Investigating the Role of Quercetin in Increasing the Rate of Cisplatin-Induced Apoptosis Via the NF-κB Pathway in MG-63 Cancer Cells

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ABSTRACT

Introduction Numerous studies suggest that the co-treatment of chemotherapeutic agents with flavonoids such as Quercetin (Que) may enhance tumor cells' susceptibility to these agents. Hence, in the current study, we investigated Que's role in combination with Cisplatin to promote cell apoptosis by focusing on the NF-κB signaling pathway in the osteosarcoma cell lines.

Methods The Que, Cisplatin, and their combination's general cytotoxicity effects were evaluated using an MTT assay for 72 hrs. The protein expression levels of NF-κB were detected by an enzyme-linked immunosorbent assay (ELISA) Kit. Flow cytometry was used to evaluate cell apoptosis.

Results Que considerably elevated the cytotoxicity of Cisplatin ($P < 0.05$). Que also dramatically down-regulated the expression levels of NF-κB in MG-63 cells compared to mono-treatment ($P < 0.05$). Besides, Que promotes cisplatin-induced apoptosis in MG-63 cells.

Conclusion Our study's findings provide an exact point in the field of adjuvant therapy in osteosarcoma. In other words, this study could provide new insights into a better understanding of the role of Que in elevating cisplatin-induced apoptosis with NF-κB down-regulation.

ABBREVIATIONS

OS	osteosarcoma
NSCLC	non-small cell lung cancer
ELISA	enzyme-linked immunosorbent assay
Que	Quercetin
SD	standard deviation
DMSO	dimethyl sulfoxide
DR.	Death receptor
MAPK	mitogen-activated protein kinase
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
PBS	phosphate-buffered saline
PI3K	phosphatidylinositol 3-kinase
FBS	fetal bovine serum
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Introduction

Treatment of patients with osteosarcoma (OS) remains a major clinical challenge, the second leading factor of tumor-related mortality in the pediatric age. Patients have a median overall survival of less than one year, and a standard treatment approach is yet to be established [1–3]. However, even when patients with high-grade OS undergo intensive chemotherapy, the survival rate is only 50 % to 80 % [4]. Treatment of OS patients with Cisplatin as a single chemotherapeutic drug may cause ototoxicity. Therefore, developing molecularly targeted agents with high tumor specificity is essential to address these problems [5].

Nuclear factor-kappa B (NF-κB) is associated with chemotherapy reply and resistance in malignancies. Constitutive NF-κB activation has been recognized in various malignancies [6]. Therefore, inhibiting NF-κB can hinder tumor cell survival, metastasis, and chemoresistance. Hence, targeting NF-κB has the probability of improving the efficacy of chemotherapeutics agents. Diverse NF-κB blockers exist, including proteasome blockers and antioxidants, directly or indirectly repressing the NF-κB pathway [7]. Previously, Ryan et al. revealed that NF-κB is a potential therapeutic target in cisplatin-resistant non-small cell lung cancer (NSCLC) [8].

Natural compounds such as Quercetin (Que) have been documented as dramatic agents for impeding and healing cancerous because of their predictable performance, high therapeutic potential, and low cytotoxicity [9]. Que is a class of flavonoid compounds with the hydroxyl-flavone backbone, a potent antitumor agent due to its pro-apoptotic, anti-proliferative, anti-angiogenic, and anti-inflammatory properties. Previous studies' findings implied that Que explicitly targeted Bcl-2 family members, HIF-1α, PI3K, and p21-related anti-apoptotic pathways [10]. Also, Youn and co-workers' findings suggested that Que mediates growth inhibition of NSCLC tumor cells by inhibiting the NF-κB and enhancing the expression of death receptors and cell cycle blockers [11]. However, whether Que affects the cisplatin-induced apoptosis via the NF-κB pathway in OS cells remains unclear.

Overall, we sought to evaluate the underlying mechanisms governing the phenomenon; wherein Que affects the cisplatin-induced apoptosis in OS cells, focusing on the NF-κB pathway.

Material and methods

In the present study, cell culture flask and plate were purchased from Corning Co, Trypsin EDTA, trypan blue, and Que were bought from Sigma-Aldrich Co., Penicillin G/Streptomycin (Pen/Strep) was obtained from YEASEN. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was provided by Bio Basic Co., RPMI-1640 was purchased from GIBCO Life Technology, fetal bovine serum (FBS) was obtained from Hyclone (Logan, UT).

