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Sesamol induced apoptotic effect in lung adenocarcinoma cells through both intrinsic and extrinsic pathways

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ABSTRACT

Sesamol is a phenolic lignan found in sesame seeds (*Sesamum indicum* L.) and sesame oil. The anticancer effects and molecular mechanisms underlying its apoptosis-inducing effect were investigated in human lung adenocarcinoma (SK-LU-1) cells. Sesamol inhibited SK-LU-1 cell growth with an IC_{50} value of 2.7 mM and exhibited less toxicity toward normal Vero cells after 48 h of treatment (Selective index = 3). Apoptotic bodies—the hallmark of apoptosis—were observed in sesamol-treated SK-LU-1 cells, stained with DAPI. Sesamol increased the activity of caspase 8, 9, and 3/7, indicating that apoptotic cell death occurred through both extrinsic and intrinsic pathways. Sesamol caused the loss of mitochondrial transmembrane potential signifying intrinsic apoptosis induction. Decreasing Bid expression revealed crosstalk between the intrinsic and extrinsic apoptotic pathways; demonstrating clearly that sesamol induces apoptosis through both pathways in human lung adenocarcinoma (SK-LU-1) cells.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths in the world. Non-small cell lung cancer (NSCLC) is the most commonly found, accounting for 85–90% of lung cancer patients [1]. About 60% of patients diagnosed with lung cancer die within a year of diagnosis and five-year survival—for newly diagnosed cases—is only 15% [2,3]. Although a combination of chemotherapy and radiotherapy can improve survival, most patients die because of disease progression and acquired or intrinsic resistance to chemotherapeutic drugs [4]. The exploration and development of novel or more effective bioactive agents that can target the molecules associated with tumor growth and apoptosis resistance is needed to improve outcome in lung cancer patients [5].

Anticancer agents have been shown to cause drug-induced cellular lesions through cytotoxic mechanisms. Some agents can trigger apoptosis or programmed cell death, leading to the rapid elimination of tumor cells [6]. The characteristics of apoptosis include membrane blebbing, chromatin condensation, DNA fragmentation, loss of mitochondrial membrane potential ($\Delta\psi m$), and phosphatidylserine translocation to the outer cell surface [6]. There are two pathways of apoptosis—viz., *(a)* the extrinsic (death receptor) pathway, involving the activation of caspase 8, and *(b)* the intrinsic (mitochondria-dependent) pathway, involving the activation of caspase 9. The expression and translocation of the Bcl-2 family proteins—release of cytochrome *c* from mitochondria and activation of caspase 3—is required for apoptosis [7]. The Bcl-2 family proteins (i.e., Bax, Bcl-2, Bcl-XL) are the regulators of apoptosis and are associated with the intrinsic pathway; while the expression of death receptors (i.e.,TRAIL-R1, TRAIL-R2) are associated with the extrinsic pathway. Moreover, TRAIL-induced apoptosis activation leads to cross-talk between the extrinsic and intrinsic apoptotic pathways via Bid cleavage. This so-called "amplification loop" encompasses activation of the intrinsic or mitochondrial apoptotic pathway; as a result of activation of the extrinsic apoptotic pathway [8]. TRAIL engages its receptors via the extrinsic apoptotic pathway and recruits caspase 8, which is cleaved to its active form. Activated caspase 8 then cleaves Bid, which in turn interacts with mitochondrial anti- and pro-apoptotic molecules [9].

Currently, natural plant products offer promising new options for the development of more effective chemotherapeutic strategies for cancer of various organs [5]. In fact, more than 60% of anticancer drugs are of natural origin [10–13]. Sesame (*Sesamum indicum* L.)—a member of the Pedaliaceae family—is an important oil seed crop.

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It is widely cultivated in Thailand, China, India, Mexico, Sri Lanka, Saudi Arabia and Turkey. Sesame has long been used as a food or traditional medicine [14].

The major lignan compounds found in sesame seed are sesamin and sesamolin. Roasted (always below 170 °F, or ~76 °C) sesame is popular in Asian foods. When roasting sesame, sesamolin is converted to sesamol in the presence of moisture. Sesamol—a phenolic component of lignan—is a known major antioxidant present in sesame oil [15,16]. Sesamol possesses anti-inflammatory, antioxidant, radio-protective effects against DNA damage [17–19], as well as anticancer activities. The anticancer effect of sesamol was found against hepatocellular carcinoma, leukemia, and mouse Leydig tumor cells [20–22]. To date, the apoptosis induction effect that suggests a chemopreventive effect of sesamol on human lung adenocarcinoma cells has not been defined. We, therefore, investigated the apoptotic effect of sesamol on lung cancer and the molecular mechanism of sesamol-induced apoptosis in human lung adenocarcinoma cell lines (SK-LU-1).

