ANTICANCER RESEARCH *40*: 5611-5620 (2020)
doi:10.21873/anticanres.14574

Role of PrP^C in Cancer Stem Cell Characteristics and Drug Resistance in Colon Cancer Cells

GYEONGYUN GO[1], CHUL WON YUN[2], YEO MIN YOON[2], JI HO LIM[2], JUN HEE LEE[1,3,4] and SANG HUN LEE[1,2]

[1]*Department of Biochemistry, Soonchunhyang University College of Medicine, Cheonan, Republic of Korea;*
[2]*Medical Science Research Institute, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea;*
[3]*College of Science and Technology, Dankook University, Cheonan, Republic of Korea;*
[4]*Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan, Republic of Korea*

Abstract. *Background/Aim: Cancer stem cell characteristics and drug resistance of colorectal cancer are associated with failure of cancer treatment. In this study, we investigated the effects of PrP^C on cancer stem cell characteristics, migration, invasion, and drug resistance of 5FU-resistant CRC cells. Materials and Methods: PrP^C negative and PrP^C positive cells were isolated from 5FU-resistant CRC cells using magnetic activated cell sorting. Sphere formation, cancer stem cell marker expression, migration, invasion, and drug resistance were analyzed. Results: PrP^C positive cells showed increased sphere formation capacity and increased expression of cancer stem cell markers compared to PrP^C negative cells. In addition, PrP^C positive cells showed increased migration, invasion and drug resistance compared to PrP^C negative cells. Furthermore, knockdown of PrP^C abolished these effects. Conclusion: PrP^C expression is important in CRC cell behavior, such as sphere formation, migration, invasion, and drug resistance. PrP^C is an important therapeutic target for the treatment of CRC.*

In addition to lung cancer, liver cancer and pancreatic cancer, colorectal cancer (CRC) is one of the leading causes of cancer-related deaths (1). Although treatments for CRC have been developed, they are still limited. One of the major limiting factors in cancer treatment is the existence of cancer stem cells (CSCs). CSCs is a subpopulation of self-renewing cells that are responsible for tumor development and therapeutic failure (2, 3). The expression of cancer stem cell markers is also associated to tumor progression. For example, the expression of cancer stem cell markers such as ALDH1A, Oct4, and Nanog induces cancer cell stemness, increases metastasis and inhibits apoptosis of cancer cells (4). Furthermore, drug resistance of cancer cells is also responsible for CRC treatment failure. Drug resistance restricts the chemotherapeutic effect and is associated with improved DNA repair process and drug efflux pump mechanisms (5, 6). Recent studies have indicated that molecular-targeted therapies may be effective treatments for CRC (7-9). Therefore, the discovery of new targets and the development of novel treatment approaches are essential for the treatment of CRC.

Cellular prion protein (PrP^C) is a glycosylphosphatidylinositol-anchored protein that is expressed in nerve and other tissues, regulating diverse cellular processes such as cell death, survival, proliferation, and differentiation (10, 11). The misfolding of PrP^C is associated with neurodegenerative diseases, such as transmissible spongiform encephalopathy and prion diseases (12). Accumulating evidence has shown that PrP^C has a significant effect on the functions of cancer cells such as proliferation, metastasis, and drug resistance in many types of cancer (13, 14). A recent study has shown that hypoxia increases the expression of PrP^C, which regulates cancer stem cell markers in CRC cells (15). The correlation between PrP^C expression, cancer cell invasion, and lymph node metastasis has also been demonstrated in patients with gastric cancers (16). In addition, the formation of the PrP^C/P-glycoprotein (P-gp) complex is also known to increase drug resistance of breast cancer cells against paclitaxel (17). Although many studies have been conducted on the effects of prions on proliferation, metastasis, and drug resistance of cancer cells, studies on the effects of PrP^C on cancer stem cell marker expression, migration, invasion, and drug resistance in CRC cells are still insufficient.

In this study, the effects of PrP^C on cancer stem cell characteristics, such as formation of spheroids and the expression of cancer stem cell markers, were examined. In addition, the effects of prion proteins on migration and invasion, and drug resistance of CRC cells were also investigated. The

Correspondence to: Sang Hun Lee, Soonchunhyang 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: ykckss1114@nate.com

Key Words: Prion protein, PrP^C, cancer stem cell characteristics, anti-cancer drug resistance, colorectal cancer.

