Platelet-derived growth factor receptor-β gene expression relates to recurrence in colorectal cancer

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Received September 22, 2017; Accepted February 12, 2018

DOI: 10.3892/or.2018.6290

Abstract. Platelet-derived growth factor receptor-β (PDGFR-β) in epithelial tumors is mainly expressed by stromal cells. High expression of *PDGFR-β* has been related to poor prognosis in several cancers, however its significance in colorectal cancer (CRC) remains unknown. The present study aimed to clarify the prognostic impact of *PDGFR-β* in CRC patients. The study included 194 patients who underwent surgery for CRC. *PDGFR-β* expression was examined by real-time reverse transcription-polymerase chain reaction and immunohistochemistry and the expression levels were correlated with various clinical parameters. The biological significance was evaluated by knockdown experiments in CRC cell lines and the specific PDGFR inhibitor, crenolanib. *PDGFR-β* mRNA and protein expression levels were positively correlated with each other. Low *PDGFR-β* expression was associated with significantly better disease-free survival after curative surgical resection, than high *PDGFR-β* expression, according to univariate and multivariate analyses. The assessment of *PDGFR-β* knockdown in two cell lines revealed that small interfering RNA (siRNA) inhibition resulted in statistically significant reductions in cell growth and invasion. PDGFR inhibitor suppressed CRC cell proliferation *in vitro* in a dose-dependent manner. In conclusion, *PDGFR-β* expression was a risk factor for recurrence in patients with CRC and PDGFR inhibitor may be a useful therapeutic agent for CRC.

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Abbreviations: PDGFR, platelet-derived growth factor receptor; CRC, colorectal cancer; DFS, disease-free survival; OS, overall survival

Key words: colorectal cancer, metastasis, tyrosine kinase inhibitor, *PDGFR-β*, crenolanib, PDGFR inhibitor

Introduction

Cancer is a leading cause of death worldwide and both the numbers of cancer cases and cancer-related deaths are expected to continue to rise. There are currently an estimated 17 million deaths worldwide due to cancer per year (1), with colon, lung, breast, liver and stomach cancer being responsible for most cancer-related deaths. Colorectal cancer (CRC) is the second most frequent cancer in Europe (2) and the second most common cause of cancer-related deaths in the United States (3). CRC was also the leading cause of cancer-related deaths among women and the third leading cause among men in Japan as of 2013 and its incidence continues to increase (4). Surgical resection of the primary tumor and regional lymph nodes is an important treatment strategy for CRC and 5-year survival rates of 92% of patients in stage I, 85% in stage II and 72% in stage III have been reported following complete resection (5,6). However, recurrence occurred in 17.3% of these patients and distant metastases were the major cause of death in CRC patients, with a 5-year survival rate of only 19% in stage IV patients with distant metastases.

It is necessary to identify the genes responsible for CRC in order to identify new therapeutic targets. Multiple receptor tyrosine kinases and their growth factor ligands have recently been reported to play important roles in cancer progression and metastasis (2). Platelet-derived growth factor receptors (PDGFRs) belong to a family of cell surface type III receptor tyrosine kinases and have been reported to increase proliferation and migration in several malignant tumors (7-11). CRC tissue expresses PDGFR-α and PDGFR-β (12) and these factors were revealed to stimulate invasion and liver-metastasis formation in mice (13). Crenolanib is a highly selective PDGFR inhibitor (14) and low micromolar concentrations in plasma were achieved with no significant myelosuppression in a phase I study in patients with advanced cancer (15).

The present study examined the correlation between the expression of *PDGFR-β* in CRC tissues and clinicopathological factors and also examined the possible use of PDGFR inhibitors for the treatment of CRC.

