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PrPC Regulates the Cancer Stem Cell Properties *via* Interaction With c-Met in Colorectal Cancer Cells

JI HO LIM[1,2], GYEONGYUN GO[1,3] and SANG HUN LEE[1,2,3,4]

[1]*Department of Biochemistry, BK21FOUR Project2, College of Medicine, Soonchunhyang University, Cheonan, Republic of Korea;*
[2]*Medical Science Research Institute, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea;*
[3]*Department of Biochemistry, Soonchunhyang University College of Medicine, Cheonan, Republic of Korea;*
[4]*Stembio Ltd., Entrepreneur 306, Asan, Republic of Korea*

Abstract. *Background/Aim: Studies have reported that the expression of c-Met and PrP^C improves tumor progression. However, not much is known about their relationship. We hypothesized that c-Met and PrP^C interact with each other, and enhance cancer stem cell (CSC) characteristics. Materials and Methods: Magnetic activated cell sorting was used to examine the interaction between c-Met and PrP^C. The effects of the interaction on downstream signals, stem cell marker expression, and sphere formation of colorectal cancer (CRC) cells were investigated. Results: We demonstrated the increased expression and binding levels of c-Met and PrP^C in CRC cells compared to normal colon epithelial cells. We revealed that the c-Met and PrP^C interaction induced the ERK activation and Oct4 upregulation. The inhibition of c-Met by crizotinib reduced ERK activation and Oct4 expression and suppressed CSC properties. Conclusion: c-Met and PrP^C interact with each other, and targeting c-Met using crizotinib could be a powerful strategy for CRC therapy.*

Colorectal cancer (CRC) is a critical global health problem since it is the second most common cause of cancer death worldwide (1, 2). The 5-year survival rate for patients with early diagnosis increased to more than about 90%, while those diagnosed with metastases decreased by as low as 14% (1). Traditional treatments for colorectal cancer include surgery, targeted therapy, radiotherapy, and chemotherapy. However, several issues, such as drug resistance and recurrence, remain a limitation in cancer treatment, which decreases the survival rate of CRC patients (3). In addition, cancer cells are composed of heterogeneous populations and include cancer stem cells as a subpopulation, which have self-renewing ability. These features of cancers are related to tumor development and progression, and therapeutic failure (4, 5). Therefore, the discovery and development of novel therapies and molecular targets are desirable for developing efficient therapeutic strategies against the treatment of colorectal cancer.

The tyrosine-protein kinase Met (c-Met) is a heterodimeric transmembrane tyrosine kinase receptor, which is known as hepatocyte growth factor receptor (HGFR) and encoded by MET proto-oncogene. c-Met activated multiple signal transduction pathways, such as Ras GTPase (RAS), phosphatidylinositol 3-kinases (PI3K), and Notch signaling pathways, and modulates the proliferation, survival, and motility of cancer cells *via* them (6). Also, abnormal expression of c-Met has been reported in a variety of cancers, such as CRC, non-small lung cancer, gastric cancer, and breast cancer (7-10). In addition, c-Met over-expression is related to the proliferation, invasion, and angiogenesis of tumors (11, 12). Crizotinib is a small molecule targeting inhibitor of diverse receptor tyrosine kinases (RTKs), such as c-Met, anaplastic lymphoma kinase (ALK), and ROS1 receptor (13). In addition, crizotinib has shown anticancer effects in non-small cell lung cancer (NSCLC), colon cancer, gastric cancer, and glioma *via* the inhibition of downstream effector functions and induction of apoptosis (14). Furthermore, the inhibition of c-Met by crizotinib has increased the sensitivity of CRC to radiotherapy *via* attenuation of downstream signaling pathways and cell cycle arrest (15).

Cellular prion protein (PrP^C) is a glycosylphosphatidylinositol linked cell surface protein expressed in diverse cell types, including nerve cells (16), and related to multiple cellular functions, such as stress protection, proliferation, and cellular

Correspondence to: S.H. Lee, Medical Science Research Institute, Soonchunhyang University Seoul Hospital, 59, Daesagwan-ro (657 Hannam-dong), Yongsan-gu, Seoul, 04401, Republic of Korea. Tel: +82 027099029, Fax: +82 027925812, e-mail: shlee0551@gmail.com

Key Words: c-Met, PrPC, colorectal cancer, crizotinib, cancer stem cell.

