



# Fisetin targets phosphatidylinositol-3-kinase and induces apoptosis of human B lymphoma Raji cells



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## ABSTRACT

Aberrant regulation of phosphatidylinositol-3-kinases (PI3Ks) is known to be involved in the progression of cancers. PI3K-binding flavonoids such as quercetin and myricetin have been shown to inhibit PI3K activity, but the direct targeting of fisetin to PI3K has not been established. Here, we carried out an *in silico* investigation of fisetin binding to PI3K and determined fisetin's inhibitory activity in enzymatic and cell-based assays. In addition, fisetin induced apoptosis in human Burkitt's lymphoma Raji cells by inhibiting both PI3Ks and mammalian target of rapamycin (mTOR). Our results indicate that fisetin may serve as a natural backbone for the development of novel dual inhibitors of PI3Ks and mTOR for the treatment of cancer.

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## 1. Introduction

Phosphatidylinositol-3-kinases (PI3Ks) constitute a family of lipid kinases characterized by their ability to phosphorylate the 3'-OH group on the inositol ring of inositol phospholipids to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate. PI3Ks participate in various cellular functions, including proliferation, migration, and survival, by regulating the phosphorylation of Akt. Aberrant regulation of PI3K has been implicated in the induction and progression of several diseases, including cancer [44]; PI3K-mediated cell growth, proliferation, malignant transformation, and resistance to apoptosis have all been reported to be connected with hyperactivation of Akt [18,47]. PI3K is well known to be critically involved in lymphocyte growth and survival during B-cell tumor immortalization [1,5,6,9,14,19,36,42]. Therefore, as expected, inhibitors of the PI3K/Akt pathway induce apoptosis in leukemia, lymphoma, and myeloma cells [20].

Fisetin (3, 7, 3', 4'-tetrahydroxy flavone; Fig. 1A) is commonly found in various fruits (e.g. strawberries and grapes) and vegeta-

bles (e.g. onions and cucumbers) at concentrations of 2–160 µg/g [16,23,33,41]. Several studies have demonstrated fisetin's wide variety of activities, which include anti-proliferative, anti-oxidant, anti-angiogenic, and anti-cancer activities [22,24,29,38,43,52–54]. Fisetin belongs to the same flavonol subgroup as PI3K-binding flavonoids such as quercetin and myricetin [48]. Interestingly, fisetin exhibits anti-metastatic potential accompanied by the inhibition of PI3K protein expression in prostate cancer cells [8], but its direct targeting of PI3Ks and its anti-cancer activity in blood cancer cells have not been established. Therefore, in this study, we carried out an *in silico* investigation of the binding of fisetin to PI3K and evaluated whether fisetin inhibited PI3K activity at the enzymatic and cellular levels in human B lymphoma Raji cells. The pro-apoptotic activity of fisetin and its inhibition of mammalian target of rapamycin (mTOR) in Raji cells were also assessed to provide insight into the possibility of using fisetin as an initial backbone for the development of dual inhibitors of PI3Ks and mTOR.