Cell culture

Mg-63 of the human OS cell line, purchased from Pasteur Institute (Tehran, Iran), were cultured in RPMI 1640 medium supplemented with 10 % FBS, 100 unit/ml, and 100 µg/ml of Pen/Strep. These cell lines were incubated at 37°C in the presence of 5 % CO_2. The viable cells were detected by conducting the trypan blue dye exclusion test.

Cell viability assay

The induction of the cytotoxicity effect of Que on Cisplatin in MG-63 cells was assessed by MTT assay. In brief, cells were plated in 96-well microplates at the density of 5×10^3 cells/200 µl/well and exposed to different concentrations of Que and Cisplatin for 72 h. Subsequently, cells were exposed to 5 mg/mL of MTT (20 µl) in a 37°C humidified incubator for 4 h. Then, the formazan product was dissolved at 175 µL DMSO. Absorbance was measured at 490 nm by a Benchmark Plus microplate spectrometer (Bio-Rad, California).

Measurement of NF-κB expression

MG-63 cell lines were plated at a density of 2×10^4 cells per well in twenty-four-well microplates and then exposed to Cisplatin and Que (IC_{50} dose) and their combination of inappropriate drug concentrations. Followed 48 h incubation, according to the manufacturer's direction, NF-κB was identified by a colorimetric NF-κB expression assay kit (MBS2023291). Then the absorbance was detected using a plate auto-reader at 412 nm.

Flow cytometry analysis

After treatment with Que and Cisplatin, MG-63 cells were detached from the plates with 0.25 % trypsin without EDTA and washed with PBS before being blocked with 0.5 % BSA in 1x PBS for 30 minutes at 4 °C. After washing with ice-cold PBS, the cells were resuspended in 500 µl of binding buffer and then 5 µl annexin V-FITC and 5 µl propidium iodide (EXBIO Diagnostics, Czech Republic). The stained cells were collected an analyzed by FACScan cytometer (Becton Dickinson, Heidelberg, Germany).

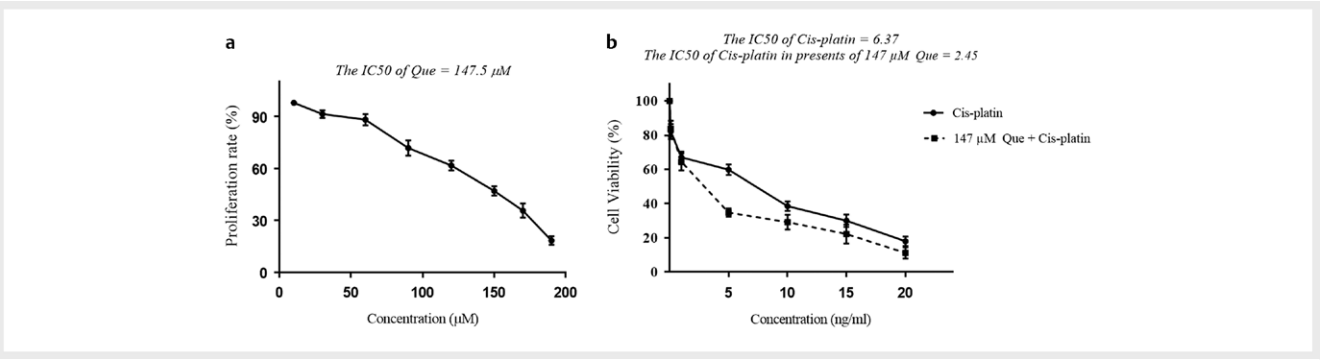
Statistical Analysis

All data analyses were presented as the mean ± standard deviation (SD). The significant differences among diverse groups were determined by one-way analysis of variance (ANOVA) at least three independent experiments. Statistical analysis was done by using GraphPad Prism 7.04 software. *P*-values < 0.05 were considered statistically significant unless mentioned otherwise.