Materials and methods

Clinical tissue samples for the analysis of PDGFR-β. A total of 194 patients with CRC were registered and

underwent resection of CRC and any distant metastases at Osaka International Cancer Institute from 2009 to 2013. None of the patients received chemotherapy or radiotherapy prior to surgery and none died of any other cancer. Primary CRC specimens and adjacent normal colorectal mucosa were obtained from the patients after obtaining their informed written consent, in accordance with the ethical guidelines of the Osaka International Cancer Institute. The surgical specimens were fixed in formalin, processed through graded ethanols, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). The degree of histological differentiation, lymphatic and venous invasion was examined. Pieces of all specimens were also frozen in liquid nitrogen immediately after resection and kept at -80˚C for RNA extraction. After surgery, the patients underwent follow-up blood examinations to assess tumor markers (serum carcinoembryonic antigen and cancer antigen 19-9) and imaging examinations (including abdominal ultrasonography, computed tomography and chest X-rays) every 3-6 months. Patients with stage III and stage IV lesions with no residual tumor (R0)-operation received adjuvant postoperative chemotherapy according to the Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines (5), following informed patient consent. The clinicopathological factors were assessed according to the tumor node metastasis (TNM) classification of the International Union Against Cancer (UICC) (16). The Review Board and Animal Research Committee of the Osaka International Cancer Institute approved the present study and written informed consents for the study were obtained from all participants according to the ethics guidelines of the Osaka International Cancer Institute.

RNA preparation and expression analysis. Total RNA was prepared using an RNA Purification kit (Qiagen GmbH, Hilden, Germany). Reverse transcription was performed with a Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics, Tokyo, Japan). A 92-bp *PDGFR-β* fragment was amplified. Two human *PDGFR-β* oligonucleotide primers were designed for the polymerase chain reaction (PCR) as follows: forward 5'-CAACTTCGAGTGGACATACCC-3' and reverse, 5'-AGCGGATGTGGTAAGGCATA-3'. PCR was also performed using primers specific for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, to normalize gene expression levels. The GAPDH primers (forward, 5'-AGC CACATCGCTCAGACAC-3' and reverse 5'-GCCCAATAC GACCAAATCC-3') produced a 66-bp amplicon. cDNA from the Human Reference Total RNA (Clontech Laboratories; Takara Bio USA, Inc., Palo Alto, CA, USA) and RNA extracted from NTERA-2 cancer cells were studied concurrently as positive controls. Quantitative assessment was performed by real-time reverse transcription-polymerase chain reaction (RT-PCR) using a Universal ProbeLibrary platform (Roche Diagnostics) and a FASTStart TaqMan Probe Master (Roche Diagnostics) for the cDNA amplification of the target genes (Table I). The expression ratios of *PDGFR-β* mRNA copies in tumor and normal tissues were calculated after normalization against GAPDH mRNA expression.

Immunohistochemistry. Twenty-one formalin-fixed, paraffin-embedded CRC surgical specimens were selected randomly for immunohistochemical detection of PDGFR-β. After deparaffinization and blocking, the sections were incubated with primary anti-PDGFR-β rabbit polyclonal antibody (cat. no. 4564; Cell Signaling Technology Inc. Danvers, MA, USA) at a dilution of 1:50 overnight at 4˚C. The signal was detected using Vectastain Universal Elite kit (Vector Laboratories, Burlingame, CA, USA). Diaminobenzidine was used for color modification. All sections were counterstained with hematoxylin.

Culture of CRC cell lines. The colorectal tumor cell lines, HCT116, DLD-1 and RKO gifted by Dr Bert Vongelstein (Johns Hopkins University, Baltimore, MD, USA), were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific Inc., Waltham, MA, USA), 1% GlutaMAX-I (Thermo Fisher Scientific Inc.), 1% penicillin/streptomycin/amphotericin B (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The cells were kept at 37˚C in a humidified atmosphere containing 5% CO_2.

Primary culture of CRC cells. CRC tissue was minced into 1-mm pieces using scissors, dissociated with 1 mg/ml collagenase (C6885; Sigma-Aldrich, St. Louis, MO, USA) in DMEM (Sigma-Aldrich) and shaken using a BioShaker BR-13FP (Taitec Co, Saitama, Japan) at 6 x g for 15 min at 37˚C. The dissociated tissue was filtered through custom-made filters (Sansho Co. Ltd., Tokyo, Japan). The collected cells were then centrifuged at 400 x g for 5 min at room temperature and the cell pellet was resuspended in 2 ml culture medium (modified embryonic stem cell culture medium containing fibroblast growth factor 2 and transforming growth factor-β). Suspended primary culture cells (603iCC and 821iCC) were seeded on plates coated with 0.03% Matrigel (Corning Inc., Corning, NY, USA) in DMEM/F12 (Sigma-Aldrich) and the medium was changed every two days. After the cells had spread over more than 50% of the plate, they were passaged using Accutase (Nacalai Tesque, Kyoto, Japan) for 3-5 min and checked at 1-min intervals. The primary culture cells were then collected and resuspended in the medium and seeded on a Matrigel-coated plate for passage.