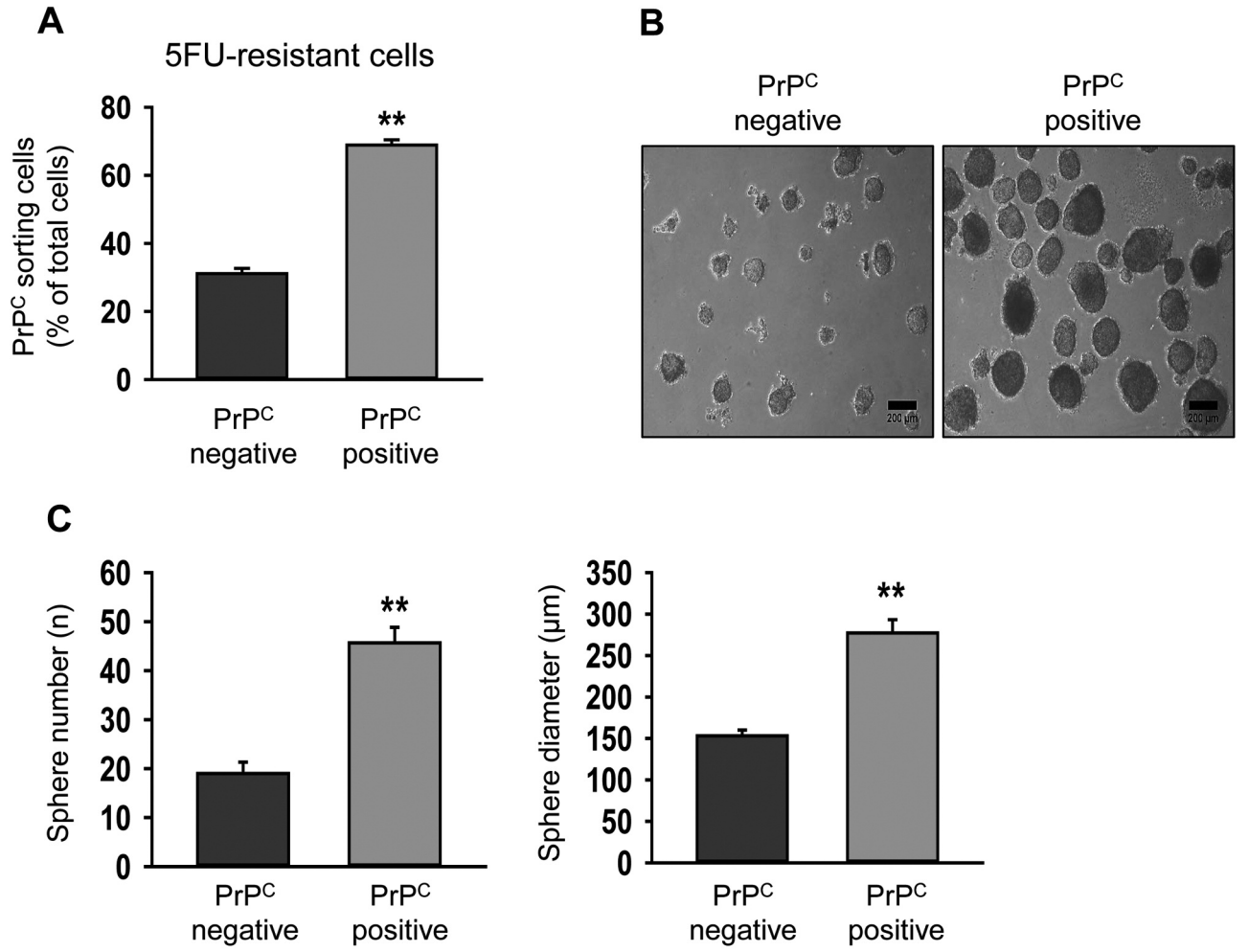

Figure 1. *PrP^C is associated with sphere formation capacity of 5FU-resistant CRC cells. (A) Quantification of PrP^C negative and PrP^C positive cells in 5FU-resistant CRC cells (SNU-C5/5FUR) after cell isolation using MACS (n=3). The values are presented as the mean±SEM. **p<0.01. (B) Sphere formation assay of PrP^C negative and PrP^C positive 5FU-resistant CRC cells. PrP^C negative and PrP^C positive 5FU-resistant CRC cells were grown in ultra-low attachment plates for 2 weeks. (C) Quantification of the number and diameter of spheres (n=3). The values are presented as the mean±SEM. **p<0.01.*

results demonstrated that PrP^C plays a pivotal role in cancer stem cell characteristics of CRC, as well as migration, invasion, and drug resistance of CRC cells. Therefore, PrP^C could be a promising therapeutic target for CRC treatment.

Materials and Methods

Culture of human colorectal cancer cell line. The 5FU-resistant human colon cancer cell line SNU-C5/5FUR was obtained from the Chosun University Research Center for Resistant Cells (Gwangju, Republic of Korea). The cells were grown in Roswell Park Memorial Institute 1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) containing with 10% FBS, L-glutamine, and antibiotics (Thermo Fisher Scientific) at 37˚C in a 5% CO_2 humidified incubator.

Isolation of PrP^C positive cells using magnetic activated cell sorting (MACS). The PrP^C positive cells were sorted using manual MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. The 5FU-resistant CRC cells were incubated with the human CD230 (PrP)-Biotin primary antibody (Miltenyi Biotec). Then, the cells were washed with MACS rinsing solution containing 5% BSA and attached with anti-Biotin MicroBeads secondary antibody. The cells were washed with MACS rinsing solution and sorted with a MACS LS column using a magnetic field.

Spheroid culture. The 5FU-resistant CRC cells were cultured in ultra-low attachment six-well plates (Corning, Corning, NY, USA) for spheroid formation. The 5FU-resistant CRC cells were incubated in RPMI1640 media and grown at 37˚C in a 5% CO_2 atmosphere. Spheroids were grown for 14 days and observed using a optical inverted microscope (Olympus, Tokyo, Japan).

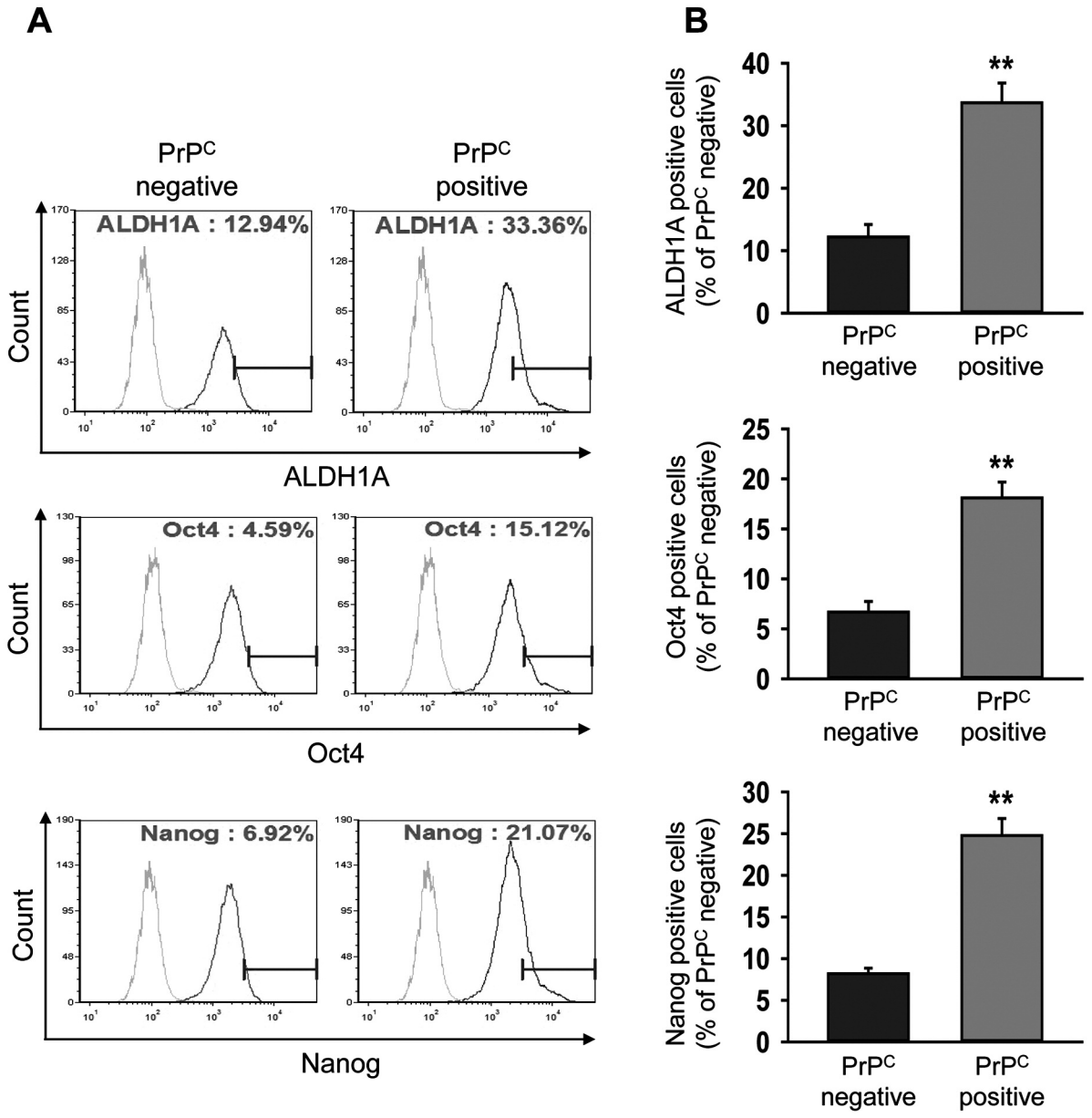

Figure 2. *The expression of cancer stem cell markers was increased in PrP^C positive 5FU-resistant CRC cells. (A) Flow cytometry analysis of ALDH1A, Oc4, and Nanog in PrP^C negative and PrP^C positive 5FU-resistant CRC cells. (B) Percentages of ALDH1A, Oct4, Nanog positive cells in PrP^C negative and PrP^C positive cells (n=3). The values are presented as the mean±SEM. **p<0.01.*

Flow cytometry analysis. Flow cytometry analysis of ALDH1A, Oct4, and Nanog was used to identify cancer stem cells. By comparing the results with the corresponding negative controls, the percentage of stained cells was calculated.

