World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i12.1465

World J Gastroenterol 2010 March 28; 16(12): 1465-1472
ISSN 1007-9327 (print)

ORIGINAL ARTICLE

Expression and functional characterization of platelet-derived growth factor receptor-like gene

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Supported by The National Natural Science Foundation of China, No. 30672352
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Received: November 14, 2009 Revised: December 19, 2009
Accepted: December 26, 2009
Published online: March 28, 2010

Abstract

AIM: To investigate the role of platelet-derived growth factor receptor-like gene (*PDGFRL*) in the anti-cancer therapy for colorectal cancers (CRC).

METHODS: *PDGFRL* mRNA and protein levels were measured by reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry in CRC and colorectal normal tissues. *PDGFRL* prokaryotic expression vector was carried out in *Escherichia coli* (*E. coli*), and purified by immobilized metal affinity chromatography. The effect of *PDGFRL* protein on CRC HCT-116 cells was detected by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), clone counting, cell cycle, and wound healing assay.

RESULTS: Both RT-PCR and immunohistochemistry showed that the expression of *PDGFRL* in colorectal normal tissues was higher than in cancer tissues. Recombinant *pET22b-PDGFRL* prokaryotic expression vector was successfully expressed in *E. coli*, and the target protein was expressed in the form of inclusion bodies. After purification and refolding, recombinant human *PDGFRL* (*rhPDGFRL*) could efficiently inhibit the proliferation and invasion of CRC HCT-116 cells detected by MTT, clone counting and wound healing assay. Moreover, *rhPDGFRL* arrested HCT-116 cell cycling at the G0/G1 phase.

CONCLUSION: *PDGFRL* is a potential gene for application in the anti-cancer therapy for CRC.

Key words: Platelet-derived growth factor receptor-like; Colorectal cancer; Prokaryotic expression; Reverse transcription-polymerase chain reaction; Immunohistochemistry

Peer reviewers: Dr. Lucia Ricci Vitiani, Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Viale Regina Elena, 299, Rome, 00161, Italy; Dr. Abdel-Majid Khatib, PhD, INSERM, UMRS 940, Equipe Avenir, Cibles Thérapeutiques, IGM 27 rue Juliette Dodu, 75010 Paris, France

Guo FJ, Zhang WJ, Li YL, Liu Y, Li YH, Huang J, Wang JJ, Xie PL, Li GC. Expression and functional characterization of platelet-derived growth factor receptor-like gene. *World J Gastroenterol* 2010; 16(12): 1465-1472 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i12/1465.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i12.1465

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors in the world. It is estimated that 783 000 new cases are diagnosed each year, and the number has increased rapidly since 1975[1]. CRC is the third most common cancer and the second leading cause of cancer related mortality in the Western world. The incidence of CRC in China has increased rapidly over the past few decades[2]. The molecular mechanism of carcinogenesis and development of CRC is still not fully understood.

Tumor suppressor genes are genes that can slow down cell division, repair DNA errors, and tell cells when to die (a process known as apoptosis or programmed cell death). When tumor suppressor genes do not work properly, cells can grow out of control, leading to cancer. About 30 tumor suppressor genes have been identified, including *p53*, *BRCA1*, *BRCA2*, *APC*, RB1, and platelet-derived growth factor receptor-like gene (*PDGFRL)*. Tumor suppressor genes cause cancers when they are inactivated, and encode proteins that normally serve as a brake on cell growth. When such genes are mutated, the brake may be lifted, resulting in the runaway cell growth known as cancer. Gain of oncogene function associated with loss of tumor suppressors is now widely accepted as a hallmark of cancer initiation and progression[3].

PDGFRL is located in chromosome 8p21.3-8p22, which is commonly deleted in sporadic hepatocellular carcinoma, CRC, breast cancer, and non-small cell lung cancers[4,5]. While its precise biological function is not known, *PDGFRL* encodes a 375aa product with significant sequence similarity to the ligand-binding domain of platelet-derived growth factor receptor β. Mutations in *PDGFRL* have been found in individual cancer samples[6-9]. An in-depth study using micro-cell-mediated chromosome transfer found that *PDGFRL* expression is decreased in the majority of breast cancer cells[10]. Recently, *PDGFRL* is identified to play a central role in the tumor suppressor network by adopting a network perspective[11]. Furthermore, *PDGFRL* was found to be involved in the suppression of the tumor metastatic phenotype as a strong candidate gene[12].

To further identify new genes involved in the pathogenesis of CRC, we analyzed the expression of *PDGFRL* in CRC tissues and normal tissues by both reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry, and found that *PDGFRL* was expressed at higher levels in normal tissues than in CRC tissues. We constructed *pET22b-PDGFRL* recombinant prokaryotic expression vector before expression and purification of the *PDGFRL* recombinant protein were completed. The biological features of protein were identified to inhibit the proliferation and invasion of CRC HCT-116 cells by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), clone counting, and wound healing assay. In addition, *rhPDGFRL* arrested HCT-116 cell cycling at the G0/G1 phase.