Small interfering RNA inhibition of cultured cells. CRC cell lines (HCT116, DLD-1 and RKO) and primary cultured cells were used. For small interfering RNA (siRNA) inhibition, double-stranded RNA duplexes targeting human *PDGFR-β* were purchased as a Validated Stealth RNAi kit (Thermo Fisher Scientific Inc.) and a negative control siRNA (cat. no. 12935-112; Stealth RNAi Negative Control, Med GC Duplex; Thermo Fisher Scientific Inc.). CRC cell lines were transfected with siRNA at a concentration of 20 nM using lipofectamine RNAiMAX (Thermo Fisher Scientific Inc.), incubated in glucose-free Opti-MEM (Thermo Fisher Scientific Inc.) and analyzed.

Cell proliferation assay in vitro. *PDGFR-β* knockdown cells (PDGFR-β siRNA), negative control cells (NC siRNA) and wild-type cells (WT) were seeded on 96-well plates. The cell proliferation was analysed using Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies,

Table I. Primer sequences corresponding to universal probe libraries.

Primer	Sequence (5'-3')	UPL no.	Applications
PDGFR-β	F: CAACTTCGAGTGGACATACCC	28	*PDGFR-β*
PDGFR-β	R: AGCGGATGTGGTAAGGCATA		RT-PCR
GAPDH	F: AGCCACATCGCTCAGACAC	60	*GAPDH*
GAPDH	R: GCCCAATACGACCAAATCC		RT-PCR

F, forward; R, reverse; UPL, universal probe library.

Inc., Kumamoto, Japan). The values are presented as the means ± standard deviation (SD) from all independent experiments performed six times.

Cell invasion assay in vitro. The cells ($5x10^4$; PDGFR-β siRNA and NC siRNA) suspended with DMEM (Sigma-Aldrich) were seeded on 24-well insert chambers [Corning® BioCoat™ Matrigel® Invasion Chamber (cat.no. 354480); Corning] and DMEM supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific Inc.) was added to each well. The cells were kept at 37˚C in a humidified atmosphere containing 5% CO_2 for 24 h. The cells on the lower surface of the membrane were stained with DAPI (ProLong® Gold; Thermo Fisher Scientific Inc.) and counted by four parts of the membrane. The values are presented as the means ± SD from all independent experiments performed in triplicate.

Drug-sensitivity assay in vitro. The cells were harvested using 0.25% Trypsin-EDTA (Thermo Fisher Scientific Inc.). Primary cultured cells ($1x10^4$/well) and cell lines ($5x10^3$/well) were added to 96-well plates and exposed to crenolanib (Selleck Chemicals LLC, Houston, TX, USA) and PDGFR-α antibody (MAB322-500; R&D Systems, Abingdon, UK) 72 h later. The percentage of viable cells was determined after 96 h using a TACS XTT Cell Proliferation assay (Trevigen, Gaithersburg, MD, USA).

Statistical analysis. *PDGFR-β* expression levels in CRC and normal colorectal mucosa, and the relationships between *PDGFR-β* expression levels and clinicopathological factors were analysed using Wilcoxon's rank sum and χ^2 tests. Kaplan-Meier survival curves were plotted and compared using the generalized log-rank test. Prognostic factors were identified by univariate and multivariate analyses using a Cox proportional hazards regression model. *In vitro* assay results were analysed using Wilcoxon's rank test. All test results were analysed using JMP software version 11.2 (SAS Institute, Cary, NC, USA). A P value of <0.05 was considered to indicate a statistically significant difference.