Migration assay. The PrP^C positive 5FU-resistant CRC cells were added in 60 mm cell culture plates and grown at 90% confluency in 4 ml of culture medium. A scratch was made in the cell layer by using sterile pipette tip and treated with 5FU (140 μM) and cultured for 24 h at 37˚C. The cells were transfected with siPRNP or control siRNA prior to the 5FU treatment. The images were obtained using a microscope (Eclipse TE300, Nikon, Tokyo, Japan).

Invasion assay. Matrigel-coated transwell cell culture chambers (8-μm pore size; Sigma-Aldrich, St. Louis, MO, USA) were used to assess the invasion of the PrP^C positive 5FU-resistant CRC cells. The cells were first treated with 5FU, siPRNP, and 5FU + siPRNP, then collected and resuspended in serum-free RPMI1640 medium. Subsequently, 30,000 cells were added in the inserts of transwell chambers. RPMI1640 medium containing 10% FBS was added in

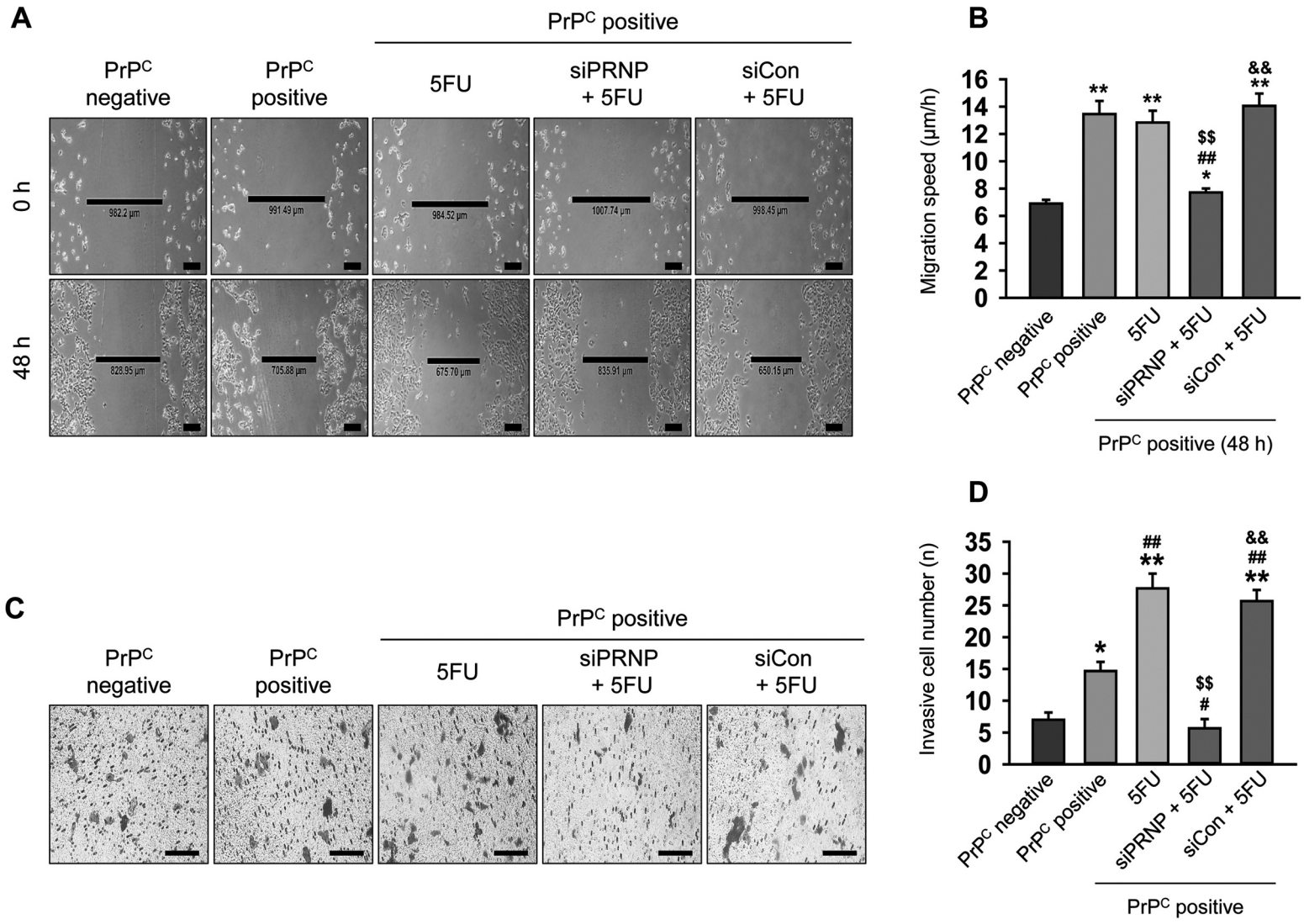

Figure 3. *The effect of PrP^C on the migration and invasion of 5FU-resistant CRC cells. (A) Representative migration assay of PrP^C negative and PrP^C positive 5FU-resistant CRC cells after treatment with 5FU (140 μM, 48 h). The PrP^C positive cells were transfected with siPRNP or control siRNA prior to the 5FU treatment. Scale bar=200 μm. (B) Bar graph of migration speed (n=3). The values are presented as the mean±SEM. **p<0.01 vs. PrP^C negative, ##p<0.01 vs. PrP^C positive, $$p<0.01 vs. PrP^C positive cells treated with 5FU, &&p<0.01 vs. knockdown of PrP^C in PrP^C positive cells treated with 5FU. (C) Representative invasion analysis of PrP^C negative and PrP^C positive 5FU-resistant CRC cells after treatment with 5FU (140 μM, 72 h). The PrP^C positive cells were transfected with siPRNP or control siRNA prior to the 5FU treatment. Scale bar=200 μm. (D) Bar graph of number of invasive cells (n=3). The values are presented as the mean±SEM. *p<0.05, **p<0.01 vs. PrP^C negative. ##p<0.01 vs. PrP^C positive. $$p<0.01 vs. PrP^C positive cells treated with 5FU, &&p<0.01 vs. knockdown of PrP^C in PrP^C positive cells treated with 5FU.*

