INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed cancer among males and females, the second leading cause of cancer related death among males, and third leading cause among females (Siegel et al., 2017). Although surgical techniques, chemotherapies, and molecular therapies have improved, clinical outcomes have not kept pace. This lag is mostly because of changes in lifestyle, local recurrence, distal metastasis, and resistance to chemotherapeutics and molecularly targeted therapies (Holohan et al., 2013; Oliphant et al., 2013). Among these hurdles, drug resistance limits therapeutic efficacy, because chemotherapy is one of the principal modes of cancer therapy (Holohan et al., 2013). In particular, chemotherapy failure caused by anti-cancer drug resistance induces most cancer related deaths due to decreases in drug delivery and changes in metabolic enzymes (Hammond et al., 2016). Therefore, understanding the mechanisms underlying drug resistance plays a pivotal role in developing reversal strategies and effective cancer therapeutic combinations.

5-fluorouracil (5-FU) is widely used in the treatment for several cancers, particularly for CRC. It is an antimetabolite drug that inhibits thymidylate synthase and is incorporated into RNA, single DNA, and double DNA helix, leading to cancer cell death (Longley et al., 2003). Several clinical studies have shown that 5-FU-based chemotherapies and chemoradiotherapies increased the survival of patients with several cancers (Pignon et al., 2000; Adelstein et al., 2006; Tsukuda et al., 2010). However, despite the benefits of 5-FU to cancer therapy, acquired resistance to 5-FU is a major clinical problem. Accumulated evidence shows that abnormally high activities of thymidylate synthase, deoxyuridine triphosphatase, Bcl-2, Bcl-XL, and Mcl-1 lead to 5-FU resistance (Zhang et al., 2008). However, the detailed molecular mechanisms for 5-FU chemoresistance in CRC require further investigation.

Cellular prion protein (PrPC) is highly expressed in a variety of cells, such as lymphocytes, and tissues, including muscle, heart, skin, and nervous tissues (Liang and Kong, 2012). Although studies on PrP C have initially focused on the nervous system, recent evidence indicates that PrPC regulates not only self-renewal of stem/progenitor cells and stem cell fate but also proliferation and resistance to apoptosis in cancer cells. Anti-cancer drug resistance is a major problem in colorectal cancer (CRC) research. Although several studies have revealed the mechanism of cancer drug resistance, molecular targets for chemotherapeutic combinations remain elusive. To address this issue, we focused on the expression of cellular prion protein (PrP C) in 5-FU-resistant CRC cells. In 5-FU-resistant CRC cells, PrP C expression is significantly increased, compared with that in normal CRC cells. In the presence of 5-FU, PrP C increased CRC cell survival and proliferation by maintaining the activation of the PI3K-Akt signaling pathway and the expression of cell cycle-associated proteins, including cyclin E, CDK2, cyclin D1, and CDK4. In addition, PrP C inhibited the activation of the stress-associated proteins p38, JNK, and p53. Moreover, after treatment of 5-FU-resistant CRC cells with 5-FU, silencing of PrP C triggered apoptosis via the activation of caspase-3. These results indicate that PrP C plays a key role in CRC drug resistance. The novel strategy of combining chemotherapy with PrP C targeting may yield efficacious treatments of colorectal cancer.

Key Words: Colorectal cancer, Prion protein, Drug resistance, 5-fluorouracil, Anticancer
In CRC, PrP<sup>C</sup> promotes cancer cell survival by increasing uptake of glucose (Li et al., 2011). PrP<sup>C</sup> augmented CRC metastasis through the Fyn-SP1-SATB1 pathway (Wang et al., 2012). Our previous study reveals that silencing PrP<sup>C</sup> inhibits colon cancer cell growth (Yun et al., 2016). However, there is little evidence of a relationship between PrP<sup>C</sup> and anti-cancer chemoresistance in CRC cells. In this study, we investigated the effect of PrP<sup>C</sup> on proliferation and survival in 5-FU resistant CRC cells. Moreover, we explored the underlying mechanism of resistance to 5-FU in CRC cells through regulation of PrP<sup>C</sup> expression.

**MATERIALS AND METHODS**

**Preparation of 5-fluorouracil and oxaliplatin**

5-Fluorouracil and oxaliplatin were obtained from Sigma (St. Louis, MO, USA). 5-Fluorouracil and oxaliplatin powder were dissolved in dimethyl sulfoxide (DMSO), and aliquots were stored at 4°C until use.