## 2. Materials and methods

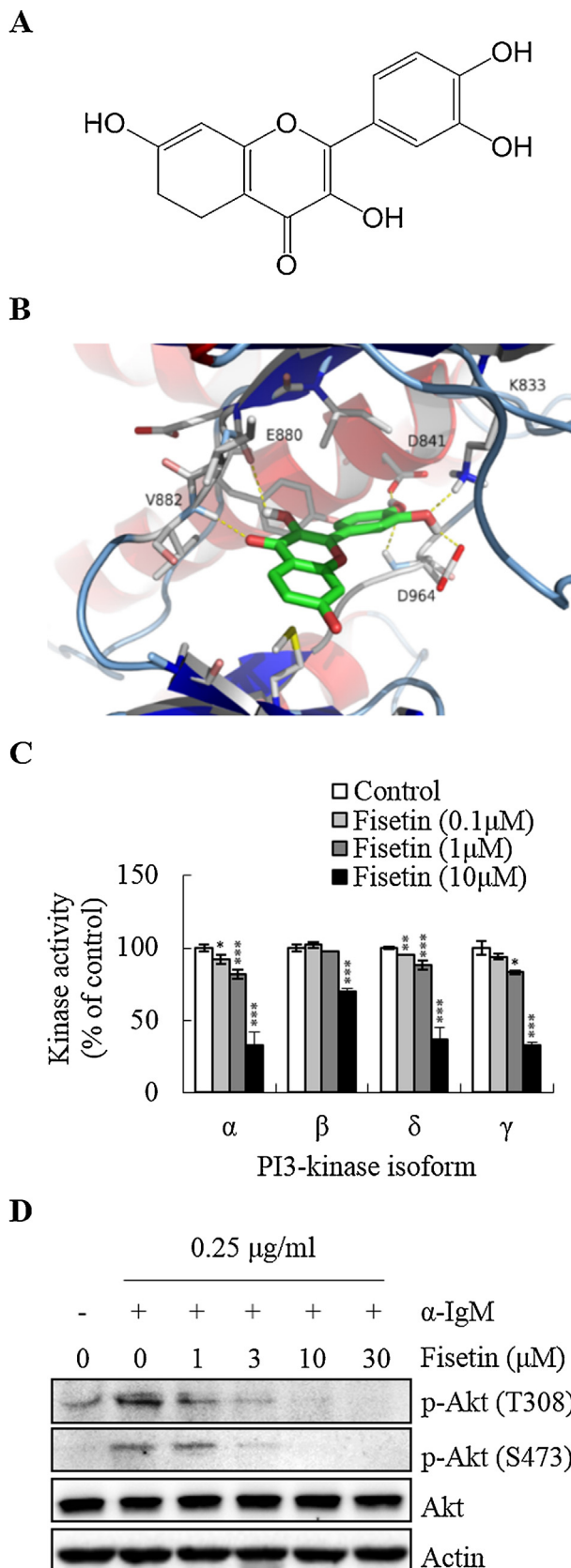
### 2.1. Materials

Fisetin was purchased from Sigma–Aldrich (MO), dissolved in dimethyl sulfoxide (DMSO) as 30 mM stock, and stored at –20 °C.

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**Fig. 1.** Fisetin targets and inhibits PI3K activity. (A) Chemical structure of fisetin. (B) Proposed binding mode of fisetin (green) to the ATP-binding pocket of PI3K. PI3K and interacting residues are represented by ribbons and sticks, respectively. Hydrogen-bonding interactions appear as dashed yellow lines; all hydrogen atoms except those involved in hydrogen bonding were omitted for clarity. (C) Assay of PI3K kinase activity in the presence of fisetin (Materials and methods). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . (D) Immunoblotting assay of PI3K activity (Materials and methods).

Mouse F(ab')<sub>2</sub> anti-human α-immunoglobulin M (α-IgM, μ chain specific) was purchased from Southern Biotech (AL).

## 2.2. Computational modeling

Molecular docking analysis was performed using the Maestro modeling environment (Schrödinger, CA). The crystal structure of human PI3Kγ (PDB: 1E8Y; [48]) was preprocessed with the Protein Preparation Wizard by adding hydrogens, removing water molecules, assigning partial charges using the OPLS 2005 force field and protonation states, and restraining minimization. All optimized ligands were prepared using LigPrep, which verifies proper ionization states, tautomeric forms, stereochemistry, and ring conformations.

Based on a grid box of 20 Å × 20 Å × 20 Å, ligands were docked into the active site of PI3Kγ using Glide in Extra Precision (XP) mode. All default parameters were used for XP docking. To account for flexibility in both the ligand and the receptor, we employed the Induced Fit Docking (IFD) protocol implemented in Maestro. All residues within 5.0 Å of ligand poses were included in the Prime refinement. Each ligand was re-docked into every refined active site of PI3Kγ in Glide XP mode with a van der Waals scaling of 0.50 for both protein and ligand non-polar atoms.

## 2.3. Assay of kinase activity

PI3K activity was assayed in Millipore (CA). Briefly, human PI3K isoforms were diluted in buffer consisting of 20 mM 3-(N-morpholino)propanesulfonic acid, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.01% Brij-35, 5% glycerol, 0.1% β-mercaptoethanol, and 1 mg/mL bovine serum albumin and mixed with the reaction mixture containing the compound. The reaction was initiated by adding the Mg-ATP mix. After incubation for 30 min at room temperature, the reaction was stopped by adding stop solution containing EDTA and biotinylated phosphatidylinositol-3,4,5-trisphosphate. Finally, the solution was exposed to detection buffer, which contains europium-labeled anti-glutathione S-transferase monoclonal antibody, glutathione S-transferase-tagged GRP1 pleckstrin homology domain, and streptavidin-allophycocyanin. The plate was read in time-resolved fluorescence mode and the homogenous time-resolved fluorescence signal was calculated as 10,000 × (emission at 665 nm/emission at 620 nm).

## 2.4. Cell culture

All materials for cell culture were purchased from Hyclone (UT). Human B lymphoma Raji cells were purchased from American Type Culture Collection (VA). Cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C.

## 2.5. Cell-based PI3K assay

Raji cells were seeded at density of 1 × 10<sup>6</sup>/mL and cultured for 24 h. Cells were pre-treated with fisetin at the indicated concentrations for 90 min and treated with α-IgM (0.25 μg/mL) for 30 min. Then, proteins were prepared for immunoblotting in order to detect the levels of phosphorylated Akt (p-Akt).