MATERIALS AND METHODS

Cell culture

HCT-116 CRC cells and human umbilical vein endothelial cells (HUVEC) were maintained by our lab and cultured in Dulbecco-modified Eagle medium (DMEM; Gibco) supplemented with 10% bovine calf serum (BCS) (Gibco). Cells were maintained at 37℃ in an atmosphere of humidified air with 50 mL/L CO_2.

Collection of tissues

Fifteen samples of CRC tissues and paired non-cancerous tissues (5 cm away from tumor) were obtained from the First Hospital of Changsha. Written consent was obtained from the patients, who agreed with the collection of tissue samples. The resected tissue samples were immediately cut into small pieces, and snap frozen in liquid nitrogen until use. All tumor tissue and paired non-cancerous tissue samples were pathologically confirmed.

RT-PCR

RNA isolated from tissues and cells was reversibly transcribed and amplified using the RT-PCR System (Fermentas). Primer sequences used were sense 5'-CAAGAAGGTGAAGCCCAAAAT-3' and antisense 5'-ACAAGGAACCACAGCCTGTCT-3' for *PDGFRL*. A 587-bp Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) fragment was amplified as an internal control. For *GAPDH*, the forward primer 5'-AATCCCA TCACCATCTTCCA-3', and the reverse primer 5'-CC TGCTTCACCACCTTCTTG-3' were used. After heating at 95℃ for 1 min, PCRs were exposed to 30 cycles (*GAPDH*, 25 cycles) of 95℃ for 30 s, 60℃ for 30 s, and 68℃ for 1 min and 30 s with a final extension at 68℃ for 10 min. The relative mRNA levels were normalized to that of *GAPDH* and the ratio of *PDGFRL* to *GAPDH* was calculated.

Immunohistochemistry

Paraffin sections were deparaffinized with xylene and rehydrated in graded alcohol. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide at reverse transcription (RT) for 10 min. Non-specific binding was blocked with phosphate buffered saline Tween-20 (PBST) containing 10% goat serum for 2 h at RT. *PDGFRL* antibody (Abcam) was added to each slide and incubated at 4℃ overnight. Following three washes, slides were incubated with Envision (DAKO) for 40 min at RT. Diaminobenzidine was used as a chromogen. Sections were counterstained with hematoxylin, dehydrated, and mounted. Evaluation of immunohistochemical slides was done with a Nikon Eclipse E800 microscope at × 100 magnification. The intensity of the staining was scored on a scale of 0 to 3+ where 0, 1, 2 and 3 represented no staining and weak, moderate or strong staining, respectively. The mean staining scores for tissues and the mean fold change in protein expression was calculated.

Plasmid construction

A DNA fragment encoding the gene *PDGFRL* was amplified by PCR using a sense primer, 5'-TGAG CCATGGATCAACACCTTCC-3', and an anti-sense primer, 5'-AAGCTCGAGGGAAAACTCAACAGT-3'. The primers were introduced to an *Nco*I site (sense) and an *Xho*I site (anti-sense), respectively. The amplification

