CHAPTER 13

Polysaccharides from *Capsosiphon fulvescens* Stimulate the Growth of Gastrointestinal Cells

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Contents

Abstract

Capsosiphon fulvescens is a green alga that is abundant along the southwest coast of South Korea. Although it is consumed for its purported health-enhancing properties, particularly as a treatment for stomach disorders and hangovers, the health effects of dietary *C. fulvescens* remain unclear. Polysaccharides extracted from *C. fulvescens* (*Cf*-PS) are investigated for their effects on the proliferation of rat small intestinal epithelial IEC-6 cells. *Cf*-PS stimulated IEC-6 cell proliferation in a dose-dependent manner. Further, *Cf*-PS treatment induced the translocation of β-catenin, an effector of the Wnt signaling pathway, from the cytosol to the nucleus and increased the expression of cyclinD1 and c-myc. *Cf*-PS also induced ERK1/2 phosphorylation, which is activated by mitogenic and proliferative stimuli such as growth factors, but the phosphorylation of JNK and p38 was not enhanced. Therefore, this chapter discusses the effect of *Cf*-PS on the growth of gastrointestinal cells.

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I. INTRODUCTION

Capsosiphon fulvescens is a green alga that is rich in vitamins A and C and minerals such as iron, potassium, and iodine. Hence, it has great potential as a human food and it is indeed consumed in Korea. Moreover, the alga is often used to treat stomach disorders and hangovers and shows immunostimulatory, anticancer, and antiatherosclerotic effects. However, in contrast to other sea vegetables, there are few reports about the potential benefits of *C. fulvescens*. For example, Cho *et al*. (2010) observed that the EtOH extract of *C. fulvescens* has the antioxidant effect. They suggested that the antioxidant activities were correlated with total phenolic and flavonoid contents. The water-soluble polysaccharide of *C. fulvescens* (SPS-CF) has also immunostimulating activity (Na *et al*., 2010). That is, they determined that SPS-CF stimulates the release of inflammatory cytokines, TNF-α, IL-6, i-NOS, COX-2, and PGE2. Moreover, Sheu *et al.* (1996) and Yamamoto *et al.* (1987) reported that oral consumption of several seaweeds significantly decreased the incidence of carcinogenesis *in vivo*. Alekseyenko *et al*. (2007) suggested that polysaccharide from *Fucus evanescens* has antitumor and antimetastatic activity in C57BI/6 mice transplanted Lewis lung adenocarcinoma. Downregulating tissue factor expression, *Grateloupia longifolia* polysaccharide inhibits angiogenesis in HMEC-1 endothelial cells (Zhang *et al*., 2006). A study of *Porphyra yezoensis* polysaccharide (Guo *et al*., 2007) has shown that it has protective effect against carbon tetrachloride-induced hepatotoxicity in mice. Jung *et al.* (2008) have investigated the effect of the extract (CFE: C. fulvescens extract) on the liver tissue and fecal cholesterol content in rats. CFE diet induced the increase of bile acid, dietary fiber, and cholesterol excretion in feces and the improvement of lipid metabolism in Sprague Dawley rats. The extract consisted of 68.32% dietary fiber and they suggested the effect of CFE were due to the fiber. Cho *et al*. (2010) observed that the EtOH extract of *C. fulvescens* has the antioxidant effect. They suggested that the antioxidant activities were correlated with total phenolic and flavonoid content. In this chapter, we demonstrated that polysaccharides extracted from *C. fulvescens* (*Cf*-PS) stimulated the growth of rat small intestine epithelial IEC-6 cells.

Cell proliferation depends on intracellular signal transduction, mediated by receptors such as enzyme-linked receptors (e.g., tyrosine kinase) and protein degradation dependent receptors (e.g., secretory Wnt protein). The Wnt signaling pathway plays an important role in a number of development processes, including β-catenin stabilization and translocation to the nucleus, where it associates with TCF/LEF family transcription factors, resulting in activation of specific target genes such as cyclinD1 and *c-myc*. In the intestine, the canonical Wnt signaling cascade plays a crucial role in driving proliferation of epithelial cells (Greogrieff *et al*., 2005). The signaling pathway also plays an important

role in a number of developmental processes, including body axis formation, development of the central nervous system, axial specification in limb development, and mouse mammary gland development (Akiyama, 2000). Other signaling pathways related to cell proliferation include the growth factor signaling pathways. The EGF signaling pathway is activated in a variety of tissues of epithelial, mesenchymal, and neuronal origin, where it plays fundamental roles in development, proliferation, and differentiation (Olayioye *et al.*, 2000). The MAPK signaling pathway is a major signaling cascade downstream of activated epidermal growth factor receptor (EGFR) receptors involved in regulation of cellular proliferation and differentiation. Once activated, MAPK can translocate to the nucleus, where it presumably regulates expression of different transcription factors (Garrington and Johnson, 1999; Velarde *et al.*, 1999).