## 2.6. Immunoblotting

Raji cells were seeded at density of 1 × 10<sup>6</sup>/mL and cultured for 24 h. Cells were treated with fisetin for the indicated times and harvested, rinsed with cold phosphate-buffered saline (Gibco, NY), and lysed with RIPA buffer (Elpis Biotech, Korea; 50 mM

Tris–HCl [pH 8.0], 5 mM EDTA, 150 mM NaCl, 1% NP-40, and 1 mM phenylmethanesulfonyl fluoride). After incubation on ice for 1 h and centrifugation at  $18,000 \times g$  for 15 min at  $4^\circ\text{C}$ , the protein content of the supernatant was quantified with the RC DC Protein Assay Kit (Bio-Rad, CA). Proteins were mixed with  $5 \times$  sample buffer (Elpis Biotech; 0.375 M Tris–HCl [pH 6.8], 5% sodium dodecyl sulfate, 5%  $\beta$ -mercaptoethanol, 50% glycerol, and 0.05% bromophenol blue), denatured by boiling at  $100^\circ\text{C}$  for 5 min, loaded onto a 10% sodium dodecyl sulfate polyacrylamide gel, separated via electrophoresis, and transferred to a polyvinylidene difluoride membrane (Millipore). The membrane was washed with TBST (10 mM Tris–HCl [pH 7.5], 150 mM NaCl, and 0.1% Tween-20), blocked with TBST containing 5% skim milk (BD Difco, NJ), and incubated with 1:1000 primary antibody at  $4^\circ\text{C}$  overnight. After three washes with TBST for 30 min, the membrane was incubated with secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, CA) for 2 h. After another three washes with TBST for 30 min, the membrane was developed using Pierce SuperSignal West Femto Maximum Sensitivity Substrate (Fisher Scientific, PA) in a LAS-3000 Luminescent Image Analyzer (Fuji Photo Film Co., Ltd., Japan). Primary antibodies against p-Akt (S473 or T308), Akt, p-mTOR (S2448), mTOR, p-p70S6K (S371), p-p70S6K (T389), p-4E-BP1 (T37/46), p-histone H2A.X ( $\gamma$ H2A.X, S139), and Bax were purchased from Cell Signaling Technology Inc. (MA). Primary antibodies against actin, cIAP-2, and cIAP-1 were purchased from Santa Cruz Biotechnology. Primary antibodies against Bcl-2, Bcl-x, Bad, and Bak were purchased from Epitomics (CA). Actin was used as a loading control.

### 2.7. Assay of cell viability

Cells ( $1 \times 10^4$ /well) were seeded, incubated in a 96-well plate for 24 h, and treated with fisetin or vehicle control (0.1% DMSO) for an additional 24, 48, or 72 h. Cell viability was subsequently determined with the EZ-CYTOX Kit (Dogen, Korea) according to the manufacturer's protocol. Absorbance was measured with a Wallac EnVision microplate reader (PerkinElmer, Finland). All experiments were performed in triplicate.

### 2.8. Analysis of apoptosis

Cells ( $1 \times 10^6$ /well) were plated in a 6-well plate, incubated for 24 h, and treated with fisetin or vehicle control (0.1% DMSO) for an additional 24, 48, or 72 h. Then, cells were harvested, subjected to the Muse Annexin V and Dead Cell Assay Kit (Millipore), and evaluated with the Muse Cell Analyzer (Millipore) according to the manufacturer's protocol.

### 2.9. Statistical analysis

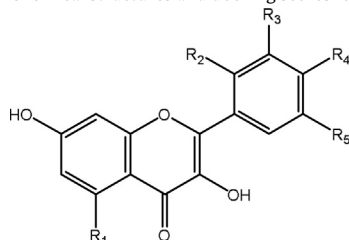
Data are presented as mean  $\pm$  standard deviation. Significance was determined using Student's *t* test (two-tailed) and differences were considered significant when  $P < 0.05$ .

## 3. Results and discussion

### 3.1. Fisetin targets PI3K and inhibits its activity

The hypothesis that fisetin directly binds PI3K was computationally evaluated in comparison with other members of fisetin's flavonol subgroup, which includes PI3K-binding flavonoids such as quercetin and myricetin [48]. Fisetin exhibited a binding mode that was similar to that of the quercetin crystal structure (Fig. 1B). The C3 hydroxyl and C4 carbonyl groups of fisetin formed two hydrogen bonds with E880 and V882 at the hinge region. The C3 substituent was critical to the binding of fisetin to the active site of PI3K, as it formed two bivalent hydrogen bonds with K833, D841, and D964. In

**Table 1**  
Chemical structures and docking scores for selected flavonols.



Compound <sup>a</sup>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	XP <sup>a</sup>	IFD <sup>b</sup>
Fisetin	H	H	H	OH	OH	-11.00	-1845.11
Myricetin	OH	H	OH	OH	OH	-12.49	-1847.62
Quercetin	OH	H	OH	OH	H	-11.90	-1846.74
Morin	OH	OH	H	OH	H	-10.74	-1843.82
Kaempferol	OH	H	H	OH	H	-10.73	-1843.29
Galangin	OH	H	H	H	H	-10.56	-1842.78

<sup>a</sup> Glide XP docking scores computed with rigid docking in which only ligands are considered flexible; the protein is kept fixed during docking.

<sup>b</sup> IFD scores computed with the IFD protocol for protein flexibility. Docking scores indicate how well the ligands are predicted to bind to the target.

addition, the Glide XP and IFD scores of fisetin were comparable to those of quercetin and myricetin (Table 1). These observations suggest that fisetin may bind in a mode analogous to that of quercetin to PI3K. Fisetin inhibited the kinase activities of PI3K $\alpha$ ,  $\gamma$ , and  $\delta$  in a dose-dependent manner and weakly inhibited the activity of PI3K $\beta$  only at  $10 \mu\text{M}$  (Fig. 1C).