**Cell culture**

The human colon cancer cell line (SNU-C5/WT), 5-FU resistant cell line (SNU-C5/5FUR) and oxaliplatin-resistant cell line (SNU-C5/Oxal-R) were obtained from the Chosun University Research Center for Resistant Cells (Gwangju, Korea). The cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, l-glutamine, and antibiotics (Biological Industries, Beit Haemek, Israel) at 37°C with 5% CO<sub>2</sub> in a humidified incubator.

**Western blot analysis**

Total cell protein was extracted by utilizing RIPA lysis buffer (Thermo Fisher Scientific, Rockford, IL, USA). Cell lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and proteins were transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk and incubated with primary antibodies against PrP<sup>C</sup>, phospho-phosphatidylinositol-3-kinase (PI3K), total PI3K, phospho-AKT, total AKT, cyclin dependent kinase (CDK) 2, CDK4, cyclin D1, cyclin E, phosphor-p38, total p38, phosphor-c-JUN N-terminal kinase (JNK), total JNK, cleaved caspase-3, cleaved poly [ADP-ribose] polymerase 1 (PARP1), β-actin (Santa Cruz Biotechnology, Dallas, TX, USA), phospho-p53, total p53 (Cell Signaling Technology, Danvers, MA, USA), phospho-ataxia-telangiectasia mutated (ATM), and total ATM (Thermo Fisher Scientific). After incubation of membranes with peroxidase-conjugated goat anti-mouse or anti-rabbit IgG secondary antibodies (Santa Cruz biotechnology), bands were detected by utilizing enhanced chemiluminescence reagents (Amersham Biosciences, Uppsala, Sweden).

**Flow cytometry**

SNU-C5/WT and SNU-C5/5FUR cells were subjected to flow cytometry analysis using anti-PrP<sup>C</sup> antibody (Santa Cruz Biotechnology). Flow cytometry was performed using a Cyflow Cube 8 (Partec, Münster, Germany). Data analysis was per-
formed using standard FSC Express (De Novo software; Los Angeles, USA).

**Immunocytochemistry**

SNU-C5/WT and SNU-C5/5FUR cells on the cover glass slide were fixed in 4% paraformaldehyde solution for 10 min. After washing three times in PBS, cells were blocked with 10% goat serum for 1 h, and then incubated with primary antibodies against PrP<sup>C</sup> (Santa Cruz Biotechnology) at 4°C for 24 h. After washing three times in PBS, cells were incubated with secondary antibodies conjugated with Alexa-488 (Thermo Fisher Scientific) for 1 h. Immunostained slides were imaged by fluorescent microscope (ZEISS, Oberkochen, Germany).

**Silencing of PrP<sup>C</sup> expression by small interfering RNA**

SNU-C5/5FUR and SNU-C5/Oxal-R cells were seeded in 60 mm plates and grown up to 75% confluence. Cells were transfected with siRNA in serum-free Opti-MEM (Thermo Fisher Scientific), using Lipofectamine 2000 (Thermo Fisher Scientific) by following the manufacturer's protocols. At 48 h after transfection, the level of PrP<sup>C</sup> was analyzed by western blot. The siRNA used to target PRNP and a scrambled sequence were synthesized by Dharmacon (Lafayette, CO, USA).