Results

Quercetin enhances cisplatin-induced cytotoxicity in MG-63 cells

The cytotoxic effects of Que and Cisplatin on MG-63 cells were determined using an MTT assay. As shown in ▶ **Fig. 1a** and **b**, different concentrations of Que (0 to 20 µM) and Cisplatin (0 to 200 µM) were used to determine the IC_{50} of Que and Cisplatin in MG-63 cells. IC_{50} values for Que were 147.5 µM and for Cisplatin 6.37 µM in MG-63 cells. After combination in IC_{50} dose for Que and in a concentration-dependent manner of Cisplatin, the IC_{50} values 2.45 µM decreased compared to DOX alone (reduced 2.5-fold, $P < 0.05$). Indeed, co-treatment Que plus Cisplatin enhances cytotoxicity and decreases the survival rate of MG-63 cells compared to treatment with Cisplatin alone.



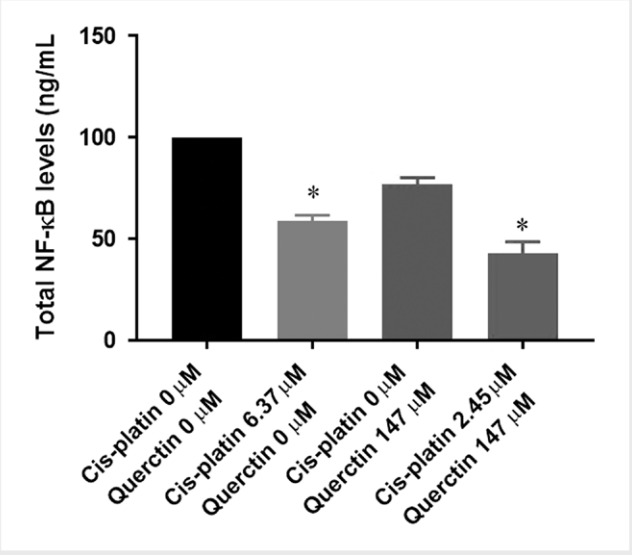
▶ **Fig. 1** Cell viability in different concentrations of Quercetin and Cisplatin alone or in combination in MG-63 cells. **a**) Cytotoxicity of various concentrations of Que for 72 hrs. **b**) Cytotoxicity of different concentrations of Cisplatin, and in combination with Que in IC50 dose for 72 hrs. Each point is the mean ± SD of three times independent experiments; * $P<0.05$.

Quercetin and cisplatin co-treatment could significantly alleviate NF-κB expression in MG-63 cells

To clarify Que's mechanism in augments Cisplatin-induced apoptosis, we surveyed NF-κB protein expression levels enhanced in OS cells. As revealed in ▶ **Fig. 2**, we observed that co-treatment Cisplatin plus Que significantly diminished NF-κβ levels in MG-63 cells compared to treatment with mono-treatment. Indeed, Que administration intensifies the effect of Cisplatin in reducing NF-κB levels ($P<0.05$).

Quercetin exacerbates cisplatin-induced cell apoptosis in MG-63 tumor cells

Flow cytometric analysis was conducted to determine whether alleviated cell viability by inducing apoptosis upon exposure with Que or Cisplatin alone or in combination with HepG2 cells after 48 hrs. The results demonstrate that after 48 hrs of exposure to Que with Cisplatin in MG-63 cells, the apoptosis rate increased by 10.56-folds compared to the control group (▶ **Fig. 3**). Also, this combination therapy significantly increases the apoptosis rate compared to treatment with Cisplatin or Que alone. These findings suggest that Que may synergistically enhance the cisplatin-induced apoptosis in MG-63 cells.



