Ellagic acid inhibits melanoma growth in vitro

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Abstract

Ellagic is a polyphenolic compound with anti-fibrotic and antioxidant properties, and exhibits antitumor properties against various cancer cells in vitro. There are few studies, however, which examine the effects of ellagic acid on melanoma. In the present study, we observe effects of ellagic acid on melanoma cells in vitro. Three metastatic melanoma cell lines (1205Lu, WM852c and A375) were examined to determine the effects of ellagic acid on melanoma cell viability, cell-cycle, apoptosis, NF-κB activity, and IL-1β & IL-8 secretion. Cell viability assays demonstrated that ellagic acid possesses an inhibitory effect on cell proliferation at concentrations between 25 and 100 μM. In addition, ellagic acid promoted G1 cell cycle arrest, increased levels of apoptosis and decreased synthesis of IL-1β and IL-8 in melanoma cells. Ellagic acid also decreased NF-κB activity, suggesting at least one potential mechanism by which ellagic acid may exert its effects in melanoma cells. Our findings support further investigation into prospective roles for ellagic acid as a therapeutic, adjuvant, or preventive agent for melanoma.

Introduction

Ellagic acid (EA) is a polyphenolic compound found in various types of fruit, including berries, pomegranates, and nuts, and is becoming a popular dietary supplement. It possesses anti-fibrotic,1,2 anti-proliferative,3,4 and antitumorigenic5,6 properties. EA is known to be protective against several types of cancer. EA has been shown to induce apoptosis in human melanoma cells in vitro.7 Few studies have investigated the effects of EA on melanoma, however, and the mechanism of action of EA in this setting is not well defined. In vitro studies have shown that EA decreases tyrosinase activity in mouse melanoma cells by chelating the copper tyrosinase cofactor.8 We are not aware of any other studies that have defined a mechanism by which EA exerts its effects in melanoma.

The exact mechanism by which EA exerts its effects in other types of cancer is also unclear, but several potential mechanisms have been suggested. EA has been shown to modulate a variety of signaling pathways in cancer cells, including NF-κB, iNOS, and Wnt. Alternative mechanisms through which EA exerts anti-cancer effects have also been proposed. One group9 showed that EA prevented copper and catecholamine transmitter-mediated oxidative DNA damage, thus suggesting a protective role for EA in preventing reactive oxygen species production, lipid peroxidation, and DNA strand breaks. EA has also been shown to induce apoptosis via caspase pathways as well as potentiating trans-retinoic acid-mediated cell-differentiation on human leukemia cell lines.10 Another study showed that EA inhibits components of Wnt signaling pathways known to play a pivotal role in human colon carcinogenesis.11 Additionally, EA has been shown to reduce hepatic phase I CYP enzymes responsible for converting estrogen to harmful metabolites implicated in mammary tumorigenesis.12 EA has been shown to downregulate iNOS, COX-2, TNF-α and IL-6 secretion by inhibiting nuclear factor-kappa B (NF-κB) in colon and pancreatic cancers.13 Pomegranate fruit extracts (including EA) decrease NF-κB expression in UVB-stimulated keratinocytes, decreasing skin tumorigenesis.14,15 Here we report on the effect of EA on melanoma cells, including inhibition of the NF-κB pathway.

Materials and Methods

Tissue culture

Human metastatic melanoma cell lines (1205Lu, WM852c and A375) were cultured in RPMI 1640 medium (GIBCO BRL, Gaithersburg, Maryland, USA) supplemented with 10% fetal bovine serum (FBS, Gemini Bio-Products, Inc., Woodland, CA, USA) and antibiotics (penicillin (10,000 IU/mL), streptomycin (10,000 IU/mL), and amphotericin B (25 microg/mL, Celigro, Manassas, VA, USA) and were incubated at 37°C and 5% CO₂.

Ellagic acid treatment

EA was obtained from MP Biochemicals, Solon, OH, USA) and dissolved in sterile DMSO (5 mM) and stored at -20°C. Separate, fresh 100 μl aliquots of EA were used for each experiment and excess reagent was disposed of according to protocol.

Cell cycle analysis

4×10⁶ cells were seeded on 10 cm tissue culture dish and incubated overnight prior to treatment with EA. Cells were treated with 0 or 50 μM EA and incubated at 37°C for 48 h. Cells were then detached from the plate, stained with propidium iodide (PI) and allowed to incubate overnight at 4°C. Cells were then analyzed with Beckman Coulter FC500 flow cytometer (Beckman Coulter). Apoptotic cells were defined as being positive for Annexin V.

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Materials and Methods

Ellagic acid (EA) is a polyphenolic compound obtained from various sources such as berries, pomegranates, and nuts. It exhibits anti-fibrotic and antioxidant properties. In the present study, we aimed to investigate the effects of ellagic acid on melanoma cell lines in vitro. Three metastatic melanoma cell lines (1205Lu, WM852c and A375) were treated with ellagic acid at concentrations between 25 and 100 μM. Cell viability assays showed that ellagic acid inhibited cell proliferation in a concentration-dependent manner.

Ellagic acid also induced apoptosis and arrested cell cycle progression by promoting G1 cell cycle arrest. It increased levels of apoptosis and decreased synthesis of IL-1β and IL-8 in melanoma cells. Ellagic acid downregulated NF-κB activity, suggesting a potential mechanism by which ellagic acid may exert its effects in melanoma cells.

