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Research Article

Involvement of Exportin 1 Mediated c-Myc and Cullin 4A Signaling in Apoptotic Effect of Fisetin in Non-small Cell Lung Cancer Cells

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Abstract

Though Fisetin was known to exhibit antitumor effect in prostate, colon, breast, stomach, and lung cancers, the underlying antitumor mechanism is not fully understood in non-small cell lung cancers (NSCLCs). Thus, the aim of the present work is to elucidate the antitumor mechanism of Fisetin in A549 and H460 NSCLC cells in association with ribosomal biogenesis and ubiquitin ligase proteins. Fisetin showed cytotoxic and antiproliferative effects in a concentration and time dependent manner in A549 and H460 cells. Also, Fisetin increased the number of Annexin V/PI positive apoptotic portion and sub G1 accumulation in A549 and H460 cells. Additionally, Fisetin cleaved Poly ADP-ribose polymerase (PARP) and caspase 3 and increased p53, attenuated the expression of G1 phase related proteins such as cyclin D1, cyclin E and CDK2 and ribosomal biogenesis related gene Exportin 1 (XPO1) in A549 and H460 cells. Of note, Fisetin abrogated the expression of driver oncogenses such as c-Myc, S-Phase Kinase Associated Protein 2 (SKP2) and Cullin 4A (CUL4A) in A549 and H460 cells. Conversely, depletion of XPO1 enhanced p53 and PARP cleavage, while depletion of CUL4A promoted PARP cleavage without p53 upregulation in H460 cells. Overall, these findings highlight evidence that XPO1 mediated c-Myc and CUL4A signaling is critically involved in Fisetin induced apoptotic effect in NSCLCs as a potent antitumor candidate

Keywords: Fisetin; Non-small Cell Lung Cancer; c-Myc; p53; XPO1; CUL4A

Introduction

Non-small-cell lung cancer (NSCLC) is one of the most common malignant diseases in humans worldwide [1]. T he three major types of NSCLC are adenocarcinoma (ADC), Squamous Cell Carcinoma (SCC) and Large Cell Carcinoma (LCC) [2,3]. However, current treatments have a drawback to cause a variety of side effects. For instance, cisplatin is known to induce adverse reactions such as anemia, joint pain, face swelling, convulsions and trouble in walking [4]. Recently molecular target therapy is attractive for effective cancer therapy via regulation of specific molecules.

It is well documented that the Myc family gene consisting of c-Myc, n-Myc and L-Myc work as oncogenes in several cancers, while p53 acts as one of tumor suppressors [5]. Furthermore, the cullin 4 subfamily of genes including CUL4A and CUL4B ubiquitin ligases or *C*ullin *R*ING ligase (CRL) complexes are often overexpressed in human malignancies [6,7]. Also, exportin1 (XPO1) or Chromosome region maintenance 1 (CRM1), is often overexpressed or mutated in several cancer by regulation of

nuclear export of proteins and RNAs, and ribosome biogenesis for cancer growth and survival [8,9]. Furthermore, in terms of lung cancer chemoprevention, some natural compounds are of interests to selectively induce apoptosis and growth arrest in lung cancer cells without severe cytotoxicity in normal cells [10]. For instance, sulforaphane [11], resveratrol [12], tanshinone IIA [13], shikonin [14] and quercetin [15].

In the same line, Fisetin, one of flavonol [16], was known to have anti-inflammatory [17], anti-aging [18], anti-angiogenic [19], anti-proliferative [20] and anti-tumor [21, 22] effects. In particular, Fisetin induced apoptosis in prostate [23], pancreatic [24], glioblastoma [25], gastric [26], breast [27], colon [28], ovarian [29] cancer cells. It also exerted antitumor effect in lung cancer cells via modulation of ERK1/2[30, 31] and AKT/PI3K/mTOR [32] signaling pathway. Nevertheless, the underlying antitumor mechanism of Fisetin is not fully understood in non-small cell lung cancer cells so far. Thus, in the present study, the apoptotic mechanism of Fisetin was explored in association with c-Myc/p53 signaling mediated by XPO1 ribosomal protein and ubiquitin ligase CUL4A in non-small cell lung cancer cells.

Materials and Methods

Cell culture and reagents

H460 and A549 cells (human epithelial non-small cell lung cancers; NSCLCs) were purchased from the American Type Culture Collection (ATCC, USA). The cells were cultured in RPMI containing 10% FBS (Fetal bovine serum) and 1% antibiotic (Welgene, South Korea) and incubated at 37 ℃ with 5% CO_2. Fisetin ((3, 7, 3′, 4′-tetrahydroxyflavone) was purchased from Sigma Chemical Co., (St. Louis, MO, USA) and dissolved in DMSO (dimethyl sulfoxide) as a 100 mM stock.

Cell viability assay

Cell viability was assessed by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, St. Louis, MO, USA) according to the manufacturer's instruction. After H460 and A549 cells were seeded onto 96-well microplate, the cells were treated by various concentrations (0, 10, 20, 40, 60, 80 μM) of Fisetin for 24 h. For time or concentration dependent study, the cells were exposed to Fisetin at 40 and 80 μM for 24 h, 48 h, and 72 h. MTT solution (1 mg/ml) was added and formazan was dissolved with DMSO (dimethyl sulfoxide). To measure the optical density, microplate reader (TECAN, Austria) was used at 570 nm. Cell viability was determined as a percentage of viable cells in Fisetin treated group versus untreated control.

Colony formation assay

H460 and A549 cells (1,000 cells) were seeded in 6-well plates RPMI 1640 medium containing 10% serum. The cells were treated with Fisetin (40, 80 μM) for 24 h and replaced by new media. The cells were incubated at 37 ℃ with 5% CO_2 for 10 days, the colonies were fixed and stained with Diff-Quick solution (Sysmex, Japan). Colonies containing ≥50 cells were counted using ImageJ software.

Annexin-V-FITC and propidium iodide staining for apoptotic portion analysis

H460 and A549 cells were treated with Fisetin (40, 80 μM) for 24 h. Then apoptosis was measured by Annexin V staining-based FACS analysis by using FITC Annexin V Apoptosis Detection Kit (BD Biosciences, Korea).