Results

Expression of PDGFR-β in clinical tissue specimens. We determined *PDGFR-β* mRNA expression levels in primary CRC and adjacent normal colorectal mucosa by quantitative RT-PCR. *PDGFR-β* mRNA expression levels were calculated as *PDGFR-β/GAPDH* expression for each sample (Fig. 1A).

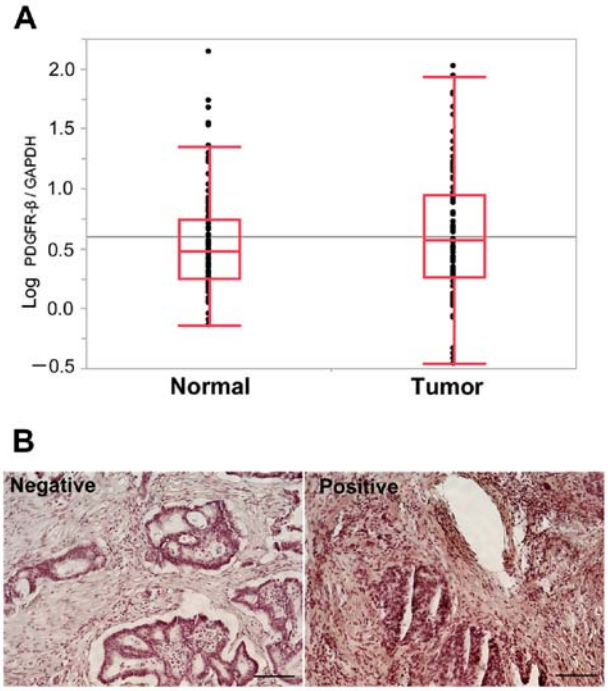

Figure 1. PDGFR-β mRNA expression and PDGFR-β protein expression in clinical tissue specimens. (A) PDGFR-β mRNA expression in clinical tissue specimens. Quantitative real-time RT-PCR analysis of mean PDGFR-β mRNA expression levels in 95 paired clinical samples revealed no significant difference between tumor tissues (normalized by GAPDH gene expression) and the corresponding normal tissues (P=0.223, Wilcoxon's rank test). (B) PDGFR-β protein expression in clinical tissue specimens. PDGFR-β protein staining in a negative sample (left) and PDGFR-β staining mostly in the cytoplasm and stromal cells in cancer tissue (right). Scale bar, 100 μm.

There was no significant difference in *PDGFR-β* mRNA expression levels between tumor and normal tissues. The median *PDGFR-β/GAPDH* mRNA expression ratio in tumor tissue was 3.01 (range, 0.16-105.97). Patients were then divided into high- and low-expression groups according to the median calculated *PDGFR-β* expression level.

Immunohistochemical detection of PDGFR-β expression. PDGFR-β protein staining was observed in the cytoplasm and cellular membrane of cancer cells (Fig. 1B). All sections were examined independently for protein expression and scored as positive when >50% of tissues in the examined area were stained. Among the 21 CRC specimens, five exhibited higher expression of the PDGFR-β protein and 16 lower expression in cancer tissues (data not shown).

The frequency of high PDGFR-β expression was in accordance with the results for *PDGFR-β* mRNA expression. The RT-PCR confirmed that all five of the tumors with high protein expression levels, also had higher *PDGFR-β* mRNA expression levels, whereas 12 of the 16 tumors with low protein expression had lower mRNA levels, indicating that high expression of *PDGFR-β* mRNA was associated with PDGFR-β protein expression (P=0.003; χ^2 test). We concluded that *PDGFR-β* mRNA and protein levels were associated in patients with CRC.

Expression of PDGFR-β and clinicopathological characteristics. We divided the samples into two groups according to the *PDGFR-β* expression status for clinicopathological evaluation. The relationships between the clinicopathological factors and *PDGFR-β* expression status in the 194 patients are summarized in Table II. *PDGFR-β* expression was not significantly correlated with any of the examined clinicopathological factors.