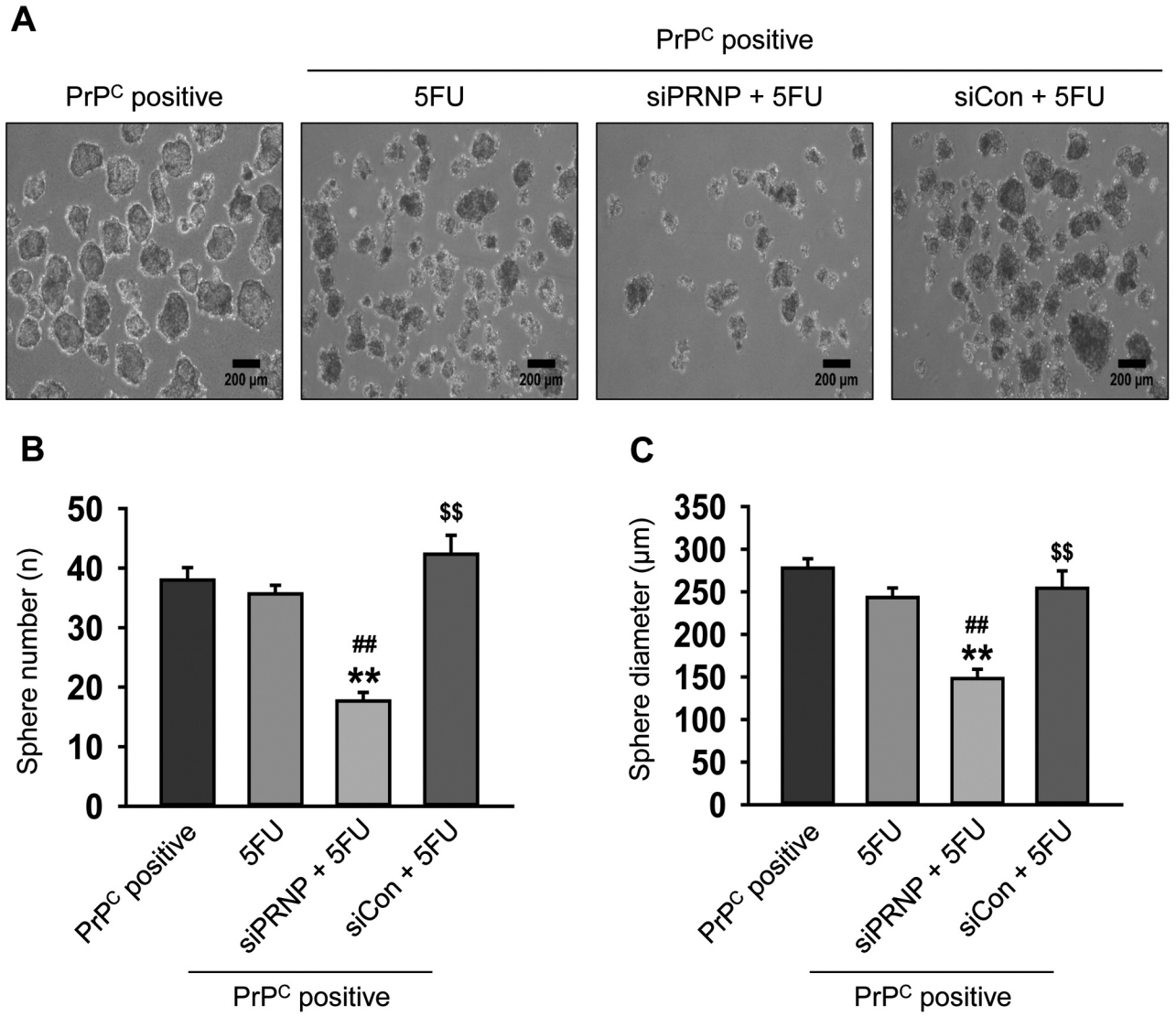

Figure 4. *Knockdown of PrP^C decreased sphere formation capacity of PrP^C positive 5FU-resistant CRC cells. (A) Sphere formation assay of PrP^C positive 5FU-resistant CRC cells after treatment with 5FU (140 μM, 7 days). The PrP^C positive cells were transfected with siPRNP or control siRNA prior to the 5FU treatment. Scale bar=200 μm. PrP^C negative and PrP^C positive 5FU-resistant CRC cells were grown in ultra-low attachment plates for 2 weeks. (B, C) Quantification of the number and diameter of spheres (n=3). The values are represented as the mean±SEM. **p<0.01 vs. PrP^C positive. ##p<0.01 vs. PrP^C positive cells treated with 5FU, $$p<0.01 vs. knockdown of PrP^C in PrP^C positive cells treated with 5FU.*

the lower chamber. The cells were incubated in a 37˚C incubator for 72 h. In the transwell chambers, the cells were fixed with 4% PFA (Affymetrix, Santa Clara, CA, USA) in PBS and stained with 2% crystal violet in 2% ethanol for 10 min at room temperature. After the cells were stained, the transwell chamber was thoroughly washed with distilled water. Non-invasive cells were removed using a cotton swab and the invasive cells in the lower surface of the transwell chamber were imaged and quantified using a light microscope.

Inhibition of PrP^C expression using siRNA. The PrP^C positive 5FU-resistant cells were seeded (2.5×10^5 cells) in 60 mm culture plate. The cells were transfected with siRNA against PrPc using using Lipofectamine 2000, according to the manufacturer's instructions (Thermo Fisher Scientific). The control siRNA and siPRNP were purchased from Bioneer (Daejeon, Republic of Korea).

Cell survival assay. PrP^C negative and PrP^C positive 5FU-resistant cells were seeded in 96-well culture plates (5,000 cells/well) for 48 h. After treatment with 5FU, cell survival was determined using a modified MTT assay, in which the tetrazolium salt was converted to formazan by mitochondrial dehydrogenase. The formazan was dissolved in DMSO and the intensity of the color was quantified by measuring the absorbance at 450 nm using a microplate reader (Varioskan LUX, Thermo Fisher Scientific).

