Tropical Journal of Pharmaceutical Research April 2022; 21 (4): 707-714
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i4.4

Original Research Article

AKR1C1 overexpression attenuates the inhibitory effect of glycyrrhizic acid on gastric cancer cell proliferation and migration

Lian Qiao[1], Cheng Shi[2], Junxia Gao[1], Yalei Liu[1], Qian Zheng[1]*
[1]Experimental Center, [2]Department of Clinical Pharmacy, Hebei University of Chinese Medicine, Shijiazhuang City, Hebei Province 050000, China

For correspondence:* ***Email:* *zhengqian920@163.com;* ***Tel:*** *+86-0311-89926205*

Sent for review: 5 January 2022 *Revised accepted: 17 March 2022*

Abstract

Purpose: *To investigate the involvement of aldo-keto reductase family 1 member C1 (AKR1C1) in glycyrrhizic acid-mediated gastric cancer.*
Methods: *Immunohistochemistry, quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), and western blot were used to assess AKR1C1 expression in gastric cancer. Cell proliferation was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and colony formation assay. Apoptosis was evaluated by flow cytometry. Transwell and wound healing assays were performed to investigate cell invasion and migration, respectively.*
Results: *AKR1C1 was significantly upregulated in gastric cancer tissues and cells ($p < 0.01$). AKR1C1 knockdown suppressed cell proliferation, migration, and invasion of gastric cancer, but promoted cell apoptosis. Glycyrrhizic acid treatment reduced AKR1C1 expression in gastric cancer cells ($p < 0.05$). AKR1C1 overexpression attenuated the glycyrrhizic acid-induced increase in gastric cancer cell apoptosis as well as the decrease in cell proliferation, migration, and invasion.*
Conclusion: *AKR1C1 contributes to gastric cancer cell proliferation and metastasis and counteracts the suppressive effects of glycyrrhizic acid on gastric cancer cell proliferation and metastasis.*

Keywords: *AKR1C1, Gastric cancer, Proliferation, Metastasis, Glycyrrhizic acid*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Gastric cancer is one of the most prevalent malignancies and a leading cause of cancer-related death worldwide [1]. Advanced gastric cancer is a major health issue, with a patient five-year survival rate of less than fifty percent [2]. Therefore, more effective therapeutic strategies to treat gastric cancer are urgently required.

Aldo-keto reductase (AKR) family one member C1 (AKR1C1) belongs to the AKR family, functions as a reduced nicotinamide-adenine dinucleotide phosphate (NADPH)-dependent enzyme in the catalyzation of progesterone into 20-alpha-hydroxy-progesterone, and participates in hormone metabolism [3]. AKR1C1 overexpression promotes non-small-cell lung cancer cell metastasis [4], whereas AKR1C1 inhibition suppresses cervical cancer [5].

Moreover, AKR1C1 has been implicated in the drug-resistant pathogenesis of cancer [6], and AKR1C1 overexpression enhances metastatic bladder cancer cell resistance to cisplatin [7]. Furthermore, AKR1C1 is dysregulated in gastric cancer tissues [8]. However, the role of AKR1C1 in gastric cancer carcinogen metabolism remains unknown.

Glycyrrhizic acid, a bioactive triterpene glycoside isolated from *Glycyrrhiza glabra*, has an antitumor effect [9] and is toxic to gastric cancer cells [10]. An integrated approach has shown that AKR family one member C2 (AKR1C2) is a potential target of licorice in liver protection [11]. Furthermore, flavonoids, wogonin, and chrysin regulate AKR1C1 expression in drug-resistant non-small cell lung cancer cells [12]. Therefore, glycyrrhizic acid is hypothesized to regulate AKR1C1 expression in the progression of gastric cancer. In this study, the effects of AKR1C1 on the biological functions of gastric cancer cells were investigated, and the relationship between AKR1C1 and glycyrrhizic acid on gastric cancer progression was determined.

METHODS

Tissue samples and immunohistochemistry

Patients diagnosed with gastric cancer (n = 70) were recruited at the Hebei University of Chinese Medicine. Tumor and para-carcinoma tissues were collected from patients via surgery. This study was approved by the Hebei University of Chinese Medicine Ethics Committee (approval no. YXLL2018004) in accordance with the World Medical Association Declaration of Helsinki [13]. Written informed consent was acquired from the patients. The tissues were fixed in 10 % formalin and embedded in paraffin. The embedded tissues were then sliced into sections and subsequently incubated in citrate buffer (pH 6.0) and treated with 3 % hydrogen peroxide. The sections were blocked in normal rabbit serum and then probed with an anti-AKR1C1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Following incubation with peroxidase-labeled secondary antibodies (Santa Cruz Biotechnology) and 3,3'-diaminobenzidine, the sections were observed under an inverted microscope (Olympus, Tokyo, Japan).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Tissues were lysed and total RNA was extracted using the TRIzol method (Invitrogen, Carlsbad, CA, USA). The extracted RNAs were transcribed into cDNAs using the Reverse Transcription System (Applied Biosystems, Carlsbad, CA, USA). The resulting cDNA was used as a template for qRT-PCR analysis using SYBR Green Master Mix (Roche, Mannheim, Germany) and gene-specific primers. The sequences for primers that were used are as follows: AKR1C1 (Forward: 5'-GCCATATTGATTCTGCTCATTTAT-3' and Reverse: 5'-TGGGAATTGCTCCAAAGC-3') and GAPDH (Forward: 5'-GAAGGTGAAGGTCGGAGTC-3' and Reverse: 5'-GAAGATGGTGATGGGATTTC-3'). AKR1C1 gene expression was normalized to the endogenous control, GAPDH, using the $2^{-\Delta\Delta Ct}$ method.