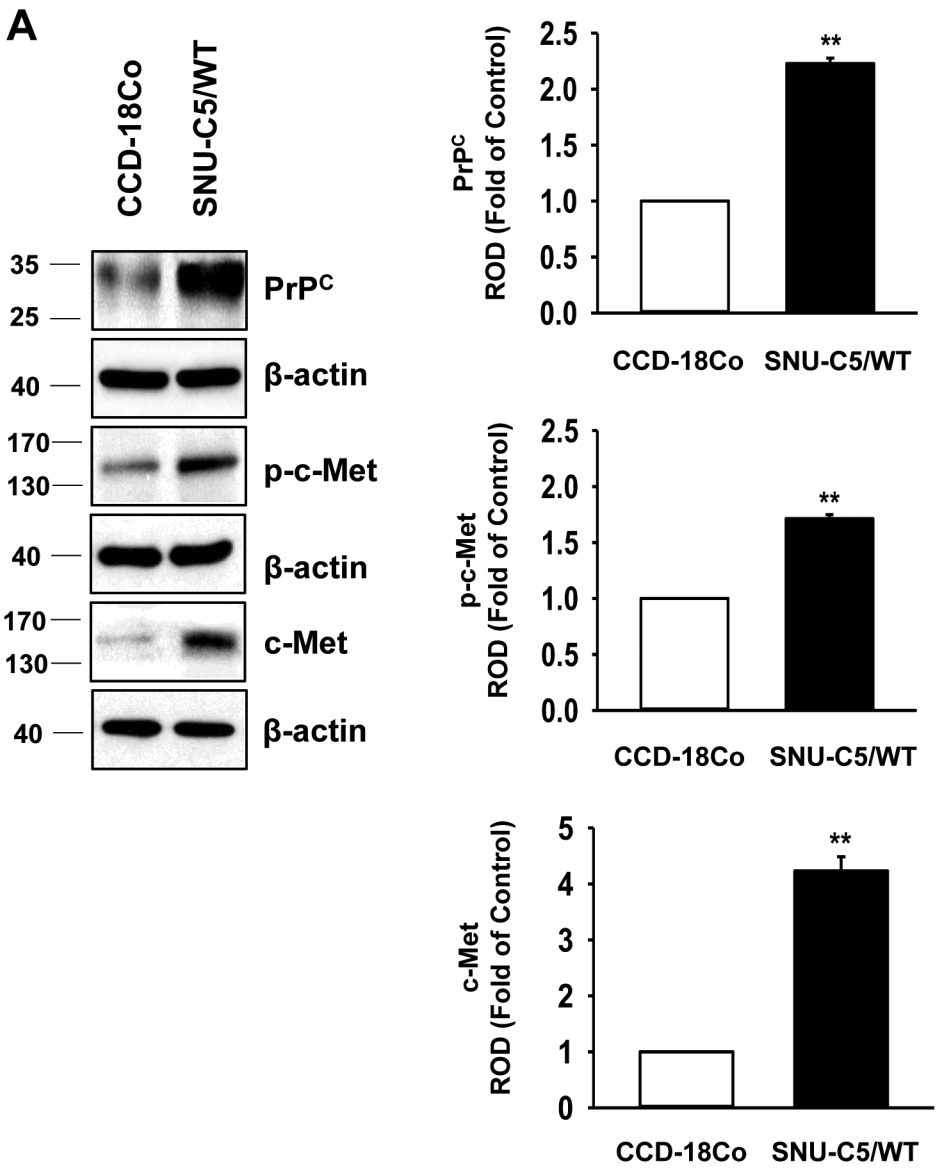

Figure 1. *Continued*

differentiation (17-19). Recent studies have reported that PrP^C is related to cancer cell proliferation, survival, drug resistance, the features of cancer stem cells, and metastasis (20-23). Also, previous studies have shown that PrP^C is related to the expression of stem cell markers, such as octamer-binding transcription factor 4 (Oct4), Homeobox protein NANOG (Nanog), SRY-box 2 (Sox2), and Aldehyde dehydrogenase 1 family member A1 (ALDH1A), which contribute to the self-renewing capacity and pluripotency of cancer stem cells (22, 24). In addition, over-expression of PrP^C prevents the suppression of Oct4 expression by various drugs including melatonin and 5-fluorouracil (5-FU) (22). Furthermore, c-Met interacts with PrP^C and regulates cell proliferation, survival, and migration *via* the Akt/MAPK signaling pathway in mesenchymal stem cells (MSCs) (25). However, the interaction between c-Met and PrP^C in cancer cells has not been well elucidated, and further investigation is needed to identify whether these interactions are related to tumor development and cancer stem cell properties. In this study, the expression levels of c-Met and PrP^C in colorectal cancer cells were examined by comparing them with those of normal colorectal cells, and the effects of their interactions on cell signaling and cancer stem cell properties in CRC cells were investigated. Moreover, the effects of crizotinib on cell viability and cancer stem cell properties in CRC were also studied.

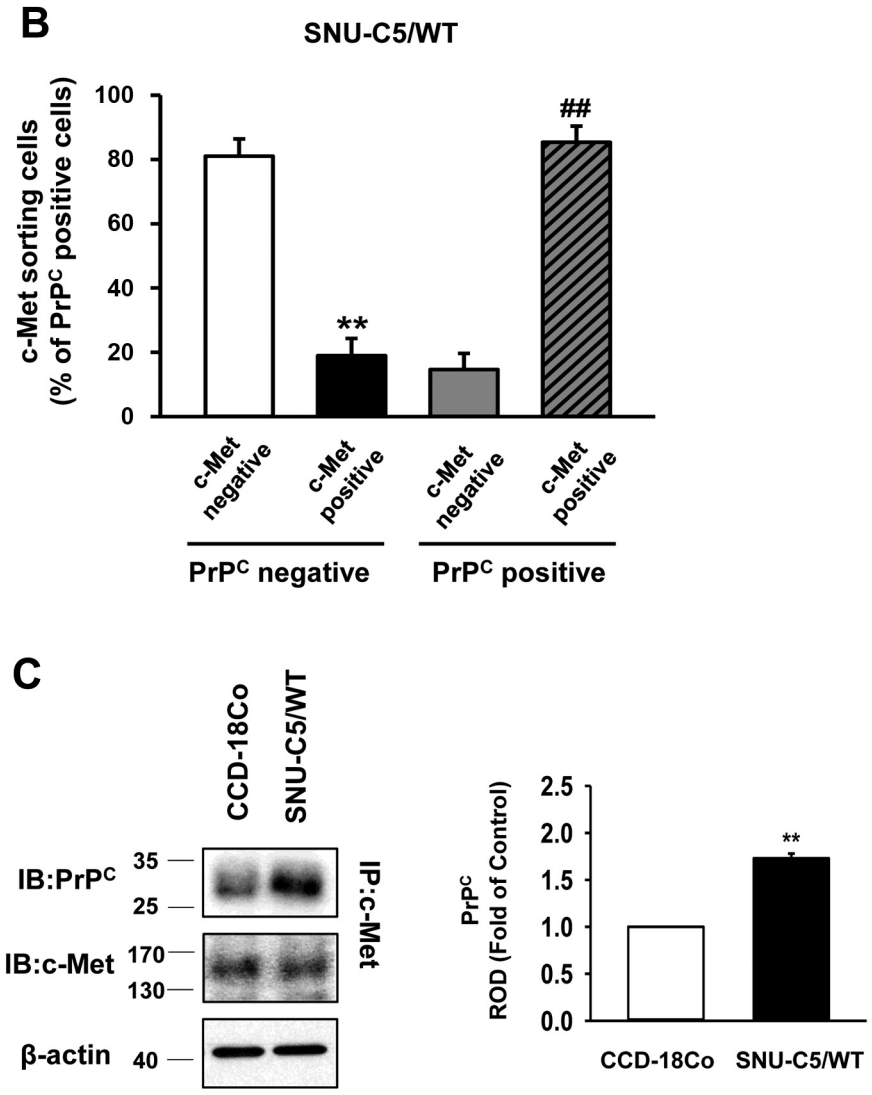

Figure 1. *PrP^C interacts with c-Met in colorectal cancer cells (CRC). (A) The level of PrP^C, phospho-c-Met (p-c-Met), and c-Met in CCD-18Co and SNU-C5/WT cells. The expression levels of PrP^C, p-c-Met, and c-Met were determined by densitometry, normalized relative to β-actin expression. Values represent mean±SEM. **p<0.01 vs. CCD-18Co. (B) Quantification of PrP^C negative/c-Met negative, PrP^C negative/c-Met positive, PrP^C positive/c-Met negative, and PrP^C positive/c-Met positive in SNU-C5/WT cells after cell isolation using MACS (n=3). Values represent mean±SEM. **p<0.01 vs. PrP^C negative/c-Met negative. ##p<0.01 vs. PrP^C positive/c-Met negative. (C) Co-immunoprecipitation analysis of c-Met bound to PrP^C in CCD-18Co and SNU-C5/WT cells. The expression level of PrP^C was determined by densitometry, normalized relative to β-actin expression. Values represent mean±SEM. **p<0.01 vs. CCD-18Co.*

