Chapter -8

Cytotoxic, anti-proliferative and apoptotic activities of PPC-Pr

Synopsis

In this study, the in vitro short-term cytotoxic activity against Dalton’s lymphoma ascites (DLA) and Ehrlich’s ascites carcinoma (EAC) cell lines, and long-term cytotoxic, anti-proliferative and apoptotic properties of PPC-Pr were examined using human colon cancer cell line HCT116 as a model system. Short-term cytotoxic activity against DLA and EAC cell lines was studied using Trypan blue exclusion method and PPC-Pr did not show any cytotoxicity in the short term assay. In the long-term cytotoxic assay, HCT116 cells were cultured in the presence of PPC-Pr at various concentrations (100-1000 µg/ml) for 24-96 h and the percentage of cell viability was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide) assay. The results showed that PPC-Pr inhibited the cells viability in time and concentration-dependent manner. The anti-proliferative effect of PPC-Pr was associated with apoptosis on HCT116 cells. The morphological changes in the PPC-Pr treated HCT116 cells were analyzed by fluorescent DNA-microscopy with 4’, 6-Diamidino-2-phenylindole dihydrochloride hydrate (DAPI) and Acridine orange-ethidium bromide (AO/EB) staining. The oligonucleosomal DNA fragmentation in the PPC-Pr treated cells was studied using Comet assay. These investigations indicate that the polysaccharide-protein complex from P.rimosus possessed cytotoxic, anti-proliferative activity and induced marked apoptosis in tumor cells in vitro.

8.1. Introduction

Apoptosis has recently become a focus of interest in oncology that may also shed light on cancer therapy (Marx, 1993). Programmed cell death or apoptosis plays an integral role in a variety of biological events, including morphogenesis, tissue homeostasis, and removal of unwanted or harmful cells (Schultz and Harrington, 2003). Failure to accurately undergo apoptosis can cause severe anomalies in humans, either due to the accumulation or due to the
deficiency of a particular cell type. Abnormal inhibition of apoptosis is a hallmark of cancer and autoimmune diseases, whereas excessive cell death has been implicated in a number of neurodegenerative disorders (Thompson, 1995). Programmed cell death is a physiological and crucial process that is regarded as the preferred way to eliminate cancer cells (Mukhtar and Ahmad, 1999).

Polysaccharides or polysaccharide-peptide (protein) complexes are the major bioactive ingredients of various mushrooms. They exert their anti-tumor action via activation of the immune response of the host organism; hence they are regarded as biological response modifiers (BRMs) (Kima et al., 2004; Ruan et al., 2005). However, increasing experimental evidences confirm that the polysaccharides or their complexes could have cytotoxic or cytostatic effect on various tumor cell lines in vitro by inducing cell apoptosis, whereas less toxic to normal cells (Lavi et al., 2006; Lee et al., 2005; Yang et al., 2005; Li et al., 2004; Takako et al., 2004). Polysaccharide-peptide (PSP) isolated from the fruiting body of *Coriolus versicolor* (Lin et al., 2004), and polysaccharides obtained from the fruiting body of *Ganoderma lucidum* (Jiang et al., 2004) were shown to have growth inhibitory effects on cancer cells mediated by cell cycle arrest and/or induction of apoptosis. A polysaccharide-peptide complex (PSP) extracted from *Trametes versicolor* significantly reduced proliferation of MAD-MB-231 breast cancer cells by increasing p21 expression and decreasing cell-cycle protein cyclin D1 expression (Chow et al., 2003). A protein-bound polysaccharide (PBP) from *Phellinus linteus* had an anti-proliferative effect on SW480 human colon cancer cells mediated by inducing apoptosis and G2/M cell cycle arrest with a decrease of cytochrome c release and reduced cyclin B1 expression (Li et al., 2004). In addition, a water soluble β-glucan isolated from *Poria cocos* was shown to have growth-inhibitory effects on human breast carcinoma MCF-7 cells mediated by cell-cycle arrest and apoptosis induction (Zhang et al., 2006). Therefore anti-tumor activities of mushroom polysaccharides are not only mediated by the immunopotentiation (Zaidman et al., 2005), but can also be resulted from a direct inhibition on the tumor cells. However, structure of mushroom polysaccharides are very diverse in terms of their monosaccharide composition, linkages in the
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main chain, degree of branching, percentage of non-carbohydrate components such as protein or peptide percentage as well as functional group modification. Thus, the correlation of the chemical structure of mushroom polysaccharides and the mechanisms of their anti-proliferation activities is still unclear.

