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REVIEW

Potential roles of longan flower and seed extracts for anti-cancer

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Abstract

Polyphenol-rich plants are known to possess benefits to human health. Recent studies have revealed that many Traditional Chinese Medicines (TCMs) are rich sources of polyphenols and exhibit antioxidant and anti-inflammatory activities, and these TCMs have been shown experimentally to overcome some chronic diseases, including cancer. Longan flowers and seeds, two TCMs traditionally used for relieving pain and urinary diseases, have been revealed in our recent reports and other studies to possess rich amounts of polyphenolic species and exhibit strong anti-oxidant activity, and these could be applied for the treatment of diabetes and cancer. Herein, we review the recent findings regarding the benefits of these two TCMs in the treatment of human cancer and the possible cellular and molecular mechanisms of both substances.

Key words: Longan flower; Longan seed; Cancer; Oncoprotein; Tumor suppressor; Traditional Chinese Medicine

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INTRODUCTION

Cancer has become the most common disease threatening public health, which has led to interventions for the prevention and treatment of this disease. Based on the fact that cancer exhibits a slow, stepwise development, and requires several years to become a life-threatening disease, it is regarded largely as a preventable disease[1-3]. Although improvements in medical techniques have been made in recent years, some types of cancer are still difficult to cure, even following advanced treatment. The challenge mainly arises owing to the recurrence, chemoresistance and distal metastasis of progressing cancer, which have become the important focuses of novel detection methods and treatment strategies[4]. Epidemiological studies show a correlation between increasing consumption of phenolic compounds and a reduced risk of cancer[5-8]. Plants are the primary source of polyphenols, and some have been regarded as forming part of a healthy diet for many years, such as tea, soybean, pome-

granate, and pine nuts[9]. Traditional Chinese Medicine (TCM) has been developed in China for more than two thousand years. TCMs comprise various forms of herbal medicine and complemental therapy such as acupuncture, massage (Tui na), exercise (qigong), and dietary therapy. The pharmacopoeia of TCM, named the Compendium of Materia Medica, records hundreds of medicinal substances such as plants, minerals and animal products, and their health beneficial action in the body. Different parts of plants, such as the leaves, roots, stems, flowers, and seeds, are used. Several clinically-used chemotherapeutic drugs are derived from TCMs, such as camptothecin, isolated from the "happy tree" (*Camptotheca accuminata*); etoposide, semi-synthesized from a compound of *Podophyllum emodi var. chinense*; vincristin and vinblastin, isolated from the Madagascar periwinkle (*Catharanthus roseus*); and paclitaxel, purified from *Taxus chinensis*[10,11]. Recent studies have further revealed that some TCMs or their components exhibit anti-tumor activities towards several types of cancer, such as liver[12], lung[13], gastric[14], nasopharyngeal[15] and colorectal cancer[16]. Non-steroidal anti-inflammatory drugs have been documented in animal and human studies to reduce the risk of colorectal cancer and adenomatous polyps and have been implicated as a cancer prevention and treatment strategy[1,17-19]. Longan flowers and seeds have been analyzed and were found to possess rich amounts of polyphenols, including proanthocyanidin A2, (-)-epicatechin, gallic acid and ellagic acid, and exhibit strong antioxidant and inflammatory activities[20-24]. Recently, several studies by our research group have further revealed that longan flower and seed extracts exhibit an anti-cancer activity towards colorectal, liver, lung, cervical and breast cancer. Herein, we review the recent findings regarding the benefits of these two TCMs in the treatment of human cancer and the possible cellular and molecular mechanisms of both substances.

LONGAN FLOWERS AND SEEDS IN TCM

Longan (*Dimocarpus Longan* Lour.) is a subtropical fruit grown throughout Asia, with southern China including Taiwan being the main center of commercial production. The longan fruit is a famous summer fruit and is used as a TCM as a stomachic, febrifuge, and vermifuge, and also as an antidote to poison[24]. In agriculture, off-season induction of flowering in longan trees is a desirable economic goal and is accomplished through the application of gibberellin biosynthesis inhibitors[25]. To increase the size and quality of the fruit, an important operation is to prune or remove flower spikes in the cluster. Longan flowers are sold in herb markets and due to their fresh and fruity aroma are mainly used to prepare an infusion that is drunk for pleasure in Taiwan. As described in a TCM pharmacopoeia named Herbal Quanzhou, drinking the water extract of longan flowers could overcome micturition, urgency and voiding dysfunction. The powder of dried longan seeds can be used for treating bleeding, dampness, hernia, lymphomegaly of the neck and armpit, odour, scabies and eczema, as described in another TCM pharmacopoeia named Chinese Herbal Medicine. The National Herbal Compendium of China also records that longan seed powder is generally applied for stomach pain and as a styptic. The multiple medical functions of longan flowers and seeds, and especially the reduction of swelling as recorded in the TCM pharmacopoeia, imply that these two TCMs can be applied in cases of microbial infection, inflammation, and metabolic diseases. Evidence to this end has been revealed by current scientific methods during the past decade.

Anti-oxidant activity and effect on inflammation and metabolic disorders of longan flower extracts

Although multiple medicinal applications of longan flowers are recorded in TCM pharmacopoeia, the scientific evidence related to their effect on human health has been accumulating over recent years. We have demonstrated that the hot water reflux or ethanol extract of the longan flower, contained abundant proantocyanidins and rarely anthocyanins, suppresses nitric oxide and prostaglandin E2 production in lipopolysaccharide -stimulated macrophage cell line RAW264.7 and may be the potential source of natural dietary anti-oxidants and anti-inflammatory agent[20]. The longan flower extract (LFE), analyzed by Professor Hwang and colleagues, exhibits a strong anti-oxidant activity, which is mainly due to (-)epicatechin and proanthocyanidin A2[26]. Proanthocyanidin-rich substances such as grape seed extracts have been implicated as preventive agents against cardiovascular disease and cancer, which are strongly associated with chronic inflammation diseases[9,27,28]. In another study by Professor Hwang, consumption of LFE reduced the blood pressure and oxidative markers such as plasma thiobarbituric acid and liver antioxidant enzyme activity in fructose-fed rats. Insulin action signaling, such as insulin receptor substrate-1 and glucose transporter 4, is enhanced in these rats, indicating the effect of LFE on overcoming insulin resistance[29]. Recent study has also revealed that feeding LFE to high-caloric-diet rats results in reduction of body weight, size of epididymal fat, serum triglycerides and atherogenic index. The main mechanism is downregulation of pancreas lipase, sterol regulatory element binding protein-1c and fatty acid synthase, and upregulation of low-density lipoprotein receptor and peroxisome proliferator-activated-receptor α expression, as well as promotion of fecal triglyceride excretion[30]. These studies indicate that LFE can not only be applied for the treatment of urinary disorders, but also has the potential for use as a preventive or treatment agent for metabolic diseases.

