functions including cell spreading, cell migration, cell proliferation, cell survival and apoptosis, and FAK has been identified as a key player in integrin signaling pathway and cytoskeletal proteins signaling pathway. Serrero has reported that progranulin has been found to stimulate VEGF expression in breast cancer cells. VEGF is a potent tumor angiogenic factor and plays an important role in tumor progress. Since progranulin can activate a variety of signalling pathways, it was presumption that progranulin specific receptor may exist on the cell membrane, but there is still no defined reports about progranulin receptor. Tumor cell migration and invasion are regulated by multiple factors. However, the mechanism of the promotion of progranulin on human colon cancer cells migration and invasion still needs further study.

Neamine is a degradation product of neomycin. The toxicity profile of neamine is ~20-fold less toxic than neomycin, which is close to that of streptomycin and kanamycin. It means that neamine is suitable for being an agent. It has been reported that neamine has an antiangiogenic activity, and the nuclear translocation of angiogenin was blocked by neamine in endothelial cells and PC-3 cells. It has also been reported that progranulin is involved in regulation of transcription. Progranulin can directly transfer to the nucleus, then combine to transcription elongation factor P-TEFb and promote phosphorylation of RNase II. Whether neamine affects nuclear translocation of progranulin or affects downstream kinase activity such as FAK, or there are some interactions between progranulin and angiogenin, the mechanism that neamine inhibits progranulin-induced migration and invasion is still undefined, and this will be investigated in our further work.

CONCLUSION

Progranulin promotes the growth, migration and invasion of human colon cancer cells and suppressed progranulin expression or stimulated by neamine induces obvious decrease of growth, migration, and invasion. These results suggest that progranulin may serve as a molecular target in the treatment of human colon cancer and neamine might be a promising candidate as a therapeutic agent.

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REFERENCES


