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Protective Effect of Genistein on Cyclin D1 Expression in Malignant Ocular Melanoma Cells

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ABSTRACT

Background: Ocular melanoma is a disorder that is rarely found but is deadly. Four tissues in the eye that can be attacked by melanoma include the uveal tract, conjunctiva, eyelids, and orbit. Uveal melanoma is the most common case, while melanoma conjunctiva is very rare. **Objective:** This study aimed to investigate the effect of giving genistein on cyclin D1 expression in malignant melanoma. **Methods:** When confluent, CRL1872 malignant melanoma cells will be divided into treatment groups, the group giving genistein dose 25 μ M, the group giving genistein a dose of 50 μ M, and the group giving genistein a dose of 100 μ M. Cyclin D1 analysis was measured by immunofluorescence using confocal laser scan microscopy. **Results:** There was a significant increase in the expression of cyclin D1, in the group given genistein 25 μ M and 50 μ M ($p < 0.05$). For the administration of the genistein dose of 100 μ M, cyclin D1 expression decreased significantly compared to the control group ($p < 0.05$). **Conclusion:** It was concluded that genistein had a biphasic effect on cyclin D1 expression in malignant melanoma cells. Thus, genistein at the right dose can be a treatment of malignant melanoma.

Keywords: ocular melanoma, soybeans, chemotherapy, biphasic effect.

1. BACKGROUND

Ocular melanoma is a disorder that is rarely found but is deadly. Four tissues in the eye that can be attacked by melanoma include the uveal tract, conjunctiva, eyelids, and orbit. Uveal melanoma is the most common case, while melanoma conjunctiva is very rare. Melanoma is a primary ocular tumor in adults. The white skin incidence is 6.3 per million, in Hispanic 0.9, and 0.24 per million in blacks. Increased cases of melanoma conjunctiva are coming from environmental exposure, including exposure to ultraviolet light (1-3). Although diagnostic modalities and their treatment have developed, they involve conservative methods and maintain the eyeball, even to the assessment of oncogene status, but survival rates are still low (4-6).

Cyclin D1 is a nuclear protein encoded by the CCND1 gene. Cyclin D1 functions for growth promoters and tumor cell survival factors (7, 8). In skin melanoma, there is an amplification or overexpression of cyclin D1. This amplification is related to the progression and high proliferation of these tumors (9-12). This excess expression is caused by a degradation defect that increases its stability (13, 14). The growth of melanoma can be activated by a decrease in p-16 and/or p-21 proteins so that it fails in cyclin D1 blockade (15). To the knowledge of the researchers, until now there have not been many studies evaluating the increase in Cyclin D1 expression in ocular melanoma. Previous studies have shown an increase in cyclin D1 expression associated with methylation and inactivation of the INK4 gene (gene for encoding p-16 tumor suppressor proteins) in uveal melanoma (16, 17).

Many studies state that the consumption of soybeans can inhibit the growth of several types of cancer, including breast, prostate, and colon cancer. This claim is referred to genistein content in the range of 1.9-2.99 mg/gram soybeans. In healthy cells, genistein is not toxic (18-20). In some cancer cell lines, genistein can down-regulate cyclin D1. Down-regulation is associated with the arrest of G2/M (21-23). In melanoma cells, genistein induces cell arrest G0/G1 (24). Until now, as far as we know, not many studies have applied genistein to the expression of cyclin D1 in malignant melanoma cells. There-

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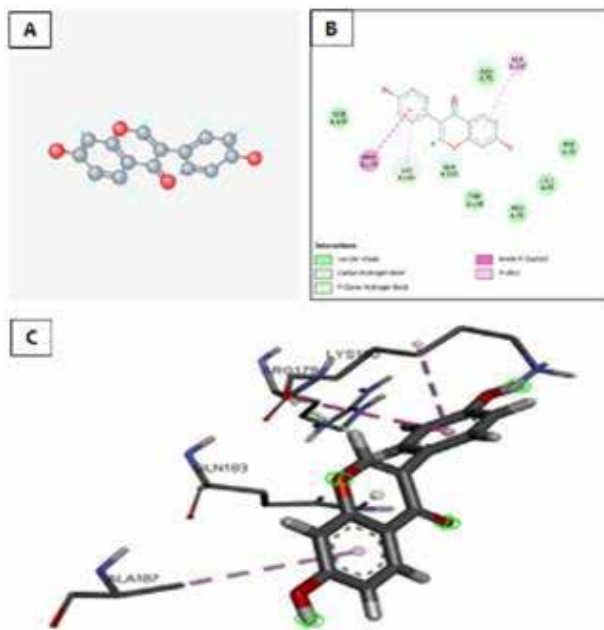


Figure 2. Chemical structure of genistein (A), two-dimensional structure of daidzein interaction with cyclin D1 (B), and three-dimensional structure of daidzein docking with cyclin D1 (C).

fore, this study aims to investigate the effect of genistein treatment on cyclin D1 expression in malignant melanoma.