Cell cycle analysis for sub G1 population

H460 and A549 cells were treated with Fisetin (40 or 80 μM) for 24 h. The cells were washed using PBS and fixed with ethanol (75%), incubated with 1mg/ml RNase A in PBS at 37 ˚C for 30 min, and stained in PBS containing 50 μg/mL PI (propidium iodide). The DNA contents of the stained cells were analyzed by using Cell Quest Software (BD Biosciences, San Jose, CA, USA) with the FACS Calibur flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA).

Western blotting

H460 and A549 cells were exposed to various concentrations of Fisetin for 24h. The cells were lysed in RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, and 1% TritonX-100) containing protease inhibitors (Roche, Germany), and phosphatase inhibitors (Sigma, USA). Total cell lysates were separated in 8 to 15% SDS-PAGE (SDS-polyacrylamide gels), and electrophoretically transferred to nitrocellulose membranes. The antibodies against p53, cyclin D1, CDK4, CDK2, Bcl-2, XPO1 were obtained from Santa Cruz Biotechnology. Cyclin E, Poly (ADP-ribose) polymerase (PARP), and cleaved-PARP were purchased from Cell signaling (Beverly, MA, USA). β-actin antibody was from Sigma-Aldrich (St. Louis, MO, USA), and c-Myc was bought from Abcam (Cambridge, England). The antibodies were diluted in 3% BSA in PBS-Tween20 (1:500-1:2000), and the membranes were incubated overnight at 4 ˚C. Then the membranes were washed three times with PBS-Tween20, and incubated with HRP-conjugated secondary antibodies. The expression was visualized by using ECL Western blotting detection reagent (GE Healthcare, UK).

Small interfering RNA (siRNA) transfection assay

H460 or A549 cells were transfected with scrambled siRNA, XPO1 siRNA or CUL4A siRNA (Bioneer, Korea) with Interferin™ transfection reagent (Polyplus-transfection Inc., New York, NY, USA). The mixtures of XPO1 siRNA with Interferin™ transfection reagent were incubated for 10 min at room temperature and the cells were incubated at 37 ˚C for 48 h before exposure to Fiseitn 80 μM for 24 h.

Statistical analysis

Data were presented as the means ± standard deviation. Statistical significance of the differences was determined by Student's t-test using Sigma Plot Software (Systat Software Inc., USA).

Results

Fisetin exerted cytotoxic and antiproliferative effect in H460 and A549 non-small cell lung cancer cells

To evaluate the cytotoxic and antiproliferative effect of Fisetin (Figure 1A), MTT assay and colony formation assay were performed in H460 and A549 cells. Here Fisetin significantly reduced the viability of H460 and A549 cells in a concentration and time dependent fashion (Figure 1B and 1C). Also, colony formation assay showed that the sizes and number of colonies were reduced in Fisetin treated group compared to untreated control (Figure 1D).

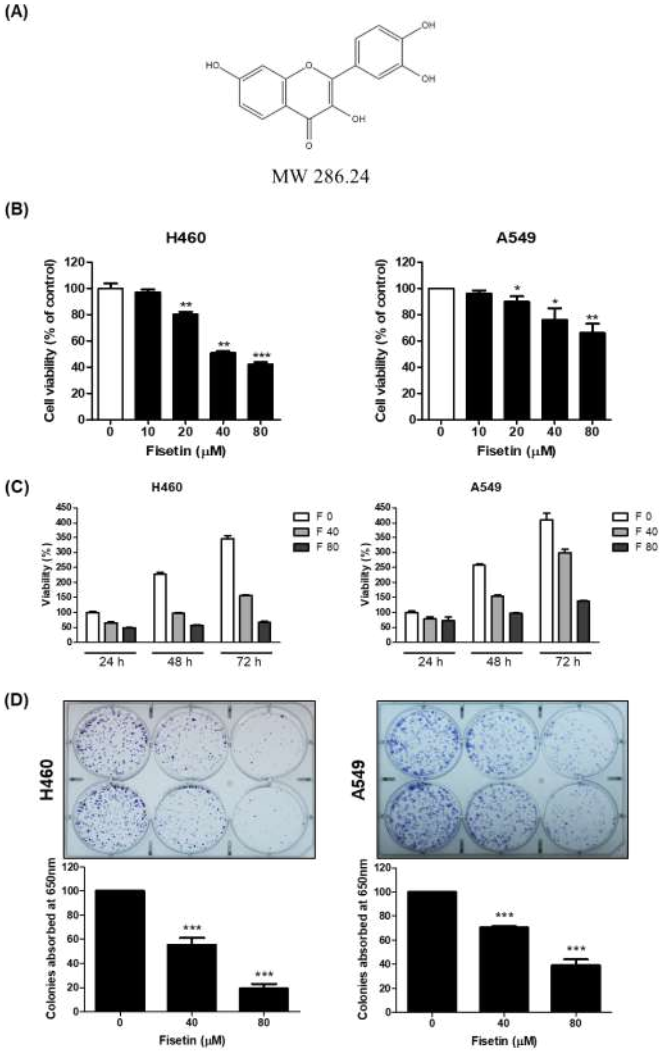

Figure 1: Effect of Fisetin on cytotoxicity and colony formation in H460 and A549 cells**.** (A) Chemical structure of Fisetin (B). Concentration dependent cytotoxicity of Fisetin in H460 and A549 cells. The cell viability by Fisetin was assessed by MTT assay after exposure to Fisetin for 24 h. *, $p<0.05$, **, $p<0.01$ vs untreated control. (C) Time dependent cytotoxicity of Fisetin in H460 and A549 cells by MTT assay after exposure to Fisetin for 24 h. (D) Effect of Fisetin on colony formation in H460 and A549 cells. Ten days after exposure to Fisetin (40, and 80 μM) for 24 h, the colonies were stained with Diff Quick Solution to visualize, and then the colonies were counted. ***, $p<0.001$ vs untreated control.