**Kinase assays**

The cells were lysed using RIPA lysis buffer (Thermo Fisher Scientific). Cdk4 kinase assays were performed using a CKD4 Kinase Assay Kit (Cusabio, Baltimore, USA). Briefly, 10 mM ATP was added to 1.25 ml of 6 µM substrate peptide. The mixture was diluted with dH<sub>2</sub>O to 2.5 ml to yield a 2x ATP/substrate cocktail. Then 1 ml of 10x kinase buffer was added to 1.5 ml of dH<sub>2</sub>O to yield a 2.5 ml 4x reaction buffer and the enzyme was diluted in reaction buffer to give the reaction cocktail. The reaction cocktail was added to 12.5 µl/well of prediluted compound of interest (usually approximately 10 µM) and incubated for 5 min at room temperature. ATP/substrate cocktail was added to 25 µl/well preincubated reaction cocktail/compound. The final assay conditions for a 50-µl reaction were therefore 25 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 5 mM b-glycerophosphate, 0.1 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM DTT, 200 µM ATP, 1.5 µM peptide, and 50 ng CDK4 kinase. The reaction was incubated at room temperature for 30 min, then the stop buffer was added. From each reaction, 25 µl was transferred to a 96-well streptavidin-coated plate and incubated at room temperature for 1 h. Plates were washed three times with PBS. Phospho-Rb (Ser780) antibody was added at 100 µl/well and incubated at room temperature for 2 h. Plates were
Fig. 3. The effect of PrP<sup>C</sup> on proliferation in colorectal cancer cells. (A) The level of cyclin E, CDK2, cyclin D1, and CDK4 in SNU-C5/5FUR cells transfected with si-scr or si-PRNP after treatment with PBS or 5-FU (140 µM) for 48 h. (B) The expression level of cyclin E, CDK2, cyclin D1, and CDK4 was determined by densitometry, relative to β-actin expression. (C) The kinase activity of CDK4 in SNU-C5/5FUR cells transfected with si-scr or si-PRNP after treatment with PBS or 5-FU (140 µM) for 48 h. (D) The percentage of G1, S, and G2/M phase in SNU-C5/5FUR cells transfected with si-scr or si-PRNP after treatment with PBS or 5-FU was determined by flow cytometry analysis of PI-stained cells. (E) The quantitation of the percentage of cells in S phase. (B, C, E) Data are expressed as the mean ± SEM of three independent experiments. *p<0.05, **p<0.01 vs. scrambled siRNA-transfected SNUC5/5FUR (si-scr) treated with PBS, #p<0.01 vs. si-PRNP treated with PBS, and ##p<0.01 vs. si-scr treated with 5-FU.

Statistical analyses

Data are expressed as means ± SEM. Statistical significance was assessed using the Student’s t test, where p<0.05 was considered significant.

RESULTS

Expression of PrP<sup>C</sup> in drug resistant CRC cells

Several studies have shown that the expression of PrP<sup>C</sup> is increased in gastric, breast, and CRC cells (Yang et al., 2014).
Fig. 4. The effect of PrP<sup>C</sup> on cell stress-associated protein in colorectal cancer cells. (A) The phosphorylation of p38 in SNU-C5/5FUR cells transfected with si-scr or si-PRNP after treatment with PBS or 5-FU (140 µM) for 48 h. (B) The level of p-p38 was determined by densitometry, relative to total p38 expression. (C) The phosphorylation of JNK in SNU-C5/5FUR cells transfected with si-scr or si-PRNP after treatment with PBS or 5-FU (140 µM) for 48 h. (D) The level of p-JNK was determined by densitometry, relative to total JNK expression. (E) The phosphorylation of p53 in SNU-C5/5FUR cells transfected with si-scr or si-PRNP after treatment with PBS or 5-FU (140 µM) for 48 h. (F) The level of p-p53 was determined by densitometry, relative to total p53 expression. (G) The phosphorylation of ATM in SNU-C5/5FUR cells transfected with si-scr or si-PRNP after treatment with PBS or 5-FU (140 µM) for 48 h. (H) The level of p-ATM was determined by densitometry, relative to total ATM expression. (B, D, F, H) Data are expressed as the mean ± SEM of three independent experiments. ** p<0.01 vs. scrambled siRNA-transfected SNU-C5/5FUR (si-scr) treated with PBS, ***p<0.01 vs. si-PRNP treated with PBS, and **p<0.01 vs. si-scr treated with 5-FU.

In addition, PrP<sup>C</sup> is associated with multidrug resistance in gastric and breast cancer cells (Liang et al., 2009; Cheng et al., 2014). To explore whether PrP<sup>C</sup> is upregulated in drug resistant CRC cells, we analyzed the expression of PrP<sup>C</sup> in wild type (SNU-C5/WT) and 5-FU resistant colorectal cancer cell lines (SNU-C5/5FUR) by western blot assay (Fig. 1A). The expression level of PrP<sup>C</sup> was significantly increased in SNU-C5/5FUR, compared with that in SNU-C5/WT (Fig. 1B). In addition, flow cytometry analysis showed that the ratio of PrP<sup>C</sup> positive cells was significantly increased in SNU-C5/5FUR (Fig. 1C, 1D). Immunofluorescent staining also revealed an increased level of PrP<sup>C</sup> in SNU-C5/5FUR (Fig. 1E). These results indicate that 5-FU-induced drug resistant CRC cells express high levels of PrP<sup>C</sup>.