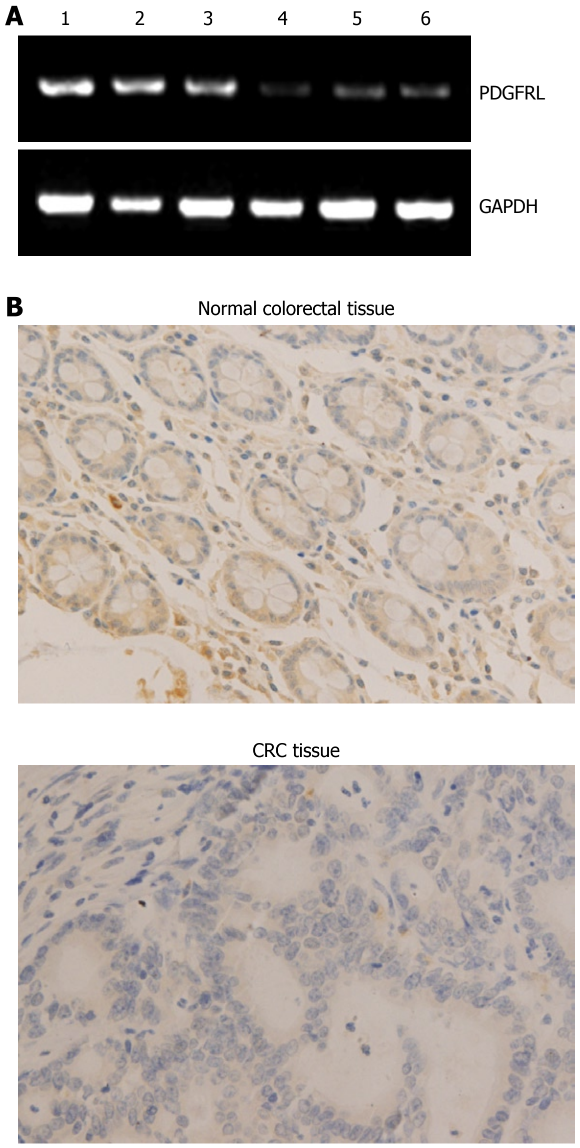

Figure 1 Expression of platelet-derived growth factor receptor-like gene (*PDGFRL*) in colorectal cancer and normal tissues. A: Reverse transcription-polymerase chain reaction (RT-PCR) analysis shows that mRNA levels in colorectal cancer tissues were lower than in normal tissues. 1-3: Normal colorectal tissues; 4-6: Colorectal cancer tissues; B: Immunohistochemical analysis illustrates that immunoreaction signal of *PDGFRL* in cancer tissues was weak compared with normal tissues. (Magnification, × 100). GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; CRC: Colorectal cancers.

was performed using 2 × pfu PCR MasterMix (MBI Fermantas) on an Eppendorf PCR instrument. The PCR products were purified by 1% agarose gel electrophoresis and double digested and ligated into the expression vector pET22b(+), resulting in a *pET22b-PDGFRL* plasmid with the sequence encoding the gene *PDGFRL*. The constructed plasmid was transformed into competent *Escherichia coli* (*E. coli*). DH5α cells and the transformed strains were grown in Luria-Bertani (LB) broth supplemented with ampicillin (100 μg/mL). All strains were incubated at 37℃ with constant shaking (220 r/min). The recombinant was identified by PCR, double endonuclease digestion and DNA sequencing.

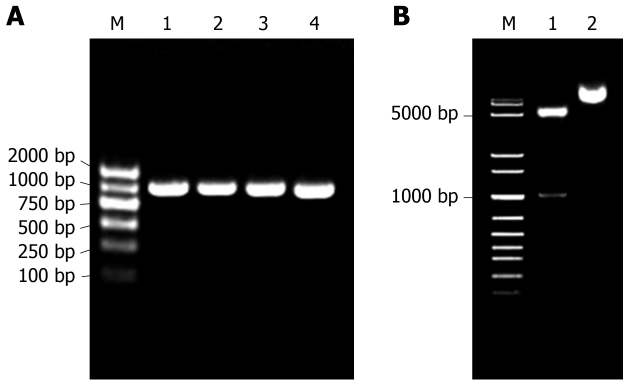

Figure 2 Construction of *pET22b-PDGFRL*. A: Bacterial colony PCR for detection of DH5a clones with prokaryotic recombinant expression vector *pET22b-PDGFRL*. M: DNA ladder; 1-4: Positive bacterial colonies; B: Double endonuclease digestion of the recombinant vector *pET22b-PDGFRL*. M: DNA ladder; 1: Double digestion with *NcoI/XhoI*; 2: *pET22b-PDGFRL* without digestion.

Bacterial expression of recombinant human PDGFRL (rhPDGFRL)

The recombinant plasmid *pET22b-PDGFRL* was transformed into *E. coli* expression strain BL21 (DE3) cells. One colony was picked up and was grown in 3 mL LB rich medium containing 100 mg/L ampicillin. After 8 h, 1 mL of the BL21 (DE3) cells were introduced into 100 mL of LB medium containing 100 mg/L ampicillin. Bacteria were grown at 37℃ until an A_{600} of 0.6 was reached. Then, isopropyl-b-D-thiogalactopyranoside (IPTG) was added to induce protein expression at 30℃. To check the expression of *pET22b-PDGFRL*, *E. coli* were induced at different final concentrations of IPTG such as 0.1, 0.5, 1.0, 1.5 and 2.0 mmol/L, and different time points such as 1, 2, 4 and 6 h, respectively. The cells were harvested by centrifugation at 4800 × *g* for 30 min and the pellet was resuspended in 50 mmol/L sodium phosphate, 0.3 mol/L NaCl, pH 8.0. The resuspended cells were lysed by sonication. The cells after lysis were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Purification of of rhPDGFRL

The protein was further purified by immobilized metal affinity chromatography (MagExtractor® His-tag protein purification kit, TOYOBO). The extracts were fractionated by gel filtration column chromatography (AKTA explorer 10S with HiLoad 16/60 Superdex 75 pg column, GE Healthcare) in 50 mmol/L Tris-HCl (pH 7.5) and 100 mmol/L NaCl. These fractionated extracts were desalinized using Slide-A-Lyzer® dialysis cassettes (Pierce Biotechnology), separated by denaturing SDS-PAGE, and stained with Coomassie brilliant blue (CBB). Western blotting was performed using an anti -His antibody (Abcam)[13].