Materials and Methods

Cells and cell culture. The human CRC cell line SNU-C5/WT was obtained from the Chosun University Research Center for Resistant Cells (Gwangju, Korea) and cultured in Roswell Park Memorial Institute 1640 media (RPMI 1640; Hyclone, Logan, UT, USA) containing 10% fetal bovine serum (FBS), L-glutamine, and antibiotic supplements (Hyclone). The human colon normal cell line (CCD-18Co) was obtained from the Korean Cell Line Bank (Seoul, Republic of Korea) and cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone) with 10% FBS, L-glutamine, and antibiotic supplements (Hyclone). All cells were cultured in a humidified incubator at 37˚C with 5% CO_2.

Preparation of crizotinib. Crizotinib was purchased from Selleckchem (Houston, TX, USA). The Crizotinib powder was dissolved in dimethyl sulfoxide (DMSO), filter-sterilized using a 0.45-μm pore filter (Sartorius Biotech GmbH, Gottingen, Germany), and aliquots were stored at –70˚C until use.

Isolation of PrP^C positive and PrP^C/c-Met double positive SNU-C5/WT cells using magnetic activated cell sorting (MACS). The PrP^C positive SNU-C5/WT cells were sorted using manual MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. The cells were incubated with the human CD230 (PrP)-Biotin primary antibody (Miltenyi Biotec) for 10 min at 4˚C. Then, the cells were washed with MACS rinsing solution containing 5% bovine serum albumin (BSA) and attached with anti-Biotin MicroBeads secondary antibody after incubation for 15 min at 4˚C. After that, the cells were again washed with MACS rinsing solution and sorted with a MACS LS column using a magnetic field. For PrP^C/c-Met double positive cell sorting, sorted cells (PrP^C negative and positive) were again incubated with the c-Met-Biotin primary antibody (Novus Biological, Centennial, CO, USA) for 10 min at 4˚C. Then, the same steps were repeated. Finally, cells were sorted again with a MACS LS column using a magnetic field.

Western blot analysis. Cells were lysed with RIPA lysis buffer (Thermo Fisher Scientific, Rockford, IL, USA), and total cell proteins were extracted. Then, total cell homogenates (30 μg protein) in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer were separated by 8-12% SDS-PAGE and the proteins were transferred to polyvinylidene fluoride membranes (Sigma Aldrich, St. Louis, MO, USA). The membranes were washed with Tris-buffered saline/Tween-20 buffer (0.05% Tween-20, 150 mM NaCl, 10 mM Tris-HCl; pH 7.6) and blocked with 5% skim milk. After that, they were incubated with primary antibodies against PrP^C (Santa Cruz Biotechnology, CA, USA), c-Met (Novus Biological, Centennial, CO, USA), p-c-Met (Santa Cruz Biotechnology), protein kinase B (Akt; R&D systems, Minneapolis, MN, USA), phospho-Akt (p-Akt; Santa Cruz Biotechnology), extracellular signal-regulated kinase (ERK; R&D systems), phospho-ERK (p-ERK; Santa Cruz Biotechnology), cyclin-dependent kinase 2 (CDK2; Santa Cruz Biotechnology), CDK4 (Santa Cruz Biotechnology), Oct4, Nanog (Santa Cruz Biotechnology), ALDH1A (Santa Cruz Biotechnology), and β-actin (Santa Cruz Biotechnology). After incubation with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology), the bands were visualized with enhanced chemiluminescence (ECL) reagents (Thermo Fisher Scientific) in a dark room.

Co-immunoprecipitation. The co-immunoprecipitation of c-Met and PrP^C in SNU-C5/WT cells was analyzed as follows: After culture at 37˚C for 24 h, SNU-C5/WT cells were disrupted *via* sonication in a co-immunoprecipitation buffer. The c-Met- PrP^C complexes were precipitated for 4 h at 4˚C with anti-c-Met antibody-conjugated agarose beads (Santa Cruz Biotechnology) from the total cell lysate (500μg protein) and incubated additionally at 4˚C for 12 h. The beads were washed 3 times and proteins were released from the beads by boiling for 7 min is SDS-PAGE sample buffer. Subsequently, the precipitated proteins were analyzed by western blot using anti-PrP^C antibody (Santa Cruz Biotechnology).

Tumor sphere culture. The SNU-C5/WT cells were cultured in ultra-low attachment six-well plates (Corning, NY, USA) for generation of tumor spheres. After cells were sorted by manual MACS (Miltenyi Biotec), PrP^C negative and positive SNU-C5/WT cells were incubated in RPMI 1640 media (Hyclone) with 10% FBS, L-glutamine, and antibiotic supplements (Hyclone). They were grown in a humidified incubator with 5% CO_2 at 37˚C. Spheres were grown for 14 days, and treated with crizotinib at day 7 in case of the PrP^C positive + Crizotinib group. Spheres were observed by a visual inverted microscope (Olympus, Tokyo, Japan).

Flow cytometry analysis. PrP^C negative and positive SNU-C5/WT cells were identified by flow cytometry analysis of Oct4. Two-color flow cytometry system (BD FACS Canto II; BD, Franklin Lakes, NJ, USA) was used to detect the immunostained cells. By comparing the results with the corresponding negative controls, the percentage of stained cells was calculated.

Cell proliferation assay. PrP^C negative and positive SNU-C5/WT cells were seeded in 96-well culture plates (5,000 cells/well) for 24 h and treated with crizotinib (0, 12, and 16 μM) at 37˚C for 24 h. Then, a cell proliferation assay was performed. The assay used was a modified MTT assay, based on the conversion of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2-tetrazolium to formazan by mitochondrial NAD(P)H-dependent oxidoreductase enzymes. The formazan levels were quantified by measuring the absorbance at 575 nm with a microplate reader (Tecan, Männedorf, Switzerland).

Statistical analysis. All data are expressed as mean±standard error of the mean (SEM). Statistical significance between groups was calculated by Two-tailed Student's *t*-test or one-way analysis of variance (ANOVA). Comparisons of three or more groups were made using the Dunnett's or Tukey's *post-hoc* test. All results were considered as statistically significant when p-values <0.05.