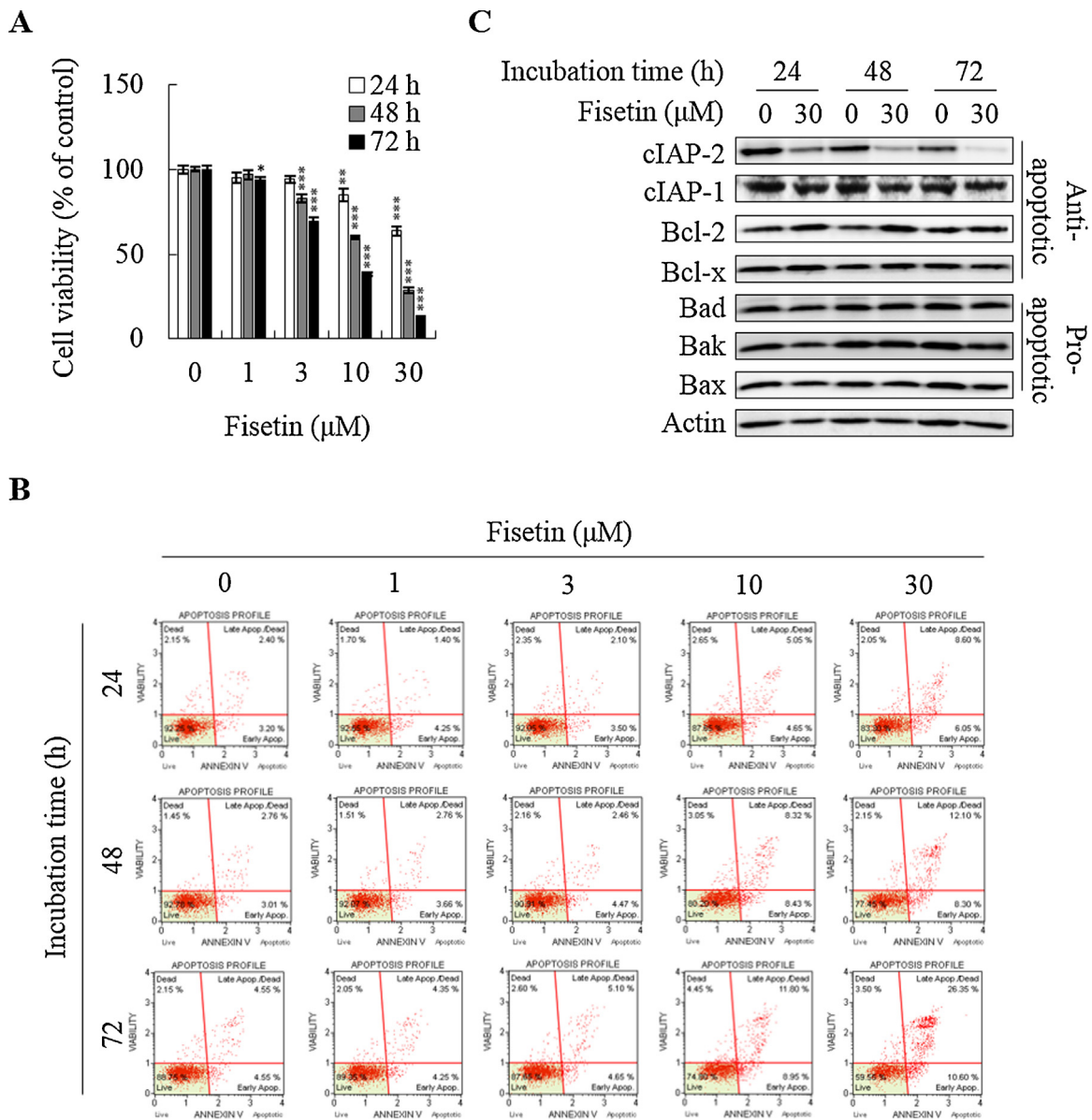
The inhibitory effect of fisetin on anti-IgM-induced activation of PI3K was evaluated in Raji cells. IgM, an antigen receptor on B lymphocytes, is a membrane-bound immunoglobulin [15,46]; immunoglobulin cross-linking on the cell surface triggers B cells to proliferate and differentiate by activating kinases, including PI3Ks [3,7,39]. Therefore, in Raji cells, the anti-IgM-stimulated phosphorylation of Akt at T308 and/or S473 enables assessment of the ability of compounds to inhibit PI3K [49,51]. Here, fisetin inhibited anti-IgM-stimulated phosphorylation of Akt in Raji cells in a dose-dependent manner (Fig. 1D).

All isoforms of PI3K are expressed in exponentially growing Raji cells, but the  $\gamma$  and  $\delta$  isoforms are more highly expressed than the  $\alpha$  and  $\beta$  isoforms [49]. In addition, since PI3K $\delta$  activity was recently suggested to predominate in Raji cells [49], PI3K $\delta$  may be a major target via which fisetin induces the apoptosis of Raji cells, although fisetin inhibited the enzymatic activity of all isoforms of PI3K (Fig. 1C). Given that the sensitivity of Raji cells to chemotherapy was previously enhanced by blocking the PI3K/Akt pathway [12], the phytochemical fisetin may enhance chemotherapeutic efficacy in patients with B cell lymphoma.