MTT assay

MTT assay was performed to measure cell viability and proliferation in external factors. The 3rd generation

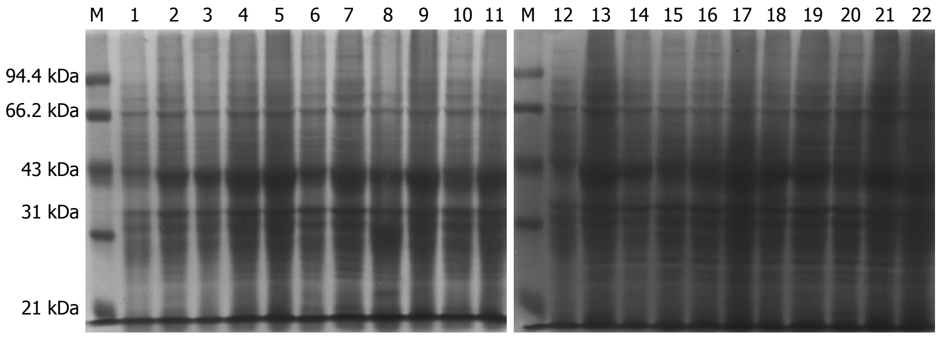

Figure 3 Protein expression in *Escherichia coli* containing *pET22b-PDGFRL*. Time course and different concentrations of isopropyl-b-D-thiogalactopyranoside (IPTG) analysis of *pET22b-PDGFRL* protein expression by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). M: Molecular weight standards; 1, 12: Uninduced bacterial lysate; 1-h (2-6), 2-h (7-11), 3-h (13-17), 4-h (18-22) induced samples at different concentrations of IPTG culture (0.1, 0.5, 1.0, 1.5, 2.0 μg/mL).

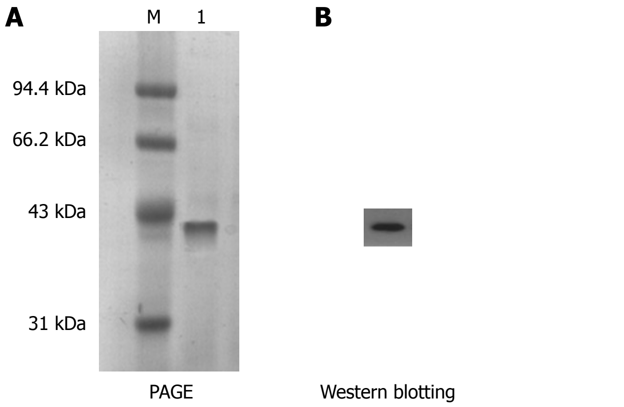

Figure 4 Purification of His-*PDGFRL* recombinant protein. A: Purification of His-tagged His-*PDGFRL* recombinant protein by immobilized metal affinity chromatography. M: Molecular weight standards; 1: The purified protein; B: Purification of recombinant protein confirmed by immunoblotting using anti-His-Tag antibody.

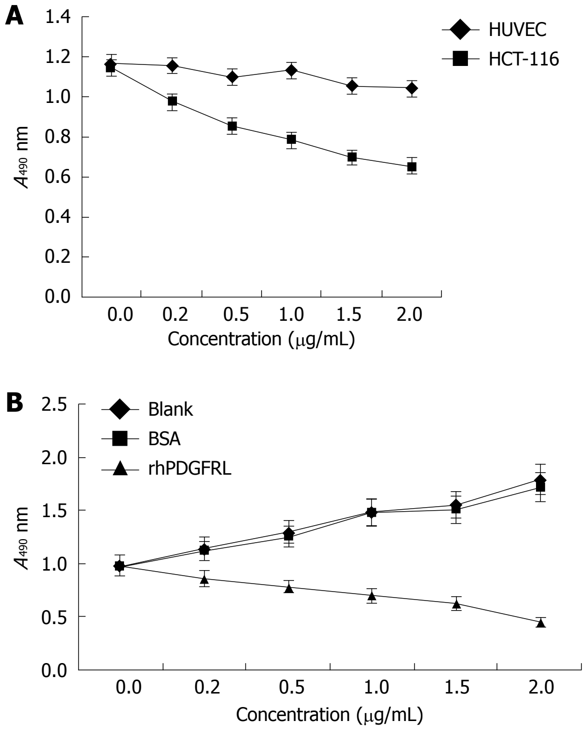

Figure 5 *PDGFRL* inhibits HCT-116 cell proliferation. A: MTT assay shows that HCT-116 cells grew slowly compared with human umbilical vein endothelial cells (HUVEC) cells after *PDGFRL* protein treatment, P < 0.05, HCT116 *vs* HUVEC; B: HCT-116 cells were treated with *rhPDGFRL* (HCT-116/*rhPDGFRL*) or bovine serum albumin (BSA) (HCT-116/BSA), P < 0.05, rhPDGFRL *vs* BSA. Data are expressed as mean ± SD of three independent experiments.

human HUVEC and HCT-116 cells were made into cell suspension with a density of 1 × 10^4/mL, which was inoculated into 96-well plates separately. The purified *rhPDGFRL* with different concentrations (0, 0.5, 1.0, 1.5 and 2.0 μg/mL, suspended in DMEM plus 1% BCS) was added to HUVEC and HCT-116 cells. After two days, 5 mg/mL MTT was added into the wells and incubated at 37℃ for 3 h. The supernatant was blotted and added with DMSO (dimethyl sulfoxide). Absorbance of the dye was measured at a wavelength of 490 nm on a Microplate Reader. HCT-116 cells were then treated with *rhPDGFRL* or bovine serum albumin (BSA) (suspended in DMEM plus 1% BCS) and MTT assay was performed.