PrP<sup>C</sup> regulates CRC cell survival via the PI3K-Akt axis

The PI3K-Akt signaling pathway is a pivotal regulator and central node in cell survival signaling downstream of growth factor, cytokines, and several cellular stimuli (Luo et al., 2003; Manning and Cantley, 2007). To investigate whether PrP<sup>C</sup> regulates the PI3K-Akt signaling pathway, we evaluated the phosphorylation of PI3K and Akt in SNU-C5/WT and SNU-C5/5FUR (Fig. 2A). The phosphorylation of PI3K and Akt were significantly enhanced in SNU-C5/5FUR, compared with that in SNU-C5/WT (Fig. 2B). To investigate whether the expression of PrP<sup>C</sup> is associated with regulation of the PI3K-Akt signaling pathway in 5-FU-resistant CRC cells, we confirmed activation of PI3K-Akt in the presence or absence of 5-FU (140 µM). Treatment of SNU-C5/WT with 5-FU increased the level of PrP<sup>C</sup> (Fig. 2C, 2D). In addition, the level of PrP<sup>C</sup> was significantly increased in SNU-C5/5FUR in the presence and absence of 5-FU, compared with in SNU-C5/WT (Fig. 2C, 2D). The expression of PrP<sup>C</sup> was suppressed using PRNP siRNA (si-PRNP) in SNU-C5/5FUR (Fig. 2E, 2F). After treatment of SNU-C5/5FUR with 5-FU, the suppression of PrP<sup>C</sup> induced a decrease in phosphorylation of PI3K and Akt (Fig. 2G, 2H). These data suggest that PrP<sup>C</sup> regulates the PI3K-Akt signaling pathway in 5-FU resistant CRC cells.

PrP<sup>C</sup> is involved in cancer cell proliferation through the regulation of cell cycle-associated proteins

To confirm the effect of PrP<sup>C</sup> on cell proliferation in 5-FU resistant CRC cells, the expression of cell cycle-associated proteins, such as cyclin E, CDK2, cyclin D1, and CDK4, was analyzed by western blot assay after treatment of SNU-C5/5FUR with 5-FU (Fig. 3A). SNU-C5/5FUR transfected with PRNP siRNA had a significant decrease in the levels of cyclin E, CDK2, cyclin D1, and CDK4 after treatment with 5-FU (Fig. 3B). In addition, SNU-C5/5FUR transfected with PRNP siRNA showed a significant decrease in CDK4 kinase activity after treatment with 5-FU (Fig. 3C). Furthermore, flow cytometry analysis for PI staining showed that PrP<sup>C</sup>-knockdown SNU-C5/5FUR exhibited a significant decrease in the ratio of S phase (Fig. 3D, 3E) after treatment with 5-FU. These findings suggest that PrP<sup>C</sup> regulates proliferation of 5-FU resistant CRC cells.
The level of C-PARP1 was determined by densitometry, relative to treatment with PBS or 5-FU (140 µM) for 48 h. (B) The level of C-Caspase3 was determined by densitometry, relative to β-actin expression. (C) The level of C-PARP1 was determined by densitometry, relative to β-actin expression. (D) Apoptosis was measured using flow cytometry analysis for PI/Annexin V staining. (E) The quantitation of the percentage of apoptotic cells. Data are expressed as the mean ± SEM of three independent experiments. **p<0.01 vs. scrambled siRNA-transfected SNU-C5/5FUR (siscr) treated with PBS, *p<0.05, *p<0.01 vs. si-PRNP treated with PBS, and **p<0.01 vs. si-scr treated with 5-FU.

C-Caspase3 and C-PARP1 were assessed using a western blot after treatment of SNU-C5/5FUR with 5-FU (Fig. 5A). The levels of cleaved caspase-3 (C-Caspase-3) and PARP1 (C-PARP1) were significantly increased in treatment with 5-FU, compared with those in treatment with PBS (Fig. 5B, 5C). SNU-C5/5FUR transfected with PRNP siRNA had a significant increase in the levels of C-Caspase-3 and C-PARP1 after treatment with 5-FU (Fig. 5B, 5C). In addition, flow cytometry for PI/Annexin V staining showed that silencing of PrPC significantly increased in the percentage of early and late apoptotic cells after treatment with 5-FU (Fig. 5D, 5E). These data indicate that PrPC inhibits apoptosis of CRC cells in anti-cancer drug conditions via suppression of caspase-3 activation and PARP1 cleavage. In addition, SNU-C5/5FUR transfected with PRNP siRNA showed a significant decrease in the kinase activity of CDK4 after treatment with 5-FU (Fig. 3C).