Fisetin induced apoptosis and increased the accumulation of sub-G1 population in H460 and A549 cells

To examine the apoptotic effect of Fisetin, cell cycle analysis and Western blotting were carried out in Fisetin treated H460 and A549 cells. Apoptotic assay by using Annexin V/propidium iodide staining reveals that Fisetin increased apoptotic portion to 14.82% and 14.02% at 80 μM compared to untreated control in H460 and A549 cells, respectively (Figure 2A). Consistently, Fisetin increased sub G1 population to 22.5 and 12.87% cells compared to untreated control in H460 and A549 cells, respectively (Figure 2B). Also, to confirm above apoptotic effect of Fisetin, Western blotting was conducted. As expected, Fisetin effectively cleaved PARP and caspase 3 in H460 and A549 cells (Figure 2C, 2D).

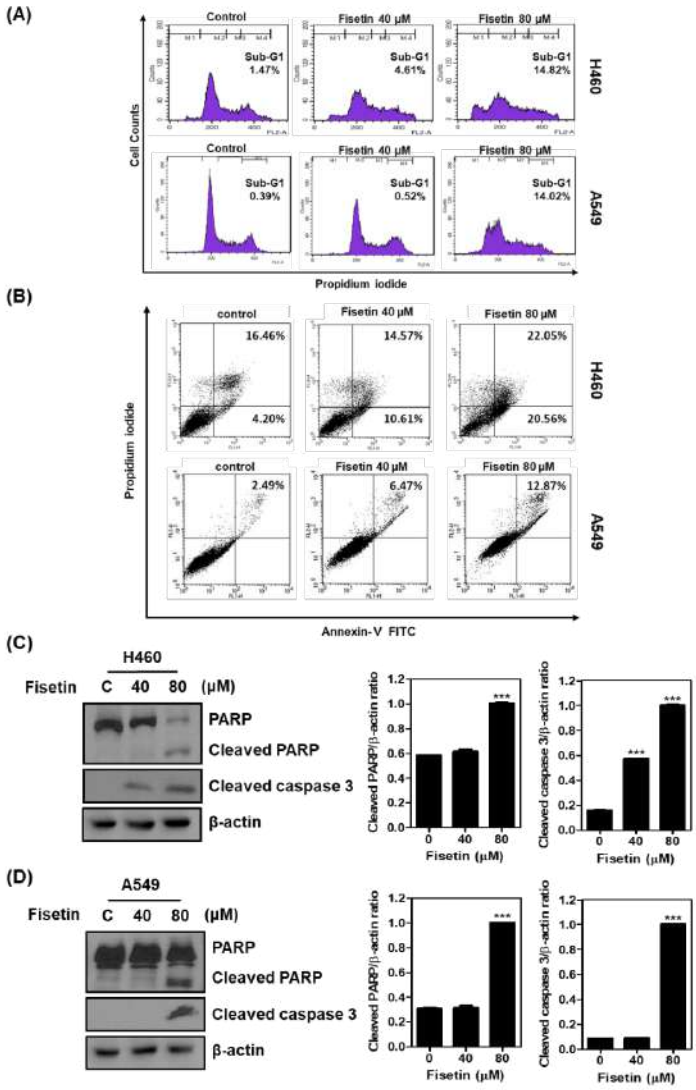

Figure 2: Effect of Fisetin on Annexin V/PI stained apoptotic portion and sub G1 population in H460 and A549 cells. (A) Apoptotic effect of Fisetin on Sub G1 population in H460 and A549 cells. Sub G1 population was evaluated in Fisetin treated H460 and A549 cells by FACS analysis. The cells were pretreated with 40 and 80 μM of Fisetin for 24 h and stained with PI. Then the data were analyzed by using Cell Quest Software. (B) Apoptotic effect of Fisetin in H460 and A549 cells by flow cytometric analysis using Annexin V/PI. After H460 and A549 cells were treated by Fisetin at 40 and 80 μM for 24 h, the apoptotic portion was evaluated in H460 and A549 by FACS analysis. (C) Effect of Fisetin on apoptosis related proteins in H460 cells. The cells were treated with Fisetin for 24 h and were subjected to Western blotting. ***, $p<0.001$ vs untreated control. (D) Effect of Fisetin on apoptosis related proteins in A549 cells. The cells were treated with Fisetin for 24 h and were subjected to Western blotting. ***, $p<0.001$ vs untreated control.

Fisetin down-regulated cell cycle related proteins in H460 and A549 cells.

To confirm whether Fisetin affects cell cycle related proteins, Western blotting was performed. As shown in Figure 3A, Fisetin activated p53 and p21, and attenuated the expression of cyclin D1, cyclin E, CDK2 compared to untreated control in H460 and A549 cells (Figure 3B).