Colony formation assay

Crystal violet (CV) staining of cells and clone counting were used to measure cell proliferation. Dilute the HCT-116 cells into a 6-well plate separately and each pole contained 1000 cells. The *rhPDGFRL* or BSA (1.5 μg/mL) was added to HCT-116 cells. After 2 wk, 0.05% CV was added into the plates. The cells were fixed for 10 min with 4% Paraformaldehyde (PFA) and stained for 30 min with 0.05% CV. The plates were carefully rinsed in ddH2O until no color appeared. Clone forming efficiency for individual type of cells was calculated according to the number of colonies/number of inoculated cells × 100%.

Flow cytometry of cell cycle

The impact of *rhPDGFRL* on the HCT-116 cell cycling was examined by flow cytometry. HCT-116 cells indicated were seeded into a 6-well plate at a density of 3.5 × 10^5 cell/well. Once the cells were grown at 70%-80% confluence, HCT-116 cells were treated with the *rhPDGFRL* or BSA (1.5 μg/mL). Cells were harvested at 48 h and resuspended in fixation fluid at a density of 10^6/mL, 1500 μL propidium iodide (PI) solution was added, and

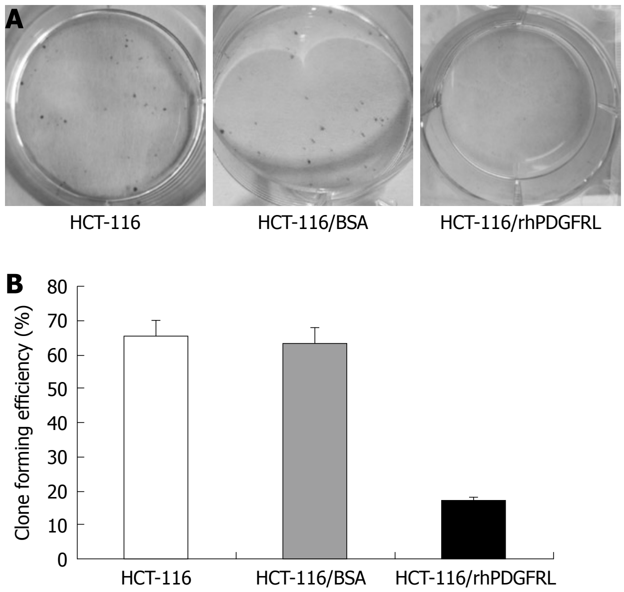

Figure 6 Crystal violet (CV) staining of cells and clone counting assay. A: CV staining of HCT-116 cells; B: Quantitative analysis of colony formation. Data are expressed as the efficiency of colony formation (%) and expressed as the mean ± SD of three separated experiments, $P < 0.05$, HCT-116/rhPDGFRL *vs* HCT-116/BSA.

the cell cycle was detected by FACS Caliber (Becton Dickinson).

Monolayer wound healing assay

Wound healing assay was applied to measure cell invasion. HCT-116 cells indicated were seeded into a 6-well plate at a density of 3.5×10^5 cell/well. Once the cells were grown to a monolayer, a wound was made and the *rhPDGFRL* or BSA (1.5 μg/mL) was added to the HCT-116 cells. The distance of cell migration was calculated by subtracting the distance between the lesion edges at 48 h from the distance measured at 0 h. The relative migrating distance of cells was measured by the distance of cell migration/the distance measured at 0 h.

RESULTS

Expression of PDGFRL in CRC and normal tissues

To verify *PDGFRL* in CRC and normal tissues, we did RT-PCR and immunohistochemical analysis in 15 human CRC and adjacent normal tissue samples. In RT-PCR analysis, mRNA levels in CRC tissues were lower than in normal tissues (Figure 1A). By immunohistochemical analysis, immunoreaction signal of *PDGFRL* in cancer tissues was weak compared with normal tissues (Figure 1B). Semi-quantitative analysis of mRNA and protein expression for *PDGFRL* was performed in CRC and adjacent normal tissue samples. The relative mRNA expression of PDGFRL in CRC tissues was 0.11, but the one in the adjacent normal tissues was 0.78. Likewise, the relative protein level of PDGFRL in adjacent normal tissues was 8.2, but the one in CRC tissues was only 1.3. Both RT-PCR and immunohistochemistry showed that the expression of *PDGFRL* in colorectal normal tissues was higher than in cancer tissues based on the identification of *PDGFRL* as a tumor suppressor.