siRRNP enhanced apoptosis in oxaliplatin-resistant CRC cells

We also confirmed apoptosis inhibition by the level of PrPC expression in other drug-resistant CRC cells. We analyzed the expression of PrPC in wild-type (SNU-C5/WT) and oxaliplatin-resistant colorectal cancer cell lines (SNU-C5/Oxal-R) by western blot assay (Fig. 6A, 6B). SNU-C5/Oxal-R transfected with PRNP siRNA showed a significant decrease in CDK4 kinase activity after treatment with oxaliplatin (Fig. 6C). In addition, flow cytometry for PI/Annexin V staining showed that

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**PrPc regulates anti-cancer drug-induced stress-associated signaling**

The main mechanism of 5-FU is the inhibition of DNA synthesis and mRNA translation (Alvarez et al., 2012). To explore whether PrPc regulates stress-associated proteins in the presence of this drug, we assessed the phosphorylation of stress-associated proteins, including p38, JNK, p53, and ATM, after treatment of SNU-C5/5FUR with 5-FU. SNU-C5/5FUR transfected with PRNP siRNA exhibited a significant increase in the phosphorylation of p38, JNK, and p53 (Fig. 4A-4F) after treatment with 5-FU. However, although knockdown of PrPc induced the phosphorylation of ATM in the PBS-treated group, there was no significant increase in the phosphorylation of ATM after treatment with 5-FU, implying that PrPc is not involved in ATM signaling in the presence of 5-FU (Fig. 4G, 4H). These results indicate that PrPc inhibits the activation of stress-associated signaling against an anti-cancer drug through suppression of p38, JNK, and p53 phosphorylation.

**PrPc inhibits apoptosis of anti-cancer drug resistant CRC cells via suppression of caspase-3 activation and PARP1 cleavage**

To investigate whether PrPc is involved in apoptosis of 5-FU resistant CRC cells, activation of caspase-3 and cleavage of PARP1 were assessed using a western blot after treatment of SNU-C5/5FUR with 5-FU (Fig. 5A). The levels of cleaved caspase-3 (C-Caspase-3) and PARP1 (C-PARP1) were significantly increased in treatment with 5-FU, compared with those in treatment with PBS (Fig. 5B, 5C). SNU-C5/5FUR transfected with PRNP siRNA had a significant increase in the levels of C-Caspase-3 and C-PARP1 after treatment with 5-FU (Fig. 5B, 5C). In addition, flow cytometry for PI/Annexin V staining showed that silencing of PrPC significantly increased in the percentage of early and late apoptotic cells after treatment with 5-FU (Fig. 5D, 5E). These data indicate that PrPc inhibits apoptosis of CRC cells in anti-cancer drug conditions via suppression of caspase-3 activation and PARP1 cleavage. In addition, SNU-C5/5FUR transfected with PRNP siRNA showed a significant decrease in the kinase activity of CDK4 after treatment with 5-FU (Fig. 3C).
silencing of PrP<sup>C</sup> significantly increased the percentage of early and late apoptotic cells after treatment with oxaliplatin (Fig. 6D).

**DISCUSSION**

In this study, we demonstrated that the level of PrP<sup>C</sup> is increased in 5-FU resistant CRC cells, implying that PrP<sup>C</sup> increased cell survival in anti-cancer drug conditions by regulating survival and apoptosis signaling pathways. After 5-FU treatment of SNU-C5/5FUR, a 5-FU resistant colorectal cancer cell line, silencing of PrP<sup>C</sup> yielded the opposite effects, including inhibition of proliferation and augmentation of apoptosis. Previous studies have shown that PrP<sup>C</sup> contributes to protection from oxidative stress, regulation of copper metabolism, cell cycle control, and cell adhesion (Brown et al., 1997; McLennan et al., 2004; Liang et al., 2007; Malaga-Trillo et al., 2009). In addition, PrP<sup>C</sup> promotes pro-survival and anti-apoptotic effects in stem cells, such as hematopoietic stem cells, neural stem cells, mesenchymal stem cells, and human embryonic stem cells (Martin-Lanneree et al., 2014). In cancer cell biology, PrP<sup>C</sup> accelerates cell proliferation, invasion, metastasis, and resistance to cytotoxic drugs (Liang et al., 2007; Mehrpour and Codogno, 2010; Wang et al., 2012; Du et al., 2013). Our results revealed for the first time that 5-FU resistant CRC cells express high levels of PrP<sup>C</sup>. In gastric cancer cells, PrP<sup>C</sup> expression enhanced drug resistance via an increase in Bcl-2 expression (Wang et al., 2011). Moreover, treatment of HCT116 CRC cells with PrP-specific antibodies increased cell survival in anti-cancer drug conditions by regulating PrPC expression enhanced drug resistance via an increase in PI3K-Akt signaling and promoted the effects of anti-cancer drugs such as 5-FU, cisplatin, and doxorubicin (McElwain et al., 2009). We also confirmed that the level of PrP<sup>C</sup> expression was significantly increased in oxaliplatin-resistant SNU-C5 cells, compared with wild-type cells (Fig. 6A, 6B). Silencing of PrP<sup>C</sup> decreased the kinase activity of CDK4 (Fig. 6C) and induced cell death in oxaliplatin-resistant CRC cells after treatment with oxaliplatin (Fig. 6D). These findings suggest that PrP<sup>C</sup> could be a key protein for resistance to anti-cancer drugs in CRC cells.