Anti-colorectal cancer effects of LFE

Polyphenol-rich extracts have been demonstrated to exert anti-cancer effects[31-33]. Owing to the rich amount of phenolics in LFE, our research team explored its possible role in human malignancy diseases. We selected colorectal cancer as the first subject, because it has been

the most common cancer type in Taiwan since 2007, when the dietary behavior of Taiwanese became more westernized[34]. Recent studies have revealed that the water reflux extract of longan flowers is enriched with two major compounds, (-)-epicatechin and proanthocyanidin A2[26], which are also found in grape seed extract as active anti-colorectal cancer agents[35,36]. Based on these reports, we proposed that LFE could play a possible role in colorectal cancer (CRC) prevention and treatment. In our recent study[37], we treated two CRC cell lines, SW480 and Colo 320DM, which are derived from Duke's B and Duke's C patients respectively, with LFE, and found an inhibitory effect on the proliferation of these two cell lines in a dose- and time-dependent manner. LFE treatment also affected the anchorage-independent growth of these two cell lines in soft agar, an *in vitro* assay to test the cancer cells clonogenic growth, and anoikis resistance, and was closely correlated with *in vivo* tumorigenesis[38,39]. The uncontrolled cell division, clonogenic growth and anoikis resistance are the main malignant properties leading cancer cells to unlimited growth and tumorigenesis *in vivo*[38-40]. The results strongly indicate that LFE is capable of influencing the malignant potential of CRC cells, which implies a role of LFE in the prevention and treatment of colorectal cancer. The mechanisms of LFE in anti-CRC growth are mainly due to cell-cycle arrest in the S phase and induction of mitochondria-mediated apoptosis. Previously, Kaur *et al*[36] reported that proanthocyanidin-rich grape seed extracts inhibited the proliferation of colorectal cancer cells due to their ability to cease G1-phase arrest of the cell cycle. However, LFE elevated cyclin E levels and downregulated cyclin A levels and arrested CRC cells in the DNA synthesis phase of the cell cycle. The elevation of cyclin E levels in both LFE-treated CRC cells was correlated with S-phase cell accumulation. Cyclin A is synthesized during the S phase and its functional disruption can inhibit chromosomal DNA replication[41-43]. Together with the accumulation of cyclin E, we conclude that the effect of LFE on the cell division cycle is mainly due to hampered DNA synthesis. LFE also exhibits selected apoptosis induction in Colo 320DM rather than SW480. Induction of apoptosis is another possible mechanism by which the anti-proliferative effect of LFE on colorectal cancer cells may be exerted. In the present study, we show that LFE treatment could induce apoptosis in one of the tested CRC cells (Colo 320DM). The apoptotic cells represented DNA fragmentation, mitochondrial membrane potential loss and the activation of caspase 3. The Bcl-2 family has been demonstrated as the main mechanism of naturally-occurring phytochemicals-induced apoptosis in cancer cells[44-47]. Bcl-2 inhibits Bax action and protects cytochrome C preservation in mitochondria, which leads to the maintenance of mitochondria membrane potential and keeping cell survive. The decrease in Bcl-2 level in LFE-treated Colo 320DM cells was correlated with apoptosis, indicating that the mechanism of apoptosis induction by LFE in this cell line is mainly due to suppression of the anti-apoptotic protein Bcl-2. LFE failed to induce apoptosis in SW480 cells, which may be associated with some DNA damage repair mechanisms and tumor suppressor gene mutations such as *p53* in SW480. This is an intriguing issue worthy of further investigation.

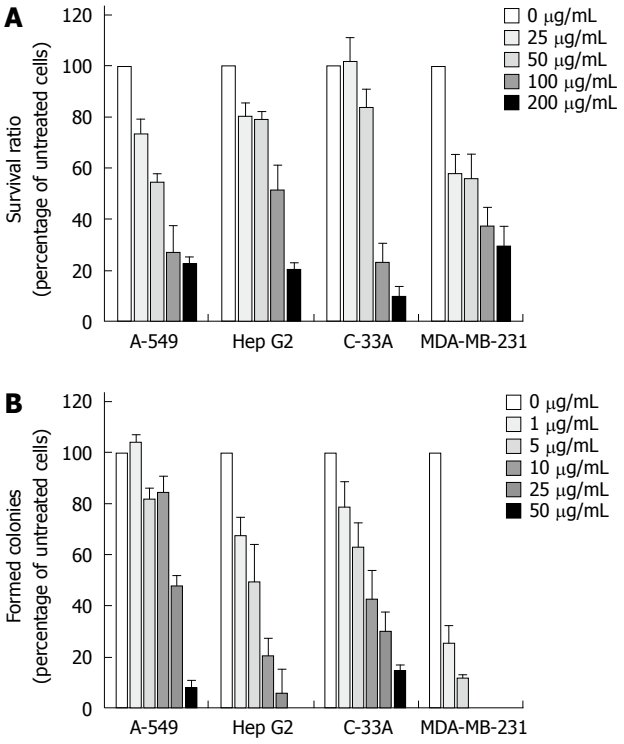

Figure 1 The inhibitory effect of longan seed extract on the growth of cancer cells. The longan seed extract (LSE)-inhibited growth assessed herein consists of cancer cell proliferation by (A) trypan blue assay and (B) colony forming activity. For the cell proliferation assay, 100 000 cancer cells as indicated were seeded in 60-mm sterile plastic dishes and treated with different concentrations of LSE. After incubation at 37 ℃ for 48 h, cells were detached by treatment with trypsin, and the suspended cells were stained with trypan blue. The viable cells were then counted under a phase contrast microscope. Colony formation activity was assessed by seeding 200 cells into 60-mm dishes then treating these cells with different concentrations of LSE. After incubation for 14 d, the formed colonies that contained more than 50 cells were regarded as one colony, and the numbers of colonies in the dishes were counted. The data represent the average of three independent experiments and are expressed as the mean ± SD.