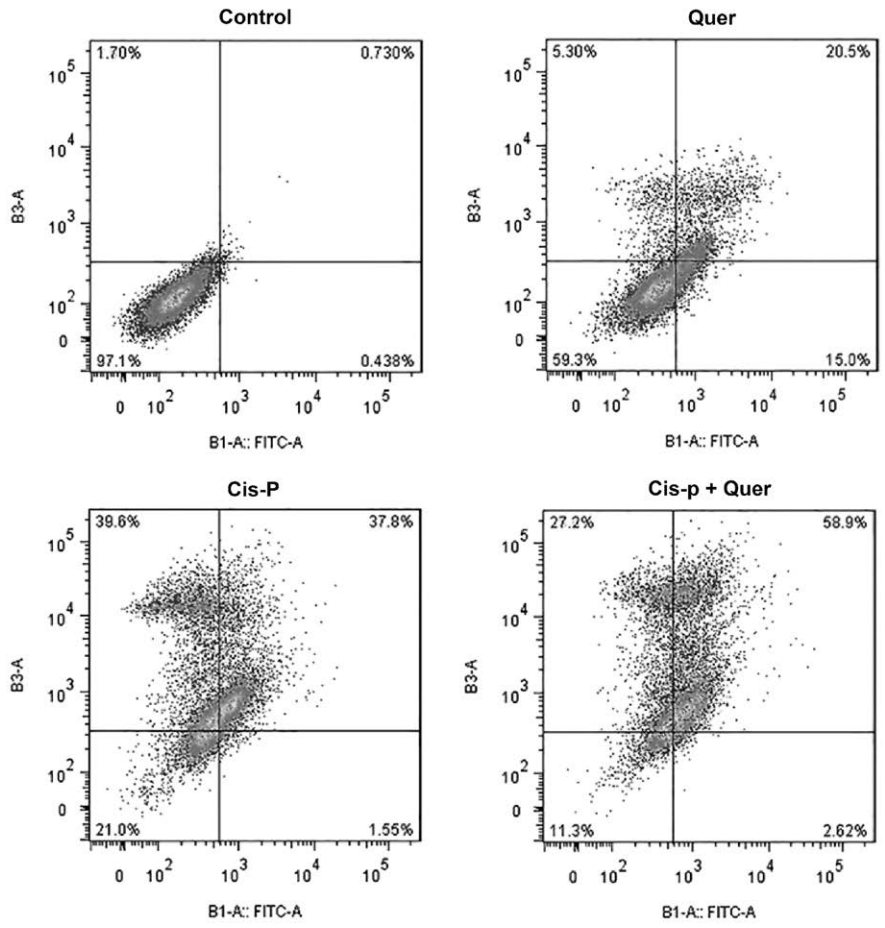
▶ **Fig. 2** Changes in Cisplatin-induced apoptosis through NF-κB protein expression levels in response to Que and Cisplatin alone or co-treatment in MG-63 cells. Results are expressed as mean ± SD implemented in triplicate independent tests; * $P<0.05$ vs. control.

Discussion

In the present study, we will address whether Que enhanced the rate of cisplatin-induced apoptosis through the NF-κB pathway in OS patients. Our results reflected three key findings to understand the reply to this question better. i) Que enhances cisplatin-induced cytotoxicity in MG-63 cells in a time-dependent manner. ii) Co-treatment of Cisplatin with Que results in significant down-regulation of the NF-κB pathway. iii) Also, Que exacerbates cisplatin-induced cell apoptosis in MG-63 tumor cells.

Cisplatin-based chemotherapeutic regimens are applied as adjuvant treatments against OS patients, especially for malignancies with aggressive characteristics [12, 13]. Notwithstanding, Cisplatin resistance is a significant hindrance to its clinical application. On the other hand, the NF-κB activation by chemotherapeutic drugs is significant for tumor cell chemoresistance strategies [14]. Therefore, repression of the activation of NF-κB expression may improve the efficacy of chemotherapy. Hence, co-treatment with other sensitizing agents with minimum toxicity on normal cells and maximum efficacy on tumor cells is a practical approach to overcome Cisplatin resistance.

A sheer number of studies have proved that Que's capability to interfere with different targets distinguished as "hallmarks of tumor" makes this agent, together with many other phytochemicals, a multi-target inhibitor with pleiotropic and synergistic impacts in malignant cells [10]. In this regard, Pan et al. demonstrated that Que hindered the viability and migration and augmented the senescence and apoptosis of glioma cells by inhibiting the Ras/MAPK/ERK and PI3K/AKT cascades. Indeed, they revealed that Que might be a potential candidate for glioma clinical treatment [15].



▶ **Fig. 3** Quercetin and impacts on Cisplatin-induced apoptosis in MG-63 cells after 48 hrs using flow cytometry technique. Apoptosis was detected after double-staining with annexin V-FITC/PI; control, Que, Cisplatin, Que plus Cisplatin.

Pozsgai and colleagues suggested that temozolomide's co-treatment with Que enhances temozolomide's efficacy in glioblastoma via synergism in apoptosis induction through cleavage of caspase-3 and PARP-1 and by the repression of the activation of the Akt pathway [16]. Along with previous studies, our cytotoxicity assay results reveal that Que synergistically enhanced Cisplatin's cytotoxicity effects by alleviating the viability of MG-63 cells and suggesting that Que-triggered cytotoxicity is independent of Cisplatin in tumor cells.