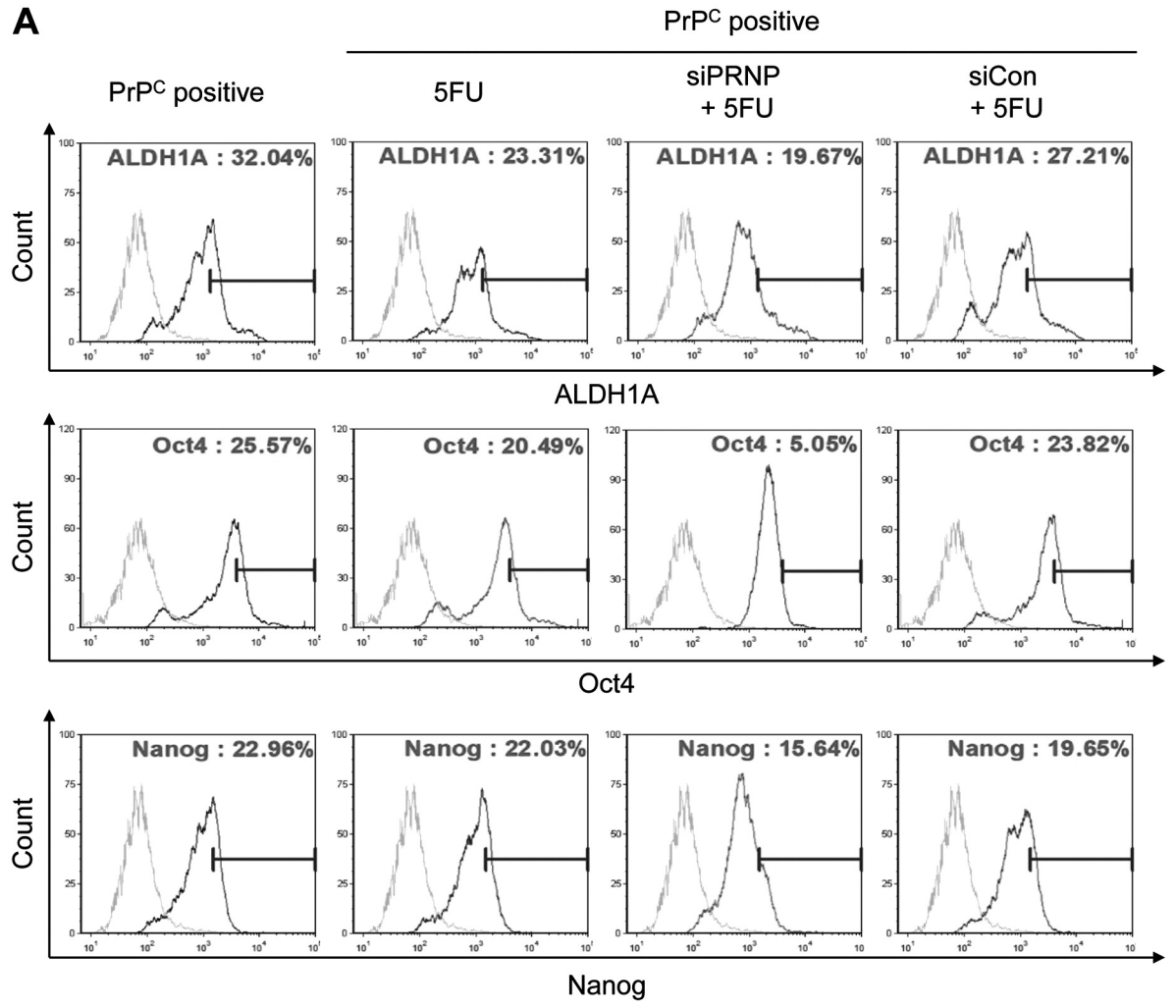

Figure 5. *Continued*

PI/Annexin V flow cytometric analysis. Apoptosis of PrP^C positive 5FU resistant cells was assessed by staining the cells with propidium iodide (PI) and annexin V-fluorescein isothiocyanate (FITC) (De Novo Software, Los Angeles, CA, USA). Each sample was analyzed using a CyFlow Cube 8 (Sysmex Partec, Münster, Germany). The data were analyzed using the FSC Express software (De Novo Software).

Statistical analysis. All values are represented as mean±standard error of the mean (SEM) with the indicated sample size. Statistical significance was determined by two-tailed Student's *t*-test or one-way analysis of variance (ANOVA). Comparisons of ≥3 groups were made using the Dunnett's or Tukey's *post-hoc* test. *p*-Values <0.05 were considered as statistically significant.

Results

Cancer stem cell characteristics are increased in PrP^C positive 5FU-resistant CRC cells. To determine whether PrP^C is involved in cancer stem cell characteristics in CRC, we first isolated PrP^C negative and PrP^C positive cells from 5FU-resistant CRC cells using MACS. In 5FU-resistant cells, the number of PrP^C positive cells was higher than that of PrP^C negative cells (Figure 1A). Next, we performed sphere formation assay by culturing the cells in ultra-low attachment six-well plates for 14 days. The sphere formation capacity, such as the number and diameter of spheres, were significantly enhanced in PrP^C positive cells (Figure 1B and 1C). To further explore whether PrP^C has an impact on cancer stem cell characteristics in 5FU-resistant CRC cells, we investigated the expression of cancer stem cell markers, including ALDH1A, Oct4, and Nanog, in PrP^C negative and PrP^C positive cells. We found that the expression of cancer stem cell markers was higher in PrP^C positive cells compared to PrP^C negative cells (Figure 2A and 2B). These results indicate that PrP^C expression is related with cancer stem cell characteristics of 5FU-resistant CRC cells.

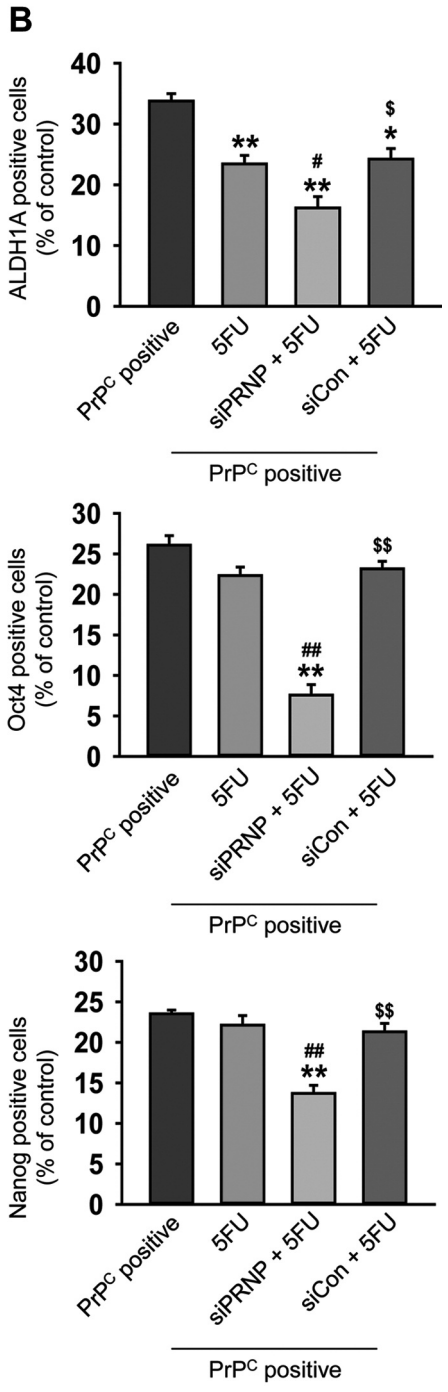

Figure 5. *Knockdown of PrP^C decreases cancer stem cell markers in PrP^C positive 5FU-resistant cells. (A) Flow cytometry analysis for ALDH1A, Oc4, and Nanog in PrP^C positive 5FU-resistant CRC cells after treatment with 5FU (140 μM, 7 days). The PrP^C positive cells were transfected with siPRNP or control siRNA prior to the 5FU treatment. (B) Percentages of ALDH1A, Oct4, Nanog in PrP^C positive cells treated with 5FU. The PrP^C positive cells were transfected with siPRNP or control siRNA prior to the 5FU treatment (n=3). The values are presented as the mean±SEM. **p<0.01 vs. PrP^C positive, $^{\#}$p<0.05, $^{\#\#}$p<0.01 vs. PrP^C positive cells treated with 5FU, $^{\$}$p<0.05, $^{\$\$}$p<0.01 vs. knockdown of PrP^C in PrP^C positive cells treated with 5FU.*

PrP^C is involved in the migration and invasion of CRC cells. Cancer metastasis, which involves migration and invasion of cancer cells, is the leading cause of cancer-related mortality. A previous study has shown that PrP^C expression affects the metastasis of CRC cells in a tumor xenograft model (18). To determine the effect of PrP^C expression on the migration of 5FU-resistant CRC cells, we performed a scratch wound-healing assay. PrP^C positive cells showed increased migration capacity compared to the PrP^C negative cells (Figure 3A and B). Next, we assessed the effect of PrP^C expression on the invasion of CRC cells. The invasion capacity of PrP^C positive cells was higher compared to PrP^C negative cells (Figure 3C and D). However, downregulation of PrP^C by siPRNP transfection significantly blocked the effect of PrP^C on the migration and invasion of CRC cells (Figure 3A-D). These results indicate that PrP^C is involved in the migration and invasion of CRC cells, and may potentially promote tumor metastasis and progression.

