Antiproliferation and Apoptosis-Inducing Effects of Quercetin in Human Melanoma CHL-1 Cells

INTRODUCTION
Melanoma is the most dangerous form of skin cancer with an incidence of the fastest growing in all cancers. According to WHO’s GLOBOCAN2012 database, the number of new cases of melanoma worldwide reached 232,000 and the number of deaths was 55,000 in 2012. Mainly this aggressive disease is believed caused by a specific mutational signature in skin cells that triggered by UV radiation damage evident. With the development of antitumor drugs, treatment for melanoma patient has more alternative administrations than before. Dacarbazine [5(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide] is the primary agent used in melanoma and also the first Food and drug administration (FDA)-approved chemotherapy for metastatic melanoma. This cytotoxic chemotherapy drug has been used in clinical for decades yet with low overall response rate and most patient with bad side effects or even got the risk of developing a blood cancer. In the early 21st century, scientist had found that the serine/threonine kinase B-type Raf proto-oncogene (BRAF) is commonly activated by somatic point mutation in human cancer, and after that people surprisingly discovered that nearly 50% of cutaneous melanoma patients got BRAF mutation. The following targeted molecular inhibitor for BRAF (vemurafenib, dabrafenib) therapy drug shows benefit in terms of overall survival than chemotherapy. BRAF inhibitor often combined with mitogen-activated protein kinasekinase (MEK) inhibitor for melanoma patient treatment. However, there are still nearly 20% melanoma patients do not respond and even those who do, most eventually acquired resistance. And for those BRAF wild type melanoma patients still they are waiting for more effective treatments.

Natural compounds are always considered as a lager library for researchers to find candidate anticancer agents. Flavonoid is one of the most studied natural compound group that contains antioxidant effects. Recently, researchers have found that quercetin (3,5,7,30,40-pentalhydroxyflavone) exhibited a good antitumor effect by inducing apoptosis in various kinds of cancer cells, including colon cancer, breast cancer, prostate cancer, leukemia and leukemia.

ABSTRACT
Although the present study have small molecular target drug to treat melanoma patient with BRAF mutation still study faces the issue of treatment resistance. For BRAF wild type, there is no efficient targeted therapy. In this paper, the antiproliferation of quercetin in human melanoma BRAF wild type CHL-1 cells through (MTT), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay has been examined.

The IC50 values of quercetin against CHL-1 cells for 24, 48 and 72 h were 17.41 ± 1.58, 14.62 ± 1.53 and 6.83 ± 1.21 μM. The inverted microscopy images also showed that the CHL-1 cells were collapsed, and the cell nucleus were fragmented after 24 h treatment. From western blot assay it was found that after quercetin treatment 24 h, the expression of Bcl-2, one of pro-apoptotic protein, was increased, while Bax, one of anti-apoptosis protein was decreased. These results indicated that quercetin possessed.

KEYWORDS antiproliferation, apoptosis, CHL-1, melanoma, quercetin

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Recent studies have shown that quercetin has a good antiproliferation effect in melanoma cell lines A375 and A2058. Both of these two melanoma cell lines contain BRAF mutation, and then we want to know if this nature compound also have antiproliferation effect in none BRAF mutation melanoma cell line and inducing those apoptosis. So, we chose a none BRAF mutation melanoma cell line CHL-1 to test quercetin. After finishing MTT assay, morphology observation and western blot assay, our studies indicated that quercetin could suppress CHL-1 cell proliferation and induce apoptosis.

**MATERIALS AND METHODS**

**Cell culture**

CHL-1 was purchased from Chinese Academy of Sciences Cell Bank (Shanghai, China) and maintained in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% FBS (Gibco, South America, USA), 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified incubator with 5% CO₂.

**MTT assay**

CHL-1 cells were plated in 96-well plates at a seeding density of 2,000 cells/well then incubated in a 37°C incubator with 5% CO₂ overnight. After that cells were treated with different concentration of quercetin (1.23, 3.70, 11.11, 33.33, or 100.00 μM) for 24, 48 or 72 h. Next, add 20 μl of MTT (0.5 g/L) in each well incubated in a 37°C incubator with 5% CO₂ for 4 h. The media were then discarded and 200 μl of Dimethyl sulfoxide (DMSO) were added per well. After 10 min vibration, the absorbance at 570 nm was determined with an ELx808 micro-plate reader (BioTek, Winooski, VT, USA). The cell inhibition and IC₅₀ were then calculated using GraphPad Prism 6.0.

**Western blot**

Cells were seeded in a 6-well plate at the density of 2 × 10⁵ cells per well and incubated for 24 h. The indicated concentrations of quercetin (20 and 40 μM) were then added to each well. After 24 h incubation, total cell protein was extracted in RIPA buffer. Protein concentrations were quantified by the Bio-Rad Protein Assay Kit. Cell lysates (15 μl per lane) were separated using 10% SDS-PAGE and transferred electrophoretically to polyvinylidene difluoride membranes. Membranes were blocked with tris-buffered saline with 0.1% Tween-20 containing 5% bovine serum albumin and then incubated overnight at 4°C with primary antibodies (1:1000). Membranes were washed three times with TBST and incubated for 2 h at room temperature with the appropriate secondary antibody conjugated to goat anti-rabbit horseradish peroxidase (1:2000). Membranes were then washed three times with TBST, and the immune blots were visualized by chemiluminescence.