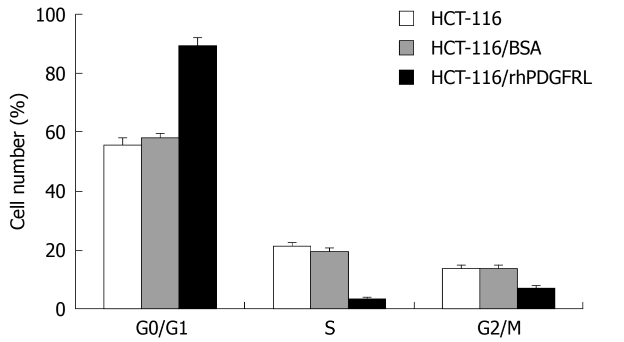

Figure 7 Analysis of cell cycle. HCT-116, HCT-116/BSA, and HCT-116/*rhPDGFRL* cells were fixed with 70% ethanol and stained with PI, followed by FACS analysis. Data are expressed as mean ± SD of three independent experiments, $P < 0.05$, HCT-116/rhPDGFRL *vs* HCT-116/BSA.

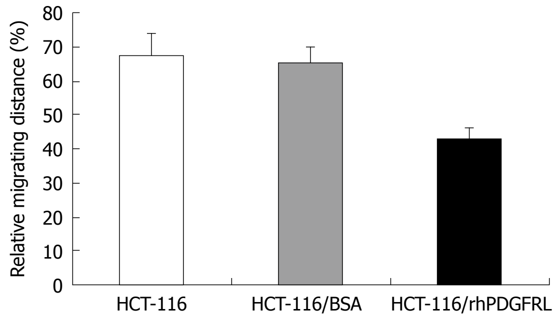

Figure 8 Measurement of migration distance in HCT-116, HCT-116/BSA, and HCT-116/*rhPDGFRL* cells. Data are expressed as the mean ± SD of three independent experiments, $P < 0.05$, HCT-116/rhPDGFRL *vs* HCT-116/BSA.

Construction of pET22b-PDGFRL

As shown in Figure 2, the prokaryotic expression recombinant *pET22b-PDGFRL* was successfully constructed using bacterial colony PCR (Figure 2A), restriction enzyme digestions (Figure 2B) and complete sequencing (data not shown).

Expression and purification of pET22b-PDGFRL recombinant protein

The prokaryotic expressive vector *pET22b-PDGFRL* was transformed into the *E. coli* BL21 (DE3) expression host strain for protein over-expression. Several potential clones were identified with DNA sequencing (data not shown). The expression and purification were identified by, respectively, running the crude lysate and the elution fractions on a 12% SDS-PAGE gel and subsequently staining with Coomassie brilliant blue. Experiments of IPTG concentration and time course were performed to determine the kinetics of protein expression in the bacterial culture (Figure 3). As a result, the cells should be harvested 4 h after 0.1 mmol/L IPTG induction, as the largest amount of the correct 42 kDa size *pET22b-PDGFRL* protein was produced at this time and concentration point.