Results

c-Met and PrP^C are over-expressed in CRC cells and correlated to each other. Several studies have reported that the expression levels of c-Met and PrP^C are increased in various cancer cells and affect various cell functions such as proliferation, metastasis, and drug resistance (21, 26, 27). In addition, it has been shown that c-Met of MSCs and PrP^C of myoblasts bind to each other in co-culturing system, and their interaction increases cell mobilization (25). First, to explore whether c-Met and PrP^C are upregulated in CRC cells, the expression levels of c-Met and PrP^C in human colon normal cell line (CCD-18Co) and human CRC cell line (SNU-C5/WT) were assessed using western blot assay. c-Met and PrP^C expression levels were significantly increased in SNU-C5/WT, compared to CCD-18Co (Figure 1A). To investigate whether c-Met and PrP^C are related in CRC cells, we used double-sorting method using MACS. We first isolated PrP^C negative and positive cells from SNU-C5/WT cells, and again isolated c-Met negative and positive cells from PrP^C negative and positive cells respectively. The number of c-Met positive cells among PrP^C positive cells was much higher than that of c-Met positive cells among PrP^C negative cells (Figure 1B). To further demonstrate the interaction of c-Met and PrP^C, co-immunoprecipitation assay was performed. The interaction of c-Met and PrP^C was increased in SNU-C5/WT, compared to CCD-18Co,

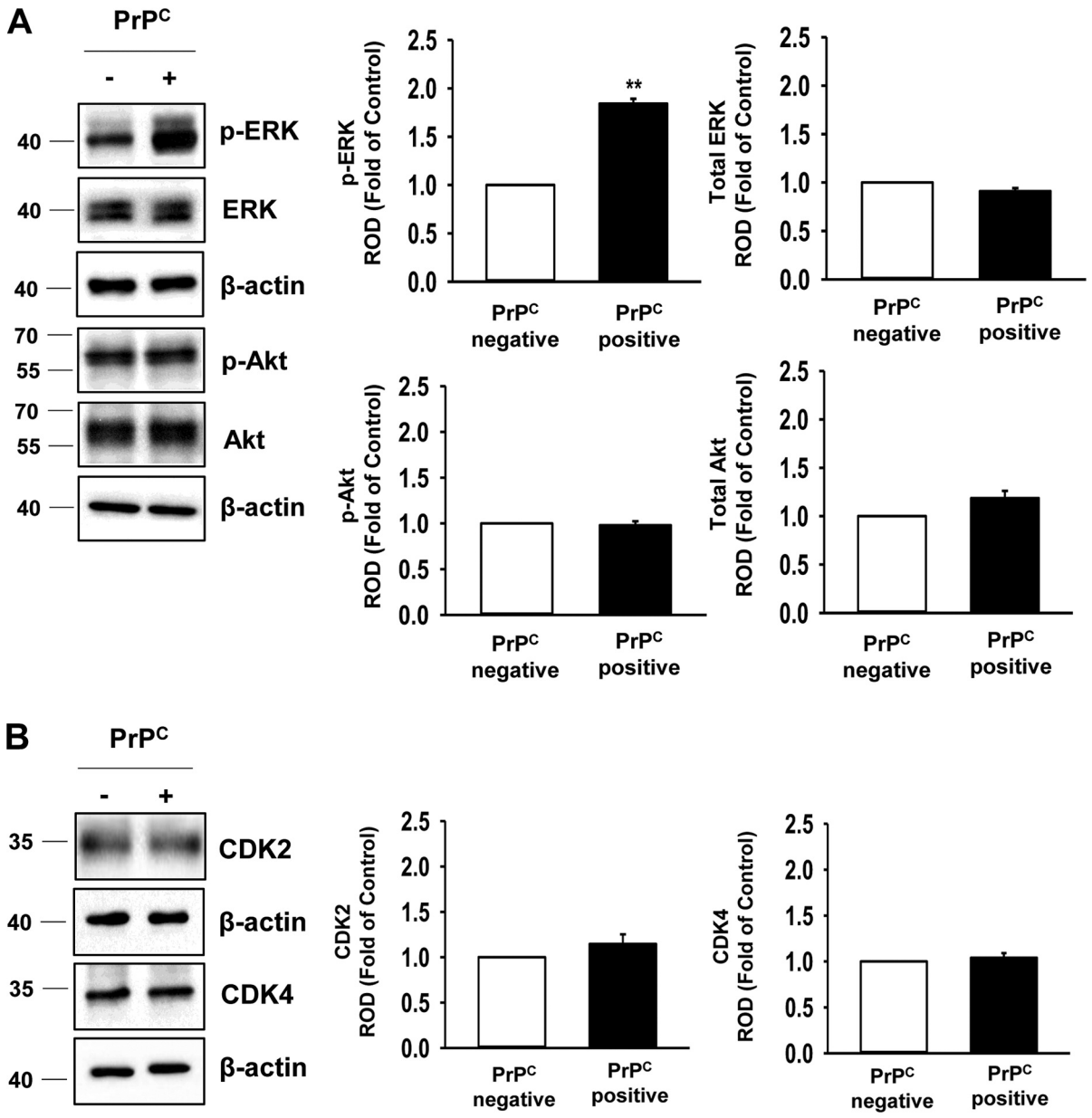

Figure 2. *Changes in the PrP^C-mediated downstream signaling pathways in CRC cells. (A) The level of phospho-Akt (p-Akt), phospho-ERK (p-ERK), total Akt, and total ERK in PrP^C negative and positive CRC cells. The expression levels of p-Akt, p-ERK, total Akt, and total ERK were determined by densitometry, normalized relative to β-actin expression. Values represent mean±SEM. **p<0.01 vs. PrP^C negative. (B) The level of CDK2 and CDK4 in PrP^C negative and positive CRC cells. The expression levels of CDK2 and CDK4 were determined by densitometry, normalized relative to β-actin expression. Values represent mean±SEM. **p<0.01 vs. PrP^C negative.*

indicating that c-Met binds to PrP^C in CRC cells (Figure 1C). These results show that c-Met and PrP^C are highly co-expressed in CRC cells and correlate with each other.