2. OBJECTIVE

This study aimed to investigate the effect of giving genistein on cyclin D1 expression in malignant melanoma.

3. METHODS

In silico analysis

The chemical structure of genistein and daidzein was obtained from the PubChem database. The three-dimensional structure of cyclin D1 was obtained from the Protein Data Bank. The energy forming this compound was minimized, then the conversion of the format of GDP into SDF is done with OpenBabel 2.4.1 software. Molecular docking was done with Hex software. Ligand-protein interactions were analyzed by Discovery Client Studio 2019 software.

Cell line

Human melanoma cells CRL1872 were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM/Ham's F-12 medium containing 10% serum fetal bovine (FBS), supplemented with 5 µg/ml amphotericin, 100 µg/ml streptomycin, and 100 U/ml penicillin. Cells were grown in 24 cm² flasks at 37°C and 5% CO₂ (25).

Cells that had reached confluence will be divided into four study groups (n = 6 per group), including the control group (without any treatment), the group was given a genistein dose of 25 µM, the group given genistein dose of 50 µM, and the group given genistein dose of 100 µM.

Genistein

Genistein 10 mg (MP Biomedicals) was dissolved in sterile aquadest so that a stock solution of 1 mg/ml was

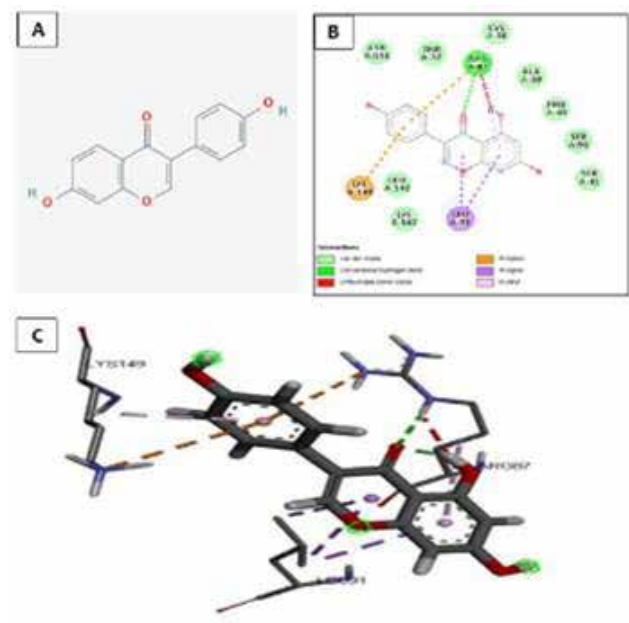


Figure 1. Chemical structure of genistein (A), two-dimensional structure of genistein interaction with cyclin D1 (B), and three-dimensional structure of genistein docking with cyclin D1 (C).

obtained. The stock solution was stored at 0°C. The stock solution will be diluted again using sterile aquadest to the desired dosage, covering 25 µM, 50 µM, and 100 µM.

Cyclin D1 analysis

Cyclin D1 analysis was carried out by immunofluorescent technique. The primary antibody used is anti-cyclin D1. The secondary antibody used is FITC. Cyclin D1 expression was assessed based on luminous density. Protein visualization was examined using confocal laser scanning microscopy Olympus fluo FV 10-ASW type 1.7. The units were expressed as intensity/mm².

Statistical analysis

Data was presented in mean ± standard deviation. Data were analyzed by ANOVA test using SPSS version 16 for Windows. In addition, a correlation test with the Pearson test was also carried out.

Ethics

This study was approved by the local Ethics committee Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia.

4. RESULTS

Figure 1 shows that genistein forms bonds with the A cyclin D1 domain on several active sites, including ARG87, LEU91, LYS149, THR37, CYS38, ALA39, PRO40, SER41, ASN151, LYS147, and LEU148. This bond is composed of conventional hydrogen bonds. Also, Pi-sigma (LEU91), Pi-carbon (LYS149), and Van der Waals (THR37, CYS38, ALA39, PRO40, SER41, ASN151, LYS147, and LEU148) were also formed. The docking bond energy between genistein and cyclin D1 is -241.2 kJ/mol.

The bond between Daidzein and Cyclin D1 can be seen in Figure 2. Interactions between Daidzein and Cyclin D1 have several active sites, including ARG179,

ALA187, ALA65, PHE78, PRO79, GLU75, GLN176, GLN176, LYS180, GLN183, and THR184. For ARG179 an Amide Pi bond is formed. The pi-alkyl bond is formed in ALA187. Meanwhile, the van der Waals bond was also formed in ALA65, PHE78, PRO79, GLU75, GLN176, LYS180, GLN183, and THR184. This interaction has a docking bond energy of - 235.9 kJ/ mol.