DISCUSSION AND RESULTS

Potential role of longan seed extract in anti-CRC and other types of carcinomas

Longan seed extract (LSE) has been shown to consist of gallic acid, corilagin and ellagic acid as the main active components, resulting in anti-oxidant and tyrosinase activities[21]. As gallic acid, ellagic acid and corilagin have been investigated in terms of their effects against different types of human cancer, we proposed that LSE, rich in these three components, could exhibit anti-cancer activity. Our recent study showed that LSE is capable of inhibiting cell proliferation and clonogenic growth in SW480, HT-29 and Colo 320DM, indicating a similar role of LSE to that of LFE in anti-CRC cells[48]. Recently, we further tested LSE with regards to the growth inhibition

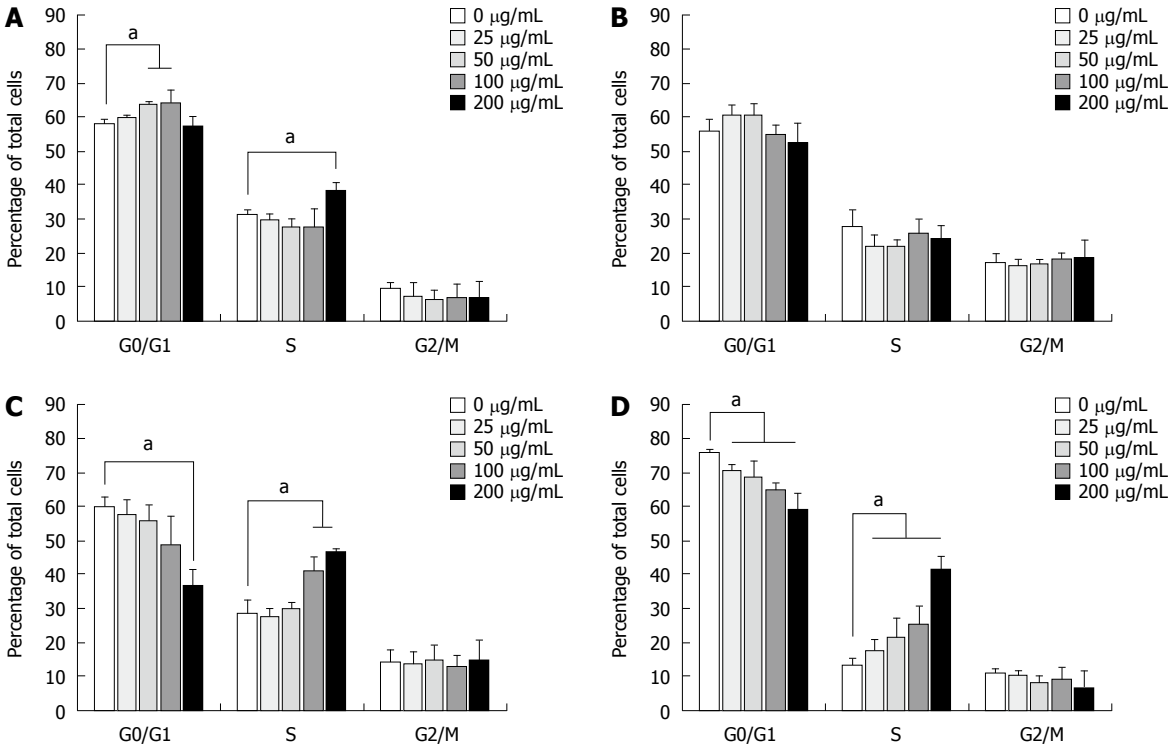

Figure 2 Cell cycle analysis of longan seed extract -treated cancer cells. About 1 × 10^6 cells in 10 mL medium in 100 mm plate were treated with increasing concentrations of longan seed extract as indicated, then incubated at 37 ℃ for 48 h. The (A) A-549, (B) Hep G2, (C) C-33A and (D) MDA-MB-231 cells harvested by trypsinization were fixed in 70% alcohol at -20 ℃ for 2 h and then reconstituted in phosphate-buffered saline. The cells were stained with propidium iodide solution in the dark at room temperature for 30 min. The stained cells were then analyzed using the FL-2A parameter of a flow cytometer to obtain the DNA content of the cells, and the distribution in each cell-cycle phase was determined using Modfit software. Data are expressed as a percentage of the total cells. Data represent the average of three independent experiments, and are expressed as the mean ± SD. $^aP < 0.05$.