**RESULTS**

**Antiproliferative effect of quercetin on CHL-1 cells**

MTT assay was used to verify the antiproliferation effects of quercetin on CHL-1 cells. As the time of quercetin administration extended, the inhibition rate of the same concentration group was increased, and the cell inhibition rate in higher concentration groups are higher than lower concentration groups (Fig. 1 and Table 1). These results indicated that quercetin could dose- and time-dependently inhibit the proliferation of CHL-1 cells. The IC₅₀ values of quercetin on CHL-1 cells at 24, 48, and 72 h were, respectively, 17.41 ± 1.58, 14.62 ± 1.53 and 6.83 ± 1.21 μM.

**Table 1** The antiproliferation effect of quercetin on CHL-1 cells.

<table>
<thead>
<tr>
<th>Quercetin concentration (μM)</th>
<th>n</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
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<tbody>
<tr>
<td>1.23</td>
<td>3</td>
<td>5.70 ± 2.03</td>
<td>6.99 ± 1.85</td>
<td>27.83 ± 0.56</td>
</tr>
<tr>
<td>3.70</td>
<td>3</td>
<td>5.74 ± 2.70</td>
<td>10.37 ± 0.37</td>
<td>27.86 ± 1.79</td>
</tr>
<tr>
<td>11.11</td>
<td>3</td>
<td>24.21 ± 2.12</td>
<td>31.67 ± 4.04</td>
<td>59.9 ± 2.28</td>
</tr>
<tr>
<td>33.33</td>
<td>3</td>
<td>86.62 ± 6.16</td>
<td>88.67 ± 4.19</td>
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</tr>
<tr>
<td>100.00</td>
<td>3</td>
<td>89.84 ± 6.36</td>
<td>92.38 ± 1.04</td>
<td>91.48 ± 8.20</td>
</tr>
</tbody>
</table>

Fig. 1 The antiproliferative effect of quercetin on CHL-1 cells.
Antiproliferation and apoptosis-inducing effects of quercetin in human melanoma CHL-1 cells

We use inverted microscope to observe the morphology of CHL-1 cells under different concentration of quercetin treatments. After treated for 24 h, CHL-1 cells in the control group were full in shape and evenly distributed showed normal morphological characteristics, while cells under 20 μM quercetin were different both in morphology characteristics and cell numbers. As the quercetin concentration increased, the cells in 40 μM treated group were much less than those in the control group, and most of the cells were in round shape (Fig. 2a).

Western blot assay was used to test the expression of Bcl-2, one of pro-apoptotic protein, and Bax, one of anti-apoptosis protein. The results showed that as the concentration of quercetin increased the expression of Bcl-2 was increased and the expression of Bax was decreased (Fig. 2b). The results suggested that quercetin could dose-dependently induce apoptosis of CHL-1 cells.

DISCUSSION AND CONCLUSION

Quercetin is a flavonoid widely existing in plants, fruits and vegetables. It has been widely studied and used for metabolic and inflammatory disorders treatment. Multiple effects have made quercetin a powerful flavonoid. Previous studies have shown that pre-treatment of quercetin could protect against radiocontrast medium toxicity in human renal proximal tubular cells due to its anti-oxidation effects. The anti-inflammation of quercetin may be related to its cytoprotective and cell stabilization effect. Quercetin possesses the immunosuppressive effect that could inhibit maturation and function in dendritic cells, and has the potential to be a therapeutic application in inflammatory diseases. Besides these, quercetin also has broad-spectrum anti-proliferation effects in various kinds of cancer cells including leukemia, breast cancer and melanoma.

Nearly 50% of cutaneous melanoma patients have BRAF V600E mutation. For these patients, using BRAF inhibitors such as vemurafenib and dabrafenib seems to be the best way for treatment. However, this treatment will get resistant from patient. The possible resistance mechanism may connect with BRAF/MEK/ERK dependent or MAPK-independent reactivation. To improve melanoma treatment program and for those BRAF wild type melanoma patients it is necessary to explore new anti-melanoma drugs.

Previous study already indicated that quercetin could significantly inhibit proliferation in BRAF mutated melanoma cell lines, while no anti-melanoma research in BRAF wild type melanoma cell lines. In this paper, we selected a BRAF wild type human melanoma cell line, CHL-1, as study subject, using MTT to verify the antiproliferation effect of quercetin in CHL-1 cells. And the results indicated that quercetin could dose- and time-dependently inhibit CHL-1 proliferation. After treated with quercetin for 24 h, CHL-1 cells got a total different morphology compared with those in control group. Cells are more like a round shape rather than a normal square shape, cells are not connected to others and apoptotic body could be seen. Based on the morphology images we suggested that quercetin may induce CHL-1 apoptosis. Then we checked the expression of two apoptosis-related proteins in all groups. As we expected, western blot results indicated that quercetin-inducing apoptosis in CHL-1 cells was accompanied by decreased expression of anti-apoptotic protein, Bcl-2, and increased expression of pro-apoptotic protein, Bax.

Above all we found that quercetin has a good antiproliferation effect in human none BRAF mutated melanoma cell line, CHL-1, and could induce it apoptosis. These results suggested that quercetin may be a candidate of anti-melanoma drug.

REFERENCES

3. Kim T, Amaria RN, Spencer C, Reuben A, Cooper ZA, Wargo JA. Combining targeted therapy and immune checkpoint