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Peanut testa extracts possessing histone deacetylase inhibitory activity induce apoptosis in cholangiocarcinoma cells

Somprasong Saenglee[a], Gulsiri Senawong[a], Sanun Jogloy[b], Banchob Sripa[c], Thanaset Senawong[a,d,*]

[a] Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[c] Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[d] Natural Product Research Unit, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

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ABSTRACT

Previous studies demonstrated that peanut testa extracts (KK4 and ICG15042) containing natural histone deacetylase (HDAC) inhibitors inhibited the growth of several human cancer cell lines via apoptosis induction. The aims of this study were to investigate the anti-proliferative effects and the mechanism(s) responsible for apoptosis induction mediated by these peanut testa extracts in human cholangiocarcinoma cell lines (KKU-M214 and KKU-100). The anti-proliferative effects were assessed by MTT assay. Apoptotic cell death and cell cycle arrest were analyzed by flow cytometry. The caspase activities were studied using colorimetric caspase activity assay and western blot analysis. Our results revealed that KK4 and ICG15042 extracts inhibited cell proliferation of both KKU-M214 and KKU-100 cells in a dose- and time-dependent manner, with IC_{50} values of 38.28 ± 0.29 (KK4), 43.91 ± 1.94 (ICG15042) μg/mL for KKU-M214 and 78.40 ± 1.74 (KK4), 82.77 ± 0.94 (ICG15042) μg/mL for KKU-100 at 72 h. Apoptosis induction by these peanut testa extracts were observed in both KKU-M214 and KKU-100 cells in a concentration-dependent manner. Moreover, the percentage of cells in the sub-G1 phase was significantly increased in both KKU-M214 and KKU-100 cells. Cell cycle arrest was not observed in other cell cycle phases. Activation of caspases 8 and 3 were apparent integral parts of apoptosis induction in both cells. Both peanut testa extracts also caused down-regulation of p53, p21, Bcl-2 and pERK1/2 protein expression in these cells. These results suggest that peanut testa extracts may be potential anti-cancer agents for cholangiocarcinoma chemoprevention or chemotherapy.

1. Introduction

Cholangiocarcinoma (CCA), also known as bile duct cancer, is a rare form of cancer. The incidence of CCA has been reported worldwide including Eastern Europe, East Asia and Southeast Asian countries (Thailand, Lao People's Democratic Republic, Vietnam and Cambodia) [1]. Specifically, the incidence of CCA in the northeast of Thailand is the highest in the world [2]. Gene mutation, chronic infection and environment are risk factors involved in cholangiocarcinoma development [3]. CCA development caused by parasitic infections such as liver fluke infection (*Opisthorchis viverrini*, *O. felineus* and *Clonorchis sinensis*) has been reported [[3],4]. Oxidative stress induced by liver fluke infection contributes to lipid peroxidation and DNA damage, leading to mutation and cholangiocarcinoma development [5]. Moreover, oxidative stress and DNA injuries induced by various carcinogens such as tobacco, alcohol or virus infections in humans are understood to cause numerous chronic degenerative diseases including cholangiocarcinoma [6]. Occasionally, DNA damage affects expression or is a mutation in a protein such as in Bcl-2 oncoprotein or tumor-suppressor proteins (p21 and p53), which results in activation of cell cycle checkpoints or induction of apoptosis [7]. The activation of oncoproteins can cause the development and progression of cancer cells, which suppresses apoptosis during carcinogenesis [8]. Cancer drugs have been developed to inhibit these impaired pathways in tumor cells. The DNA damage response pathways are recruited to protect against tumor progression through activation of specifically targeted members of the DNA damage response and apoptosis pathways [9].

Presently, apoptosis is a therapeutic goal of cancer therapy because its targeting is more advantageous than other cell death mechanisms [10]. This machinery is associated with the function of a cysteine protease family known as caspases that cleave their target proteins at specific aspartic acid regions in the early stages of apoptosis [11,12].

* Corresponding author at: Dr Thanaset Senawong, Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
E-mail address: sthanaset@kku.ac.th (T. Senawong).