Development of non-invasive treatments of cancer is currently required, as the traditional cancer treatments are often toxic to normal cells and can cause serious side effects (Wasser, 2002). Search for anti-cancer drugs with higher bioactivities and lower toxicity from nature has been very intensive in recent years. The protein bound polysaccharides isolated from mushrooms are shown to exhibit a variety of pharmacological properties without causing adverse effects. In this study cytotoxic activity of PPC-Pr against the EAC and DLA murine cancer cell line was evaluated. Also the effect of PPC-Pr on the proliferation of HCT116 human colon cancer cells was studied and, ultimately, assessing the putative mechanism underlying the inhibition of colon cancer cell growth in vitro. This is the first report on the direct cytotoxic effect of PPC-Pr from P. rimosus on HCT116 human colon cancer cells through the induction of apoptosis as studied by cell morphology and DNA analysis.

8.2. Materials and methods

8.2.1. Isolation of PPC-Pr from P. rimosus

PPC-Pr was isolated from the hot water extract of P. rimosus as described in section 4.2.1.

8.2.2. Cell lines

Ehrlich’s ascites carcinoma (EAC) and Dalton’s lymphoma ascites (DLA) cells diluted to 1x10^6 viable cells/0.1 ml as described in 5.2.2., were used for the Trypan blue exclusion method. HCT 116 cells (human colon cancer cell line) were maintained as described in section 3.1.2. PPC-Pr dissolved in the basal medium as a stock solution of 10 mg/ml, was further diluted with the basal medium to the desired concentrations and used for MTT assay.

8.2.3. Short-term cytotoxicity assay using Trypan blue exclusion method

Tumor cell lines (EAC and DLA) in 0.1 ml of Phosphate buffered saline (PBS) (0.2 M, pH 7.4); various concentrations of PPC-Pr (50µg/ml-2mg/ml) and
PBS in a final volume of 1ml were incubated in clean sterile tubes for 3h at 37°C. After incubation, 100µl of 1% trypan blue solution was added and the survival rate was assayed using a haemocytometer by counting living cells that excluded the dye. Dead cells take up the stain and appear in blue color. Percentage of cytotoxicity was calculated as:

\[
\text{% of cytotoxicity} = \frac{T_{\text{Dead}} - C_{\text{Dead}}}{T_{\text{Total}}} \times 100
\]

where, \(T_{\text{Dead}}\) is the number of dead cells in the PPC-Pr treated tube, \(C_{\text{Dead}}\) is the number of dead cells in the control tube and \(T_{\text{Total}}\) is the number of dead and live cells in PPC-Pr treated tube (Ajith and Janardhanan 2003).

### 8.2.4. Long-term cytotoxicity and cell proliferation assay

Cell density of HCT 116 was determined following exposure to various concentrations of PPC-Pr for evaluating the effect of PPC-Pr to modulate cell proliferation (Anto et al., 2000). Briefly, cells were seeded at a density of 5 x 10^3 cells/well into 96-well plates. After 24 h, PPC-Pr was added to the medium at the concentrations of 100, 500 and 1000 µg/ml, incubated for 24 to 96 h as indicated. At the end of the incubation, 100 µl of 3-(4-5 dimethylthiozo-2-yl) 2-5 diphenyltetrazolium bromide (MTT) (2mg/ml) per well was added to each well and incubated for another 2 h. The formazan crystals formed were solubilized in acidified isopropanol after aspirating the medium. The extent of MTT reduction was measured spectrophotometrically at 570 nm using an ELISA reader (Bio-Tek, Winooski, VT). All determinations were carried out in triplicate. The cell survival was calculated by following formula:

\[
\text{CS (\%)} = \left( \frac{\text{OD}}{\text{Mean OD}_0} \right) \times 100
\]

where OD and OD₀ indicated the absorbance of treated cells and untreated cells, respectively. A graph was plotted with percentage viability in the Y-axis and concentrations of PPC-Pr in the X-axis. The value of IC₅₀, which is the concentration of PPC-Pr required to inhibit the colon cancer cells by 50% of the control level, was estimated from the plot.
8.2.5. Morphological studies of apoptotic cell