Knockdown of PrP^C decreased cancer stem cell characteristics in PrP^C positive CRC cells. To confirm the effect of PrP^C on cancer stem cell characteristics in 5FU-resistant CRC cells, a sphere formation assay was performed after transfection of PrP^C positive 5FU-resistant CRC cells with PRNP siRNA. The PrP^C positive 5FU-resistant CRC cells transfected with PRNP siRNA showed decreased sphere formation capacity compared to non-transfected cells (Figure 4A-C). Furthermore, flow cytometry analysis of the expression of cancer stem cell markers showed that PrP^C knockdown also decreased the expression of ALDH1A, Oct4, and Nanog in PrP^C positive 5FU-resistant CRC cells after treatment with 5FU (Figure 5A and B). These findings suggest that PrP^C regulates cancer stem cell characteristics in 5FU-resistant CRC cells.

Knockdown of PrP^C increases anti-cancer drug susceptibility of 5FU-resistant CRC cells. To determine whether PrP^C is related to the drug susceptibility of CRC cells, PrP^C negative and PrP^C positive cells were treated with 5FU. The 5FU treatment of PrP^C positive cells did not affect cell viability, whereas treatment with 5FU of PrP^C negative cells significantly decreased cell viability (Figure 6A). To further investigate whether knockdown of PrP^C increases the anti-cancer drug sensitivity in 5FU-resistant CRC cells, we performed annexin V/propidium iodide (PI) flow cytometry analysis. Treatment with 5FU increased the percentage of early and late apoptotic cells to 5.2% compared to the percentage in the control group (3.2%). The percentage of apoptotic cells was further increased in populations transfected with siPRNP and treated with 5FU (10.7%) (Figure 6B and C). These results indicate that PrP^C expression is involved in drug resistance in 5FU-resistant CRC cells.

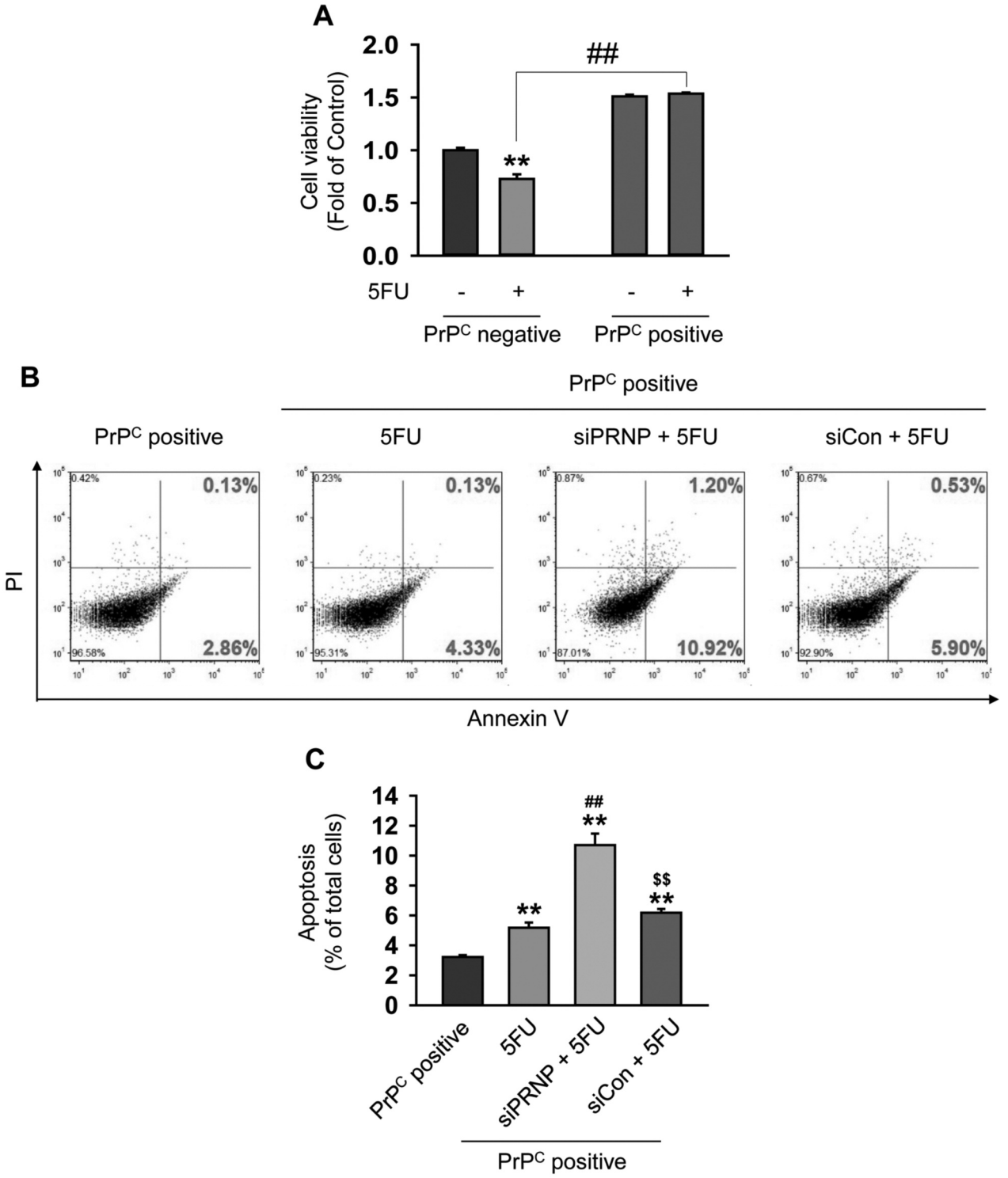

Figure 6. *Knockdown of PrP^C enhances the drug susceptibility of PrP^C positive 5FU-resistant CRC cells. (A) Cell viability assay of PrP^C negative and PrP^C positive cells treated with or without 5FU (140 μM, 24 h) (n=3). The values are presented as the mean±SEM. **p<0.01 vs. PrP^C negative without 5FU treatment. ##p<0.01 vs. PrP^C negative cells after 5FU treatment. (B) Flow cytometry analysis of Annexin V and PI stained PrP^C positive 5FU-resistant cells after treatment with 5FU. The PrP^C positive cells were transfected with siPRNP or control siRNA prior to the 5FU treatment. (C) Percentage of apoptotic PrP^C positive 5FU-resistant cells after treatment with 5FU. The PrP^C positive cells were transfected with siPRNP or control siRNA prior to the 5FU treatment. The values are presented as the mean±SEM. **p<0.01 vs. PrP^C positive, ##p<0.01 vs. PrP^C positive cells treated with 5FU, $$p<0.01 vs. knockdown of PrP^C in PrP^C positive cells treated with 5FU.*