Materials and Methods

**Tissue culture**

Human metastatic melanoma cell lines (1205Lu, WM852c and A375) were cultured in RPMI 1640 medium (GIBCO BRL, Gaithersburg, Maryland, USA) supplemented with 10% fetal bovine serum (FBS, Gemini Bio-Products, Inc., Woodland, CA, USA) and antibiotics (penicillin (10,000 IU/mL), streptomycin (10,000 IU/mL), and amphotericin B (25 microg/mL, Celigro, Manassas, VA, USA) and were incubated at 37°C and 5% CO₂.

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NF-κB luciferase reporter assay
2.5x10^4 cells were seeded into 24-well plates and were transfected after 12 h of incubation. Transfection with pNFκB-MetLuc2 Reporter Vector (Clontech, Mountain View, CA, USA) was performed according to protocol described by the Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA, USA). Cells were then treated with 0, 25, 50 and 100 µM EA and incubated for 24 or 48 h. Luminescence was then measured with a luminometer (Promega, Madison, WI, USA).

Measurement of gene expression (IL-1β, IL-8)
1205Lu metastatic melanoma cells were seeded and incubated for 24 h under previously described conditions. Cells were then treated with 0 or 50 µM EA for 48 h. Cells were detached from culture dishes and RNA was extracted from treated and untreated cells using the RNAqueous-Micro kit (Ambion, Austin, TX, USA), and subsequently reverse transcribed using random primers and MMLV reverse transcriptase (Promega, Madison, WI, USA). Real-time quantitative reverse transcription-PCR (qRT-PCR) was performed with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on the MX3000P PCR system (Stratagene, La Jolla, CA, USA). Primers were designed to generate a PCR product of 50 to 150 bp. Thermal cycling conditions were 95°C for 10 min followed by 42 cycles of 15 s at 95°C, 1 min at 60°C. GAPDH was used as a control to normalize the results.

Statistical analysis
The viabilities of each treated cell line were compared to control was evaluated using a Student’s t-test. Values of P < 0.05 were considered to be statistically significant.

Results
Melanoma cell viability is inhibited by Ellagic acid
Cells were incubated with different concentrations of EA for 24, 48, and 72 h and cell viability was measured using the MTS assay (Figure 1A, B). Inhibition of cell growth was detected as early as 24 h at 50 and 100 µM concentrations EA for 1205Lu cells (P < 0.01 and P < 0.05, respectively) and at 25, 50, and 100 µM concentrations EA for WM852c cells (P < 0.05, P < 0.05 and P < 0.01, respectively). EA continued to inhibit cell growth for a 72 h period at 25, 50 and 100 µM (P < 0.01 for 1205Lu, P < 0.01 for all concentrations tested except for 25 µM in 1205Lu (for 1205Lu, P < 0.01 for 50 and 100 µM concentrations; for WM852, P < 0.01 for 50 µM, P < 0.001 for 25 and 100 µM EA). At 48 h, there was decreased expression of NF-κB with EA treatment at 25, 50 and 100 µM (for 1205Lu, P < 0.01 for all concentrations; for WM852, P < 0.05, P < 0.01 and P < 0.01 for 25, 50 and 100 µM EA, respectively).

Ellagic acid decreases synthesis of IL-1β and IL-8 in metastatic melanoma cells
Downstream effectors of the NF-κB pathway which control cell proliferation and apoptosis were measured using quantitative RT-PCR. As indicated in Figure 4, mRNA coding for IL-1β and IL-8 were significantly decreased (P < 0.05) by treatment with EA.

Discussion
This study suggests that EA induces apoptosis and G1 cell cycle arrest in melanoma cells at in vitro concentrations between 25 and 100 µM. Inhibition of the NF-κB pathway was also shown to be a potential mechanism by which EA exerts its antiproliferative effects. The NF-κB pathway frequently plays a central role in the pathogenesis of many types of cancer, and therapeutic modalities which target the NF-κB pathway and its downstream intermediates are under investigation.
setting of melanoma, a particularly treatment-resistant malignancy, is very common. Inhibition of NF-κβ has been explored as a potential therapeutic strategy against melanoma with positive results. For example, NF-κβ upregulation has been shown to promote angiogenesis in melanoma, a key mechanism in tumor growth and maintenance. NF-κβ also increases the rate of melanoma metastasis. Conversely, suppression of NF-κβ attenuates the invasive potential of tumors. Further evidence of the importance of the NF-κβ pathway in melanoma is that ablation of an upstream effector of the NF-κβ pathway, Ikappaβ kinase beta (IKKβ), inhibits melanoma tumorigenesis and increased susceptibility to chemotherapy. Furthermore, p53 is inversely related to NF-κβ expression, and p53-mediated G1 cell cycle arrest may result from downregulation of NF-κβ in cancer cell.

These findings support a potential role for EA as a therapeutic, adjuvant, or preventive agent for melanoma. Future work, however, should address the limitations of this study. While our data shows some evidence that EA treatment is associated with increased cell-cycle arrest and apoptosis, as well as decreased melanoma cell viability and NF-κβ activity, these results were not obtained in the presence of a vehicle control. Follow up work is therefore indicated to determine if EA specifically inhibits melanoma through the pathways observed in this study. Furthermore, the concentrations of EA used in our study are higher than levels that are generally sustainable by normal consumption of EA-containing foods (typically ranging from 5-15 μM,m). While the use of higher doses of EA as an adjuvant to other therapies may prove to be of some benefit to patients it is not clear from these results if EA may exert therapeutic benefits at practical dietary doses.

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