### 3.2. Fisetin induces apoptosis in Raji cells

Fisetin significantly inhibited cell viability and induced apoptosis in Raji cells in a time- and dose-dependent manner (Fig. 2A–B). Fisetin has been shown to induce apoptosis in human promyeloleukemic cells and nonsmall lung cancer cells accompanied by the specific downregulation of anti-apoptotic molecules and via a mitochondria-mediated pathway, respectively [21,25].

To better understand the pro-apoptotic action of fisetin on Raji cells, we applied a proteome array of 35 antibodies against apoptosis-related proteins. We observed decreased protein expression of cIAP-2 after  $30 \mu\text{M}$  fisetin exposure for 24 and 72 h (data not shown). However, the levels of other anti/pro-apoptotic proteins, such as cIAP-1 and Bcl-2 family members, were not changed by fisetin treatment (data not shown). The downregulation of cIAP-2 by fisetin at  $30 \mu\text{M}$  was confirmed via immunoblotting (Fig. 2C).



**Fig. 2.** Fisetin induces apoptosis in Raji cells. (A) Effect of fisetin on the viability of Raji cells (Materials and Methods). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . (B) Flow cytometry to determine the number of apoptosis cells stained with Annexin V and propidium iodide relative to the total number of cells analyzed. (C) Immunoblotting of the effect of fisetin on the expression levels of apoptosis-related proteins.

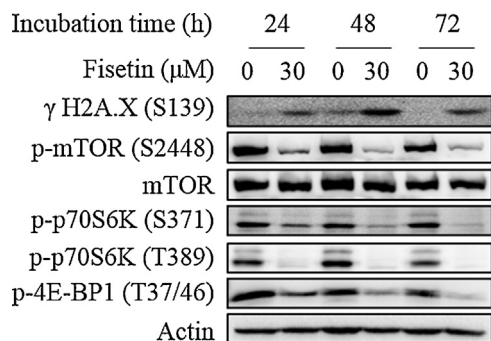
Immunoblotting also confirmed that there were no fisetin-induced changes in the levels of other anti/pro-apoptotic proteins (Fig. 2C). Fisetin specifically downregulated the expression of anti-apoptotic molecule, induced myeloid leukemia cell differentiation protein in the apoptotic process of HL-60 human myelogenous leukemia cells [25], and here it specifically downregulated the expression of cIAP-2. In addition, the pharmacological inhibition of PI3K has been shown to exhibit the downregulation of cIAP-2, suggesting that apoptotic fisetin in Raji cells could specifically downregulate the expression of cIAP-2 via its potential to inhibit PI3K [17,35].

### 3.3. Fisetin inhibits mTOR activity

In several previous studies, dual inhibition of PI3K and mTOR signaling effectively suppressed cancer progression [30,37]. The mTOR pathway appears to be critical to lymphomagenesis; its aberrant activation was detected in Burkitt's lymphoma [2,11,32,40]. Several drug candidates have been shown to inhibit the cat-

alytic subunits of both PI3K and mTOR, which contain structural similarities [31]. For example, the PI3K inhibitors LY294002 and NVP-BKM120 also inhibited mTOR [27,45]. Several clinical trials have implemented combined inhibition of PI3K and mTOR to ensure the synergistic efficacy of drug candidates for the treatment of cancers [4,10,34]. Therefore, we tested whether fisetin inhibits mTOR at 30 μM. As shown in Fig. 3, fisetin strongly inhibited the phosphorylation of mTOR (S2448) and its downstream targets p70S6K (S371 and T389) and 4E-BP1 (T37/46). Thus, in addition to fisetin's inhibition of PI3K, its inhibition of mTOR may enhance apoptosis in Raji cells [13,26].

We also examined the effect of fisetin on expression of the DNA-damage marker γH2A.X because fisetin has been shown to induce DNA damage and apoptosis at high concentrations between 50 and 250 μM in blood cancers [28,50]. The levels of γH2A.X protein increased over time in the presence of 30 μM fisetin (Fig. 3), suggesting the involvement of DNA damage in the pro-apoptotic activity of fisetin on Raji cells.



**Fig. 3.** Fisetin inhibits mTOR activity and induces DNA damage. Immunoblotting of the effects of fisetin on the expression levels of proteins related to mTOR signaling and DNA damage.

#### 4. Conclusion

In summary, we determined that fisetin targets PI3K, inhibits its activity at the enzymatic and cellular levels, and induces apoptosis in Raji cells via the downregulation of cIAP-2 protein expression. The pro-apoptotic activity of fisetin may be linked to a potential to inhibit mTOR signaling and to induce DNA damage. Since fisetin exhibits a wide variety of activities, including anti-proliferative, anti-oxidant, anti-angiogenic, and anti-cancer activities, it may provide a natural backbone for the development of novel dual inhibitors of PI3Ks and mTOR for the treatment of patients with cancer.

