EFFECT OF CHEMICALLY SYNTHESIZED PORK PEPTIDES ON PROLIFERATION OF SW480 HUMAN COLON CANCER CELLS

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Abstract – This study was performed to investigate anti-proliferative effect of bioactive peptides derived from porcine muscle. Peptides EPCLA and LVGRPRHGQ, which showed angiotensin I-converting enzyme inhibitory activity in our pervious study, were chemically synthesized and tested anti-proliferation activity on SW480 human colon cancer cells. Cell viability was significantly lower in 50 and 100 ㎍/㎖ of EPCLAT added plates than others (P<0.05). Peptide EPCLAT and LVGRPRHGQ showed no effect on expression of pro-caspase-3, apoptosis-regulated protein on SW480 cells. However, they inhibited expression of cycline D1, cycline E, phosphorylation of p42/44 mitogen-activated protein kinases (MAPKs), and MEK1/2 (MAPK kinase) in SW480 cells, which means they were able to inhibit the proliferation of SW480 cells through the inhibition of phosphorylation of MEK1/2 and p42/44 MAPK. From these results, it is considered that pork derived peptides EPCLAT and LVGRPRHGQ have potential for colon cancer prevention.

Key Words – Anti-proliferation, Colon cancer cells, Porcine peptide

I. INTRODUCTION

In recent years it has been recognized that dietary proteins provide a rich source of biologically active peptides. Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health [1]. Peptides with anti-hypertensive, anticancer, antimicrobial, and immunomodulatory activity have been found [2, 3], and several compounds with anti-carcinogenic activity are also found in relatively high concentrations in food protein [4].

In our previous study [5], we have isolated and identified 5 angiotensin I-converting enzyme (ACE) inhibitory peptides, such as LKYP, LLGR, VFPS, EPCLAT, and LVGRPRHGQ, from enzymatically proteolized pork loin [5]. In order to investigate the possibility as new anticancer agents originating from natural ACE inhibitory peptides, five peptides were chemically synthesized and examined the anticancer activity against SW480 human colon cancer cell line.

II. MATERIALS AND METHODS

Pork-derived bioactive peptides LKYP, LLGR, VFPS, EPCLA and LVGRPRHGQ were chemically synthesized by Peptron Co. (Daejeon, Korea). Peptides were diluted in phosphate buffered saline (PBS) to a concentration of 10 ㎎/㎖, and filtered using 0.2 ㎛ membrane filter. SW480 human colon cancer cell line was obtained from Korean Food Research Institute (Sungnam, Korea) and grown in RPMI-1640 medium with 10% fetal bovine serum (FBS), 100 ㎍/㎖ streptomycin, and 100 U/㎖ of penicillin.

Cell viability was assessed using 3-(4-5-dimethyl thiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) staining as per the manufacturer's instructions (Roche, Basel, Switzerland). Cells were seeded in 96 well plates at a density of 1×10^4 cells/well and incubated in a CO_2 incubator with 5% CO_2 and 37℃ for 24 h to allow the cells to adhere to the inner wall of the flask. Subsequently, 150 ㎕/well of the culture medium containing a peptide at concentrations of 12.5, 25, and 50 ㎍/㎖, respectively, were added. The treated cells were then incubated for 24 and 48 h. After incubation, the culture medium was

discarded, and 100 ㎖ of 1 ㎎/㎖ MTT was added to each well and incubated for 2 h. After incubation, the liquid was discarded, 100 ㎕ of DMSO was added to each well, and the microplate was mounted on a micromixer for 5 min to facilitate the dissolution of the blue granules. The culture plate was then loaded onto the microplate reader for absorbance reading at an excitation wavelength of 570 nm.

Whole cell lysates were extracted from the cultured cells using M-PER (Mammalian Protein Extraction Reagents, Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's instructions. The protein concentration was measured by Bradford method (Sigma-Aldrich, St. Louis, MA. USA). The whole-cell extract (20-50 ㎍) was separated by electrophoresis on SDS-polyacrylamide gel, and transferred to nitrocellulose membrane (Protran, Dassel, Germany). The blots were then incubated with antibodies p-Rb$^{ser807/811}$, p-Rb^{ser780}, p-cdc2^{tyr15}, pro-caspase-3, β-actin, p-c-Raf, p-MEK1/2, β-catenin (Cell Signalling Technology, Danvers, MA. USA), c-Myc, cyclin D1, cyclin E, cyclin B1, p21, p53 (Santa Cruz Biotechnology, Santa Cruz, CA. USA) at room temperature for 2 h or at 4□ for overnight. The blots were then incubated in horseradish peroxidase (HRP)-linked secondary antibody (Amersham Biosciences, Piscataway, NJ. USA) at room temperature for 1 h. Immunocomplexes were detected with electrchemiluminescence (ECL) system (Amersham Biosciences).

The experiments were conducted using 3 replicates, with 2 observations per replicate. Statistical analysis was performed using the SAS program for Windows ver. 9.1 (SAS Institute, Cary, NC, USA). A analysis of variance (ANOVA) between groups with Duncan's multiple range test was employed to detect any significant differences among the treatments ($P < 0.05$).