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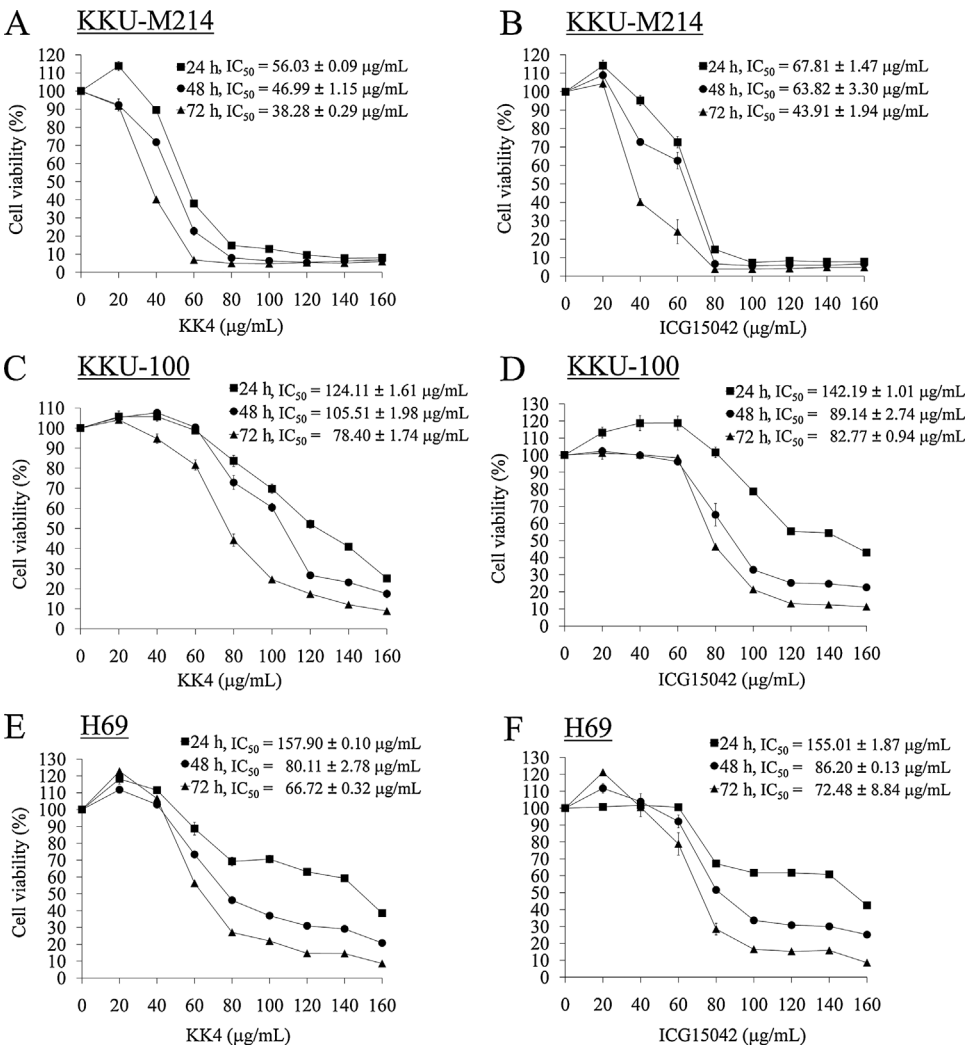

Fig. 1. Antiproliferative activity of two peanut testa extracts (KK4 and ICG15042) against KKU-M214 (A, B), KKU-100 (C, D) and non-cancer (H69) (E, F) cells treated for 24, 48 and 72 h. Data were calculated as percentages of cell viability compared with the solvent control (0.5% DMSO), which was defined as 100% survival. The results are shown as mean ± S.D. from three independent experiments. The half maximal inhibitory concentration (IC_{50}) value was estimated by plotting x–y and fitting the data with straight line (linear regression). The average of IC_{50} values from three experiments was calculated and presented along with a line graph.

Based on the length of the prodomain, caspases are categorized into 14 family members. Most of these caspases have been shown to be involved in induction of apoptosis, including caspase-2, -3, -4, -6, -7, -8, -9, and -10 [13]. Caspases are required for proteolytic cleavage of many proteins including structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes. Not only caspases but also degradative enzymes such as DNases are used in the apoptotic process.

Recently, we demonstrated that two peanut testa extracts (KK4 and ICG15042) inhibited cell proliferation and induced apoptosis in five human cancer cell lines (HeLa, HT29, HCT116, Jurkat and MCF-7 cells) [14]. In addition, both ICG15042 and KK4 testa extracts exhibited histone deacetylase (HDAC) inhibitory activity. Interestingly, HDAC inhibitors play a crucial role for restoring normal expression of silenced tumor suppressor genes and for suppressing proto-oncogenes [15]. HDAC inhibitors induced differentiation and/or apoptosis, cell cycle arrest, and inhibition of proliferation in various cancer cell lines [16], and caused DNA damage and repair mechanisms in cancer cells [17]. Although phenolic- and polyphenol-rich extracts from edible and therapeutic plants are extensively tested in clinical trials for chemoprevention and therapy [18], both KK4 and ICG15042 peanut testa extracts have not yet been investigated for their anticancer activity against cholangiocarcinoma cell lines. Thus, the objective of this study was to investigate the apoptotic induction activity of peanut testa extracts in cholangiocarcinoma cells.

2. Material and methods

2.1. Materials and reagents

The two Valencia-type peanut kernels (KK4 and ICG15042) were obtained from the Field Crop Research Station of Khon Kaen University, Thailand. The collections of peanut testa were previously described [19]. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Invitrogen, Molecular Probes products (Eugene, Oregon, USA). Annexin V-FITC and propidium iodide (PI) were purchased from Biolegend (San Diego, CA, USA) and Sigma-Aldrich Corporation (St. Louis, MO, USA), respectively. The primary antibodies, anti-cleaved caspase 3, anti-procaspase 3, anti-cleaved caspase 8, anti-procaspase 8, anti-cleaved caspase 9, anti-procaspase 9, anti-p21, anti-p53, anti-Bcl 2, anti-pERK1/2, anti-ERK1/2 and anti-β-actin, were purchased from Cell Signaling Technology (Beverly, MA, USA). Caspases 3, 8 and 9 colorimetric assay kits were purchased from BioVision, Inc. (Milpitas, CA, USA).

2.2. Cell culture

KKU-M214 (well-differentiated) and KKU-100 (poorly differentiated) cells were established from opisthorchiasis-associated Thai CCA patients. H69 cells (a non-cancer cell line; human bile duct epithelial cells) were kindly provided by Dr D. Jefferson, Tufts University, Boston, MA, USA. Both KKU-M214 and KKU-100 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, penicillin (100 U/