Relationship between PDGFR-β expression and prognosis. The median patient follow-up time was 3.78 years. Disease-free survival (DFS) was evaluated in 169 patients with R0 resection. Patients in the high-*PDGFR-β* expression group had lower disease-free survival (DFS) compared with the low-expression group (P=0.011) (Fig. 2A). According to univariate analysis, lymph node metastasis (P<0.001), positive lymphatic invasion (P=0.019), positive vascular invasion (P=0.003) and high *PDGFR-β* expression (P=0.019) were significantly correlated with DFS (Table III). Multivariate regression analysis indicated that high *PDGFR-β* expression (P=0.040), lymph node metastasis (P<0.001) and vascular invasion (P=0.010) were independent predictors of DFS.

According to univariate analysis, overall survival (OS) was significantly lower in patients with T3/4 tumor invasion (P=0.004), lymph node metastasis (P<0.001), positive lymphatic invasion (P=0.038) and positive vascular invasion (P=0.005). Multivariate regression analysis indicated that T3/4 tumor invasion (P=0.030) and lymph node metastasis (P=0.002) were independent predictors of OS (Table IV). The 5-year OS rates of patients with high and low *PDGFR-β* expression were 70 and 83%, respectively (P=0.069) (Fig. 2B), after a median follow-up of 4.31 years.

Effect of PDGFR-β inhibition in CRC cell growth and invasion. The expression of the *PDGFR-β* gene was evaluated in three CRC cell lines and six primary cultured CRC cells and all cells expressed *PDGFR-β* (Fig. 3A). CRC cell lines, HCT116 and DLD1 were subjected to siRNA knockdown. The biological role of *PDGFR-β in vitro* was analyzed in CRC, in which *PDGFR-β* expression was knocked down. Significant suppression of endogenous *PDGFR-β* expression by siRNA was confirmed by real-time RT-PCR (Fig. 3B). To determine the proliferative properties, the cells were seeded and cultured. There were significant differences in the numbers between the wild-type or negative control and *PDGFR-β* siRNA (P<0.05) in both CRC cell lines (Fig. 4A). There was no significant change between the negative control and the wild-type. In addition, in order to determine the invasive properties, an invasion assay was performed. There were significant differences in numbers between negative control and *PDGFR-β* siRNA (P<0.05) in both CRC cell lines (Fig. 4B).

Table II. Clinicopathological factors and PDGFR-β mRNA expression in 194 CRC patients.

Factors	Low expression (n=97)	High expression (n=97)	P-value
Age (years)			0.196[a]
<66	43	52	
≥66	54	45	
Sex			0.665[a]
Male	55	52	
Female	42	45	
Histological grade			0.516[a]
Well-mod	91	93	
Other[b]	6	4	
Tumor invasion			0.602[a]
T1-2	7	9	
T3-4	90	88	
Lymph node metastasis			0.378[a]
N0	41	35	
N1-2	56	62	
Lymphatic invasion			0.662[a]
Absent	55	58	
Present	42	39	
Vascular invasion			1.000[a]
Absent	22	22	
Present	75	75	
Surgical resection			0.830[a]
R0	84	85	
R1-2	13	12	

[a]NS, not significant. Well, well-differentiated adenocarcinoma; Mod, moderately differentiated adenocarcinoma; [b]Other, poorly differentiated, mucinous adenocarcinoma or squamous cell carcinoma.

Effect of crenolanib on CRC cell viability. Human CRC cell lines and primary cultured cells were both sensitive to crenolanib, according to the proliferation assay (Fig. 5A), however they were not sensitive to PDGFR-α antibody (Fig. 5B).

Discussion

The results of the present study revealed that high *PDGFR-β* expression in cancer tissue was an independent marker of poor prognosis relating to recurrence in patients with CRC. High PDGFR-β expression levels were also associated with shorter survival in patients with ovarian cancer and renal cell carcinoma (11,17). Although high *PDGFR-β* expression levels were not significantly associated with OS in the present study, OS was relatively lower in the high-expression

Table III. Univariate and multivariate analyses of disease-free survival in CRC patients after R0 resection.