Discussion

CSCs are a subset of cancer cells with the unique characteristics of self-renewal, infinite division, and ability to differentiate into multiple types of cells. These features of CSCs play an important role in the metastasis and relapse of CRC. Colorectal CSCs are characterized by a group of marker proteins, such as CD44, CD133, Nanog, Oct-4, and ALDH. In addition to being surface markers, these molecules are biologically functional. For example, ALHD1 is associated with metastasis, and drug resistance, and is related to advanced stage colorectal cancer (19, 20). Oct 4 is known to regulate stemness and is associated with lymphatic invasion and lymph node metastasis (21, 22). A recent study showed that knockdown of PrP^C in colon cancer stem cells significantly decreased the expression of ALDH1A, Oct-4, and Nanog (15). In this study, we demonstrated that the expression of cancer stem cell markers was significantly increased in PrP^C positive 5FU-resistant cells, and knockdown of PrP^C decreased the expression of cancer stem cell markers. Taken together, these results suggest that the PrP^C expression levels are strongly associated with CRC progression through regulation of cancer stem cell properties in CRC.

Cancer metastasis represents an advanced stage of malignancy and is the leading cause of cancer-related death. Metastasis involves many processes, such as the migration and invasion of cancer cells, which are features of malignancy (23). Previous studies have shown that PrP^C promotes uncontrolled cell proliferation, tumor growth, and metastasis by modifying cytoskeleton regulatory proteins such as Fyn and promoting the epithelial– mesenchymal transition (24-26). A recent study has shown that the HSP family A member 1-like (HSPA1L) protein regulates PrP^C expression and affects liver metastasis of human colon CSCs by modulating HIF-1α stability and inhibiting GP78-mediated ubiquitination of PrP^C (18). Consistent with previous findings, we found that cancer cell migration and invasion were higher in PrP^C positive cells and knockdown of PrP^C expression blocked their ability for migration and invasion. These results indicate that prion protein promotes cancer metastasis by regulating cancer cell migration and invasion.

Drug-resistant cancer cells display high proliferation and tumorigenic potential during chemotherapy (27, 28). In addition, drug-resistant cancer cells exhibit greater invasiveness and metastatic ability compared to their nonresistant counterparts, and are partly responsible for rendering the anti-cancer therapy ineffective (29). A previous study has shown that the expression of PrP^C increased drug resistance to chemotherapeutics (17). In gastric cancer cells, PrP^C expression enhanced drug resistance by increasing Bcl-2 expression (30). Moreover, treatment of HCT116 CRC cells with antibodies specific to PrP^C inhibited the growth of cancer cells and promoted the action of anticancer drugs, such as 5-FU, cisplatin, and doxorubicin (31). In this study, drug resistance was increased in PrP^C positive cells and knockdown of PrP^C decreased drug resistance and increased 5FU-induced apoptosis. These findings suggest that PrP^C could be a key protein involved in the resistance of CRC cells to anticancer drugs.

This study revealed that cancer sphere formation and the expression of cancer stem cell markers, including ALDH1A, Oct4, and Nanog, were significantly higher in PrP^C positive CRC cells. Furthermore, cancer cell migration and invasion, and drug resistance were also significantly higher in PrP^C positive CRC cells. However, downregulation of PrP^C blocked these activities. These findings indicate that PrP^C plays a pivotal role in CRC behavior and that PrP^C could be a promising therapeutic target in CRC treatment.

Conflicts of Interest

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

Authors' Contributions

Gyeongyun Go: data analysis, and drafting of manuscript; Chul Won Yun: data collection, and data analysis; Yeo Min Yoon: data collection, and data analysis; Ji Ho Lim: data collection, and data analysis; Jun Hee Lee: drafting of manuscript; Sang Hun Lee: designing the study, drafting and editing of manuscript, procurement of funding.

Acknowledgements

This work was supported by a grant from the National Research Foundation funded by the Korean government (2019M3A9H1103495).

References

1 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2020. CA Cancer J Clin 70(1): 7-30, 2020. PMID: 31912902. DOI: 10.3322/caac.21590

2 Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C and De Maria R: Identification and expansion of human colon-cancer-initiating cells. Nature *445(7123)*: 111-115, 2007. PMID: 17122771. DOI: 10.1038/nature05384

3 Kreso A and Dick JE: Evolution of the cancer stem cell model. Cell Stem Cell *14(3)*: 275-291, 2014. PMID: 24607403. DOI: 10.1016/j.stem.2014.02.006

4 Dai X, Ge J, Wang X, Qian X, Zhang C and Li X: Oct4 regulates epithelial-mesenchymal transition and its knockdown inhibits colorectal cancer cell migration and invasion. Oncol Rep *29(1)*: 155-160, 2013. PMID: 23076549. DOI: 10.3892/or.2012.2086

5 Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alakl M, Gruia G, Awad L and Rougier P: Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: A multicentre randomised trial. Lancet *355(9209)*: 1041-1047, 2000. PMID: 10744089. DOI: 10.1016/s0140-6736(00)02034-1

6 Van der Jeught K, Xu HC, Li YJ, Lu XB and Ji G: Drug resistance and new therapies in colorectal cancer. World J Gastroenterol *24(34)*: 3834-3848, 2018. PMID: 30228778. DOI: 10.3748/wjg.v24.i34.3834

7 Bartolini A, Cardaci S, Lamba S, Oddo D, Marchio C, Cassoni P, Amoreo CA, Corti G, Testori A, Bussolino F, Pasqualini R, Arap W, Cora D, Di Nicolantonio F and Marchio S: Bcam and lama5 mediate the recognition between tumor cells and the endothelium in the metastatic spreading of kras-mutant colorectal cancer. Clin Cancer Res *22(19)*: 4923-4933, 2016. PMID: 27143691. DOI: 10.1158/1078-0432.CCR-15-2664

8 Welch HG and Robertson DJ: Colorectal cancer on the decline—why screening can't explain it all. N Engl J Med *374(17)*: 1605-1607, 2016. PMID: 27119236. DOI: 10.1056/NEJMp1600448

9 Dalerba P, Sahoo D and Clarke MF: Cdx2 as a prognostic biomarker in colon cancer. N Engl J Med 374*(22)*: 2184, 2016. PMID: 27248627. DOI: 10.1056/NEJMc1602584

10 Weissmann C: The state of the prion. Nat Rev Microbiol *2(11)*: 861-871, 2004. PMID: 15494743. DOI: 10.1038/nrmicro1025

11 Zomosa-Signoret V, Arnaud JD, Fontes P, Alvarez-Martinez MT and Liautard JP: Physiological role of the cellular prion protein. Vet Res *39(4)*: 9, 2008. PMID: 18073096. DOI: 10.1051/vetres:2007048