PrP^C regulates the properties of cancer stem cells via the ERK signaling pathway in CRC cells. To investigate how the interaction between c-Met and PrP^C affects cellular functions of CRC cells, we isolated and analyzed the PrP^C positive CRC cells, where both are highly expressed and bound to each other. Some studies have reported that the ERK1/2 and PI3K/Akt signaling pathways and the subsequent transcriptional activation of cell cycle proteins contribute to

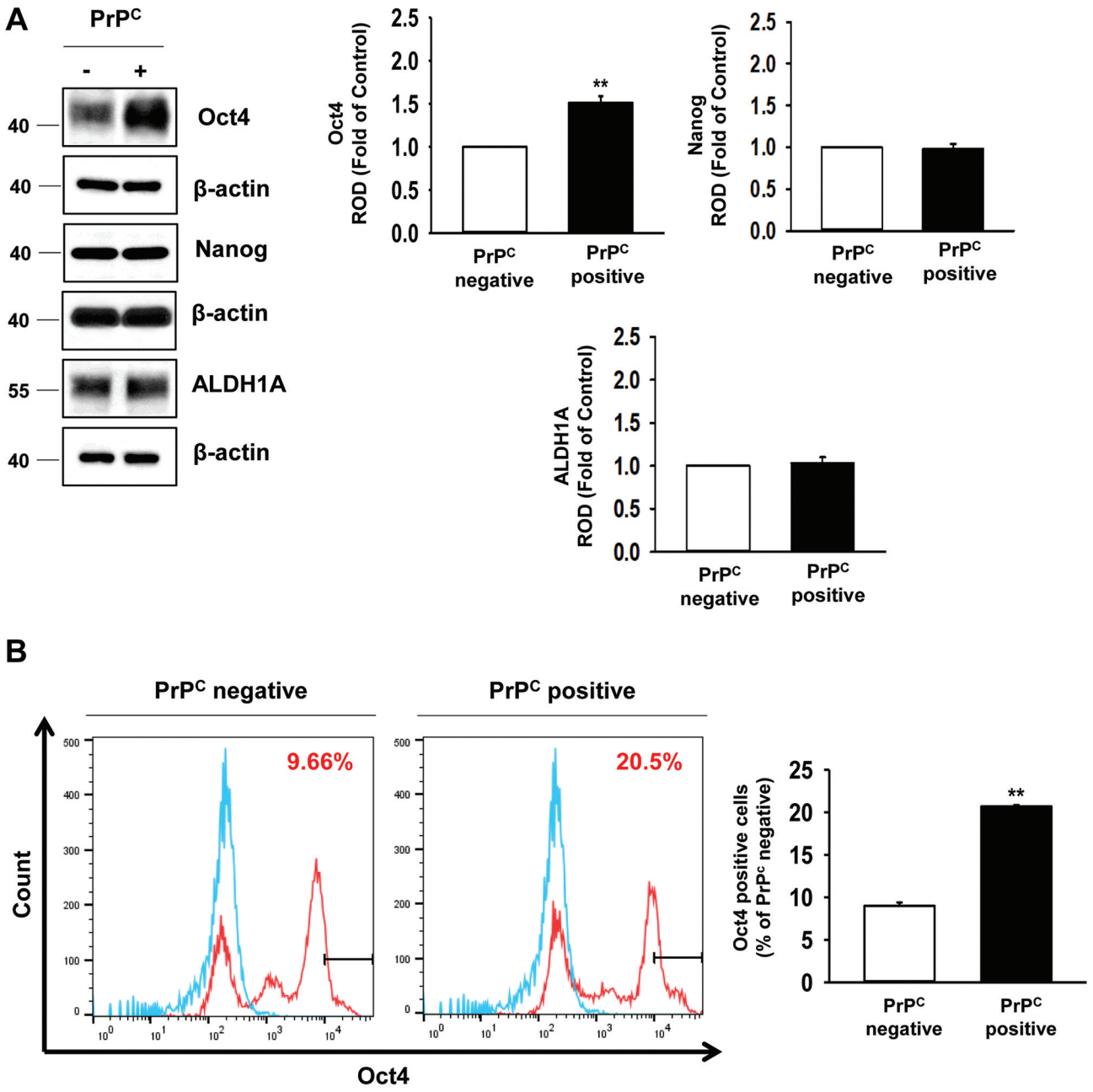

Figure 3. *Modulation of PrP^C-mediated cancer stem cell markers in CRC cells. (A) The level of stem cell markers in PrP^C negative and positive CRC cells. The expression levels of Oct4, Nanog, and ALDH1A were determined by densitometry, normalized relative to β-actin expression. Values represent mean±SEM. **p<0.01 vs. PrP^C negative. (B) Flow cytometry analysis of Oct4 in PrP^C negative and positive CRC cells. Percentages of Oct4 positive cells in PrP^C negative and positive cells (n=3). Values represent mean±SEM. **p<0.01 vs. PrP^C negative.*

the progression of cancer, and are positively correlated with PrP^C (27-29). Therefore, we evaluated the phosphorylation of Akt and ERK in PrP^C negative and positive SNU-C5/WT cells using western blot assay. The phosphorylation of ERK was significantly enhanced in PrP^C positive cells compared to PrP^C negative cells, but there was no significant difference in Akt (Figure 2A). We also analyzed the expression of the cell cycle-associated proteins CDK2 and CDK4, but there was no significant difference between PrP^C negative and positive SNU-C5/WT cells (Figure 2B). Our previous studies have

