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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

PDGFR（血小板由来成長因子受容体）はチロシンキナーゼ関連型受容体で２つの異なる受容体（PDGFRAおよびPDGFRB）を介して細胞応答を活性化させるが、PDGFRBの大腸癌における発現の意義は未だ明らかではない。大腸癌の194症例を対象として、PDGFRBの発現、臨床病理学的因子と無再発生存率、全生存率について検討したところ、単変量解析にて、リンパ節転移陽性（P<0.001）、リンパ管侵襲陽性(P=0.019)、静脈侵襲陽性(P=0.003)、PDGFRB高発現（P=0.0019）が無再発生存に関して有意な因子であった。PDGFRB高発現群は多変量解析においても独立した予後因子であり（P=0.040）、5年無再発生存率は高発現群において不良であった（P=0.011）。また、siRNAを用いてPDGFRB遺伝子発現を抑制したところ、大腸癌細胞の増殖能と浸潤能の低下が認められた。PDGFRの選択的阻害薬であるクレノラニブにても同様に大腸癌細胞の増殖の低下が認められた。以上よりPDGFRBが大腸癌において増殖や浸潤、転移に関与すること、その阻害剤であるクレノラニブが新規治療薬となる可能性を示したため、学位に値すると考える。

論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	藤野　志季
論文題名 Title	**Platelet-derived growth factor receptor-β gene expression relates to recurrence in colorectal cancer** （血小板由来成長因子受容体β遺伝子の発現は大腸癌の再発に関わる）

論文内容の要旨

Purpose

Multiple receptor tyrosine kinases and their growth factor ligands have recently been reported to play important roles in cancer progression and metastasis. 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. CRC tissue expresses PDGFR-α and PDGFR-β and these factors were shown to stimulate invasion and liver metastasis formation in mice. The current study examined the correlation between PDGFR-β expression in CRC tissues and clinicopathological factors and also examined the possible use of PDGFR inhibitors for the treatment of CRC.

Methods

This 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 expression levels were correlated with various clinical parameters. The biological significance was evaluated by knockdown experiments in colorectal cancer cell lines and the specific PDGFR inhibitor crenolanib.

Results

We determined *PDGFR-β* mRNA expression levels in primary CRC and adjacent normal colorectal mucosa by quantitative RT-PCR. 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. PDGFR-β protein staining was observed in the cytoplasm and cellular membrane of cancer cells by immunohistochemistry. The frequency of high PDGFR-β expression was in accord with the results for *PDGFR-β* mRNA expression. The relationships between the clinicopathological factors and *PDGFR-β* expression status in the 194 patients are examined, and *PDGFR-β* expression was not significantly correlated with any of the examined clinicopathological factors. 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 ($P = 0.011$). 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. The expression of *PDGFR-β* gene was evaluated it in three CRC cell lines and six primary cultured CRC cells and all cells expressed *PDGFR-β*. CRC cell lines, HCT116 and DLD1 were subjected to siRNA knockdown. Significant suppression of endogenous *PDGFR-β* expression by siRNA was confirmed by real-time RT–PCR. To determine the proliferative properties, cells were seeded and cultured. There were significant differences in numbers between wild-type or negative control and *PDGFR-β* siRNA ($P < 0.05$) in both CRC cell lines. And to determine the invasive properties, 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. Crenolanib is a highly selective PDGFR inhibitor, and effect of crenolanib on CRC cell viability was examined. Human CRC cell lines and primary cultured cells were both sensitive to crenolanib, according to proliferation assay, but not to PDGFR-α antibody. It suggests that PDGFR-β inhibitor inhibits the proliferation and crenolanib might be a promising new treatment for CRC via inhibition of PDGFR-β.

Conclusions

High PDGFR-β expression in cancer tissue was an independent marker of poor prognosis relating to recurrence in patients with CRC, and *PDGFR-β* expression was related to tumor malignancy in CRC cells. PDGFR-β may be a useful prognostic indicator and a potential therapeutic target in patients with CRC.