12 Prusiner SB: Novel proteinaceous infectious particles cause scrapie. Science 216*(4542)*: 136-144, 1982. PMID: 6801762. DOI: 10.1126/science.6801762

13 Santos TG, Lopes MH and Martins VR: Targeting prion protein interactions in cancer. Prion *9(3)*: 165-173, 2015. PMID: 26110608. DOI: 10.1080/19336896.2015.1027855

14 Mehrpour M and Codogno P: Prion protein: From physiology to cancer biology. Cancer Lett *290(1)*: 1-23, 2010. PMID: 19674833. DOI: 10.1016/j.canlet.2009.07.009

15 Lee JH, Yun CW, Han YS, Kim S, Jeong D, Kwon HY, Kim H, Baek MJ and Lee SH: Melatonin and 5-fluorouracil co-suppress colon cancer stem cells by regulating cellular prion protein-oct4 axis. J Pineal Res *65(4)*: e12519, 2018. PMID: 30091203. DOI: 10.1111/jpi.12519

16 Zhou L, Shang Y, Liu C, Li J, Hu H, Liang C, Han Y, Zhang W, Liang J and Wu K: Overexpression of prpc, combined with mgr1-ag/37lrp, is predictive of poor prognosis in gastric cancer. Int J Cancer *135(10)*: 2329-2337, 2014. PMID: 24706505. DOI: 10.1002/ijc.28883

17 Li QQ, Cao XX, Xu JD, Chen Q, Wang WJ, Tang F, Chen ZQ, Liu XP and Xu ZD: The role of p-glycoprotein/cellular prion protein interaction in multidrug-resistant breast cancer cells treated with paclitaxel. Cell Mol Life Sci *66(3)*: 504-515, 2009. PMID: 19099191. DOI: 10.1007/s00018-008-8548-6

18 Lee JH, Han YS, Yoon YM, Yun CW, Yun SP, Kim SM, Kwon HY, Jeong D, Baek MJ, Lee HJ, Lee SJ, Han HJ and Lee SH: Role of hspa1l as a cellular prion protein stabilizer in tumor progression via hif-1alpha/gp78 axis. Oncogene *36(47)*: 6555-6567, 2017. PMID: 28759037. DOI: 10.1038/onc.2017.263

19 Rassouli FB, Matin MM and Saeinasab M: Cancer stem cells in human digestive tract malignancies. Tumour Biol *37(1)*: 7-21, 2016. PMID: 26446457. DOI: 10.1007/s13277-015-4155-y

20 Hessman CJ, Bubbers EJ, Billingsley KG, Herzig DO and Wong MH: Loss of expression of the cancer stem cell marker aldehyde dehydrogenase 1 correlates with advanced-stage colorectal cancer. Am J Surg *203(5)*: 649-653, 2012. PMID: 22405917. DOI: 10.1016/j.amjsurg.2012.01.003

21 Wahab SMR, Islam F, Gopalan V and Lam AK: The identifications and clinical implications of cancer stem cells in colorectal cancer. Clin Colorectal Cancer *16(2)*: 93-102, 2017. PMID: 28237538. DOI: 10.1016/j.clcc.2017.01.011

22 Matsuoka J, Yashiro M, Sakurai K, Kubo N, Tanaka H, Muguruma K, Sawada T, Ohira M and Hirakawa K: Role of the stemness factors sox2, oct3/4, and nanog in gastric carcinoma. J Surg Res *174(1)*: 130-135, 2012. PMID: 21227461. DOI: 10.1016/j.jss.2010.11.903

23 Tahtamouni L, Ahram M, Koblinski J and Rolfo C: Molecular regulation of cancer cell migration, invasion, and metastasis. Anal Cell Pathol (Amst) *2019*: 1356508, 2019. PMID: 31218208. DOI: 10.1155/2019/1356508

24 Wang Q, Qian J, Wang F and Ma Z: Cellular prion protein accelerates colorectal cancer metastasis via the fyn-sp1-satb1 axis. Oncol Rep *28(6)*: 2029-2034, 2012. PMID: 28822989. DOI: 10.3892/or.2012.2025

25 Li QQ, Sun YP, Ruan CP, Xu XY, Ge JH, He J, Xu ZD, Wang Q and Gao WC: Cellular prion protein promotes glucose uptake through the fyn-hif-2alpha-glut1 pathway to support colorectal cancer cell survival. Cancer Sci *102(2)*: 400-406, 2011. PMID: 21265952. DOI: 10.1111/j.1349-7006.2010.01811.x

26 Du L, Rao G, Wang H, Li B, Tian W, Cui J, He L, Laffin B, Tian X, Hao C, Liu H, Sun X, Zhu Y, Tang DG, Mehrpour M, Lu Y and Chen Q: Cd44-positive cancer stem cells expressing cellular prion protein contribute to metastatic capacity in colorectal cancer. Cancer Res *73(8)*: 2682-2694, 2013. PMID: 23418321. DOI: 10.1158/0008-5472.CAN-12-3759

27 Xu YC, Liu X, Li M, Li Y, Li CY, Lu Y, Sanches J, Wang L, Du Y, Mao LM, Zuo SB, Liu HT, Shen J, Wang B, Hou L, Li LH, Tang JW, Ju JF, Guan HW and Song B: A novel mechanism of doxorubicin resistance and tumorigenesis mediated by microrna-501-5p-suppressed blid. Mol Ther Nucleic Acids *12*: 578-590, 2018. PMID: 30195794. DOI: 10.1016/j.omtn.2018.06.011

28 Margolin DA, Silinsky J, Grimes C, Spencer N, Aycock M, Green H, Cordova J, Davis NK, Driscoll T and Li L: Lymph node stromal cells enhance drug-resistant colon cancer cell tumor formation through sdf-1alpha/cxcr4 paracrine signaling. Neoplasia *13(9)*: 874-886, 2011. PMID: 21969820. DOI: 10.1593/neo.11324

29 Jeon JH, Kim DK, Shin Y, Kim HY, Song B, Lee EY, Kim JK, You HJ, Cheong H, Shin DH, Kim ST, Cheong JH, Kim SY and Jang H: Migration and invasion of drug-resistant lung adenocarcinoma cells are dependent on mitochondrial activity. Exp Mol Med *48(12)*: e277, 2016. PMID: 27932791. DOI: 10.1038/emm.2016.129

30 Wang JH, Du JP, Zhang YH, Zhao XJ, Fan RY, Wang ZH, Wu ZT and Han Y: Dynamic changes and surveillance function of prion protein expression in gastric cancer drug resistance. World J Gastroenterol *17(35)*: 3986-3993, 2011. PMID: 22046086. DOI: 10.3748/wjg.v17.i35.3986

31 McEwan JF, Windsor ML and Cullis-Hill SD: Antibodies to prion protein inhibit human colon cancer cell growth. Tumor Biol *30(3)*: 141-147, 2009. PMID: 19521145. DOI: 10.1159/000225243

Received August 3, 2020
Revised August 24, 2020
Accepted August 25, 2020