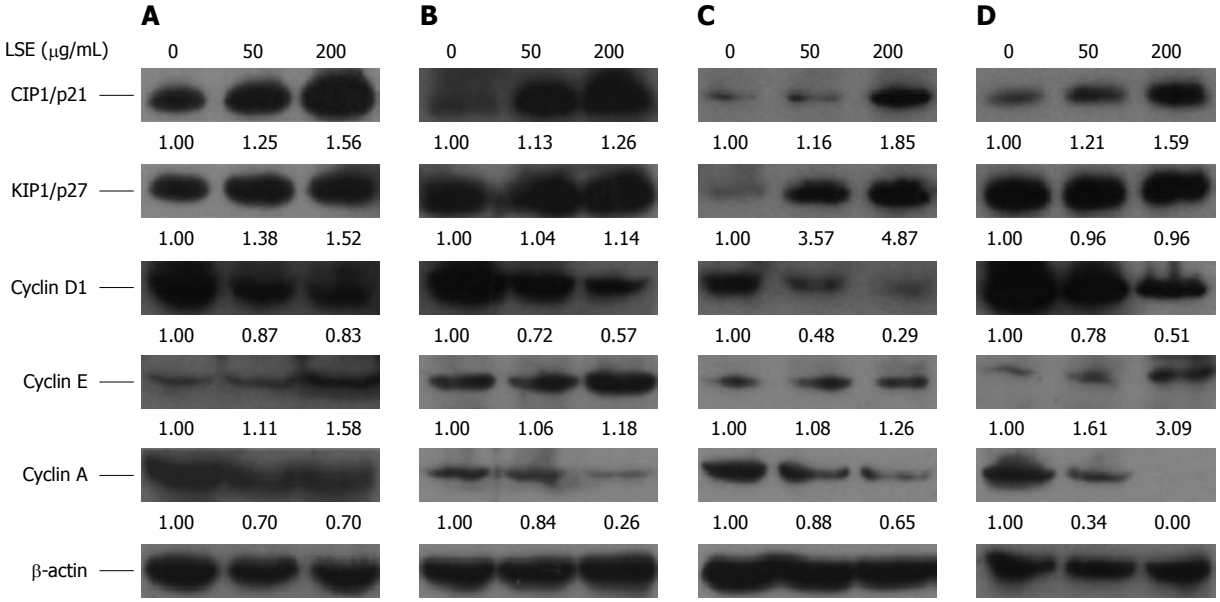

Figure 3 Immunoblots of cell cycle-controlling proteins in longan seed extract-treated cancer cells. About 50 and 200 μg/mL longan seed extract (LSE)-treated (A) A-549, (B) Hep G2, (C) C-33A and (D) MDA-MB-231 cells were incubated at 37 ℃ for 48 h. The harvested cells were lysed in Triton X 100-containing hypotonic buffer as per previous reports (Hsu *et al*[37], 2010; Chung *et al*[48], 2010) at 4 ℃ for 30 min. Cell lysates were centrifuged and the protein concentrations in the supernatants were determined using a bicinchoninic acid protein detection kit. Cell protein lysates were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes and immunoblotted to show cyclin D1, cyclin E, cyclin A, CIP1/p21 and KIP1/p27, with the β-actin level used as the loading control. The images are representative results from three independent experiments. The density of each protein band was measured using ImageJ software and protein expression was normalized to β-actin, and the relative amount of each protein band was referenced to the untreated control.

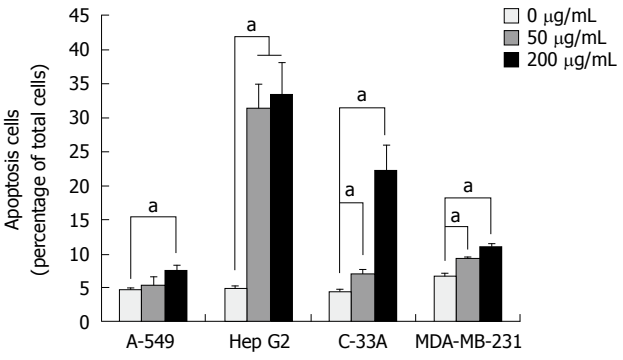

Figure 4 Detection of longan seed extract-induced apoptotic cells. Phosphatidylserine is usually distributed in the inner fleet of the plasma membrane. When cells are undergoing apoptosis, this phospholipid translocates to the outer fleet, and can be recognized by a bacteria glycoprotein named annexin V. We used annexin V conjugated with fluorescein isothiocyanate (FITC) as an apoptosis indicator and analyzed the apoptotic cells by flow cytometry. Briefly, 50 and 200 μg/mL longan seed extract-treated cells were incubated at 37 ℃ for 48 h. The treated cells were then suspended and stained with annexin V conjugated with FITC. Ten thousand cells were analyzed by flow cytometry using FL-1 as the parameter. Data are taken from the averages of three independent experiments and expressed as the mean ± SD. $^{a}P < 0.05$.

of lung adenocarcinoma cell line A549, hepatocellular carcinoma cell line Hep-G2, cervix carcinoma cell line C33A, and breast carcinoma cell line MDA-MB-231. The proliferation and colony-forming activity of these cancer cell lines were suppressed gradually by increasing LSE concentrations, and MDA-MB-231 was more sensitive to LSE than the other types of cancer cells (Figure 1). As shown in Figure 1A, the viability of MDA-MB-231 cells decreased to lower than 60% of the untreated cells following 25 μg/mL LSE treatment. A similar effect occurred in 50 μg/mL LSE-treated A549 cells and 100 μg/mL LSE-treated C33A and HepG2 cells. The colony-forming activity of these cancer cells was also suppressed. As shown in Figure 1B, the colonies of MDA-MB-231 cells were lower than 40% of the control colonies at 1 μg/mL LSE and 0% at more than 10 μg/mL LSE. Similar effects were seen in HepG2 cells at 10 and 50 μg/mL LSE, respectively. The colonies of C33A and A549 cells were lower than 40% compared to control cells at 25 μg/mL LSE, and 10%-20% compared to control cells appeared at 50 μg/mL LSE. Together, these results indicate that LSE is capable of influencing the proliferation and clonogenesis of colorectal, lung, liver, cervix and breast cancer cells.

LSE influences the expression of cell cycle-associated oncoproteins and suppressors