	Univariate analysis			Multivariate analysis		
Factors	HR	95% CI	P-value	HR	95% CI	P-value
Age (years, <66/≥66)	1.020	0.571-1.831	0.946			
Sex (male/female)	1.137	0.637-2.061	0.665			
Histological grade (other[b]/well-mod)	0.973	0.159-3.152	0.970			
Tumor invasion (T3-4/T1-2)	2.512	0.776-15.397	0.141			
Lymph node metastasis (N1-2/N0)	8.320	3.609-24.114	<0.001[a]	6.979	2.948-20.568	<0.001[a]
Lymphatic invasion (present/absent)	2.082	1.122-4.102	0.019[a]	1.234	0.656-2.466	0.523[a]
Vascular invasion (present/absent)	3.702	1.397-12.316	0.003[a]	3.170	1.279-10.559	0.010[a]
PDGFR-β expression (high/low)	2.015	1.119-3.743	0.019[a]	1.851	1.027-3.443	0.040[a]

[a]P<0.05. Cox proportional hazards regression model. CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval; Well, well-differentiated adenocarcinoma; Mod, moderately differentiated adenocarcinoma; [b]Other, poorly differentiated or mucinous adenocarcinoma.

Table IV. Univariate and multivariate analyses of overall survival in CRC patients.

	Univariate analysis			Multivariate analysis		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Age (years, <66/≥66)	1.426	0.759-2.727	0.270			
Sex (male/female)	0.930	0.496-1.764	0.822			
Histological grade (other[b]/well-mod)	1.600	0.386-4.441	0.463			
Tumor invasion (T3-4/T1-2)	NA	2.252-2.252	0.004	NA	NA	0.030
Lymph node metastasis (N1-2/N0)	7.033	2.806-23.552	<0.001[a]	5.403	2.094-18.417	0.002
Lymphatic invasion (present/absent)	2.059	1.038-4.446	0.038	1.225	0.606-2.691	0.585
Vascular invasion (present/absent)	3.989	1.438-16.548	0.005	2.733	0.974-11.10	0.057
PDGFR-β expression (high/low)	1.818	0.958-3.591	0.068			

[a]P<0.05. Cox proportional hazards regression model. HR, hazard ratio; CI, confidence interval; Well, well-differentiated adenocarcinoma; Mod, moderately differentiated adenocarcinoma; [b]Other, poorly differentiated or mucinous adenocarcinoma. NA, not available.

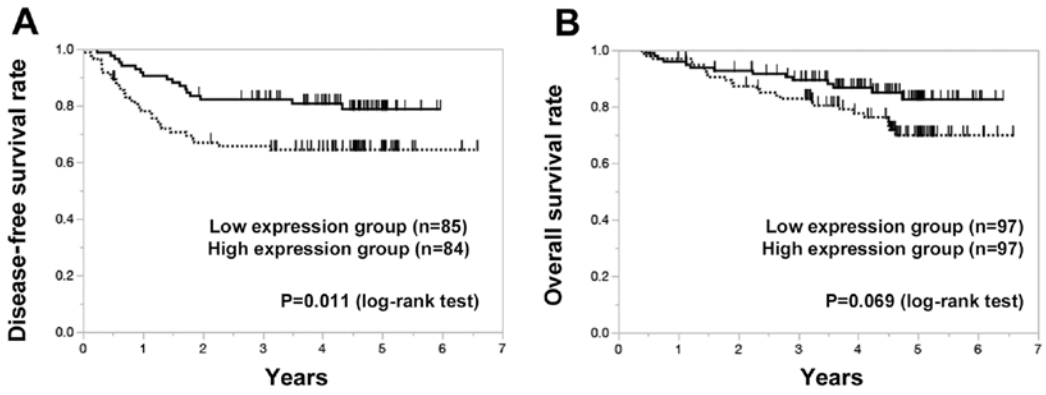

Figure 2. Disease-free survival curves and overall survival curves based on PDGFR-β mRNA expression status of CRC patients. (A) Postoperative disease-free survival was lower in patients with high PDGFR-β expression levels (n=84) compared with those with low expression levels (n=85) (P=0.011, log-rank test). (B) Five-year OS rates in patients with high and low PDGFR-β expression levels were 70 and 83%, respectively (P=0.069, log-rank test).

group. To the best of our knowledge, this findings represented the first evidence for *PDGFR-β* as a significant predictor of CRC prognosis relating to recurrence after curative resection. These results indicated the possible involvement of a PDGFR-β-dependent pathway in the progression and metastasis of CRC.