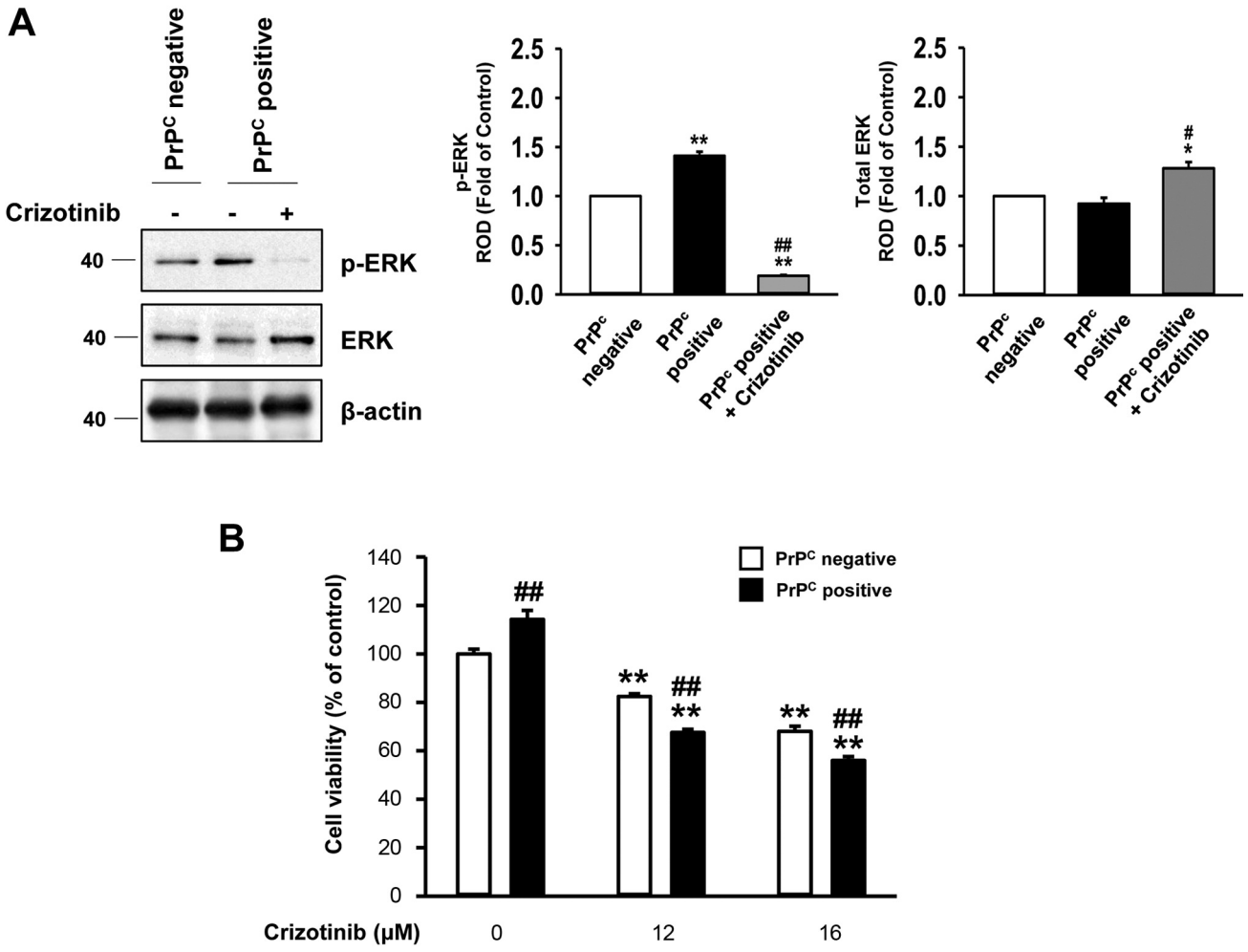

Figure 4. *Crizotinib decreases the phosphorylation of ERK and suppresses cell proliferation in PrP^C positive colorectal cancer (CRC) cells. (A) The level of phospho-ERK (p-ERK), and total ERK in PrP^C negative and positive CRC cells untreated or after treatment with crizotinib (12 μM, 1 h). The expression levels of p-ERK and total ERK were determined by densitometry, normalized relative to β-actin expression. Values represent mean±SEM. *p<0.05, **p<0.01 vs. PrP^C negative, $^{\#}$p<0.05, $^{\#\#}$p<0.01 vs. PrP^C positive. (B) PrP^C negative and positive CRC cells were treated with crizotinib (0, 12, 16 μM) for 24 h (n=3), and cell proliferation was evaluated. Values represent mean±SEM. **p<0.01 vs. control, $^{\#\#}$p<0.01 vs. PrP^C negative.*

revealed that PrP^C expression is related with cancer stem cell characteristics in drug-resistant CRC cells and human CRC tissues (22, 30), thus, we also examined the expression of cancer stem cell markers such as ALDH1A, Nanog, and Oct4 in PrP^C negative and positive SNU-C5/WT cells using western blot assay. The expression of Oct4 was significantly enhanced in PrP^C positive cells compared to PrP^C negative cells, but Nanog and ALDH1A were not significantly changed (Figure 3A). In addition, we analyzed the expression of Oct4 using flow cytometry assay, and showed that it was drastically enhanced in PrP^C positive cells (Figure 3B). These results indicate that the expression of PrP^C is related to the ERK1/2 signaling pathway and the cancer stem cell marker Oct4.

Crizotinib suppresses cell proliferation by inactivation of the ERK pathway in PrP^C positive CRC cells. To determine the effect of the interaction between c-Met and PrP^C in PrP^C positive CRC cells, we treated cells with crizotinib, a c-Met inhibitor. First, we evaluated its effect on the activation of ERK by western blot analysis in PrP^C positive CRC cells. When c-Met was inhibited by crizotinib in PrP^C positive SNU-C5/WT cells, the phosphorylation of ERK was greatly decreased, at levels significantly lower than those in PrP^C negative CRC cells (Figure 4A). Furthermore, to assess the effect of crizotinib on the proliferation of PrP^C negative and positive CRC cells, cell viability was analyzed by MTT assay after treatment of cells with several doses of crizotinib (0, 12,