Cell culture

Gastric cancer cells (AGS, HGC-27, and NCI-N87) and normal human gastric epithelial GES-1 cells were purchased from Beijing Northland Biotech Co. Ltd. (Beijing, China). Cells were cultured in a 37 °C incubator with 5 % CO_2 in RPMI-1640 medium containing 10 % fetal bovine serum (Invitrogen, Carlsbad, CA, USA).

Cell proliferation assays

AGS cells were seeded in 96-well plates (5×10^3 cells/well) and incubated for 24 hours and subsequently transfected for 48 hours with 20 nM siRNA targeting AKR1C1 (si-AKR1C1) or a negative control (si-NC) using Lipofectamine 3000 (Invitrogen). AGS cells were also transfected with 2 µg of pcDNA-AKR1C1 or pcDNA vector in the presence or absence of 4 mM glycyrrhizic acid (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). The cells were incubated for either 24, 48, or 72 hours and subsequently incubated with 10 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution (5 mg/mL) (Dojindo, Kumamoto, Japan) for another 4 hours. The medium was removed, dimethyl sulfoxide (DMSO) (150 µL) was added, and the absorbance at 490 nm was measured using a microplate reader (Sigma-Aldrich, St. Louis, MO, USA). For the colony formation assay, 8×10^2 AGS cells/well were seeded in 6-well plates and then transfected with si-AKR1C1 or si-NC. Cells were cultured in medium for 10 days, fixed, and stained with 0.1% crystal violet before measurement under a light microscope (Olympus).

Flow cytometry

AGS cells were harvested using trypsin. Harvested cells were resuspended in binding buffer from the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis kit (Nanjing KeyGen Biotech Co., Ltd.,

Nanjing, China) and subsequently stained with PI and annexin V-FITC. The stained cells were analyzed using an ACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

Cell migration and invasion assays

AGS cells were seeded in 6-well plates. Each well was scratched with a pipette tip and examined under a light microscope 24 hours later to calculate the wound width. For the transwell assay, AGS cells were harvested, resuspended in serum-free medium, and then added into the upper chamber of a matrigel (BD Biosciences)-coated well (Corning, Tewksbury, MA, USA). The lower chamber was filled with RPMI-1640 medium containing 15 % fetal bovine serum. After 24 hours, invasive cells in the lower chamber were fixed with 4 % paraformaldehyde, stained with 0.5 % crystal violet, and counted using a light microscope.

Western blot

Gastric cancer cells and tissues were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Beijing, China), and the protein concentration was determined using the bicinchoninic acid (BCA) Protein Assay Kit (Beyotime). Protein samples were separated by 10 % sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and were subsequently transferred onto a nitrocellulose membrane. The membrane was blocked in 5 % bovine serum albumin and then probed overnight at 4 °C with anti-AKR1C1 and anti-GAPDH (1:2000, Abcam, Cambridge, UK) antibodies. Following incubation with a peroxidase-conjugated secondary antibody (1: 3000, Abcam) and tetramethylbenzidine, protein bands were visualized using chemiluminescence (Sigma-Aldrich) and quantified using ImageJ (National Institutes of Health, Bethesda, MA, USA).

Statistical analysis

All data with at least three replicates is expressed as mean ± standard error of the mean (SEM), and analyzed by Student's t-test or one-way analysis of variance (ANOVA) using SPSS software. $P < 0.05$ was considered statistically significant.

RESULTS

AKR1C1 is upregulated in gastric cancer

Immunohistochemical analysis revealed higher AKR1C1 expression in gastric cancer tissues than in para-carcinoma tissues (Figure 1 A). Furthermore, elevated AKR1C1 mRNA expression was observed in tumor tissues (Figure 1 B) and cells (Figure 1 C). Gastric cancer cells (AGS, HGC-27, and NCI-N87) also expressed higher AKR1C1 protein levels than the normal human gastric epithelial GES-1 cells (Figure 1 D), demonstrating the potential relationship between AKR1C1 and the progression of gastric cancer.