Further, the MAPK signaling pathway may be related to the Wnt signaling pathway. For example, Givenni *et al.* (2003) reported that the Wnt signaling pathway activates the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway in mouse mammary epithelial cells via EGFR transactivation. In this study, we extracted polysaccharides from *C. fulvescens* and evaluated how they affect the growth of IEC-6 cells, using the Wnt and MAPK signaling pathways, which were activated by cell growth stimuli and promote cell proliferation.

II. EFFECTS OF *CF*-PS ON PROLIFERATION OF IEC-6 CELLS

Many sea vegetables have high levels of nutrients and other potentially beneficial components that may be useful for the treatment of various diseases. In particular, the antitumor activities of sea vegetables have been widely studied. For example, an algal lectin from *Galaxaura marginata* exhibits antibacterial activity (Liao *et al.*, 2003), and palmitic acid isolated from the marine alga *Colpomenia sinuosa* has antitumor activity (Heiba *et al.*, 1997). Marine algae also contain a large amount of polysaccharides such as alginate, fucoidan, carageenan, and agarose.

Active polysaccharides were extracted from *C. fulvescens* and subjected to agarose gel electrophoresis (Fig. 13.1) using two-band detection. *Cf*-PS has sulfate and 3,6-anhydrogalactose content of 28.7% and 18.6%, respectively. In addition, *Cf*-PS contains monosaccharides xylose (85%) and mannose (15%). Although there are no similar experiments replacing *Cf*-PS with each individual constituent, *Cf*-PS was evaluated to check whether it may stimulate the proliferation of normal intestinal cells. *Cf*-PS induced proliferation of IEC-6 cells, as determined by the MTS assay (Fig. 13.2A). After exposure to 0–1000 μg/ml *Cf*-PS for 24 h, relative cell numbers increased in a concentration-dependent manner because exposure to 500 μg/ml of *Cf*-PS for 24 h was sufficient to stimulate

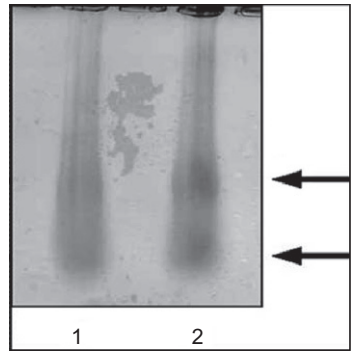

FIGURE 13.1 Agarose gel electrophoresis of the *Cf*-PS. Lane 1, 30 μg *Cf*-PS; lane 2, 60 μg *Cf*-PS.

growth. Cell morphology results showed that cells treated with *Cf*-PS appeared to increase in number compared to untreated cells (Fig. 13.2B). Further, expression of PCNA increased in treated cells (Fig. 13.2C), confirming that *Cf*-PS induced growth of IEC-6 cells.

III. EFFECTS OF *CF*-PS ON WNT SIGNALING COMPONENTS

Promotion of cell proliferation and division depends on intracellular signaling pathways such as the Wnt signal transduction pathway. In the absence of Wnt signaling, β-catenin is bound to the GSK3β/Auxin/APC complex in the cytosol; GSK3β phosphorylates β-catenin and consequently induces degradation of β-catenin through the ubiquitin proteosome pathway (Akiyama, 2000). Receptors for Wnt proteins are members of the frizzled family of transmembrane proteins, and the Wnt signal is converted to a cytoplasmic protein called disheveled (Dvl). Upon activation by the Wnt signal, GSK3β activity is inhibited by Dvl (Akiyama, 2000). This causes β-catenin to accumulate after it is translocated from the cytosol to the nucleus. In turn, β-catenin associates with TCF/LEF transcription factors and alters the expression of the Wnt signaling target genes cyclinD1 and c-myc. We believe that *Cf*-PS-induced cell proliferation was involved in the Wnt signaling pathway.