8.2.5.1. DAPI (4’, 6-Diamidino-2-phenylindole dihydrochloride hydrate) staining

Chromatin changes in the PPC-Pr treated cell line were studied by DAPI staining procedure. HCT 116 cells were cultured on cover slips at seeding density of 5 x 10^5 cells in a 12-well plate for 72 h with various concentrations of PPC-Pr. The cells were washed with 1X phosphate-buffered saline (1X PBS), fixation and permeabilisation of cells were done with acetone: methanol (1:1) for 7 min. Following 3 washes with 1X PBS, the cells were stained with DAPI (0.5µg/ml) in dark for 2 minutes. The cover slips were removed from the 12-well plates, washed with PBS, mounted on glass slides to view under a Nikon inverted fluorescent microscope (TE-Eclipse 300) attached with a camera and photographed under phase contrast and fluorescent conditions. Apoptotic cells were morphologically defined by cytoplasmic and nuclear shrinkage, and by chromatin condensation or fragmentation.

8.2.5.2. Acridine orange-ethidium bromide (AO/EB) staining

The frequency of apoptotic induction of HCT 116 cells following exposure to PPC-Pr was determined by fluorescent microscopy of nuclear stains, acridine orange and ethidium bromide. Cells were cultured and treated with PPC-Pr as in the above experiment. After treatment, washing once with 1X PBS, the cells were stained with 100 µl of a mixture (1:1) of acridine orange-ethidium bromide (4µg/ml) solutions for 3 min. The cells were immediately washed with PBS, viewed under inverted fluorescent microscope equipped with a red filter and photographed as above. Two hundred cells per sample were counted in triplicate for each dose point. Four types of cells were distinguished according to the fluorescence emission and the morphological pattern of chromatin condensation in the stained nuclei; (1) Viable normal cells have uniform bright green nuclei with organized structure; (2) early apoptotic cells (which still have intact membranes but have started to undergo DNA cleavage) have bright green nuclei, but with highly condensed or fragmented chromatin visible as bright green patches or fragments; (3) late apoptotic cells have bright orange to red nuclei with
highly condensed or fragmented chromatin; (4) Necrotic cells have a uniformly orange to red nuclei with organized structure.

**8.2.6. Analysis of DNA fragmentation by Comet assay**

For the comet (single cell gel electrophoresis) assay, initially 100 µl of 0.5% normal melting agarose (NMA) in PBS was added to the frosted microscope slides, then covered with 22 mm x 50 mm parafilm cover slips and allowed to solidify for 3-4 min at room temperature. The cover slips were gently removed after the agarose had set. HCT 116 cells were grown in culture bottles and treated with PPC-Pr in the same conditions as described above. After treatment, cells were scraped off into the ice-cold 1X PBS to yield approximately 1x10^6 cells/ml. 5 µl of the cell suspension was added to 75 µl of 0.5% low melting point agarose (LMPA) in PBS, mixed gently at 37°C by repeated pipetting using a cut micropipette tip, and added onto NMA coated slides. Cover slips were replaced on the mixture to obtain a uniform layer. Slides were placed on a tray resting on the ice packs until the agarose layer hardened (~ 3 to 5 min). Cover slips were gently removed and a final layer of 0.5% LMPA (75 µl) was placed on the slides, covered with the cover slips and returned to the slide tray to solidify. The parafilms were removed after the agarose had set and the slides were immersed in cold lysis solution (2.5 M NaCl, 100mM EDTA, 10Mm Tris, pH 10, with freshly added 1 % Triton X-100 and 10% DMSO) followed by incubation at 4°C for at least 1 h. The electrophoresis in weak alkali (0.03 M NaOH, 1 mM EDTA, pH 12.1) at 25 V and 300 mA for 20 min was preceded by a 20 min immersion of the slides in the electrophoresis buffer to promote chromatin unwinding. After electrophoresis, the slides were neutralized by drop wise addition of 0.4 M Tris buffer (pH 7.5), allowed to sit for at least 5 min and repeated twice. Drained slides were stained with Ethidium Bromide (10 µg/ml) for 5 min and rinsed with cold water to remove the excess stain. The slides were covered with fresh cover slips and examined under fluorescence microscope (TE-Eclipse 300) equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm.

Apoptosis was examined by analyzing 100 nuclei from each treatment. Nuclear phenotypes identified according to their tail length: type 1- no tail or
DNA diffusion halo, type 2- no tail but with a DNA diffusion halo, type 3- a tail and a DNA diffusion halo, type 4- a tail and not a DNA diffusion halo. Type 3 and 4 were markers for the early and late apoptotic stages, respectively.