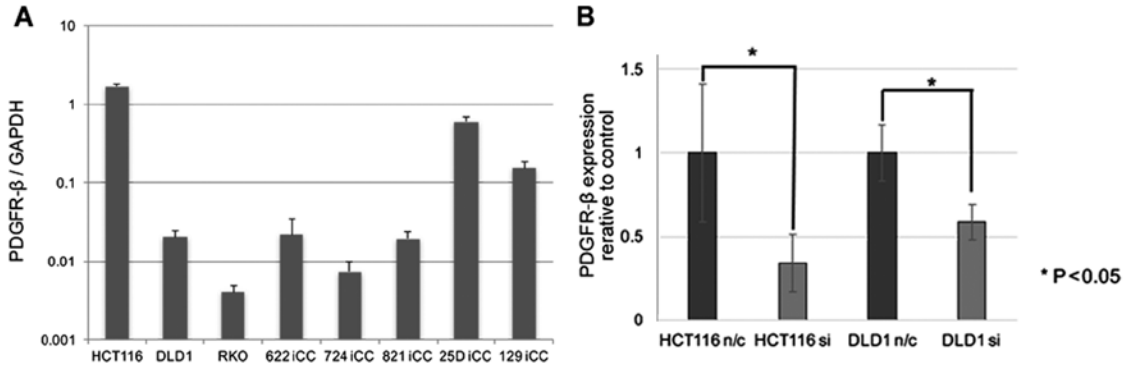

Figure 3. *PDGFR-β* mRNA expression and the siRNA inhibition of *PDGFR-β* in cultured CRC cells. (A) *PDGFR-β* mRNA expression was evaluated in three CRC cell lines (HCT116, DLD1, and RKO) and six primary cultured cells (622iCC, 724iCC, 821iCC, 25DiCC and 129iCC) normalized by GAPDH gene expression. (B) The suppression of *PDGFR-β* expression was confirmed by RT-PCR. The reduction was significant in the *PDGFR-β* siRNA experiment, compared with NC (P<0.05, Student's t-test) in two cell lines (HCT116 and DLD1). NC, negative control.

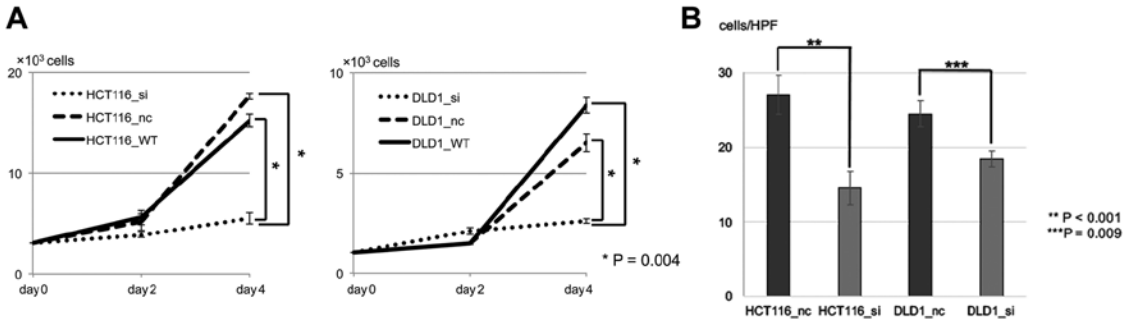

Figure 4. *PDGFR-β* inhibition in CRC *in vitro* analysis. (A) Proliferation assay with siRNA inhibition in two CRC cell lines (HCT116 and DLD1). There were significant differences between wild-type or negative control and *PDGFR-β* siRNA. The values are presented as the means ± SD. of six independent experiments. (B) Invasion assay with siRNA inhibition in two CRC cell lines (HCT116 and DLD1). There were significant differences between negative control and *PDGFR-β* siRNA. The values are presented as the means ± SD of three independent experiments. CRC, colorectal cancer; si, siRNA inhibition; nc, negative control; WT, wild-type. **P<0.001; ***P=0.009.