To identify the mechanism of *Cf*-PS-induced proliferation in IEC-6 cells, the effects of *Cf*-PS were evaluated on canonical Wnt signaling pathway proteins. Further, time-course experiment was conducted to assess the effect of *Cf*-PS on cyclinD1 and *c-myc* expression, which plays major roles in the cell cycle and in the proliferation of eukaryotic cells (He *et al.*, 1998; Sun and Jin, 2008; Tetsuo and McCormick, 1999; van de Wetering *et al.*, 2002; Willert *et al.*, 2002; Yamaguchi *et al.*, 2004).

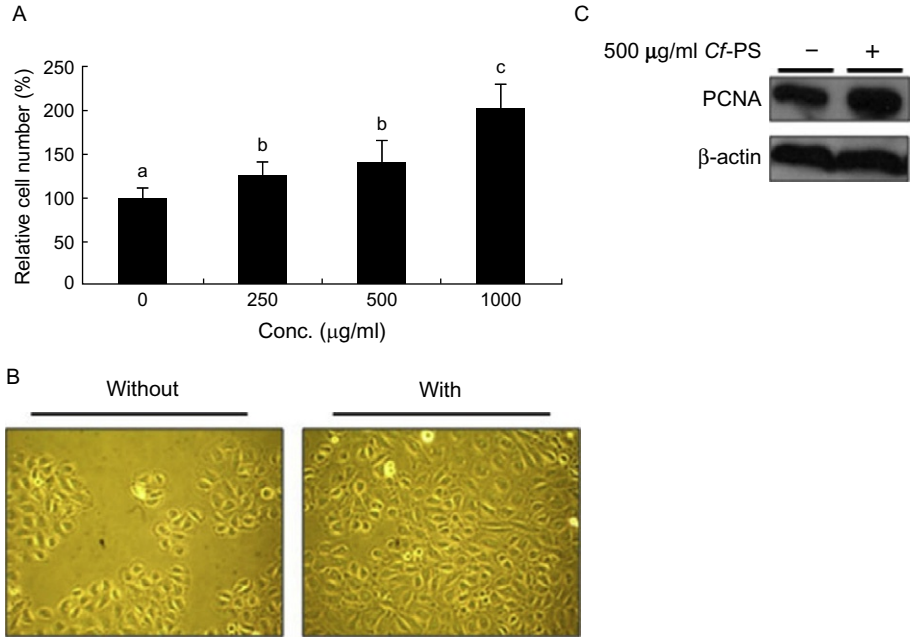

FIGURE 13.2 Effect of *Cf*-PS on the IEC-6 cell proliferation. (A) Effect of *Cf*-PS treatment on cell proliferation was assayed by MTS assay. (B) Microscopy analysis of the cells (200×). (C) Effect of *Cf*-PS on PCNA protein expression. One representative gel from three separate experiments is shown.

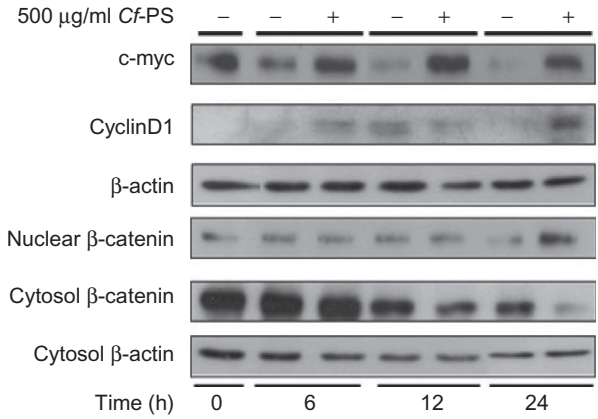

FIGURE 13.3 Effect of *Cf*-PS on Wnt signaling in IEC-6 cells. Expression of Wnt signaling pathway proteins was measured by Western blotting. One representative gel from three separate experiments is shown.