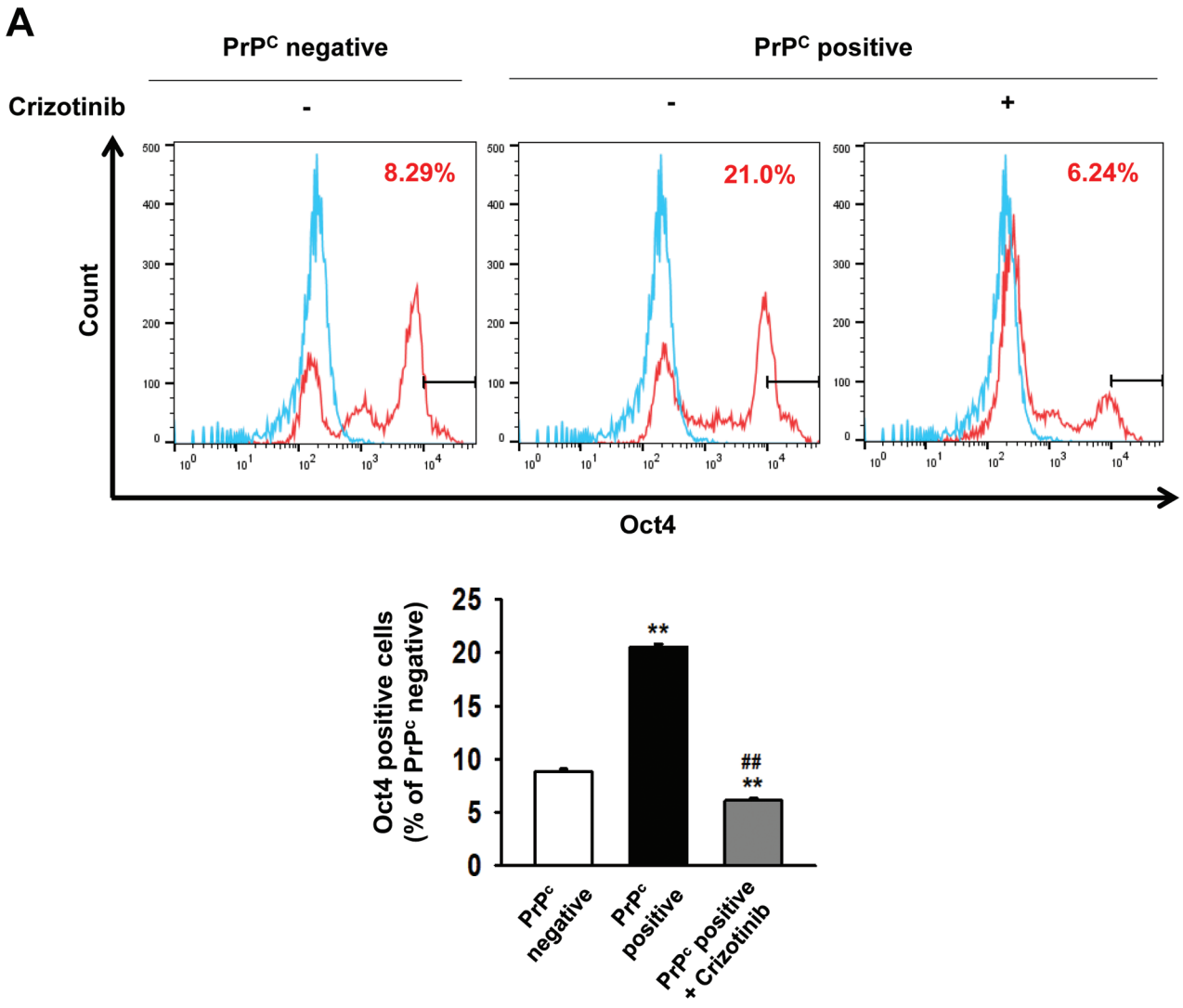

Figure 5. *Continued*

and 16 μM) for 24 h. Crizotinib reduced the viability of CRC cells in a dose-dependent manner, and it was significantly reduced in PrP^C positive SNU-C5/WT cells compared to PrP^C negative cells. (Figure 4B). These findings suggest that the interaction between c-Met and PrP^C regulates the proliferation of CRC cells *via* ERK1/2 signaling pathway.

Crizotinib decreased the characteristics of cancer stem cell in PrP^C positive CRC cells. To demonstrate the effect of crizotinib on cancer stem cell characteristics in CRC cells, we analyzed the expression of Oct4 by flow cytometry in PrP^C negative, PrP^C positive, and crizotinib-treated PrP^C positive CRC cells. The expression of the cancer stem cell marker Oct4 was significantly enhanced in PrP^C positive cells compared to PrP^C negative cells, and was greatly reduced by crizotinib treatment, at a level lower than that of PrP^C negative cells (Figure 5A). To further assess the effect of crizotinib on cancer stem cell characteristics in CRC cells, a sphere formation assay was performed. The sphere-forming capacity, expressed as the number and diameter of spheres, was significantly increased in PrP^C positive cells compared to PrP^C negative cells, and was remarkably suppressed by crizotinib treatment (Figure 5B). These results indicate that the inhibition of c-Met by crizotinib reduced the cancer stem cell characteristics in PrP^C positive CRC cells.

Discussion

In this study, the expression levels of c-Met and PrP^C in SNU-C5/WT and CCD-18Co cells were investigated. c-Met and