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#### References

- [1] N.N. Ahmed, H.L. Grimes, A. Bellacosa, T.O. Chan, P.N. Tsichlis, Transduction of interleukin-2 antiapoptotic and proliferative signals via Akt protein kinase, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 3627–3632.
- [2] P. Argyriou, P. Economopoulou, S. Papageorgiou, The role of mTOR inhibitors for the treatment of B-cell lymphomas, *Adv. Hematol.* 2012 (2012) 435342.
- [3] G.V. Baracho, A.V. Miletic, S.A. Omori, M.H. Cato, R.C. Rickert, Emergence of the PI3-kinase pathway as a central modulator of normal and aberrant B cell differentiation, *Curr. Opin. Immunol.* 23 (2011) 178–183.
- [4] P.M. Bhende, S.I. Park, M.S. Lim, D.P. Dittmer, B. Damania, The dual PI3K/mTOR inhibitor, NVP-BE225, is efficacious against follicular lymphoma, *Leukemia* 24 (2010) 1781–1784.
- [5] P. Brennan, J.W. Babbage, B.M. Burgering, B. Groner, K. Reif, D.A. Cantrell, phosphatidylinositol 3-kinase couples the interleukin-2 receptor to the cell cycle regulator E2F, *Immunity* 7 (1997) 679–689.
- [6] P. Brennan, A.M. Mehl, M. Jones, M. Rowe, Phosphatidylinositol 3-kinase is essential for the proliferation of lymphoblastoid cells, *Oncogene* 21 (2002) 1263–1271.
- [7] J.C. Cambier, J.T. Ransom, Molecular mechanisms of transmembrane signaling in B lymphocytes, *Annu. Rev. Immunol.* 5 (1987) 175–199.
- [8] C.S. Chien, K.H. Shen, J.S. Huang, S.C. Ko, Y.W. Shih, Antimetastatic potential of fisetin involves inactivation of the PI3K/Akt and JNK signaling pathways with downregulation of MMP-2/9 expressions in prostate cancer PC-3 cells, *Mol. Cell. Biochem.* 333 (2010) 169–180.
- [9] B.L. Craddock, E.A. Orchiston, H.J. Hinton, M.J. Welham, Dissociation of apoptosis from proliferation, protein kinase B activation, and BAD phosphorylation in interleukin-3-mediated phosphoinositide 3-kinase signaling, *J. Biol. Chem.* 274 (1999) 10633–10640.
- [10] A.S. Don, X.F. Zheng, Recent clinical trials of mTOR-targeted cancer therapies, *Rev. Recent Clin. Trials* 6 (2011) 24–35.
- [11] M. El-Salem, P.N. Raghunath, M. Marzec, X. Liu, M. Kasprzycka, E. Robertson, M.A. Wasik, Activation of mTORC1 signaling pathway in AIDS-related lymphomas, *Am. J. Pathol.* 175 (2009) 817–824.
- [12] X. Fang, Y. Jiang, L. Feng, H. Chen, C. Zhen, M. Ding, X. Wang, Blockade of PI3K/AKT pathway enhances sensitivity of Raji cells to chemotherapy through down-regulation of HSP70, *Cancer Cell. Int.* 13 (2013) 48.
- [13] P. Frost, E. Berlinger, V. Mysore, B. Hoang, Y. Shi, J. Gera, A. Lichtenstein, Mammalian target of rapamycin inhibitors induce tumor cell apoptosis