NF-κB is a crucial signaling pathway implicated in the pathogenesis and treatment of many malignancies [17]. Lu et al. showed that the expression of NF-κB protein in OS patients negatively correlates with the apoptotic index of OS and dramatically hindered OS cells' apoptosis [18]. Studies have currently implied that strategies, particularly versus NF-kB, may alleviate this aggressive malignancy's progression due to the influence on cell proliferation, apoptosis, and invasion [19]. Besides, investigations implied that the NF-κB activity inactivation increased tumor cells' sensitivity to chemotherapeutics [20]. However, the relationship between flavonoids and the NF-κB signaling is discrepant. Notwithstanding, Que is ideal for combining antitumor agents to improve effectiveness [21].

Youn and co-workers suggested that Que mediates growth inhibition of NSCLC tumor cells by repressing the NF-κB and enhancing the expression of death receptors (DRs) and cell cycle blockers [11]. Zou et al. revealed that Que dramatically diminished the expression of IκBα, p65, and p50, whereby repressed NF-kB signalling. Indeed, Que hinders the ZD55 TRAIL-mediated activation of NF-κB and its anti-apoptotic target genes, sensitizing human HCC cells to ZD55TRAIL-induced apoptosis [22]. Also, Li and colleagues suggested that the pretreatment of tumor cells with Que synergetically promotes both apoptosis pathways by activating caspase-8 and caspase-9. By inhibiting NF-κB, Que downregulates xIAP and sensitizes the cisplatin-induced apoptosis in oral squamous cell carcinoma. Furthermore, in vivo findings demonstrated that Que plus Cisplatin's co-treatment hinders the xenograft growth in mice [21]. Along with previous studies, our results reveal that compared to mono-treatment, Cisplatin's co-treatment with Que could statically decrease the NF-κB protein in MG-63 cells, implying the combination of Cisplatin plus Que inhibits NF-κB activation. Also, by repressing NF-κB, Que elevates cisplatin-induced apoptosis in MG-63 cells.

Que performs complex functions in different signaling pathways to regulate cancer cell metabolism, resulting in a cost-effective, safe, and promising drug for healing OS patients.

Conclusion

As a highly efficient plant extract with low cytotoxicity, Que may have utility as an auxiliary agent for OS patients' clinical improvement. Overall, our findings provide new insights into the possible underlying mechanisms of Que's combination treatment with Cisplatin significantly alleviates the intracellular signaling of NF-κB in MG-63 cells. This Approach could circumvent the limited efficiency of single-drug treatment and simultaneously overwhelm monotreatment with Cisplatin resistance in treating OS patients.

Ethical approval

All experimental procedures were applied in accordance with the approval from the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.1135).

Acknowledgment

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Italiano A, Mir O, Mathoulin-Pelissier S et al. Cabozantinib in patients with advanced Ewing sarcoma or osteosarcoma (CABONE): a multicentre, single-arm, phase 2 trial. Lancet Oncol 2020; 21: 446–455. doi:10.1016/s1470-2045(19)30825-3

[2] Smrke A, Anderson PM, Gulia A et al. Future Directions in the Treatment of Osteosarcoma. Cells 2021; 10: 172. doi:10.3390/cells10010172

[3] Bazavar M, Fazli J, Valizadeh A et al. miR-192 enhances sensitivity of methotrexate drug to MG-63 osteosarcoma cancer cells. Pathol Res Pract 2020; 216: 153176. doi:10.1016/j.prp.2020.153176

[4] Siegel RL, Miller KD, Fuchs HE et al. Cancer Statistics, 2021. CA Cancer J Clin 2021; 71: 7–33. doi:10.3322/caac.21654

[5] Zheng D, Liu W, Xie W et al. AHA1 upregulates IDH1 and metabolic activity to promote growth and metastasis and predicts prognosis in osteosarcoma. Signal Transduct Target Ther 2021; 6: 1–12. doi:10.1038/s41392-020-00387-1

[6] Asghari S, Valizadeh Dizajeykan A, Ahmadi M et al. Evaluation of ovarian cancer risk in granulosa cells treated with steroid-depleted endometriosis serum: Role of NF-κB/RelA and AKT. J Cell Physiol 2019; 234: 12011–12018. doi:10.1002/jcp.27862

[7] Gong T, Su X, Xia Q et al. Expression of NF-κB and PTEN in osteosarcoma and its clinical significance. Oncol Lett 2017; 14: 6744–6748. doi:10.3892/ol.2017.6972