Our previous study demonstrated that LSE induced the cell cycle arrest of CRC cells in the DNA synthesis phase and suppressed cyclin D1 and cyclin A expression. Recently, we examined the effect of LSE on the cell-cycle progression of lung (A549), liver (HepG2), cervix (C33A) and breast cancer cells (MDA-MB-231). The S phase obviously increased in LSE-treated C33A and MDA-MB-231 cells, while the G1 phase increased in A549 cells (Figure 2). We further analyzed cell cycle-modulating proteins including cyclin D1, cyclin E, cyclin A, CIP/p21 and KIP/p27 in LSE-treated cells, and found that LSE systemically suppressed cyclin D1 and cyclin A expression and concomitantly enhanced CIP/p21 and KIP/p27 (Figure 3). The growth inhibition effect of naturally-occurring products on human cancer cells may arise from downregulation of oncoproteins or enhancement of tumor suppressor proteins[49-51]. Overexpression of cyclin D1 has been shown to play a pivotal role in promoting cancer cell proliferation, focus formation, tumorigenesis, drug resistance and metastasis, and has long been regarded as one of the important oncoproteins[52-56]. Loss of expression or function of CIP1/p21 and KIP1/p27 has been implicated in the genesis or progression of many human malignancies[57,58]. The suppression of cyclin D1 and enhancement of CIP1/p21 and KIP1/p27 expression by LSE treatment imply that the inhibitory effects of LSE on cancer cell growth may arise from the regulation of these proteins. The related cellular events are cell-cycle arrest in LSE-treated cancer cells, as these proteins are cell cycle-controlling proteins. Cyclin D1 and cyclin E are generated after mitogen stimulation in quiescent cells. These two proteins associate with CDK4/CDK6 and CDK2 and then enter the nucleus to phosphorylate/inactivate retinoblastoma protein, which leads cells to enter into the S phase[59]. Cyclin/CDKs activity is negatively regulated by inhibitors of cyclin-dependent kinases (INKs). CIP1/p21 and KIP1/p27 belong to the Kip/Cip family of INKs, which associate with CDK4,6/cyclin D1 and CDK2/cyclin E, A complex and inactivate their kinase activity by interfering with ATP binding and the cyclin/CDK structure[60]. Fifty μg/mL LSE-treated A549 cells exhibit decreased cyclin D1 and E and elevated CIP1/p21 and KIP1/p27, indicating the molecular mechanism of the LSE-induced G1-phase arrest. However, other cancer cells treated with the same concentration of LSE show no change or increased cyclin E and exhibit S-phase arrest (MDA-MB-231) or little change in the cell cycle (C33A, HepG2). S-phase arrest of LSE-treated cells occurs in MDA-MB-231 cells treated with more than 25 μg/mL LSE and C33A and A549 cells treated with more than 200 μg/mL LSE. Cyclin A, which is synthesized in the G1/S transition and associates with CDK2 to promote the S/G2 phase, is remarkably decreased in all LSE-treated MDA-MB-231 cells and in A549 and C33A cells treated with 200 μg/mL LSE. Our previous study indicated that elevated cyclin E and decreased cyclin A may be the key mechanism leading cancer cells to arrest in the S phase[37]. The results further confirm the distribution of cyclin E and A in S-phase arrest of cancer cells. The results taken together indicate that LSE interferes with tumor-promoting activities and cell-cycle progression by suppression of oncoproteins such as cyclin D1 and A and enhancement of suppressors such as CIP1/p21 and KIP1/p27.

LSE induced mitochondria-mediated apoptosis

Apoptosis is the essential mechanism by which unwanted

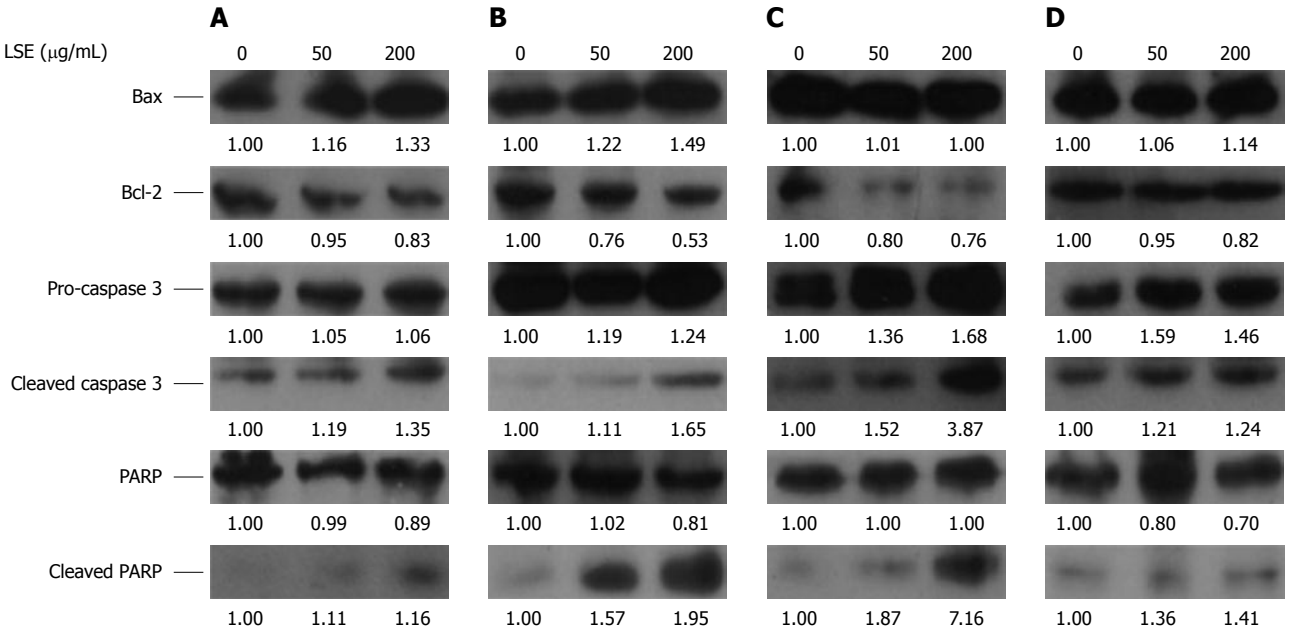

Figure 5 Immunoblots of apoptosis-associated proteins in longan seed extract-treated cancer cells. The same cell lysates from longan seed extract (LSE)-treated (A) A-549, (B) Hep G2, (C) C-33A and (D) MDA-MB-231 cells as described in Figure 3 were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes and immunoblotted to show the levels of Bax, Bcl-2, pro-Caspase 3, cleaved-caspase 3, poly (ADP-ribose) polymerase-1 (PARP-1) and cleaved-PARP. The images are representative of three independent experiments.