The expressed protein at 0.1 mmol/L IPTG for 4 h

A

1	CCTGCGTCCC	CGCCCCGCGC	AGCCGCCGCG	CTCCTGCGCT	CCGAGGTCCG	AGGTTCCCGA
61	GATGAAGGTC	TGGCTGCTGC	TTGGTCTTCT	GCTGGTGCAC	GAAGCGCTGG	AGGATGTTAC
121	TGGCCAACAC	CTTCCCAAGA	ACAAGCGTCC	AAAAGAACCA	GGAGAGAATA	GAATCAAACC
181	TACCAACAAG	AAGGTGAAGC	CCAAAATTCC	TAAAATGAAG	GACAGGGACT	CAGCCAATTC
241	AGCACCAAAG	ACGCAGTCTA	TCATGATGCA	AGTGCTGGAT	AAAGGTCGCT	TCCAGAAACC
301	CGCCGCTACC	CTGAGTCTGC	TGGCGGGGCA	AACTGTAGAG	CTTCGATGTA	AAGGGAGTAG
361	AATTGGGTGG	AGCTACCCTG	CGTATCTGGA	CACCTTTAAG	GATTCTCGCC	TCAGCGTCAA
421	GCAGAATGAG	CGCTACGGCC	AGTTGACTCT	GGTCAACTCC	ACCTCGGCAG	ACACAGGTGA
481	ATTCAGCTGC	TGGGTGCAGC	TCTGCAGCGG	CTACATCTGC	AGGAAGGACG	AGGCCAAAAC
541	GGGCTCCACC	TACATCTTTT	TTACAGAGAA	AGGAGAACTC	TTTGTACCTT	CTCCCAGCTA
601	CTTCGATGTT	GTCTACTTGA	ACCCGGACAG	ACAGGCTGTG	GTTCCTTGTC	GGGTGACCGT
661	GCTGTCGGCC	AAAGTCACGC	TCCACAGGGA	ATTCCCAGCC	AAGGAGATCC	CAGCCAATGG
721	AACGGACATT	GTTTATGACA	TGAAGCGGGG	CTTTGTGTAT	CTGCAACCTC	ATTCCGAGCA
781	CCAGGGTGTG	GTTTACTGCA	GGGCGGAGGC	CGGGGGCAGA	TCTCAGATCT	CCGTCAAGTA
841	CCAGCTGCTC	TACGTGGCGG	TTCCCAGTGG	CCCTCCCTCA	ACAACCATCT	TGGCTTCTTC
901	AAACAAAGTG	AAAAGTGGGG	ACGACATCAG	TGTGCTCTGC	ACTGTCCTGG	GGGAGCCCGA
961	TGTGGAGGTG	GAGTTCACCT	GGATCTTCCC	AGGGCAGAAG	GATGAAAGGC	CTGTGACGAT
1021	CCAAGACACT	TGGAGGTTGA	TCCACAGAGG	ACTGGGACAC	ACCACGAGAA	TCTCCCAGAG
1081	TGTCATTACA	GTGGAAGACT	TCGAGACGAT	TGATGCAGGA	TATTACATTT	GCACTGCTCA
1141	GAATCTTCAA	GGACAGACCA	CAGTAGCTAC	CACTGTTGAG	TTTTCCTGAC	TTGGAAAAGG
1201	AAATGTAATG	AACTTATGGA	AAGCCCATTT	GTGTACACAG	TCAGCTTTGG	GGTTCCTTTT
1261	ATTAGTGCTT	TGCCAGAGGC	TGATGTCAAG	CACCACACCC	CAACCCCAGC	GTCTCGTGAG
1321	TCCGACCCAG	ACATCCAAAC	TAAAAGGAAG	TCATCCAGTC	TATTCACAGA	AGTGTTAACT
1381	TTTCTAACAG	AAAGCATGAT	TTTGATTGCT	TACCTACATA	CGTGTTCCTA	GTTTTTATAC
1441	ATGTGTAAAC	AATTTTATAT	AATCAATCAT	TTCTATTAAA	TGAGCACGTT	TTTGTAAAAA
1501	AT					

B

Met	Lys	Val	Trp	Leu	Leu	Leu	Gly	Leu	Leu	Leu	Val	His	Glu	Gla	Leu	Glu	Asp	Val	Thr	Gly
ATG	AAG	GTC	TGG	CTG	CTG	CTT	GGT	CTT	CTG	CTG	GTG	CAC	GAA	GCG	CTG	GAG	GAT	GTT	ACT	GGC

Figure 9 PDGFRL protein contains a putative signal peptide of 21aa. A: *PDGFRL* cDNA sequences contain an open reading frame (ORF) of 1128-base pairs that is matched in bold; B: ORF encodes a protein of 375 amino acids (aa) with a putative signal peptide of 21aa.

was further purified by immobilized metal affinity chromatography. The electrophoretic analysis revealed that the His-tagged His-*PDGFRL* recombinant protein was purified to near homogeneity and migrated as a 42 kDa band (Figure 4A). Moreover, the purification of the recombinant protein was confirmed by immunoblotting using anti-His-Tag antibody (Figure 4B).

PDGFRL inhibits HCT-116 cell proliferation

MTT assay was performed to measure the effect on cell viability. HCT-116 cells were found to grow slowly compared with HUVEC cells after *PDGFRL* protein was added (Figure 5A). The proliferating ability of HCT-116 cells decreased gradually with an increasing concentration of *PDGFRL*, but normal cell line HUVEC had no evident change in proliferation. Next, we treated HCT-116 cells with *rhPDGFRL* or BSA, and found that the cell viability of HCT-116 treated with *rhPDGFRL* (HCT-116/*rhPDGFRL*) decreased compared with that treated with BSA (HCT-116/BSA), which was not distinguishable from blank group (Figure 5B). A similar pattern of inhibitory effect of *rhPDGFRL* in HCT-116 cells was achieved in colony formation assay (Figure 6). Following incubation for 2 wk, a few colonies from HCT-116/*rhPDGFRL* cells were generated compared with HCT-116 or HCT-116/BSA. Therefore, the low MTT activity and a small number of cell colonies from HCT-116/*rhPDGFRL* cells demonstrated that *rhPDGFRL* inhibited the growth of HCT-116 cells *in vitro*.

Impact of PDGFRL on HCT-116 cell cycle

To further explore the cause of the decrease in cell viability, we examined the effects of *PDGFRL* on cell cycle. As illustrated in Figure 7, HCT-116 cells treated with *PDGFRL* blocked the cell cycle in G1 phase. The G0/G1-phase fraction increased from 57.8% (HCT-116/BSA) to 89.4% (HCT-116/*rhPDGFRL*). These data indicated that *PDGFRL* arrested HCT-116 cell cycling at the G0/G1 phase, which may inhibit the growth of HCT-116 cells.