[8] Ryan SL, Beard S, Barr MP et al. Targeting NF-κB-mediated inflammatory pathways in cisplatin-resistant NSCLC. Lung Cancer 2019; 135: 217–227. doi:10.1016/j.lungcan.2019.07.006

[9] Hashemzaei M, Delarami Far A, Yari A et al. Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. Oncol Rep 2017; 38: 819–828. doi:10.3892/or.2017.5766

[10] Russo M, Spagnuolo C, Tedesco I et al. The flavonoid quercetin in disease prevention and therapy: facts and fancies. Biochem Pharmacol 2012; 83: 6–15. doi:10.1016/j.bcp.2011.08.010

[11] Youn H, Jeong JC, Jeong YS et al. Quercetin potentiates apoptosis by inhibiting nuclear factor-kappaB signaling in H460 lung cancer cells. Biol Pharm Bull 2013; 36: 944–951. doi:10.1248/bpb.b12-01004

[12] Almeida LO, Abrahao AC, Rosselli-Murai LK et al. NFκB mediates cisplatin resistance through histone modifications in head and neck squamous cell carcinoma (HNSCC). FEBS Open Bio 2014; 4: 96–104. doi:10.1016/j.fob.2013.12.003

[13] Chen Z, Zeng Z, Zhu S et al. Effects of different combined regimens of Cisplatin, metformin, and quercetin on nasopharyngeal carcinoma cells and subcutaneous xenografts. Sci Rep 2021; 11: 1–10. doi:10.1038/s41598-020-80198-0

[14] Chuang SE, Yeh P Y, Lu YS et al. Basal levels and patterns of anticancer drug-induced activation of nuclear factor-kappaB (NF-kappaB), and its attenuation by tamoxifen, dexamethasone, and curcumin in carcinoma cells. Biochem Pharmacol 2002; 63: 1709–1716. doi:10.1016/s0006-2952(02)00931-0

[15] Pan HC, Jiang Q, Yu Y et al. Quercetin promotes cell apoptosis and inhibits the expression of MMP-9 and fibronectin via the AKT and ERK signalling pathways in human glioma cells. Neurochem Int 2015; 80: 60–71. doi:10.1016/j.neuint.2014.12.001

[16] Pozsgai E, Bellyei S, Cseh A et al. Quercetin increases the efficacy of glioblastoma treatment compared to standard chemoradiotherapy by the suppression of PI-3-kinase-Akt pathway. Nutr Cancer 2013; 65: 1059–1066. doi:10.1080/01635581.2013.810291

[17] Sun Y, Guan Z, Liang L et al. NF-κB signaling plays irreplaceable roles in cisplatin-induced bladder cancer chemoresistance and tumor progression. Int J Oncol 2016; 48: 225–234. doi:10.3892/ijo.2015.3256

[18] Lu J, Song G, Tang Q et al. IRX1 hypomethylation promotes osteosarcoma metastasis via induction of CXCL14/NF-κB signaling. J Clin Invest 2015; 125: 1839–1856. doi:10.1172/jci78437

[19] Sun ZJ, Chen G, Hu X et al. Activation of PI3K/Akt/IKK-alpha/NF-kappaB signaling pathway is required for the apoptosis-evasion in human salivary adenoid cystic carcinoma: its inhibition by quercetin. Apoptosis 2010; 15: 850–863. doi:10.1007/s10495-010-0497-5

[20] Fu J, Xu Y, Yang Y et al. Aspirin suppresses chemoresistance and enhances antitumor activity of 5-Fu in 5-Fu-resistant colorectal cancer by abolishing 5-Fu-induced NF-κB activation. Sci Rep 2019; 9: 1–11. doi:10.1038/s41598-019-53276-1

[21] Li X, Guo S, Xiong XK et al. Combination of quercetin and Cisplatin enhances apoptosis in OSCC cells by downregulating xIAP through the NF-κB pathway. J Cancer 2019; 10: 4509–4521. doi:10.7150/jca.31045

[22] Zou H, Zheng YF, Ge W et al. Synergistic Anti-tumour Effects of Quercetin and Oncolytic Adenovirus expressing TRAIL in Human Hepatocellular Carcinoma. Sci Rep 2018; 8: 1–8. doi:10.1038/s41598-018-20213-7