The PI3K signaling pathway is involved in various cellular processes, including cell survival, metabolism, inflammation, motility, and cancer progression (Vanhaesebroeck et al., 2010). In particular, the PI3K-Akt signal axis regulates survival and proliferation in cancer cells (Yuan and Cantley, 2008). In human lung cancer cells, Akt amplification plays a pivotal role in acquiring cisplatinum resistance (Liu et al., 2007). Our results indicated that the phosphorylation of PI3K and Akt are significantly increased in 5-FU resistant CRC cells, and inhibition of PrP<sup>C</sup> expression reduced the activation of PI3K and Akt in the presence of 5-FU. Activation of Akt also promotes cell proliferation through multiple downstream targets for cell cycle regulation (Manning and Cantley, 2007). We also revealed that downregulation of PrP<sup>C</sup> inhibited proliferation by suppression of cell cycle-associated proteins, such as cyclin E, CDK2, cyclin D1, and CDK4 after 5-FU treatment of 5-FU resistant cells. PrP<sup>C</sup> accelerated the G1/S transition via the PI3K-Akt-cyclin D1 axis (Liang et al., 2007). These findings suggest that PrP<sup>C</sup> plays pivotal roles in cell survival and proliferation in drug resistant CRC cells via regulation of the PI3K-Akt signaling pathway.

Activation of PI3K-Akt pathway by PrP<sup>C</sup> expression promotes anti-apoptotic effect (Vassallo et al., 2005). Conversely, downregulation of PrP<sup>C</sup> promotes caspase-3 activation by reducing Akt activation (Weise et al., 2006). In hypoxic conditions, increased PrP<sup>C</sup> inhibits TRAIL-mediated apoptosis.
PrPC facilitates anti-cancer drug resistance by regulating stress-associated proteins, such as p38, JNK, and p53, and CRC cells. Furthermore, PrPC suppressed the activation of colorectal cancer cells expressing high levels of PrP C, and that role in the development of chemoresistance by CRC cells. This additional activation implies that PrP C inhibits drug resistance. This study may help clarify the physiological relationship between PrP C and anti-cancer drug resistance, and blockage of stress- and apoptosis-mediated protein activation.

(Park et al., 2015). Our previous study revealed that silencing of PrP C increased apoptosis of CRC cells. To explore the relationship between PrP C and anti-cancer drug resistance, we showed that treatment with 5-FU induced the activation of stress-associated proteins, such as p38, JNK, and p53, and suppression of PrP C triggered additional activation of these proteins. This additional activation implies that PrP C inhibits the activation of stress-associated proteins in drug resistant CRC cells. Furthermore, PrP C suppressed the activation of caspase-3 after 5-FU treatment of drug-resistant CRC cells. These results suggest that the level of PrP C plays an important role in the development of chemo-resistance by CRC cells.

Our findings reveal for the first time that 5-FU resistant colorectal cancer cells express high levels of PrP C, and that PrP C facilitates anti-cancer drug resistance by regulating survival, proliferation, and apoptosis signaling pathways (Fig. 7). Further studies are required to understand how the expression of PrP C is induced during the acquisition of anti-cancer drug resistance. This study may help clarify the physiological function of PrP C in drug resistant colorectal cancer cells. The strategy of targeting PrP C may enable novel molecular target combination chemotherapies.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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