2. Materials and methods

2.1. Chemicals and reagents

Sesamol (purity 98%) was purchased from Spectrum (New Brunswick, NJ, USA). Cisplatin was purchased from Boryung (Ansan, Korea). Cisplatin has been used in a chemotherapy regimen for non small cell lung cancer according to the NCCN Guidelines for patients [23]. Cisplatin was therefore used as a positive control. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), 0.25% trypsin-EDTA (1x), penicillin, and streptomycin were purchased from GIBCO® (Invitrogen, Grand Island, NY, USA). Neutral red (NR) and DAPI (4′-6-diamidino-2-phenylindole) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). FITC-conjugated annexin V and PI were from BioLegend (San Diego, CA, USA). Caspase-Glo® 3/7, 8, and 9 Assay Kits were purchased from Promega (Madison, WI, USA). 3,3′-Dihexyloxacarbocyanine iodide ($DiOC_6$) was obtained from Sigma-Aldrich Inc (Saint Louis, MO, USA). Antibodies specific to Bcl-2, Bax, Bid, TRAIL-R2, PARP, and β-actin were bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HRP-conjugated goat anti-mouse IgG and antibodies specific to TRAIL-R1 were from Abcam (Cambridge, UK).

2.2. Cell culture

The human lung adenocarcinoma cisplatin-sensitive (SK-LU-1) cell line (Cell Lines Service—CLS#300335, Eppelheim, Germany) and normal African green monkey kidney (Vero) cell line (ATCC#CCL-81) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were incubated at 37 °C with 95% air and 5% CO_2.

2.3. Antiproliferation assay

The antiproliferation effect of sesamol and cisplatin were evaluated by Neutral red (NR) assay [24]. Briefly, SK-LU-1 and Vero cells were seeded at a density of 4×10^5 cells/ml in a 96-microplate well and allowed to adhere for 24 h. Then the cells were treated with various concentrations of sesamol or cisplatin for 48 h. After incubation, the medium was replaced with NR solution (50 μg/ml) and incubated at 37 °C for 2 h. The cells were then washed with 150 μl of phosphate buffered saline (PBS) and the supernatant was decanted. Then 0.33% HCl/isopropanol was added into each well to solubilize the cells. Absorbance was measured by using a microplate reader (Tecan, Lyon, France), set at 537 nm with a reference wavelength of 650 nm. The percentage of antiproliferation was calculated compared to the control (untreated cells). The selectivity index (SI) was calculated to determine the selective antiproliferation on cancer cell lines over the normal cell line. SI of the test compound was calculated by dividing the IC_{50} of Vero cells by the IC_{50} of SK-LU-1 cells. The IC_{50} is an inhibition concentration of 50%.

2.4. Nuclei morphological change assay

The effect of sesamol on nuclei morphological changes of SK-LU-1 cells was observed by DAPI assay. DAPI (4′,6-diamidino-2-phenylindole) is a fluorescent dye which binds specifically to double-stranded DNA. This technique was used to detect the morphological changes of cell nuclei undergoing apoptosis. In brief, the cells were seeded in 24-well plates and incubated for 24 h. The cells were then treated with sesamol or cisplatin for 48 h. After treatment, the cells were washed with PBS and fixed with 100% methanol. Then the cells were stained with 1 μg/ml DAPI solution and incubated at 37 °C for 30 min. After that the excess dye was removed and PBS:Glycerin (1:1) was added to stain cells. The morphological change of nuclei was observed under inverted fluorescent microscope with 40× magnification.

2.5. Annexin V/PI double staining assay

To determine the effect of sesamol on mode of cell death, an experiment was performed using the Annexin V/PI apoptosis detection kit; as per manufacturer instructions (BioLegend, San Diego, CA, USA). Briefly, cells were seeded in 6-well plates for 24 h. Then the cells were treated with $1 \times IC_{50}$, and $2 \times IC_{50}$ of sesamol for 12, 24, and 48 h. At the end of treatment, the cells were trypsinized and washed twice with cold cell staining buffer, then resuspended in Annexin V binding buffer at a concentration of 3×10^6 cells/ml. The 100-μl cell suspension was transferred to a microcentrifuge tube, and 5 μl of FITC Annexin V and 10 μl PI added. Cells were gently vortexed and then incubated for 15 min at room temperature (25 °C) in the dark then 400 μl of Annexin V binding buffer was added to each tube. The stained cells were analyzed by flow cytometer (BD FACSCanto II, San Jose, CA, USA). Mode of cell death was analyzed based on a quadrant marker.

2.6. Caspase enzyme activity

SK-LU-1 cells (2×10^5 cells/ml) were seeded in a white opaque 96-well plate and incubated at 37 °C for 24 h. The cells were treated with $1 \times IC_{50}$ of sesamol at 12, 24, and 36 h (caspase 3/7) or 12, and 24 h (caspase 8 and 9). To determine the respective activity of caspase 8 and 9, the cells were pretreated for 6 h with 10 μM each of the specific inhibitors of caspases; 8 (z-IETD-FMK) and 9 (z-LEHD-FMK). After treated with sesamol at specific time, caspase substrate was added to treated cells. The substrates for caspases 3, 8 and 9 were luminogenic substrate containing the DEVD sequence, the IETD sequence, and the LEHD sequence, respectively. Caspase substrate and treated cells were mixed using plate shaker for 30 s and incubated for 1 h at room temperature. The respective activity of the caspases was determined based on a luminescent technique; detectable by Varioskan Flash (Thermo scientific, Waltham, MA, US). The signals generated were expressed as units of relative luminescence (RLU), proportional to caspase activity.

2.7. Alteration of mitochondrial transmembrane potential

SK-LU-1 cells (2×10^5 cells/ml) were seeded and treated with $1 \times IC_{50}$ and $2 \times IC_{50}$ of sesamol for 24 and 48 h. Concentrated hydrogen peroxide (35%, v/v) was used as the positive control. Then