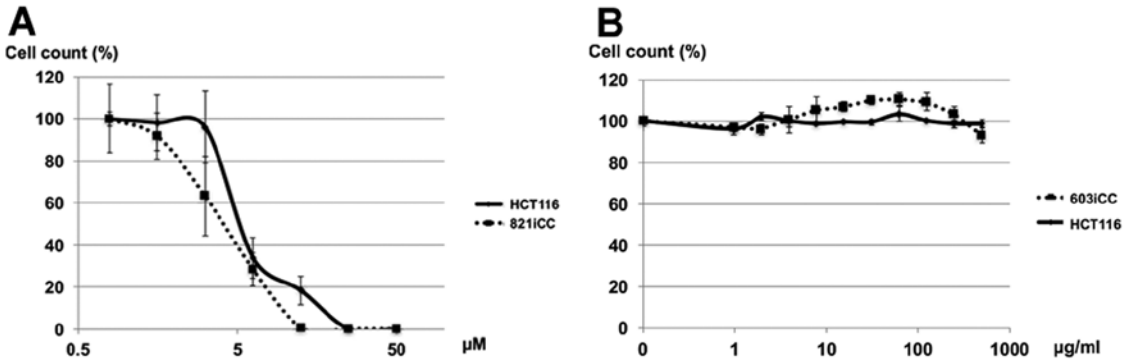

Figure 5. Drug sensitivity assay of crenolanib and PDGFR-α antibody. (A) Crenolanib decreased CRC cell proliferation. Dose-dependent inhibition of cell proliferation in a CRC cell line (HCT116) and primary cultured CRC cells (821iCC). (B) Cell proliferation was not inhibited by PDGFR-α antibody in a CRC cell line (HCT116) or in primary cultured CRC cells (603iCC).

In biological assessment, the present study revealed that *PDGFR-β* expression was related to tumor malignancy in CRC cell lines. The *in vivo* study revealed that siRNA inhibition of *PDGFR-β* resulted in a significant reduction in cell growth and invasion of CRC cell lines (P<0.05). Furthermore, PDGFR has recently been reported as a possible new therapeutic target in several solid tumors, such as breast cancer, gastrointestinal stromal tumor, lung cancer and rhabdomyosarcoma (8,18-20). A PDGFR inhibitor decreased TGF-β-induced migration in human cells *in vitro* and suppressed tumor growth *in vivo* in a mouse hepatocarcinoma model (21). PDGFR-β was also expressed in mesenchymal-like CRC cell lines *in vitro* and was related to tumor invasion and liver metastasis formation in mice (13). PDGFR-α antibody did not inhibit the proliferation of CRC, while the PDGFR inhibitor crenolanib inhibited CRC cell proliferation. These findings indicated that PDGFR-β

inhibitor inhibited cell proliferation and that crenolanib may be a promising new treatment for CRC through the inhibition of PDGFR-β.

The present study had some limitations. Notably, it was a retrospective study with a relatively small sample size, which may have limited its ability to detect a significant relationship between *PDGFR-β* expression and OS. High *PDGFR-β* expression was an independent prognostic factor in DFS, however a PDGFR-β-dependent pathway in the progression and metastasis of CRC was not clarified. Further studies with larger samples are needed to confirm these findings.

In conclusion, PDGFR-β may be a useful prognostic indicator and a potential therapeutic target in patients with CRC.

Acknowledgements

We thank Ms. Aya Ito for her technical assistance.

Funding

This research was supported by AMED under the grant no. JP17ak0101082 and a grant-in-aid for scientific research (C, 41040220, 17K16542).

Availability of data and materials

All data generated or analysed during this study are included.

Authors' contributions

SF and NM conceptualized the project, designed and performed the experiments, interpreted the results and SF wrote the manuscript. NM, MO, YT, MY, TH and CM performed the surgery and analyzed the clinical data. NM, TM, YD. and MM analyzed the data or participated in the discussions of the results.

Ethics approval and consent to participate

No. 1608057113 (Osaka International Cancer Institute).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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