Expression of cyclinD1 increased 6 and 24 h but decreased 12 h after *Cf*-PS exposure. In contrast, expression of c-myc increased at every time point after *Cf*-PS exposure (Fig. 13.3). The decreased expression of cyclinD1 at 12 h was likely an experimental error. Expression of Wnt signaling target genes is needed to transfer β-catenin from the cytosol to the nucleus. To confirm translocation of β-catenin from the cytosol to the nucleus, cytosolic-enriched fractions of *Cf*-PS-treated or untreated cells were used. Cytosolic β-catenin protein significantly decreased in the presence of *Cf*-PS as early as 12 h after exposure, and this reached a maximum by 24 h after exposure. In contrast, nuclear β-catenin increased only 24 h after *Cf*-PS exposure. There were no changes of nuclear β-catenin 12 h after exposure, but this may have been due to experimental error. These demonstrate that *Cf*-PS induced β-catenin translocation from the cytosol to the nucleus, which may increase cyclinD1 and c-myc expression. c-myc plays a major regulatory role in the cell cycle and the growth of eukaryotic cells (McMahon and Monroe, 1992; Wang *et al.*, 1993). The c-myc gene codes for a nuclear protein that functions as a transcription factor controlling cell division, differentiation, and apoptosis (Marcu *et al.*, 1992; Varmus, 1984). Moreover, c-myc modulates cell proliferation and governs cell cycle progression in the intestinal mucosa (Yamaguchi *et al.*, 2004). D-type cyclins are cell cycle regulators, among which cyclinD1 is a major regulator of the progression of cells into the proliferative stage of the cell cycle. CyclinD1 has a positive effect on cell cycle progression (Koseoglu *et al.*, 2009). *Cf*-PS-induced expression of cyclinD1 and c-myc may play a critical role in the stimulation of normal intestinal epithelial cell proliferation.

IV. *Cf*-PS INDUCES ACTIVATION OF ERK1/2 IN IEC-6 CELLS

Stimulation of cell proliferation and division also depends on multisignaling pathways by tyrosine kinases, including MAPK and phosphatidylinositol 3-kinase (PI3K) (Khandwala *et al.*, 2000; LeRoith *et al.*, 1995; Rother and Accili, 2000). The MAPK family in mammalian cells includes ERK1/2, the JNK, and p38 MAPK (Paruchuri *et al.*, 2002). To further investigate the mechanism of *Cf*-PS-induced proliferation, MAPK proteins were used to examine whether *Cf*-PS activates the EGFR signaling pathway in IEC-6 cells. In the absence of *Cf*-PS, JNK and p38 were phosphorylated in a time-dependent manner. However, *Cf*-PS treatment inhibited the activation of JNK and p38 in a time-dependent manner (Fig. 13.4). Further, ERK1/2 phosphorylation increased after treatment with *Cf*-PS as early as 0 min and reached a maximum 5 min after EGF exposure. JNK and p38 are involved in the cell death pathway and oxidative stress. Therefore, *Cf*-PS-induced cell proliferation appears to be related to the activation of MAPK (ERK1/2, p38, JNK), especially ERK1/2. Therefore, cell proliferation was analyzed after inhibition of ERK1/2 using U0126 to examine the role of ERK1/2 activation during the *Cf*-PS effect. As shown in Fig. 13.5, exposure to U0126 with *Cf*-PS inhibited cell proliferation, but viability was higher than that of untreated cells. These results indicate that ERK1/2 plays a role in the proliferation of IEC-6 cells.

Wnt peptides transactivate EGFR, presumably by increasing the availability of EGFR ligands, which in turn leads to strong stimulation of the MAPK pathway (Givenni *et al.*, 2003). Transactivation of EGFR has a specific biological effect: stimulation of cyclinD1 expression (Givenni *et al.*, 2003). Therefore, there might be cross talk between the MAPK

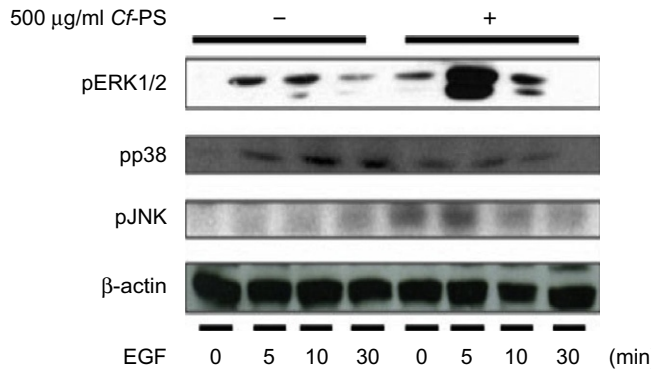

FIGURE 13.4 *Cf*-PS induces activation of ERK1/2. MAPK protein expression was measured by Western blotting. One representative gel from three separate experiments is shown.