III. RESULTS AND DISCUSSION

Pork derived ACE inhibitory peptides were chemically synthesized and treated in the media for SW480 cell line at concentrations of 12.5, 25, and 50 ㎍/㎖. The peptide EPCLAT inhibited the proliferation of SW480 cells ($P<0.05$) in dose-dependent manner, however the peptide LVGRPRGHQ, which showed the highest ACE inhibitory activity in the previous study [5] had no effect on the proliferation of SW480 cells (Fig. 1).

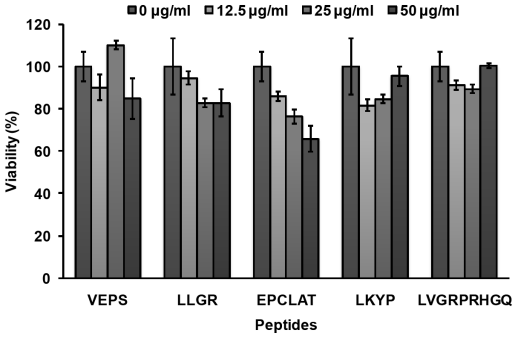

Figure 1. Anti-proliferative effect of pork-derived ACE inhibitory peptides on SW480 cell line.

The inhibition of cancer cell proliferation is induced by delay of cell cycle progression or apoptosis [6]. The phosphorylation of serine 807/811 residues of Rb protein, expression of cyclin D1 and E were inhibited by 100 ㎍/㎖ treatment of EPCLAT or LVGRPRHGQ. On the other hand, there were no differences in expressions of p-cdc2^{tyr15}, cyclin B1, p21 and pro-caspase-3 proteins (Fig. 2). This result shows that the pork-derived peptides influence on mitogen-activated protein kinases (MAPKs), upstream regulatory factor of cell cycle regulation.

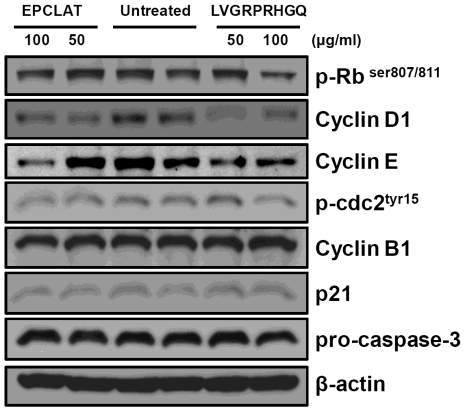

Figure 2. Effect of pork-derived peptides on the expression of cell cycle- and apoptosis-related proteins in SW480 cell line.

The phosphorylation of extracellular signal-regulated protein kinase (p-ERK) was decreased by treated pork-derived peptides. However, there was no differencein in phosphorylation of c-Jun N-terminal protein kinase (JNK) between

treatments. Pork-derived peptides inhibited MEK1/2 (MAPK kinase) phosphorylation, however, there was no significant inhibition in c-Raf phosphorylation (Fig. 3). This result means the cell cycle modulating effect of pork-derived peptides is generated by the inhibition of ERK phosphorylation, and it is not related with JNK-induced apoptosis.

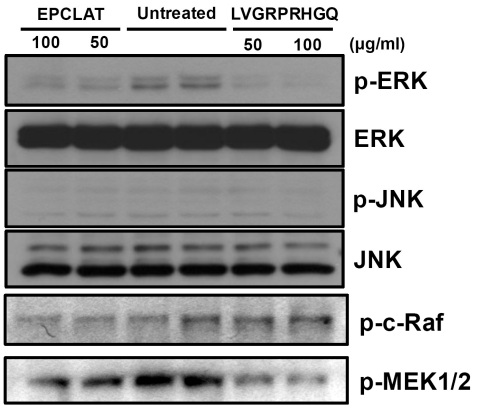

Figure 3. Effect of pork-derived peptides on the phosphorylation of ERK and JNK in SW480 cell line.

Mutations resulting in the truncation of the adenomatous polyposis coli (APC) protein are common to most colonic tumours, and β-catenin levels are usually elevated in cells with mutated APC, which changes the transcription of genes involved in differentiation and proliferation [7]. After treatment the pork-derived peptides at concentrations of 50 or 100 ㎍/㎖, there was no difference in the expression of β-catenin and c-Myc (Fig. 4), and this means that there is no relationship between pork-derived peptides and β-catenin related signaling pathway.

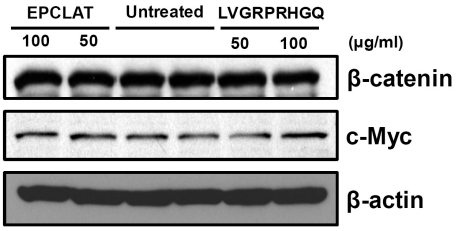

Figure 4. Effect of pork-derived peptides on the phosphorylation of c-Raf and MEK1/2 in SW480 cell line.

These results show that pork-derived peptides, EPCLAT and LVGRPRHGQ, were able to inhibit the proliferation of SW480 colon cancer cells through the inhibition of phosphorylation of MEK1/2 and p42/44 MAPK.

IV. CONCLUSION

In this study, chemically synthesized ACE inhibitory peptides derived from pork also showed anti-proliferative activity against human cancer cells. Therefore, the ACE inhibitory peptides can be expected to possess other biological functions and have potential for using as functional materials for food industry.

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