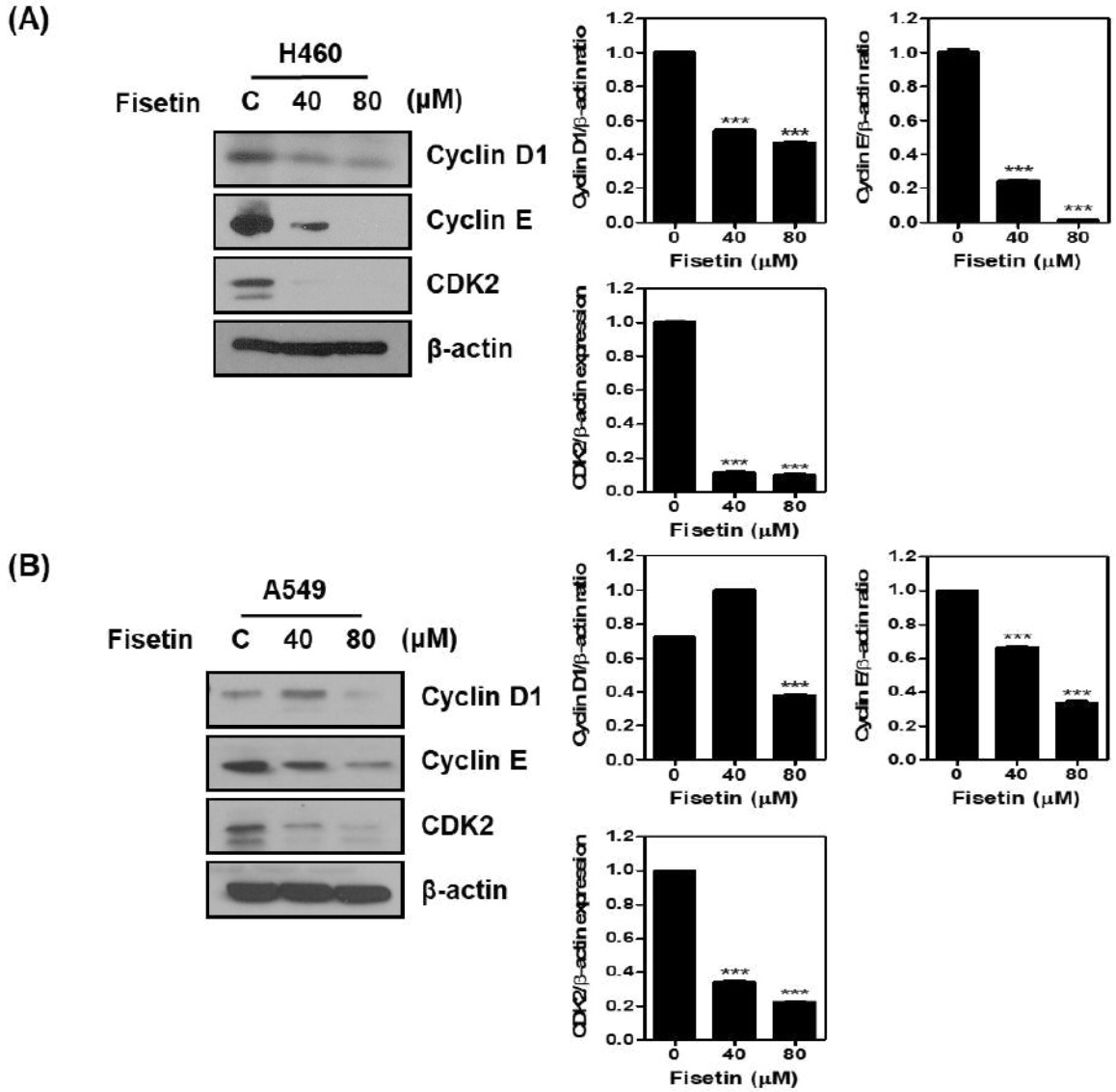

Figure 3: Effect of Fisetin on cell cycle related proteins in H460 and A549 cells. (A) Effect of Fisetin on cell cycle related proteins in H460 cells. After exposure to Fisetin at 40 and 80 μM for 24 h, cell cycle related proteins were evaluated in H460 cells by Western blotting. ***, $p<0.001$ vs untreated control. (B) Effect of Fisetin on cell cycle related proteins in A549 cells. The expression of cell cycle and ribosomal biogenesis related proteins was assessed in Fisetin treated A549 cells by Western blotting. ***, $p<0.001$ vs untreated control.

Fisetin attenuated the ubiquitination related proteins in H460 and A549 cells

Exportin-1 (XPO1) controls nuclear-cytoplasmic transport of protein, ribosomal RNA and certain mRNAs for ribosomal biogenesis, which is closely associated with ubiquitination [5,33]. The c-*Myc* is known an unstable *protein* susceptible to ubiquitination [34], *while SKP2* (S-phase kinase-associated protein 2) acts as the F-box protein of the E3 *ubiquitin ligase* complex [35] and Cullin 4A (CUL4A), an E3 ubiquitin ligase, is overexpressed in several cancers [36]. Hence, Western blotting was performed to assess the effect of Fisetin on ubiquitination related proteins in A549 and H460 cells. Here Fisetin attenuated the expression of XPO1, c-Myc, SKP2 and CUL4A compared to untreated control in A549 and H460 cells (Figure 4A and 4B). However, H460 cells were more susceptible to Fisetin than A549 cells.