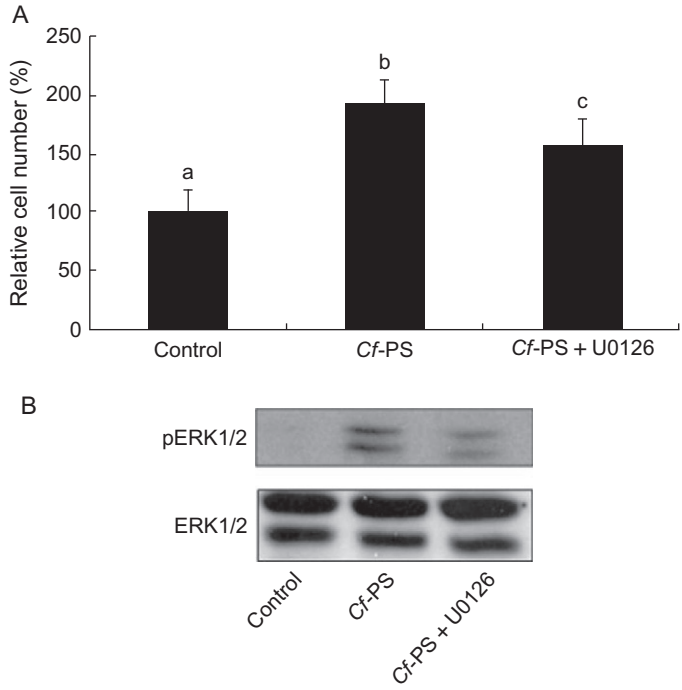

FIGURE 13.5 ERK1/2 phosphorylation affects *Cf*-PS-induced cell proliferation. (A) MTS assay was used to assess ERK1/2 activation on *Cf*-PS-induced cell proliferation. (B) Inhibition of phospho-ERK1/2 level by U0126. One representative gel from three separate experiments is shown.

signaling pathway and the Wnt signaling pathway during *Cf*-PS-induced proliferation of IEC-6 cells. To investigate this hypothesis, the activation of ERK1/2 was inhibited using U0126 and the effects of this inhibition on the Wnt signaling pathway was investigated.

Inhibition of ERK1/2 led to a decrease in the level of cytosolic β-catenin (Fig. 13.6). This decrease was also observed when cells were treated with *Cf*-PS or U0126 only (Fig. 13.3), but to a greater degree after U0126 treatment (Fig. 13.6). Further, β-catenin in the nucleus, which increased when treated with *Cf*-PS alone (Fig. 13.3), also decreased in the presence of U0126 (Fig. 13.6). These results suggest that translocation of β-catenin from the cytosol to the nucleus was inhibited, and the resulting accumulation of β-catenin in the cytosol was also inhibited by inhibition of ERK1/2. Moreover, we have made a hypothesis model of the cross talk between Wnt signaling and MAPK signaling pathways and given in Fig.13.7.

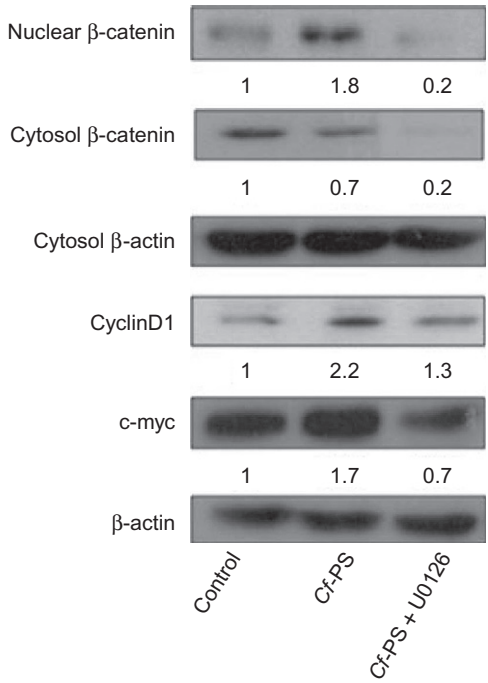

FIGURE 13.6 Effect of ERK1/2 activation on Wnt signaling. Expression of Wnt signaling proteins was measured by Western blotting. One representative gel from three separate experiments is presented. Each number is quantitative analysis based on densitometry.