8.3. Results

The PPC-Pr was not found to possess cytotoxic activity against the murine cell lines DLA and EAC up to a tested concentration of 2mg/ml in the short-term Trypan blue exclusion assay. MTT assay revealed the metabolic activity of the cells and reflected its growth potential indicating the balance between proliferation and death. The HCT 116 cells were incubated with 100, 500 and 1000µg/ml of PPC-Pr for 24, 48, 72 and 96 h. As shown in Figure 8.1., PPC-Pr inhibited colon cancer cells growth in time and dose-dependent manner (P<0.001). After 48 h treatment, PPC-Pr demonstrated distinct inhibition at concentrations of 500 and 1000µg/ml; whereas the growth inhibitory effects for 24 h treatment were not significant (Figure 8.2). The concentrations of PPC-Pr with 50 % inhibition on the cancer cell growth, i.e., IC<sub>50</sub>, were found to be 852, 456 and 420 µg/ml for 48, 72 and 96 h, respectively. The mechanism of action for the growth inhibition was further investigated after the HCT 116 cells were incubated with various concentrations of PPC-Pr for 72 h. As the percentage of inhibition did not increase for 100 µg/ml of PPC-Pr, 500 and 1000µg/ml concentrations were chosen for further experiments.

The characteristic changes of apoptosis were observed in the morphology of PPC-Pr treated cells. While the untreated HCT 116 cells (control) were well spread with flattened morphology, many round-shaped cells poorly adhered to the culture plates were noticed in those treated with PPC-Pr for 72 h. Cultures contained abundant particles and debris with reduced number of HCT 116 cells remaining intact. Significant decrease of the number of HCT 116 cells treated with 500 and 1000µg/ml of PPC-Pr was observed compared to the control group.
Figure 8.1. Cytotoxic effect of PPC-Pr on HCT 116 cells. Cells were incubated with 100, 500 and 1000µg/ml of PPC-Pr for 24, 48, 72 and 96 h. Cell viability was measured by the MTT assay. Data are averages ± S.D (bars) of triplicate determinations.

Figure 8.2. Effect of PPC-Pr on HCT 116 cell proliferation. Viable HCT116 cells were determined using the MTT method as described in Materials and methods and expressed as a percentage of the untreated control cell samples. Samples were incubated for 24, 48, 76 and 92 h in the presence or absence of various concentrations of PPC-Pr. Values are the mean ± S.D. of data from at least three independent experiments; * p<0.001, # p<0.01 compared with control group.
The morphological changes observed consequent to DAPI staining are represented in Figure 8.3. The untreated cells (control) showed uniform staining of nucleus and chromatin network (Figure 8.3a and 8.3b). The cells treated with PPC-Pr showed nuclear shrinkage and chromatin condensation. Marginalisation of chromatin network was also observed in the treated cells (Figure 8.3c - 8.3f). The apoptotic morphology in cellular bodies and the chromatin condensation were also confirmed by Acridine orange-ethidium bromide double staining. The compact masses of chromatin aggregated along the nuclear membrane. Round, compact granular masses appeared nearer the center of the nucleus and there was a reduction in nuclear volume. Cytoplasm displayed condensation and some of the nucleus degenerated into discrete spherical or ovoid fragments of highly condensed chromatin (Figure 8a-8.4f). Compared with the spontaneous apoptosis observed in control cells (early apoptotic 0.77 %; late apoptotic 4.64 %), PPC-Pr treated cells showed highly increased percentages of early apoptotic (500 µg/ml: 6.11 %; 1000 µg/ml: 3.64 %), and late apoptotic (500 µg/ml: 43.65 %; 1000 µg/ml: 65.39 %) cells, with concomitant increase in the total number of apoptotic cells (Figure 8.4g).