or damaged cells are eliminated during development or maintenance of tissue homeostasis in multiple cellular organisms[61,62]. Defective regulation of apoptosis has been closely associated with many human chronic diseases such as neural degeneration, autoimmune disease, AIDS and cancer[63]. Inducing cancer cells into apoptosis is the main mechanism by which anti-neoplasm drugs or natural products suppress cancer cell growth[62]. In our previous study, induction of apoptosis was found to be another possible mechanism of the antiproliferative activity of LSE on CRC cells[48]. However, one CRC cell line appeared to be resistant to LSE-induced apoptosis. Result of present study further showed that LSE selectively induced significant apoptosis in HepG2 and C33A cells but only a slight elevation of apoptosis in A549 and MDA-MB-231 cells (Figure 4). Caspase 3 was activated by the proteolytic cleavage of upstream apoptosome and the consequent cleavage of substrates such as PARP. Many reports suggest that the activation of caspase 3 is a common event during polyphenolics-induced apoptosis of cancer cells[35-37,64]. Our previous study showed that LSE enhances caspase 3 activity in CRC cells and induces apoptosis. Our recent results further showed that LSE induced pro-caspase 3 expression and cleavage (Figure 5). LSE-induced caspase 3 activation and apoptosis may operate through the Bcl-2 family of proteins. The Bcl-2 family members are important mediators of mitochondria-induced apoptosis in cancer cells[61,65,66]. These proteins form multimers, which act as pores in cell membranes, controlling the flow of molecules[67]. Bcl-2 has been associated with apoptosis inhibition, whereas expression of Bax has been associated with apoptosis induction[68,69]. Bcl-2 inhibits apoptosis by inhibiting the release of cytochrome c (Apaf 2) and apoptosis inducing factor (AIF) from the mitochondria to the cytoplasm, and by limiting the activation of caspase 3 by inhibiting its activator protein, Apaf 1[70]. Recent studies indicate that the ratio of Bax:Bcl-2 proteins is the determining factor in transmitting the apoptosis signal[46,67,71,72]. In our study, the Bcl-2 levels were decreased in LSE-treated cancer cells, especially in sensitive cells[48]; however, the change in Bax levels in these cells are not affected, indicating that LSE-induced apoptosis is mainly due to the regulation of the level of Bcl-2.

CONCLUSION

The longan is one of the most important fruits in China, economically speaking. The flowers and seeds of the longan were regarded as waste for a long time, and failed to be utilized. However, according to TCM pharmacopoeia, longan flowers and seeds possess multiple pharmaceutical applications. Recent advanced biotechnology and pharmacology techniques allow us to gain a deeper insight into the functions of these two TCMs using scientific methods. LFE could suppress oxidation and inflammation, decrease blood pressure, triglycerides and body weight, and overcome metabolic diseases such as diabetes mellitus. We provide data to demonstrate that LFE is also capable of inducing cell-cycle arrest and apoptosis, at least in colorectal cancer cells, implying that LFE could also be applied as an anti-neoplasm agent. LSE systemically inhibits colorectal, lung, liver, cervix and breast cancer cells, indicating the utilization of LSE in cancer prevention and treatment. LSE could suppress oncoproteins such as cyclin D1, A and Bcl-2 and elevate suppressors

such as caspase 3, Bax, CIP1/p21 and KIP1/p27. This may provide a possible role of LSE as a multiple-target therapeutic agent to control abnormal growth and malignancy in cancer. The *in vivo* efficacy of both LFE and LSE in mice tumorigenesis is the next important issue for further investigation, and indeed this research is ongoing in our research team. In conclusion, recent advanced studies have validated the novel pharmaceutical functions of LFE and LSE, especially their anti-cancer functions, and have provided scientific evidence to further the application of these two TCMs in the pharmaceutical industry.