V. CONCLUSION

Seaweeds have recently received a great deal of attention from scientific researchers. A number of investigators have found that these traditional sources of food not only provide nutritional benefits but also help to fight against diseases and contribute to the maintenance of good health. Certain types of seaweeds contain significant amounts of essential protein, vitamins, and minerals. Moreover, various polysaccharides from seaweeds have diverse biological activities, including effects on the immune system and cancer. Although *C. fulvescens*, a green alga, is consumed for its purported health-enhancing properties, particularly as a treatment for stomach disorders and hangovers, the health effects of dietary *C. fulvescens* remain unclear. There are also a few studies on the *C. fulvescens*.

In this chapter, stimulated effect of *Cf*-PS on IEC-6 cell growth was discussed. Many pharmaceutical products have been developed for cancer treatment. However, despite recent pharmaceutical advances, these

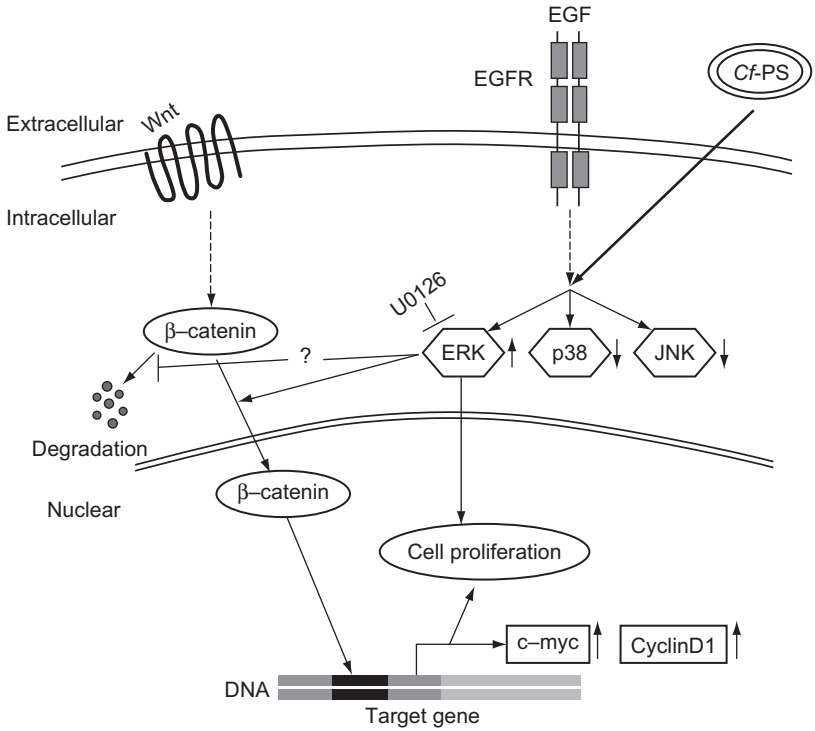

FIGURE 13.7 Schematic model of the cross talk between Wnt signaling and MAPK signaling pathways.

products that relieve pain, heal tumors, and delay malignancy are relatively expensive and are associated with various side effects. Hence, in the search for effective treatments for cancer, many researchers have begun to investigate natural products that are less likely to produce side effects. Among natural foods, seaweeds have received much research attention in past decades. In this study, *Cf*-PS stimulated IEC-6 cell proliferation in a dose-dependent manner. Further, *Cf*-PS treatment induced the translocation of β-catenin, an effector of the Wnt signaling pathway, from the cytosol to the nucleus and increased the expression of cyclinD1 and c-myc. *Cf*-PS also induced ERK1/2 phosphorylation, which is activated by mitogenic and proliferative stimuli such as growth factors, but the phosphorylation of JNK and p38 was not enhanced. Hence, this chapter confirmed that *Cf*-PS regulates proliferation via stimulating the nuclear translocation of β-catenin and ERK1/2 activation in intestinal epithelial cells.

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