Chromatin degradation into multiple internucleosomal fragments of 180-200 base pairs is a distinct biochemical hallmark for apoptosis. In order to investigate whether the morphological changes observed in PPC-Pr treated HCT 116 were consistent with chromatin degradation, the DNA damage in individual cells was studied by comet assay (Figure 8.5a, 8.5b & 8.5c). The comet assay showed that control cells had few nuclei with fragmented chromatin whereas a high number of nuclei had no detectable tail (type 1 & 2). Apoptotic nuclei (type 3 & 4) were more frequent in PPC-Pr treated cells (Figure 8.5d). These results support the observations derived from DAPI and AO/EB staining, suggesting the excessive chromatin fragmentation in PPC-Pr treated cells was the result of apoptosis.
Figure 8.3. Fluorescent microscopic analysis of apoptotic cells stained with DAPI. Cells were treated for 72 hrs without PPC-Pr (a & b), with PPC-Pr at a dose of 500µg/ml (c & d) and 1000 µg/ml (e & f). The representative cells were counted as apoptosis in fluorescence microscopy with x 200 magnification (g). Values are ± S.D; *p<0.001 compared with control group.
Figure 8.4. Fluorescent microscopic analysis of apoptotic cells stained with AO/EB. Cells were treated for 72 hrs without PPC-Pr (a & b), with PPC-Pr at a dose of 500µg/ml (c & d) and 1000 µg/ml (e & f). The representative cells were counted as apoptosis in fluorescence microscopy with x 200 magnification (g). Values are ± S.D; *p<0.001 compared with control group.
DNA fragmentation analysis of apoptotic cells. Cells were treated for 72 hrs without PPC-Pr (a), with PPC-Pr at a dose of 500 µg/ml (b) and 1000 µg/ml (c). The treated cells were subjected to comet test, stained with EB and classified by phenotypes. Control group showed a low frequency of apoptotic nuclei (type 3 & 4), in contrast to PPC-Pr treated cells (d).
8.4. Discussion

The ability to induce cell apoptosis is an important property of anti-cancer drugs (Frankfurt and Krishnan, 2003). In the past years, increasing number of reports revealed that polysaccharides, proteo-polysaccharides originated from mushrooms and other natural products exhibited direct action on the proliferation of the tumor cells in vitro at certain concentrations by modulating cell-cycle progression and inducing apoptosis. PSP, PSK, *Lycium barbarum* polysaccharide (LBP) and *Phellinus linteus* polysaccharide showed the inhibition of tumor cells proliferation by inducing the cell apoptosis (Lee et al., 2005; Li et al., 2004; Lin et al., 2003; Takako et al., 2004; Zhang et al., 2005; Yang et al., 2005).

Apoptosis modulates cell cycle and finally leads to cell death (Paulovich et al., 1997). Unlike necrosis in which dying cells fall apart releasing their contents and provoking macrophage activation and inflammation, apoptotic cells are rapidly taken up by phagocytosis and degraded in phagolysomes (Roitt et al., 2001). Various tumors undergoing the process of apoptosis were induced by treatments of radiation, chemotherapeutic agents and mild hyperthermia (Kerr et al., 1994). Cell death in tumors, whether spontaneous or treatment-induced, occurs predominantly via apoptosis rather than necrosis (Wyllie et al., 1980; Arend et al., 1990). The characteristic morphological changes of apoptosis include membrane blebbing, chromatin condensation and the formation of apoptotic bodies. In addition, apoptotic cells undergo DNA double strand cleavage into fragments of multiples of about 185-200 base pairs (Wyllie et al., 1980; Gong et al., 1994).

Apoptosis is a tightly regulated process and its mechanisms involve mainly two signaling pathways, including cell death receptor pathway and mitochondrial pathway (Reed, 2001). In the mitochondrial pathway, a variety of death signals triggers the release of several pro-apoptosis proteins. Among the numerous factors known to modulate cancer-related apoptosis, Bcl-2 family and p53 are the most extensively characterized mechanistically. Bax is a crucial mediator and as a tumor suppressor (Yamaguchi et al., 2003), it mediates the p53-induced
apoptosis and increases sensitivity to the drug-induced apoptosis. Meanwhile, Bcl-2 is an anti-apoptosis protein. When it is activated or prevalent, apoptosis is prohibited (Tsujimimoto and Shimizu, 2000). And relative expression levels of Bcl-2/Bax were reported to determine the sensitivity to apoptosis (Danial and Korsmeyer, 2004). Caspase have been shown to be activated during apoptosis in many tumor cells. Related references revealed that whatever the mitochondrial pathway and cell death receptor pathway, both finally activate caspase-3, which is essential for DNA fragmentation, the morphological change associated with apoptosis, and its activation represents a key and irreversible point in the development of apoptosis (Cui et al., 2007).