REFERENCES

1 **Algra AM**, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol* 2012; **13**: 518-527
2 **Tanaka T**, Shnimizu M, Moriwaki H. Cancer chemoprevention by carotenoids. *Molecules* 2012; **17**: 3202-3242
3 **Narayanan BA**. Chemopreventive agents alters global gene expression pattern: predicting their mode of action and targets. *Curr Cancer Drug Targets* 2006; **6**: 711-727
4 **Ribatti D**. Cancer stem cells and tumor angiogenesis. *Cancer Lett* 2012; **321**: 13-17
5 **Brown EM**, Gill CI, McDougall GJ, Stewart D. Mechanisms underlying the anti-proliferative effects of berry components in in vitro models of colon cancer. *Curr Pharm Biotechnol* 2012; **13**: 200-209
6 **Ros E**. Health benefits of nut consumption. *Nutrients* 2010; **2**: 652-682
7 **Halliwell B**. Antioxidants and human disease: a general introduction. *Nutr Rev* 1997; **55**: S44-S9; discussion S44-S9
8 **Surh YJ**. Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 2003; **3**: 768-780
9 **Kaur M**, Agarwal C, Agarwal R. Anticancer and cancer chemopreventive potential of grape seed extract and other grape-based products. *J Nutr* 2009; **139**: 1806S-1812S
10 **Efferth T**, Fu YJ, Zu YG, Schwarz G, Konkimalla VS, Wink M. Molecular target-guided tumor therapy with natural products derived from traditional Chinese medicine. *Curr Med Chem* 2007; **14**: 2024-2032
11 **Efferth T**, Li PC, Konkimalla VS, Kaina B. From traditional Chinese medicine to rational cancer therapy. *Trends Mol Med* 2007; **13**: 353-361
12 **Wu P**, Dugoua JJ, Eyawo O, Mills EJ. Traditional Chinese Medicines in the treatment of hepatocellular cancers: a systematic review and meta-analysis. *J Exp Clin Cancer Res* 2009; **28**: 112
13 **Tian G**, Guo L, Gao W. Use of compound Chinese medicine in the treatment of lung cancer. *Curr Drug Discov Technol* 2010; **7**: 32-36
14 **Wu M**, Yao B. Advances in TCM treatment of gastric cancer and studies on the apoptosis. *J Tradit Chin Med* 2002; **22**: 303-307
15 **Cho WC**, Chen HY. Clinical efficacy of traditional Chinese medicine as a concomitant therapy for nasopharyngeal carcinoma: a systematic review and meta-analysis. *Cancer Invest* 2009; **27**: 334-344
16 **Tan KY**, Liu CB, Chen AH, Ding YJ, Jin HY, Seow-Choen F. The role of traditional Chinese medicine in colorectal cancer treatment. *Tech Coloproctol* 2008; **12**: 1-6; discussion 6
17 **Cathcart MC**, Lysaght J, Pidgeon GP. Eicosanoid signalling pathways in the development and progression of colorectal cancer: novel approaches for prevention/intervention. *Cancer Metastasis Rev* 2011; **30**: 363-385
18 **Schrör K**. Pharmacology and cellular/molecular mechanisms of action of aspirin and non-aspirin NSAIDs in colorectal cancer. *Best Pract Res Clin Gastroenterol* 2011; **25**: 473-484
19 **Johnson CC**, Hayes RB, Schoen RE, Gunter MJ, Huang WY. Non-steroidal anti-inflammatory drug use and colorectal polyps in the Prostate, Lung, Colorectal, And Ovarian Cancer Screening Trial. *Am J Gastroenterol* 2010; **105**: 2646-2655
20 **Ho SC**, Hwang LS, Shen YJ, Lin CC. Suppressive effect of a proanthocyanidin-rich extract from longan (Dimocarpus longan Lour.) flowers on nitric oxide production in LPS-stimulated macrophage cells. *J Agric Food Chem* 2007; **55**: 10664-10670
21 **Rangkadilok N**, Sitthimonchai S, Worasuttayangkurn L, Mahidol C, Ruchirawat M, Satayavivad J. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. *Food Chem Toxicol* 2007; **45**: 328-336
22 **Rangkadilok N**, Worasuttayangkurn L, Bennett RN, Satayavivad J. Identification and quantification of polyphenolic compounds in Longan (Euphoria longana Lam.) fruit. *J Agric Food Chem* 2005; **53**: 1387-1392
23 **Soong YY**, Barlow PJ. Isolation and structure elucidation of phenolic compounds from longan (Dimocarpus longan Lour.) seed by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr A* 2005; **1085**: 270-277
24 **Yang B**, Jiang YM, Shi J, Chen F, Ashraf M. Extraction and pharmacological properties of bioactive compounds from longan (Dimocarpus longan Lour.) fruit - A review. *Food Research International* 2011; **44**: 1837-1842
25 **Manochai P**, Sruamsiri P, Wiriya-alongkorn W, Naphrom D, Hegele M, Bangerth F. Year around off season flower induction in longan (Dimocarpus longan, Lour.) trees by KClO3 applications: potentials and problems. *Sci Hortic* 2005; **104**: 379-390
26 **Hsieh MC**, Shen YJ, Kuo YH, Hwang LS. Antioxidative activity and active components of longan (Dimocarpus longan Lour.) flower extracts. *J Agric Food Chem* 2008; **56**: 7010-7016
27 **Pan MH**, Lai CS, Wu JC, Ho CT. Molecular mechanisms for chemoprevention of colorectal cancer by natural dietary compounds. *Mol Nutr Food Res* 2011; **55**: 32-45
28 **Kidd PM**. Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. *Altern Med Rev* 2009; **14**: 226-246
29 **Tsai HY**, Wu LY, Hwang LS. Effect of a proanthocyanidin-rich extract from longan flower on markers of metabolic syndrome in fructose-fed rats. *J Agric Food Chem* 2008; **56**: 11018-11024
30 **Yang DJ**, Chang YY, Hsu CL, Liu CW, Lin YL, Lin YH, Liu KC, Chen YC. Antiobesity and hypolipidemic effects of polyphenol-rich longan (Dimocarpus longans Lour.) flower water extract in hypercaloric-dietary rats. *J Agric Food Chem* 2010; **58**: 2020-2027
31 **Yang CS**, Wang H. Mechanistic issues concerning cancer prevention by tea catechins. *Mol Nutr Food Res* 2011; **55**: 819-831
32 **Wilken R**, Veena MS, Wang MB, Srivatsan ES. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Mol Cancer* 2011; **10**: 12
33 **Korkina LG**, De Luca C, Kostyuk VA, Pastore S. Plant polyphenols and tumors: from mechanisms to therapies, prevention, and protection against toxicity of anti-cancer treatments. *Curr Med Chem* 2009; **16**: 3943-3965
34 Department of Health. 2011. Available from: URL: http://www.doh.gov.tw/CHT2006/DM/DM2_2.aspx?now_fod_list_no=11965&class_no=440&level_no=5. Accessed on December 20, 2011
35 **Hsu CP**, Lin YH, Chou CC, Zhou SP, Hsu YC, Liu CL, Ku