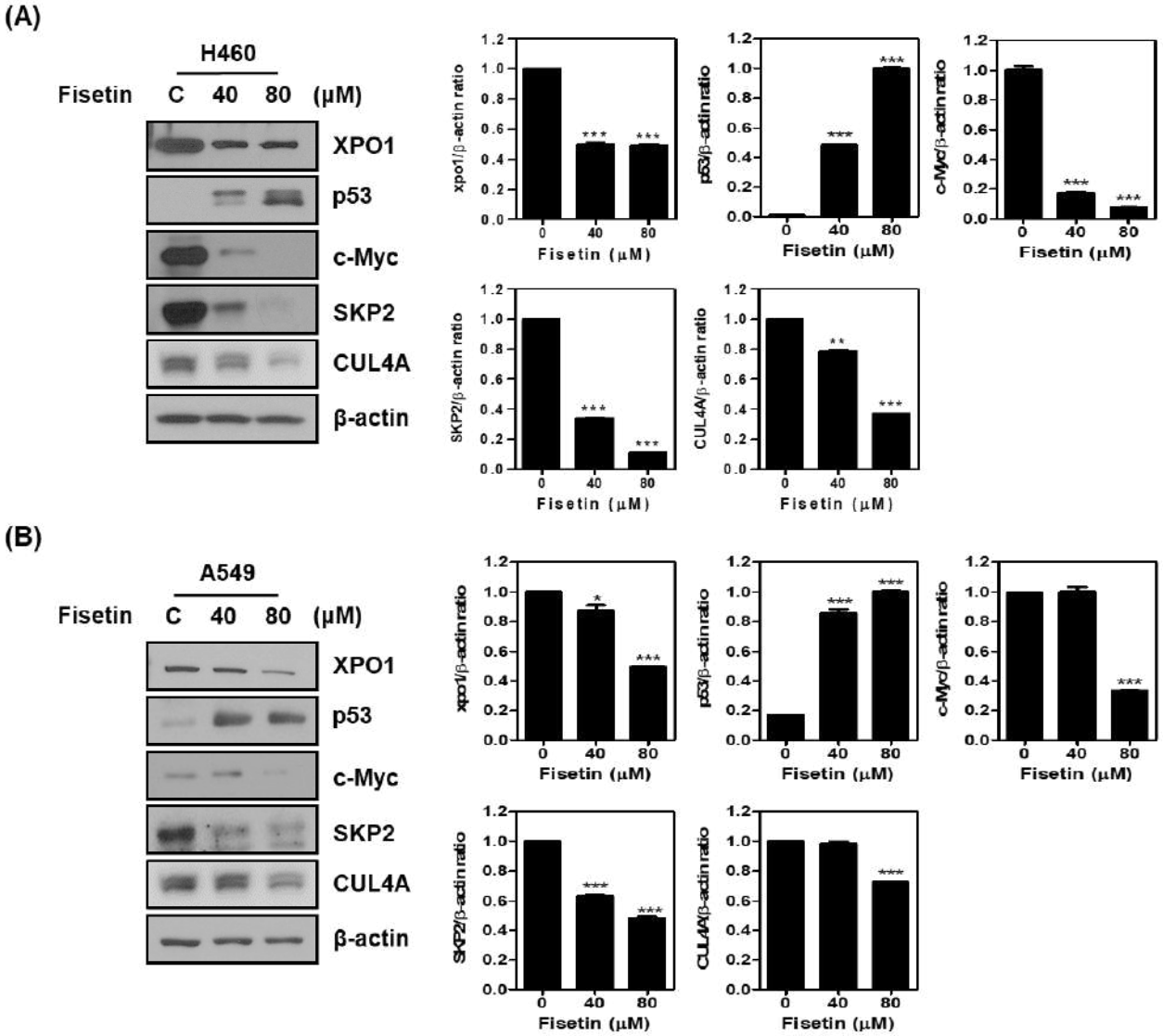

Figure 4: Effect of Fisetin on ubiquitination related proteins in H460 and A549 cells. (A) Effect of Fisetin on ubiquitination related proteins in H460 cells. After exposure to Fisetin at 40 and 80 μM for 24 h, ubiquitination related proteins were evaluated in H460 cells by Western blotting. **, p<0.01, ***, p<0.001 vs untreated control. (B) Effect of Fisetin on ubiquitination related proteins in A549 cells. *, p<0.05, ***, p<0.001 vs untreated control.

XPO1 or CUL4A depletion reduced the capacity of Fisetin to cleave PARP and induce p53 in H460 cells

To confirm the important role of XPO1 and IPO7 in Fisetin-induced apoptosis in H460 cells, Western blotting was performed in H460 cells transfected with siRNA vector, XPO1 siRNA plasmids. Herein XPO1 depletion enhanced p53 activation, while CUL4A depletion enhanced PARP cleavage in Fisetin treated H460 cells (Figure 5A and 5B).

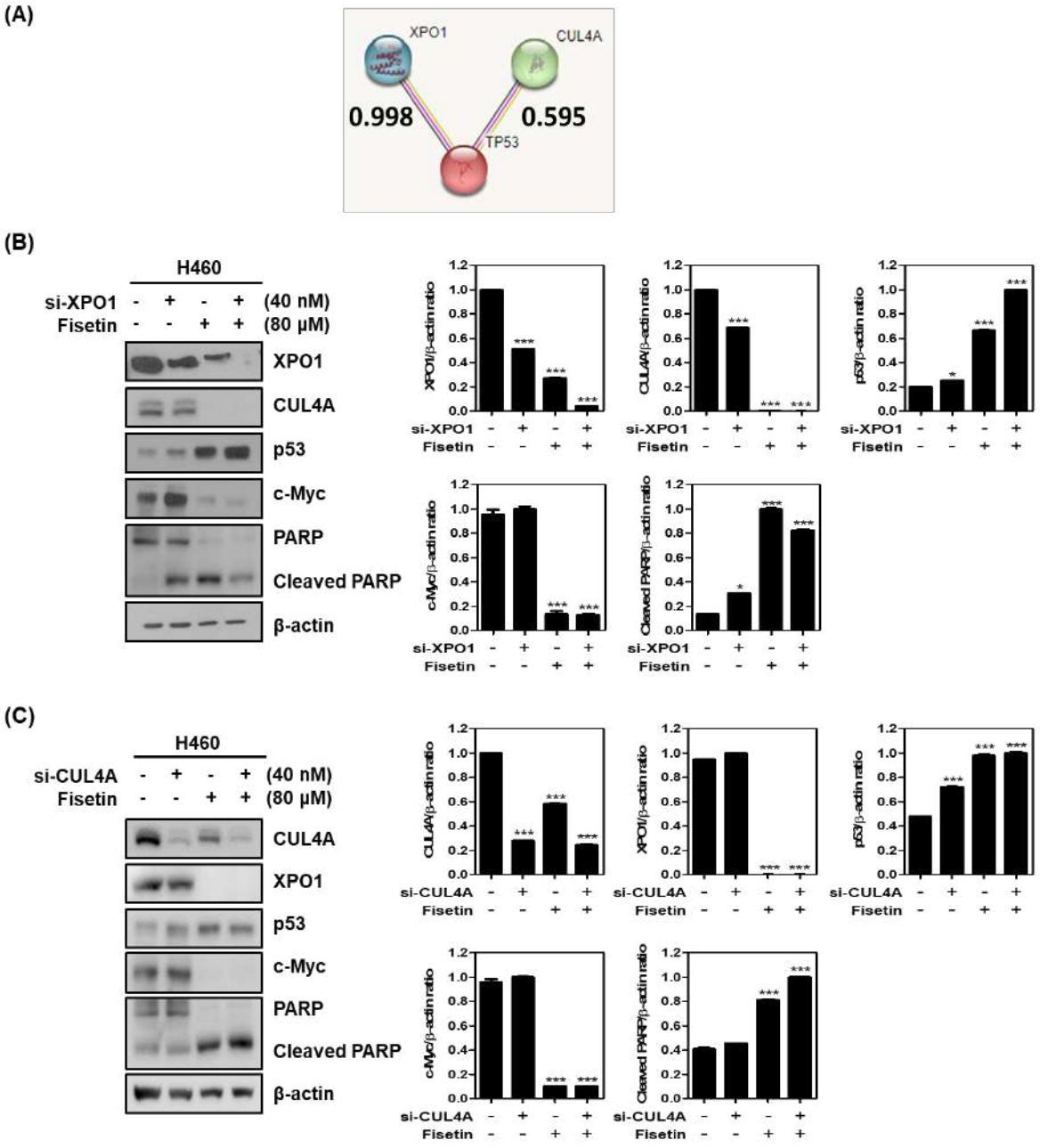

Figure 5: The pivotal role of XPO1 or CUL4A in Fisetin induced apoptosis in H460 cells. **(**A) PPI binding score between XPO1 or CUL4A and p53 by String database (B) Effect of XPO1 depletion on apoptosis related proteins in Fisetin treated H460 cells. *, $p<0.05$, ***, $p<0.001$ vs untreated control. (C) Effect of CUL4A depletion on apoptosis related proteins in Fisetin treated H460 cells ***, $p<0.001$ vs untreated control.