Figure 3 shows the expression of cyclin D1 in the control and treatment group. The expression of cyclin D1 was significantly higher in groups treated with genistein doses of 25 and 50 μm compared to the control ($p < 0.05$). There was a significant decrease in the cyclin D1 expression in the melanoma cells supplemented with a genistein dose of 100 μm compared to the control or genistein group doses of 25 and 50 μm ($p < 0.05$).

5. DISCUSSION

Cyclin D1 controls the cell cycle transition from the G1 phase to the S phase. Cyclin D1 overexpression is associated with cancer, resistance to chemotherapy, and is the target of cancer therapy (26).

In this study, we demonstrate the expression of cyclin D1 in malignant melanoma cells. The previous study proved the expression of cyclin D1 in melanocytes and melanoma cells 451 Lu cells and 1205 Lu cells (8).

In this study, the results of the in silico analysis showed that the interaction energy between genistein and cyclin D1 was more negative than the interaction between daidzein and cyclin D1. This interaction indicates that genistein is more accessible to interact with cyclin D1 than daidzein. Besides, the bonds formed in the genistein interaction are strong. Furthermore, genistein was selected in an in vitro study. This study extends previous in silico findings of the communication of genistein and daidzein against cyclin D1 (27, 28).

The application of genistein to malignant melanoma was carried out by previous researchers. A previous study has shown that genistein can inhibit the growth of highly metastatic melanoma cells, namely K1735M2 cells and WM451 cells (29). Other studies prove that genistein can upregulate p-21 as a tumor suppressor protein (30). In this study, administration of genistein doses of 25 and 50 μM significantly increased the expression of cyclin D1 compared to controls. Meanwhile, for dose 100 μM significantly lowered the expression of cyclin D1 compared to the control. This result shows that genistein has a biphasic effect on the cyclin D1 in malignant melanoma. This finding is the novelty of this study. Previous studies have shown an increase in the expression of cyclin D1 in MCF-7 cells exposed to a genistein dose of 10-5 M for 2 hours (31). In pancreatic cancer cells, genistein can downregulate cyclin D1 in doses of 10-40 μM (32). Genistein doses of 15 M and 20 M also downregulated cyclin D1 in hepatocellular carcinoma (HepG2 and Hep3B cells) (33). For the bipha-

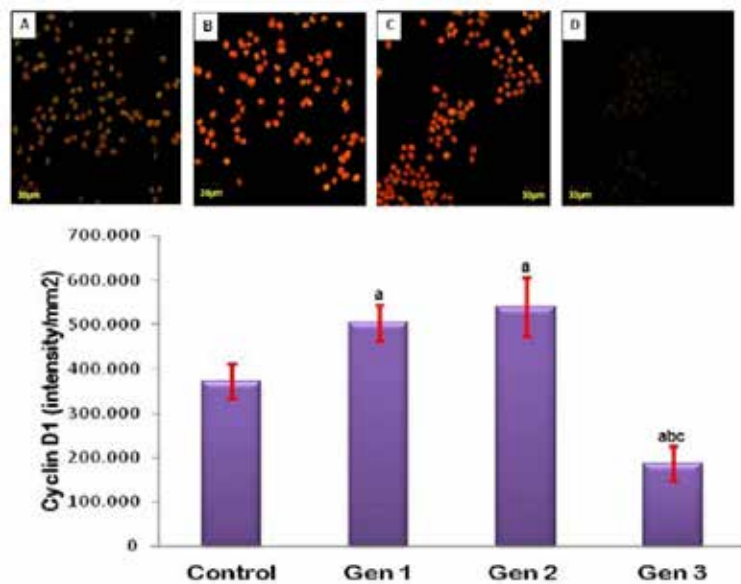


Figure 3. Representative micrograph of cyclin D1 expression in melanoma malignant cells (Upper figure). The mean value of cyclin D1 in control and treatment groups (Bottom figure). Note: control group (A); melanoma malignant cells treated by 25 m

sic genistein effect, genistein at lower than physiological levels triggering a mitogenic impact, while superior pharmacological levels induce apoptosis (34-36). Other studies prove that genistein triggers the proliferation of MC3T3-E1 cells in doses of 10-7 to 10-10 M, while at high doses (10-4 M) it will inhibit proliferation (37).

6. CONCLUSION

We conclude that genistein had a biphasic effect on cyclin D1 expression in malignant melanoma cells. Thus, genistein at the right dose can be a treatment of malignant melanoma.

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- **Conflict of interest:** None declared.
- **Financial support and sponsorship:** Nil.

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