Effects of PDGFRL on the migration of HCT-116 cells

We examined the impact of *PDGFRL* on the migration of HCT-116 cells by the wound healing assay as shown in Figure 8. Following incubation of physically wounded cells for 48 h, the mobile distance of HCT-116/*rhPDGFRL* cells was found significantly shorter than that of controls.

DISCUSSION

Despite curative surgery, nearly 4 out of 10 patients with CRC experience disease relapse, 1 of 5 will develop liver metastases, and 1 of 12 will develop pulmonary metastases[14]. The survival of CRC patients is still a key issue to address and the need for drugs curing CRC is urgent.

The tools and concept of gene therapy are being applied to the development of new effective treatment strategies for human cancer[15]. Most human cancers are associated with multiple interacting and cooperating mutations in protooncogenes and tumor suppressor genes. Cancer therapies that target oncogenes usually seek to block or reduce their action, while those aimed at tumor suppressor genes seek to restore or increase their action. In several model systems, some features of the tumor phenotype can be suppressed *in vitro* through the restoration of expression of tumor suppressor genes such as *Rb* and *p53*[16,17]. It is interesting that most investigators have found that pVHL suppresses tumourigenicity in a nude mouse assay but not *in vitro*[18,19]. Protein phosphatase-2A (PP2A) has progressively been considered as a potential tumor suppressor. PP2A activation by forskolin, 1,9-dideoxy-forskolin and FTY720 effectively antagonize leukemogenesis in both *in vitro* and *in vivo* models of these cancers[20-22]. PP2A is now a highly promising target for developing a new series of anticancer agents potentially capable of overcoming the drug-resistance[23]. It is necessary to identify new genes applied in the treatment for CRC.

In this study, both RT-PCR and immunohistochemistry showed that the expression of *PDGFRL* in colorectal normal tissues was higher than in cancer tissues based on the identification of *PDGFRL* as a tumor suppressor. To investigate the role of *PDGFRL* in the anti-cancer therapy for CRC, pET-22b (+) prokaryotic expression vector was used to construct *rhPDGFRL*.

The pET-22b (+) vector carries an N-terminal pelB signal sequence for potential periplasmic localization, plus optional C-terminal His-tag sequence. However, in our study, periplasmic secretion of *PDGFRL* protein was too small to collect the purified protein, and most of expressive proteins were produced in an insoluble form in *E. coli* (data not shown).

PDGFRL cDNA sequences contain an open reading frame (ORF) of 1128-base pairs encoding a protein of 375 amino acids (aa) with a putative signal peptide of 21aa (Figure 9). The signal peptide could not be expressed stably in prokaryotic expression vector in high yield, and therefore the signal peptide was deleted in the construction of the recombinant *pET22b-PDGFRL*[24,25].

In this report, *in vitro* bioactivity of *PDGFRL* was determined by MTT, clone counting, cell cycle and wound healing assay. When *PDGFRL* protein was added to HCT-116 cells, MTT, clone counting and wound healing assay showed that proliferation and invasion of HCT-116 cells decreased. This result indicated that *PDGFRL* as a tumor suppressor inhibited the growth of CRC cells. In addition, *PDGFRL* arrested HCT-116 cell cycling at the G0/G1 phase. The results of this study extended our previous knowledge of *PDGFRL* as a tumor suppressor in CRC. Further characterization of *PDGFRL* will provide new insights into the role of *PDGFRL* in the molecular pathogenesis and therapy of CRC.

ACKNOWLEDGMENTS

We thank Dr. Di Wang, Department of Pathology, The First Hospital of Changsha, for supplying tumor and normal adjacent tissue sections.

COMMENTS

Background

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer related mortality in the Western world. The incidence of CRC in China has increased rapidly over the past few decades. The molecular mechanism of human carcinogenesis and development of CRC is still not clear.

Research frontiers

Mutations in platelet-derived growth factor receptor-like gene (*PDGFRL*) as a tumor suppressor have been found in individual cancer samples and *PDGFRL* expression is decreased in the majority of breast cancer cells. Recently *PDGFRL* is identified to play a central role in the tumor suppressor network by adopting a network perspective. Furthermore, *PDGFRL* was found to be involved in the suppression of the tumor metastatic phenotype as a strong candidate gene.

Innovations and breakthroughs

This study extended the previous knowledge of *PDGFRL* as a tumor suppressor in CRC.

Applications

Further characterization of *PDGFRL* will provide new insights into the role of *PDGFRL* in the molecular pathogenesis and therapy of CRC.

Terminology

PDGFRL is located in chromosome 8p21.3-8p22, which is commonly deleted in sporadic hepatocellular carcinoma, CRC, breast cancer, and non-small cell lung cancer.

Peer review

By different methods, the authors analyzed the role of platelet-derived growth factor receptor-like gene in CRC and its possible application in anti-cancer therapy. The manuscript is well-written and the study is conducted appropriately.

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S- Editor Wang JL **L- Editor** Ma JY **E- Editor** Lin YP