- in vivo primarily by inhibiting VEGF expression and angiogenesis, *J. Oncol.* 2013 (2013) 897025.
- [14] D.A. Fruman, S.B. Snapper, C.M. Yballe, L. Davidson, J.Y. Yu, F.W. Alt, L.C. Cantley, Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85alpha, *Science* 283 (1999) 393–397.
- [15] J.W. Goding, D.W. Scott, J.E. Layton, Genetics, cellular expression and function of IgD and IgM receptors, *Immunol. Rev.* 37 (1977) 152–186.
- [16] F.Y. Goh, N. Upton, S. Guan, C. Cheng, M.K. Shanmugam, G. Sethi, B.P. Leung, W.S. Wong, Fisetin, a bioactive flavonol, attenuates allergic airway inflammation through negative regulation of NF-kappaB, *Eur. J. Pharmacol.* 679 (2012) 109–116.
- [17] S. Guo, A.F. Messmer-Blust, J. Wu, X. Song, M.J. Philbrick, J.L. Shie, J.S. Rana, J. Li, Role of A20 in cIAP-2 protection against tumor necrosis factor alpha (TNF-alpha)-mediated apoptosis in endothelial cells, *Int. J. Mol. Sci.* 15 (2014) 3816–3833.
- [18] N. Hay, The Akt-mTOR tango and its relevance to cancer, *Cancer Cell* 8 (2005) 179–183.
- [19] H.J. Hinton, M.J. Welham, Cytokine-induced protein kinase B activation and Bad phosphorylation do not correlate with cell survival of hemopoietic cells, *J. Immunol.* 162 (1999) 7002–7009.
- [20] Y. Huang, J. Hu, J. Zheng, J. Li, T. Wei, Z. Zheng, Y. Chen, Down-regulation of the PI3K/Akt signaling pathway and induction of apoptosis in CA46-Burkitt lymphoma cells by baicalin, *J. Exp. Clin. Cancer Res.* 31 (2012) 48.
- [21] K.A. Kang, M.J. Piao, J.W. Hyun, Fisetin induces apoptosis in human nonsmall lung cancer cells via a mitochondria-mediated pathway, *In Vitro Cell Dev. Biol. Anim.* 51 (2015) 300–309.
- [22] S.C. Kim, S.H. Kang, S.J. Jeong, S.H. Kim, H.S. Ko, Inhibition of c-Jun N-terminal kinase and nuclear factor kappa B pathways mediates fisetin-exerted anti-inflammatory activity in lipopolysaccharide-treated RAW264.7 cells, *Immunopharmacol. Immunotoxicol.* 34 (2012) 645–650.
- [23] M. Kimira, Y. Arai, K. Shimoi, S. Watanabe, Japanese intake of flavonoids and isoflavonoids from foods, *J. Epidemiol.* 8 (1998) 168–175.
- [24] S.E. Lee, S.I. Jeong, H. Yang, C.S. Park, Y.H. Jin, Y.S. Park, Fisetin induces Nrf2-mediated HO-1 expression through PKC-delta and p38 in human umbilical vein endothelial cells, *J. Cell Biochem.* 112 (2011) 2352–2360.
- [25] W.R. Lee, S.C. Shen, H.Y. Lin, W.C. Hou, L.L. Yang, Y.C. Chen, Wogonin and fisetin induce apoptosis in human promyeloleukemic cells, accompanied by a decrease of reactive oxygen species, and activation of caspase 3 and Ca(2+)-dependent endonuclease, *Biochem. Pharmacol.* 63 (2002) 225–236.
- [26] H. Li, X. Jin, Z. Zhang, Y. Xing, X. Kong, Inhibition of autophagy enhances apoptosis induced by the PI3K/AKT/mTOR inhibitor NVP-BE225 in renal cell carcinoma cells, *Cell Biochem. Funct.* 31 (2013) 427–433.
- [27] W.L. Liu, M. Gao, K.Y. Tzen, C.L. Tsai, F.M. Hsu, A.L. Cheng, J.C. Cheng, Targeting Phosphatidylinositol 3-Kinase/Akt pathway by BKM120 for radiosensitization in hepatocellular carcinoma, *Oncotarget* 5 (2014) 3662–3672.
- [28] M. Lopez-Lazaro, E. Willmore, C.A. Austin, The dietary flavonoids myricetin and fisetin act as dual inhibitors of DNA topoisomerases I and II in cells, *Mutat. Res.* 696 (2010) 41–47.
- [29] P. Maher, K.F. Salgado, J.A. Zivin, P.A. Lapchak, A novel approach to screening for new neuroprotective compounds for the treatment of stroke, *Brain Res.* 1173 (2007) 117–125.
- [30] A. Majchrzak, M. Witkowska, P. Smolewski, Inhibition of the PI3K/Akt/mTOR signaling pathway in diffuse large B-cell lymphoma: current knowledge and clinical significance, *Molecules* 19 (2014) 14304–14315.
- [31] M. Martini, E. Ciraolo, F. Gulluni, E. Hirsch, Targeting PI3K in cancer: any good news? *Front. Oncol.* 3 (2013) 108.
- [32] J.R. Mills, Y. Hippo, F. Robert, S.M. Chen, A. Malina, C.J. Lin, U. Trojahn, H.G. Wendel, A. Charest, R.T. Bronson, S.C. Kogan, R. Nadon, D.E. Housman, S.W. Lowe, J. Pelletier, mTORC1 promotes survival through translational control of Mcl-1, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 10853–10858.
- [33] H.H. Park, S. Lee, J.M. Oh, M.S. Lee, K.H. Yoon, B.H. Park, J.W. Kim, H. Song, S.H. Kim, Anti-inflammatory activity of fisetin in human mast cells (HMC-1), *Pharmacol. Res.* 55 (2007) 31–37.
- [34] A. Pavlidou, N.F. Vlahos, Molecular alterations of PI3K/Akt/mTOR pathway: a therapeutic target in endometrial cancer, *ScientificWorldJournal* 2014 (2014) 709736.
- [35] S.L. Petersen, M. Peyton, J.D. Minna, X. Wang, Overcoming cancer cell resistance to Smac mimetic induced apoptosis by modulating cIAP-2 expression, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 11936–11941.
- [36] S.L. Pogue, T. Kurosaki, J. Bolen, R. Herbst, B cell antigen receptor-induced activation of Akt promotes B cell survival and is dependent on Syk kinase, *J. Immunol.* 165 (2000) 1300–1306.
- [37] C. Porta, C. Paglino, A. Mosca, Targeting PI3K/Akt/mTOR signaling in cancer, *Front. Oncol.* 4 (2014) 64.
- [38] N. Ravichandran, G. Suresh, B. Ramesh, G.V. Siva, Fisetin, a novel flavonol attenuates benzo(a) pyrene-induced lung carcinogenesis in Swiss albino mice, *Food Chem. Toxicol.* 49 (2011) 1141–1147.
- [39] G.T. Rehe, I.M. Katona, M. Brunswick, L.M. Wahl, C.H. June, J.J. Mond, Activation of human B lymphocytes by nanogram concentrations of anti-IgM-dextran conjugates, *Eur. J. Immunol.* 20 (1990) 1837–1842.
- [40] J.E. Skeen, P.T. Bhaskar, C.C. Chen, W.S. Chen, X.D. Peng, V. Nogueira, A. Hahn-Windgassen, H. Kiyokawa, N. Hay, Akt deficiency impairs normal cell proliferation and suppresses oncogenesis in a p53-independent and mTORC1-dependent manner, *Cancer Cell* 10 (2006) 269–280.