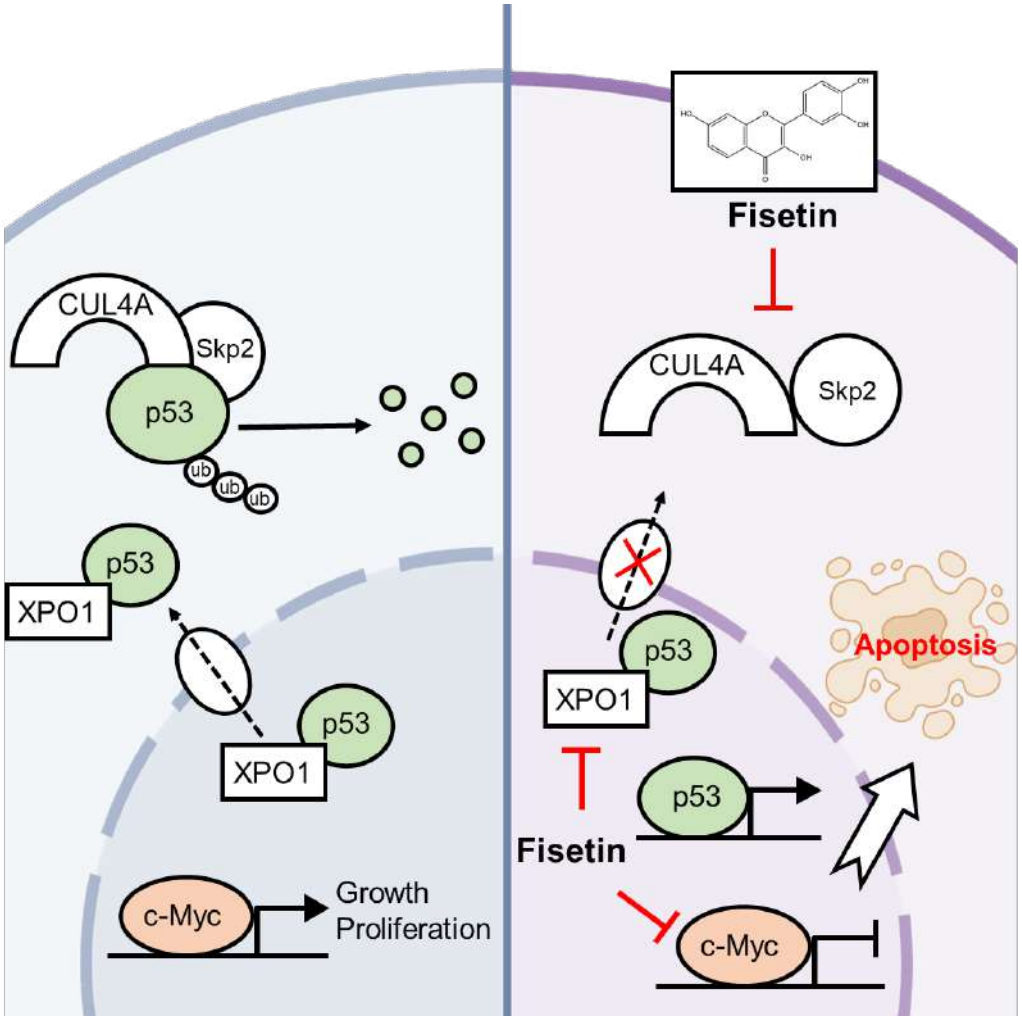

Figure 6: Apoptotic mechanism of Fisetin via modulation of XPO1 and CUL4A in non-small lung cancer cells.

Discussion

In the current study, the underlying mechanisms of Fisetin were explored in H460 and A549 cells in association with ribosomal protein XPO1 and CUL4A signaling axisis. Herein we found that Fisetin significantly suppressed the cell proliferation A549 and H460 lung cancer cells by MTT assay and colony formation assay, implying antitumor potential of Fisetin in non-small cell lung cancer cells. Also, Fisetin significantly increased the Annexin V/PI positive apoptotic portion and sub G1 portion in H460 and A549 cells, indicating the cytotoxicity of Fisetin is mediated by apoptosis in H460 and A549 cells. Consistently, several papers reported the capacity of Fisetin to induce cell cycle arrest in several cancer cells [5,37]. Additionally, Fisetin attenuated the expression of G1 related proteins such as cyclin D1, cyclin E, CDK2 and activated p53 and p21, implying G1 arrest leading to cell death by Fisetin in H460 and A549 cells.

Ribosome biogenesis is known the course of producing a variety of ribosomes in the cytoplasm and nucleus to organize the production and processing of ribosomal RNAs (rRNAs) to sustain cell homeostasis and cell survival [38]. Also, the important role of ribosomal proteins was reported as the regulators of cell cycle and proliferation [38], though ribosomal proteins require the assistance of nuclear import factors during the active nuclear import process [9]. To date, exportin 1 (XPO1) is known to mediate the nuclear export of various proteins including p53 and RPL5, RPL4,RPL6 and RPL23A [39], since ribosomal proteins increase the cell proliferation of colon cancer cells through c-Myc [40,41] and p53 [39,42]. Our data demonstrate that Fisetin effectively suppressed the expression of XPO1 and c-Myc and activated the expression of p53 in A549 and H460 cells.