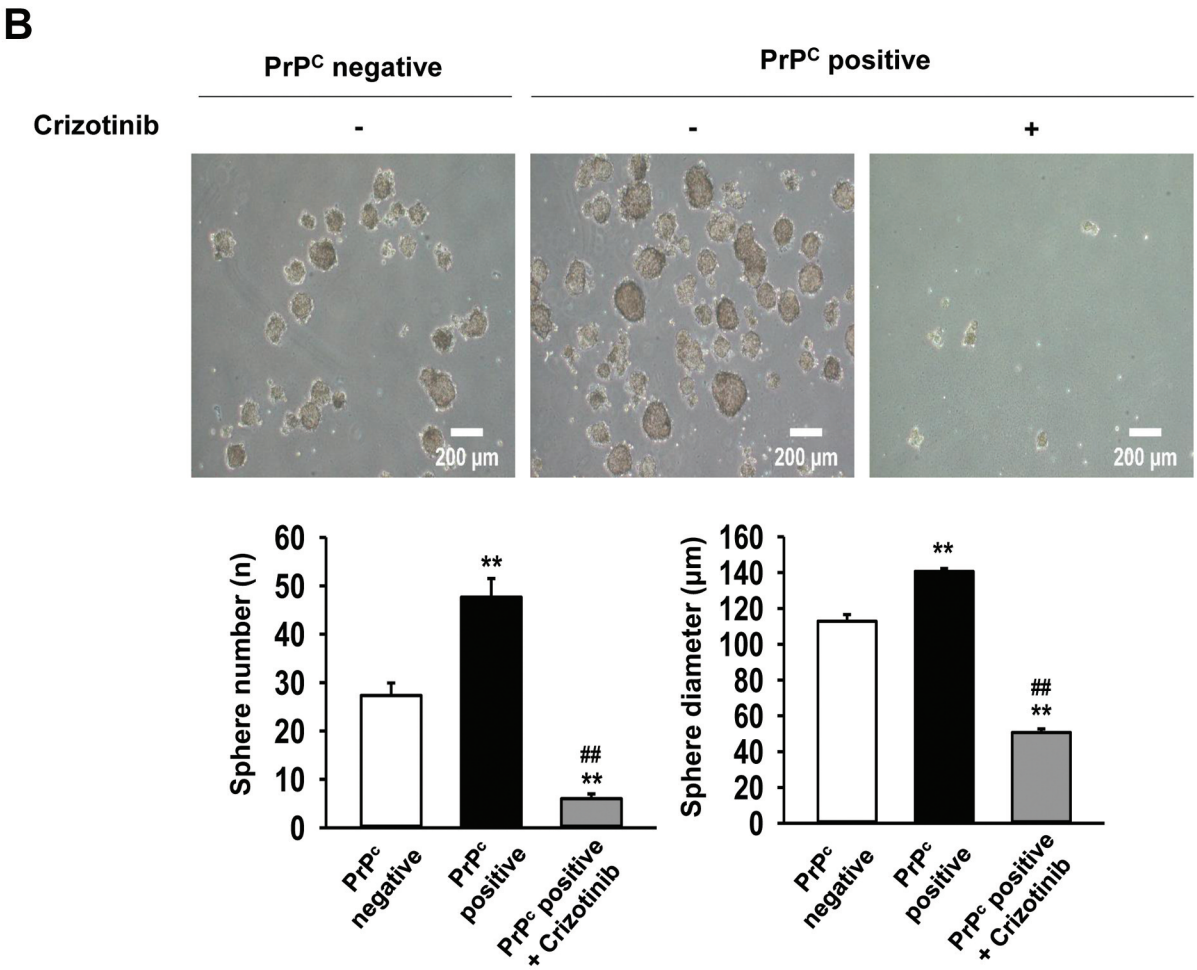

Figure 5. *Crizotinib decreases cancer stem cell properties in PrP^C positive colorectal cancer (CRC) cells. (A) Flow cytometry analysis of Oct4 in PrP^C negative and positive CRC cells untreated or after treatment with crizotinib (12 μM, 24 h). Percentages of Oct4 positive cells in PrP^C negative and positive cells untreated or treated with crizotinib (n=3). Values represent mean±SEM. $^{**}p<0.01$ vs. PrP^C negative, $^{\#\#}p<0.01$ vs. PrP^C positive. (B) Sphere formation assay of PrP^C negative and positive CRC cells untreated or after treatment with crizotinib. Cells were grown in ultra-low attachment plates for 2 weeks and crizotinib was added at day 7 (12 μM, 7 days). Scale bar=200 μm. The number and diameter of spheres were quantified (n=3). Values represent mean±SEM. $^{**}p<0.01$ vs. PrP^C negative, $^{\#\#}p<0.01$ vs. PrP^C positive.*

PrP^C expression was increased in SNU-C5/WT CRC cells compared to CCD-18Co cells. Isolation of PrP^C/c-Met positive SNU-C5/WT cells using MACS showed that the number of c-Met positive cells was much higher in PrP^C positive cells compared to PrP^C negative cells. The interaction between c-Met and PrP^C was confirmed by the co-immunoprecipitation assay. Also, p-ERK expression was increased in PrP^C positive cells compared to negative cells, but expression levels of p-Akt and the cell cycling proteins CDK2 and CDK4 were not. In addition, the expression level of Oct4 was significantly enhanced in PrP^C positive SNU-C5/WT cells. Treatment with crizotinib significantly decreased the expression levels of p-ERK, as well as cell viability in a dose-dependent manner in PrP^C positive SNU-C5/WT cells. Finally, crizotinib suppressed the expression of Oct4 and the cancer sphere-forming capacity in PrP^C positive SNU-C5/WT. Several studies have reported that ERK1/2 signaling pathway is related with cancer stem cell properties in various cancer cells (31, 32). Therefore, these findings indicate that crizotinib reduced the cancer stem cell properties *via* the ERK1/2 signaling pathway by inhibiting the interaction between c-Met and PrP^C in PrP^C positive colorectal cancer cells. Our study suggests crizotinib as a novel anti-cancer drug inhibiting the cancer stem cell properties by these mechanisms.

Crizotinib, has potential as an anti-cancer drug by inhibiting the growth and migration of cancer cells and

inducing apoptosis *via* the inhibition of c-Met phosphorylation. Also, many studies have reported that crizotinib inhibits cancer cell growth, migration, and colony formation *via* regulation of the ERK1/2 signaling pathway (33, 34). Moreover, another study demonstrated that crizotinib inhibits the cancer stem cell properties by reducing c-Met in ALDH1 and CD133 positive breast cancer stem cells (35). It is already well known that several tumor cells show over-expression of c-Met (36-38), which is involved in tumorigenesis through activation of c-Met itself and its downstream signaling pathways such as PI3K/Akt, Ras/MAPK, and JAK/STAT (39). Our findings also indicate that crizotinib treatment decreased the activation of ERK1/2 signaling pathway in PrP^C positive SNU-C5/WT CRC cells, leading to the inhibition of Oct4 expression and sphere-forming capacity.

Previous studies have reported that PrP^C plays an important role in tumorigenesis *via* various signaling pathways such as PI3K/Akt, ERK1/2, and Notch (40). Moreover, some studies have shown that over-expression of PrP^C is related to the expression of cancer stem cell markers, such as ALDH1A, Oct4, Nanog, SOX2, and tumorigenesis (22, 41). Since c-Met is also known to be related to cancer stem cell properties (35), the effects of the c-Met inhibitor crizotinib on PrP^C positive SNU-C5/WT cells were examined. We revealed that it greatly reduces the activation of ERK as well as the expression of Oct4 and sphere-forming capacity, which even reversed the effect of PrP^C expression. These results suggest that inhibition of c-Met and its downstream signaling pathways also suppress the cancer stem cell properties due to PrP^C expression in SNU-C5/WT cells.

In our previous study, it was discovered that the expression levels of c-Met in MSCs and PrP^C in myoblasts were increased under hypoxic conditions. In addition, in the co-culturing system, they bound to each other to improve cell mobility (25). However, the specific molecular mechanisms for the effects of their interactions in cancer cells are still unknown. In this study, we confirmed that the expression of c-Met and PrP^C is related, and they also bind to each other within the same SNU-C5/WT cells. Therefore, it could be assumed that the interaction between them affects the ERK1/2 signaling pathway and cancer stem cell properties in SNU-C5/WT cells. Since c-Met and PrP^C have been reported to be over-expressed in various cancer cells, targeting the interaction between them could be an effective, novel therapeutic strategy for cancer.

In summary, our results suggest that c-Met and PrP^C expression and the interaction between them regulate the ERK1/2 signaling pathway and expression of the cancer stem cell marker Oct4 in SNU-C5/WT cells. Also, in PrP^C positive SNU-C5/WT cells, inhibition of c-Met by crizotinib suppresses the activation of ERK, resulting in decreased cell viability and reduced sphere-forming capacity, which is due to decreased Oct4 expression. Further studies are necessary to understand how the interaction between c-Met and PrP^C regulates cell signaling pathways and cancer stem cell properties. This study demonstrates that understanding of the effect of the interaction between c-Met and PrP^C may contribute to the development of a novel therapeutic strategy in cancer therapy.

Conflicts of Interest

The Authors have no conflicts of interest to declare with regard to this study.

Authors' Contributions

Ji Ho Lim: data collection, data analysis, and drafting of manuscript; Gyeongyun Go: data analysis, and drafting of manuscript; Sang Hun Lee: designing the study, drafting and editing of manuscript, procurement of funding, and study supervision.

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