- [41] Y. Suh, F. Afaq, J.J. Johnson, H. Mukhtar, A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways, *Carcinogenesis* 30 (2009) 300–307.
- [42] H. Suzuki, Y. Terauchi, M. Fujiwara, S. Aizawa, Y. Yazaki, T. Kadowaki, S. Koyasu, Xid-like immunodeficiency in mice with disruption of the p85alpha subunit of phosphoinositide 3-kinase, *Science* 283 (1999) 390–392.
- [43] D.N. Syed, F. Afaq, N. Maddodi, J.J. Johnson, S. Sarfaraz, A. Ahmad, V. Setaluri, H. Mukhtar, Inhibition of human melanoma cell growth by the dietary flavonoid fisetin is associated with disruption of Wnt/beta-catenin signaling and decreased Mitf levels, *J. Invest. Dermatol.* 131 (2011) 1291–1299.
- [44] M. Thomas, C. Owen, Inhibition of PI-3 kinase for treating respiratory disease: good idea or bad idea, *Curr. Opin. Pharmacol.* 8 (2008) 267–274.
- [45] D. Trivigno, L. Bornes, S.M. Huber, J. Rudner, Regulation of protein translation initiation in response to ionizing radiation, *Radiat. Oncol.* 8 (2013) 35.
- [46] E.S. Vitetta, J.W. Uhr, Cell surface immunoglobulin. XV. The presence of IgM and an IgD-like molecule on the same cell in murine lymphoid tissue, *Eur. J. Immunol.* 6 (1976) 140–143.
- [47] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer, *Nat. Rev. Cancer* 2 (2002) 489–501.
- [48] E.H. Walker, M.E. Pacold, O. Perisic, L. Stephens, P.T. Hawkins, M.P. Wymann, R.L. Williams, Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine, *Mol. Cell* 6 (2000) 909–919.
- [49] X. Wang, J.P. Li, Y. Yang, J. Ding, L.H. Meng, A pharmacological model reveals biased dependency on PI3K isoforms for tumor cell growth, *Acta Pharmacol. Sin.* 34 (2013) 1201–1207.
- [50] W. Watjen, G. Michels, B. Steffan, P. Niering, Y. Chovolou, A. Kampkotter, Q.H. Tran-Thi, P. Proksch, R. Kahl, Low concentrations of flavonoids are protective in rat H4IIE cells whereas high concentrations cause DNA damage and apoptosis, *J. Nutr.* 135 (2005) 525–531.
- [51] D.G. Winkler, K.L. Faia, J.P. DiNitto, J.A. Ali, K.F. White, E.E. Brophy, M.M. Pink, J.L. Proctor, J. Lussier, C.M. Martin, J.G. Hoyt, B. Tillotson, E.L. Murphy, A.R. Lim, B.D. Thomas, J.R. Macdougall, P. Ren, Y. Liu, L.S. Li, K.A. Jessen, C.C. Fritz, J.L. Dunbar, J.R. Porter, C. Rommel, V.J. Palombella, P.S. Changelian, J.L. Kutok, PI3K-delta and PI3K-gamma inhibition by IPI-145 abrogates immune responses and suppresses activity in autoimmune and inflammatory disease models, *Chem. Biol.* 20 (2013) 1364–1374.
- [52] M.Y. Wu, S.K. Hung, S.L. Fu, Immunosuppressive effects of fisetin in ovalbumin-induced asthma through inhibition of NF-kappaB activity, *J. Agric. Food Chem.* 59 (2011) 10496–10504.
- [53] T.H. Ying, S.F. Yang, S.J. Tsai, S.C. Hsieh, Y.C. Huang, D.T. Bau, Y.H. Hsieh, Fisetin induces apoptosis in human cervical cancer HeLa cells through ERK1/2-mediated activation of caspase-8/-caspase-3-dependent pathway, *Arch. Toxicol.* 86 (2012) 263–273.
- [54] L.T. Zheng, J. Ock, B.M. Kwon, K. Suk, Suppressive effects of flavonoid fisetin on lipopolysaccharide-induced microglial activation and neurotoxicity, *Int. Immunopharmacol.* 8 (2008) 484–494.