DAPI is a DNA specific dye, which forms a fluorescent complex by attaching in the minor groove of consecutive (3-4 base pairs) A-T rich sequences of DNA. DAPI can penetrate through the intact plasma membrane to stain DNA and makes visible the changes in the chromatin more clearly. Staining of apoptotic cells with fluorescent dyes such as AO and EB is considered the correct method for evaluating the changed nuclear morphology. AO is used to determine the number of cells within the given population, which has undergone apoptosis, but it cannot distinguish viable from non-viable cells. To achieve this, a mixture of AO and EB was used. The differential uptake of these two dyes allows the identification of viable and non-viable cells and also the visualization of aberrant chromatin organization. Among the methods used to study apoptosis, microscopic examination of AO/EB stained cells is recommended as the most reliable, since it makes it possible to perform high-quality studies of cell morphology, nuclear and chromatin disintegration as well as to distinguish viable, early or late apoptotic and necrotic cells (Baskic et al., 2006).

Double stranded DNA fragmentation giving the characteristic “ladder” pattern on agarose gel electrophoresis is one of the most widely accepted criteria used to characterize apoptotic cell death because it is discernible from the random single stranded DNA breaks produced by necrosis (Yaoita et al., 2000). However, this technique is difficult to quantify and not particularly sensitive as an extensive number of cells (> 10^6 cells/sample) is required in synchronous fragmentation to
produce oligonucleosomal ladders (Page et al., 2000). At present, enzymatic techniques are used for detecting DNA fragmentation at the individual cell level, the most common being the terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay. But this assay also suffers from lack of specificity and can not distinguish between apoptotic and non apoptotic cell, as it labels DNA strand breaks from any insult (Singh, 2000). With heterogeneous cell population, DNA ladder formation tends to miss contributions from small sub-populations and obtained results can not be quantified. The detection of apoptosis at the single cell level requires an assay that allows analyses of individual cells for the biological changes induced by apoptosis. Comet assay or single cell gel electrophoresis (SCGE) is one such technique that can detect the fragmented DNA on a cell-by-cell basis, thereby readily allowing the detection of apoptotic cells. By virtue of its ability to detect DNA fragmentation at the level of individual cell comet assay allows analysis of sub-populations of cells (Bacso et al., 2000). The “apoptotic comet” appears usually as a ring with less DNA in the center of the head (the “pin” head) followed by a large cloud (the “puffy” tail) representing randomly cleaved DNA of different chain lengths. This is characteristic of apoptotic DNA fragmentation. Necrotic DNA damage, on the other hand, shows a comet with considerable DNA both in the head and in the tail as a result of non-random DNA cleavage. The comet assay thus can be used to detect a cell undergoing apoptosis and also for the quantitative estimation of apoptosis.

The current experimental results indicate that PPC-Pr, a novel polysaccharide-protein complex isolated from the fruiting body of P.rimosus, is able to significantly inhibit the growth of HCT116 colon cancer cell line in a time and concentration-dependent manner. The proliferation inhibition of HCT116 was the result of apoptosis induction, as evidenced by the cell morphological analyses of the PPC-Pr treated HCT 116 cells by DAPI and AO/EB staining methods. The typical DNA fragmentation of apoptosis in the treated cells is obtained in comet assay, which further indicates that PPC-Pr is able to induce the apoptosis of HCT116 cells. The mechanisms underlying these effects depend on
the initiating stimulus, but a common feature is the activation of certain endonucleotidases leading to DNA fragmentation (Barry and Eastman, 1992; Wyllie et al., 1984). Endonucleotidase activation may result from disruption of DNA supercoil structure (Murakami et al., 1995), interference with DNA repair mechanisms (Darzynkiewicz, 1995) or interference with normal cellular signaling pathways (Bergamaschi et al., 1993). It has been suggested that endonucleotidase activation depends upon the disruption of the interaction of histones with DNA. The apoptosis induced by PPC-Pr on HCT116 cells might be mediated via membrane polysaccharide receptor on cell surface, since PPC-Pr cannot penetrate cells due to its large molecular mass. Since the PPC-Pr treated HCT116 cells showed significant DNA fragmentation, the possible mechanism of PPC-Pr induced apoptosis might involve caspase-3 activation and the following cascades of reactions. However, further detailed studies are required to clarify the cellular signaling process by which PPC-Pr induces apoptosis in HCT116 cells. In conclusion, PPC-Pr inhibited the proliferation of HCT116 cells by triggering apoptosis in addition to causing DNA damage. The findings thus suggest the potential use of PPC-Pr in cancer therapy.