It is well documented that the ubiquitin-dependent proteolysis system (UPS) for protein degradation is involved in the regulation of cell proliferation, differentiation and apoptosis via the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2) and the ubiquitin-protein ligase (E3) [43,44]. S-phase kinase-associated protein 2 (Skp2) for a Skp2-SCF E3 ligase complex induces proteasome mediated proteolysis through conjugating K63-linked polyubiquitin chains to targeting Akt, LKB1 and Aurora B [45] CUL4A ubiquitin ligase that forms a complex with DNA damage binding protein 1 (DDB1) and ring of cullin 1(ROC1) is known as a promising target for cancer treatment [46]. Here Fisetin attenuated the expression of cMyc, SKP2, CUL4A in A549 and H460 cells. Of note, XPO1 depletion enhanced p53 activation, while CUL4A depletion enhanced PARP cleavage in Fisetin treated H460 cells, indicating the pivotal roles of XPO1 and CUL4A. However, considering that interaction scores were 0.998 between XPO1 and p53, 0.595 between CUL4A and p53 by String database, it can be assumed that XPO1 binds to p53, while CUL4A as E3 ubiquitine ligase enhances ubiquitination in H460 cells.

Conclusions

Fisetin increased cytotoxic and apoptotic effect by increasing the number of Annexin V/PI positive apoptotic portion and sub G1 accumulation, and the cleavages of PARP and caspase 3, activated p53/p21 and attenuating cyclin D1, cyclin E, CDK2, XPO1, cMyc, SKP2 and Cullin 4A in A549 and H460 cells. Conversely, depletion of XPO1 enhanced p53 and PARP cleavage, while depletion of UL4A promoted PARP cleavage without p53 upregulation in H460 cells. Taken together, our work strongly demonstrates the pivotal role of XPO1 mediated c-Myc and CUL4A signaling in Fisetin induced apoptotic effect in NSCLCs as a potent antitumor candidate (Figure 6). Overall, our findings suggest that Fisetin remarkably supressed c-Myc and increased p53 in a concentration-dependent manner, along with decreased IPO7 and XPO1, suggesting the involvement of IPO7 and XPO1 linked c-Myc and p53 signaling in Fisetin-induced antiproliferative effect in NSCLCs.

References

1. Bugge A, Kongerud J, Brunborg C, Solberg S, Lund MB (2017) Gender-specific survival after surgical resection for early stage non-small cell lung cancer. Acta Oncol 56:448-454.
2. Yan W, Wistuba, II, Emmert-Buck MR, Erickson HS (2011) Squamous Cell Carcinoma - Similarities and Differences among Anatomical Sites. American journal of cancer research 1: 275-300.
3. Yuan X, Wu H, Xu H, Han N, Chu Q, et al. (2015) Meta-analysis reveals the correlation of Notch signaling with non-small cell lung cancer progression and prognosis. Scientific reports 5: 10338.
4. Waissbluth S, Garnier D, Akinpelu OV, Salehi P, Daniel SJ (2017) The impact of erdosteine on cisplatin-induced ototoxicity: a proteomics approach. Eur Arch Otorhinolaryngol 274: 1365-1374.
5. Adan A, Baran Y (2015) The pleiotropic effects of fisetin and hesperetin on human acute promyelocytic leukemia cells are mediated through apoptosis, cell cycle arrest, and alterations in signaling networks. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 36: 8973-8984.
6. Jia L, Yan F, Cao W, Chen Z, Zheng H, et al. (2017) Dysregulation of CUL4A and CUL4B Ubiquitin Ligases in Lung Cancer. The Journal of biological chemistry 292: 2966-2978.
7. Hannah J, Zhou P (2015) Distinct and overlapping functions of the cullin E3 ligase scaffolding proteins CUL4A and CUL4B. Gene 573: 33-45.
8. Zhu ZC, Liu JW, Yang C, Zhao M, Xiong ZQ (2019) XPO1 inhibitor KPT-330 synergizes with Bcl-xL inhibitor to induce cancer cell apoptosis by perturbing rRNA processing and Mcl-1 protein synthesis. Cell death & disease 10: 395.
9. Kim J, McMillan E, Kim HS, Venkateswaran N, Makkar G, et al. (2016) XPO1-dependent nuclear export is a druggable vulnerability in KRAS-mutant lung cancer. Nature 538: 114-117.
10. Yong WK, Ho YF, Malek SN (2015) Xanthohumol induces apoptosis and S phase cell cycle arrest in A549 non-small cell lung cancer cells. Pharmacognosy magazine 11: S275-283.
11. Zhou L, Yao Q, Li Y, Huang YC, Jiang H, et al. (2017) Sulforaphane-induced apoptosis in Xuanwei lung adenocarcinoma cell line XWLC-05. Thoracic cancer 8: 16-25.
12. Li W, Ma X, Li N, Liu H, Dong Q, et al. (2016) Resveratrol inhibits Hexokinases II mediated glycolysis in non-small cell lung cancer via targeting Akt signaling pathway. Experimental cell research 349: 320-327.
13. Xie J, Liu JH, Liu H, Liao XZ, Chen Y, et al. (2016) Tanshinone IIA combined with adriamycin inhibited malignant biological behaviors of NSCLC A549 cell line in a synergistic way. BMC cancer 16: 899.
14. Jeung YJ, Kim HG, Ahn J, Lee HJ, Lee SB, et al. (2016) Shikonin induces apoptosis of lung cancer cells via activation of FOXO3a/EGR1/SIRT1 signaling antagonized by p300. Biochimica et biophysica acta 1863: 2584-2593.
15. Liu YH, Liu GH, Mei JJ, Wang (2016) The preventive effects of hyperoside on lung cancer in vitro by inducing apoptosis and inhibiting proliferation through Caspase-3 and P53 signaling pathway. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 83: 381-391.
16. Corina D, Bojin F, Ambrus R, Muntean D, Soica C, et al. (2017) Physico-chemical and Biological Evaluation of Flavonols: Fisetin, Quercetin and Kaempferol Alone and Incorporated in beta Cyclodextrins. Anti-cancer agents in medicinal chemistry 17: 615-626.
17. Seo SH, Jeong GS (2015) Fisetin inhibits TNF-alpha-induced inflammatory action and hydrogen peroxide-induced oxidative damage in human keratinocyte HaCaT cells through PI3K/AKT/Nrf-2-mediated heme oxygenase-1 expression. International immunopharmacology 29: 246-253.
18. Maher P (2009) Modulation of multiple pathways involved in the maintenance of neuronal function during aging by fisetin. Genes & nutrition 4: 297-307.
19. Bhat TA, Nambiar D, Pal A, Agarwal R, Singh RP (2012) Fisetin inhibits various attributes of angiogenesis in vitro and in vivo--implications for angioprevention. Carcinogenesis 33: 385-393.