FM, Chung YC. Mechanisms of grape seed procyanidin-induced apoptosis in colorectal carcinoma cells. *Anticancer Res* 2009; **29**: 283-289
36 **Kaur M**, Singh RP, Gu M, Agarwal R, Agarwal C. Grape seed extract inhibits in vitro and in vivo growth of human colorectal carcinoma cells. *Clin Cancer Res* 2006; **12**: 6194-6202
37 **Hsu CP**, Lin YH, Zhou SP, Chung YC, Lin CC, Wang SC. Longan flower extract inhibits the growth of colorectal carcinoma. *Nutr Cancer* 2010; **62**: 229-236
38 **Coates JM**, Galante JM, Bold RJ. Cancer therapy beyond apoptosis: autophagy and anoikis as mechanisms of cell death. *J Surg Res* 2010; **164**: 301-308
39 **Chiarugi P**, Giannoni E. Anoikis: a necessary death program for anchorage-dependent cells. *Biochem Pharmacol* 2008; **76**: 1352-1364
40 **Westhoff MA**, Fulda S. Adhesion-mediated apoptosis resistance in cancer. *Drug Resist Updat* 2009; **12**: 127-136
41 **Müller GA**, Engeland K. The central role of CDE/CHR promoter elements in the regulation of cell cycle-dependent gene transcription. *FEBS J* 2010; **277**: 877-893
42 **Piechaczyk M**, Farràs R. Regulation and function of JunB in cell proliferation. *Biochem Soc Trans* 2008; **36**: 864-867
43 **Pines J**, Hunter T. p34cdc2: the S and M kinase? *New Biol* 1990; **2**: 389-401
44 **Geng F**, Tang L, Li Y, Yang L, Choi KS, Kazim AL, Zhang Y. Allyl isothiocyanate arrests cancer cells in mitosis, and mitotic arrest in turn leads to apoptosis via Bcl-2 protein phosphorylation. *J Biol Chem* 2011; **286**: 32259-32267
45 **Brito PM**, Simões NF, Almeida LM, Dinis TC. Resveratrol disrupts peroxynitrite-triggered mitochondrial apoptotic pathway: a role for Bcl-2. *Apoptosis* 2008; **13**: 1043-1053
46 **Mantena SK**, Baliga MS, Katiyar SK. Grape seed proanthocyanidins induce apoptosis and inhibit metastasis of highly metastatic breast carcinoma cells. *Carcinogenesis* 2006; **27**: 1682-1691
47 **Meeran SM**, Katiyar SK. Grape seed proanthocyanidins promote apoptosis in human epidermoid carcinoma A431 cells through alterations in Cdki-Cdk-cyclin cascade, and caspase-3 activation via loss of mitochondrial membrane potential. *Exp Dermatol* 2007; **16**: 405-415
48 **Chung YC**, Lin CC, Chou CC, Hsu CP. The effect of Longan seed polyphenols on colorectal carcinoma cells. *Eur J Clin Invest* 2010; **40**: 713-721
49 **Weng JR**, Bai LY, Chiu CF, Wang YC, Tsai MH. The dietary phytochemical 3,3'-diindolylmethane induces G2/M arrest and apoptosis in oral squamous cell carcinoma by modulating Akt-NF-κB, MAPK, and p53 signaling. *Chem Biol Interact* 2012; **195**: 224-230
50 **Rajasekaran D**, Elavarasan J, Sivalingam M, Ganapathy E, Kumar A, Kalpana K, Sakthisekaran D. Resveratrol interferes with N-nitrosodiethylamine-induced hepatocellular carcinoma at early and advanced stages in male Wistar rats. *Mol Med Report* 2011; **4**: 1211-1217
51 **Melchini A**, Costa C, Traka M, Miceli N, Mithen R, De Pasquale R, Trovato A. Erucin, a new promising cancer chemopreventive agent from rocket salads, shows anti-proliferative activity on human lung carcinoma A549 cells. *Food Chem Toxicol* 2009; **47**: 1430-1436
52 **Musgrove EA**, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. *Nat Rev Cancer* 2011; **11**: 558-572
53 **Wang C**, Lisanti MP, Liao DJ. Reviewing once more the c-myc and Ras collaboration: converging at the cyclin D1-CDK4 complex and challenging basic concepts of cancer biology. *Cell Cycle* 2011; **10**: 57-67
54 **Alao JP**. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Mol Cancer* 2007; **6**: 24
55 **Fu M**, Wang C, Li Z, Sakamaki T, Pestell RG. Minireview: Cyclin D1: normal and abnormal functions. *Endocrinology* 2004; **145**: 5439-5447
56 **Tashiro E**, Tsuchiya A, Imoto M. Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. *Cancer Sci* 2007; **98**: 629-635
57 **Starostina NG**, Kipreos ET. Multiple degradation pathways regulate versatile CIP/KIP CDK inhibitors. *Trends Cell Biol* 2012; **22**: 33-41
58 **Abukhdeir AM**, Park BH. P21 and p27: roles in carcinogenesis and drug resistance. *Expert Rev Mol Med* 2008; **10**: e19
59 **Malumbres M**, Barbacid M. To cycle or not to cycle: a critical decision in cancer. *Nat Rev Cancer* 2001; **1**: 222-231
60 **Pavletich NP**. Mechanisms of cyclin-dependent kinase regulation: structures of Cdks, their cyclin activators, and Cip and INK4 inhibitors. *J Mol Biol* 1999; **287**: 821-828
61 **Park JW**, Choi YJ, Jang MA, Lee YS, Jun DY, Suh SI, Baek WK, Suh MH, Jin IN, Kwon TK. Chemopreventive agent resveratrol, a natural product derived from grapes, reversibly inhibits progression through S and G2 phases of the cell cycle in U937 cells. *Cancer Lett* 2001; **163**: 43-49
62 **Scatena R**. Mitochondria and cancer: a growing role in apoptosis, cancer cell metabolism and dedifferentiation. *Adv Exp Med Biol* 2012; **942**: 287-308
63 **Thompson CB**. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995; **267**: 1456-1462
64 **Engelbrecht AM**, Mattheyse M, Ellis B, Loos B, Thomas M, Smith R, Peters S, Smith C, Myburgh K. Proanthocyanidin from grape seeds inactivates the PI3-kinase/PKB pathway and induces apoptosis in a colon cancer cell line. *Cancer Lett* 2007; **258**: 144-153
65 **Green DR**, Reed JC. Mitochondria and apoptosis. *Science* 1998; **281**: 1309-1312
66 **Reed JC**. Double identity for proteins of the Bcl-2 family. *Nature* 1997; **387**: 773-776
67 **Reed JC**. Balancing cell life and death: bax, apoptosis, and breast cancer. *J Clin Invest* 1996; **97**: 2403-2404
68 **Oltvai ZN**, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 1993; **74**: 609-619
69 **Zhan Q**, Fan S, Bae I, Guillouf C, Liebermann DA, O'Connor PM, Fornace AJ. Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. *Oncogene* 1994; **9**: 3743-3751
70 **Rossé T**, Olivier R, Monney L, Rager M, Conus S, Fellay I, Jansen B, Borner C. Bcl-2 prolongs cell survival after Bax-induced release of cytochrome c. *Nature* 1998; **391**: 496-499
71 **Chresta CM**, Masters JR, Hickman JA. Hypersensitivity of human testicular tumors to etoposide-induced apoptosis is associated with functional p53 and a high Bax: Bcl-2 ratio. *Cancer Res* 1996; **56**: 1834-1841
72 **Reed JC**, Miyashita T, Takayama S, Wang HG, Sato T, Krajewski S, Aimé-Sempé C, Bodrug S, Kitada S, Hanada M. BCL-2 family proteins: regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. *J Cell Biochem* 1996; **60**: 23-32

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