20. Sinha R, Srivastava S, Joshi A, Joshi UJ, Govil G (2014) In-vitro anti-proliferative and anti-oxidant activity of galangin, fisetin and quercetin: role of localization and intermolecular interaction in model membrane. European journal of medicinal chemistry 79: 102-109.

21. Yang Q, Liao J, Deng X, Liang J, Long C, et al. (2014) Anti-tumor activity and safety evaluation of fisetin-loaded methoxy poly(ethylene glycol)-poly(epsilon-caprolactone) nanoparticles. Journal of biomedical nanotechnology 10: 580-591.

22. Yi C, Zhang Y, Yu Z, Xiao Y, Wang J, et al. (2014) Melatonin enhances the anti-tumor effect of fisetin by inhibiting COX-2/iNOS and NF-kappaB/p300 signaling pathways. PloS one 9: e99943.

23. Adhami VM, Syed DN, Khan N, Mukhtar H (2012) Dietary flavonoid fisetin: a novel dual inhibitor of PI3K/Akt and mTOR for prostate cancer management. Biochemical pharmacology 84: 1277-1281.

24. Jia S, Xu X, Zhou S, Chen Y, Ding G, et al. (2019) Fisetin induces autophagy in pancreatic cancer cells via endoplasmic reticulum stress- and mitochondrial stress-dependent pathways. Cell death & disease 10: 142.

25. Renault-Mahieux M, Vieillard V, Seguin J, Espeau P, Le DT, et al. (2021) Co-Encapsulation of Fisetin and Cisplatin into Liposomes for Glioma Therapy: From Formulation to Cell Evaluation. Pharmaceutics: 13.

26. Yan W, Chen S, Zhao Y, Ye X (2018) Fisetin inhibits the proliferation of gastric cancer cells and induces apoptosis through suppression of ERK 1/2 activation. Oncology letters 15: 8442-8446.

27. Guo G, Zhang W, Dang M, Yan M, Chen Z (2019) Fisetin induces apoptosis in breast cancer MDA-MB-453 cells through degradation of HER2/neu and via the PI3K/Akt pathway. Journal of biochemical and molecular toxicology 33: e22268.

28. Lim DY, Park JH (2009) Induction of p53 contributes to apoptosis of HCT-116 human colon cancer cells induced by the dietary compound fisetin. American journal of physiology Gastrointestinal and liver physiology 296: G1060-1068.

29. Liu Y, Cao H, Zhao Y, Shan L, Lan S (2022) Fisetin-induced cell death in human ovarian cancer cell lines via zbp1-mediated necroptosis. Journal of ovarian research 15: 57.

30. Wang J, Huang S (2018) Fisetin inhibits the growth and migration in the A549 human lung cancer cell line via the ERK1/2 pathway. Experimental and therapeutic medicine 15: 2667-2673.

31. Kang KA, Piao MJ, Madduma Hewage SR, Ryu YS, Oh MC, et al. (2016) Fisetin induces apoptosis and endoplasmic reticulum stress in human non-small cell lung cancer through inhibition of the MAPK signaling pathway. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 37: 9615-9624.

32. Khan N, Afaq F, Khusro FH, Mustafa Adhami V, Suh Y, et al. (2012) Dual inhibition of phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin signaling in human nonsmall cell lung cancer cells by a dietary flavonoid fisetin. International journal of cancer 130: 1695-1705.

33. Gao J, Azmi AS, Aboukameel A, Kauffman M, Shacham S, et al. (2014) Nuclear retention of Fbw7 by specific inhibitors of nuclear export leads to Notch1 degradation in pancreatic cancer. Oncotarget 5: 3444-3454.

34. Farrell AS, Sears RC (2014) MYC degradation. Cold Spring Harbor perspectives in medicine: 4.

35. Asmamaw MD, Liu Y, Zheng YC, Shi XJ, Liu HM (2020) Skp2 in the ubiquitin-proteasome system: A comprehensive review. Medicinal research reviews 40: 1920-1949.

36. Lung J, Chen YC, Lin YC, Li YC, You L, et al. (2021) The effect of cullin 4A on lung cancer cell chemosensitivity to paclitaxel through p33ING1b regulation. American journal of translational research 13: 11194-11208.

37. Poor M, Zrinyi Z, Koszegi T (2016) Structure related effects of flavonoid aglycones on cell cycle progression of HepG2 cells: Metabolic activation of fisetin and quercetin by catechol-O-methyltransferase (COMT). Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 83: 998-1005.

38. Chen FW, Ioannou YA (1999) Ribosomal proteins in cell proliferation and apoptosis. International reviews of immunology 18: 429-448.

39. Golomb L, Bublik DR, Wilder S, Nevo R, Kiss V, et al. (2012) Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis. Molecular cell 45: 222-232.

40. Eilers M, Eisenman RN (2008) Myc's broad reach. Genes & development 22: 2755-2766.

41. Yoshimura M, Ishizawa J, Ruvolo V, Dilip A, Quintás-Cardama A, et al. (2014) Induction of p53-mediated transcription and apoptosis by exportin-1 (XPO1) inhibition in mantle cell lymphoma. Cancer science 105: 795-801.

42. Golomb L, Volarevic S, Oren M (2014) p53 and ribosome biogenesis stress: the essentials. FEBS letters 588: 2571-2579.

43. Martín-Villanueva S, Gutiérrez G, Kressler D, de la Cruz J (2021) Ubiquitin and Ubiquitin-Like Proteins and Domains in Ribosome Production and Function: Chance or Necessity? International journal of molecular sciences: 22.

44. Severe N, Dieudonne FX, Marie PJ (2013) E3 ubiquitin ligase-mediated regulation of bone formation and tumorigenesis. Cell death & disease 4: e463.

45. Cai Z, Moten A, Peng D, Hsu CC, Pan BS, et al. (2020) The Skp2 Pathway: A Critical Target for Cancer Therapy. Semin Cancer Biol 67: 16-33.

46. Sharma P, Nag A (2014) CUL4A ubiquitin ligase: a promising drug target for cancer and other human diseases. Open biology 4: 130217.