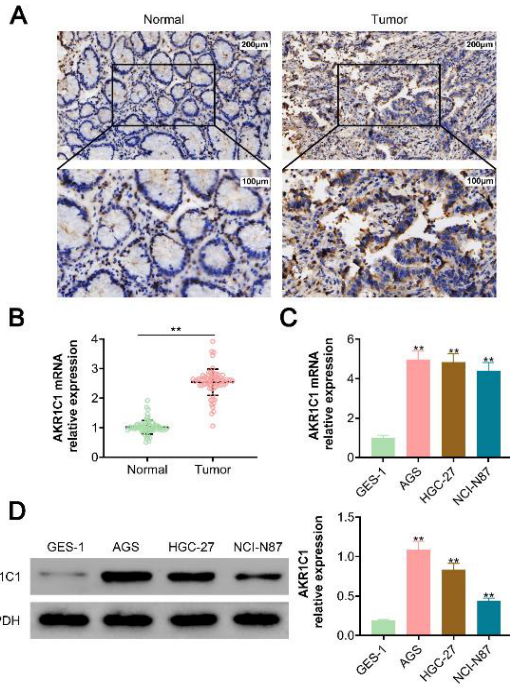

Figure 1: AKR1C1 is upregulated in gastric cancer. (A) Immunohistochemical analysis showed higher AKR1C1 expression in gastric cancer tissues than in para-carcinoma tissues. (B) Levels of AKR1C1 mRNA were elevated in gastric cancer tissues compared to para-carcinoma tissues. (C) AKR1C1 mRNA expression was upregulated in gastric cancer cells (AGS, HGC-27, and NCI-N87) relative to normal human gastric epithelial GES-1 cells. (D) Protein expression of AKR1C1 was upregulated in gastric cancer cells (AGS, HGC-27, and NCI-N87) compared to normal human gastric epithelial GES-1 cells. $^{**}P < 0.01$

AKR1C1 contributes to gastric cancer cell proliferation

Transfection with si-AKR1C1 reduced AKR1C1 protein expression (Figure 2 A). Furthermore, AKR1C1 knockdown decreased AGS cell viability (Figure 2 B) and suppressed AGS cell proliferation (Figure 2 C). Moreover, silencing AKR1C1 promoted AGC cell apoptosis (Figure 2 D), suggesting that AKR1C1 silencing has an anti-proliferative effect on gastric cancer.

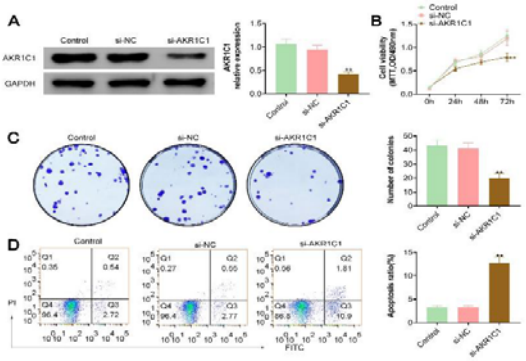

Figure 2: AKR1C1 contributes to gastric cancer cell proliferation. (A) Transfection with si-AKR1C1 reduced AKR1C1 protein expression in AGS cells. (B) AGS cell viability was reduced after si-AKR1C1 transfection. (C) Transfection with si-AKR1C1 suppressed AGS cell proliferation. (D) AGS cell apoptosis was promoted after transfection with si-AKR1C1. ***P* < 0.01

AKR1C1 contributes to gastric cancer cell metastasis

Transfection with si-AKR1C1 repressed AGC cell migration (Figure 3 A). Furthermore, AGS cell invasion was also suppressed after transfection with si-AKR1C1 (Figure 3 B). These results revealed that silencing AKR1C1 has an anti-invasive effect on gastric cancer.

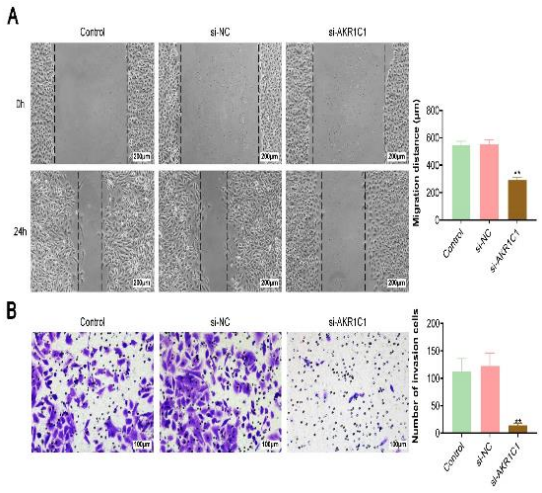

Figure 3: AKR1C1 contributes to gastric cancer cell metastasis. (A) AGS cell migration was repressed after transfection with si-AKR1C1. (B) Transfection with si-AKR1C1 repressed AGS cell invasion. ** vs. si-NC, *p* < 0.01

AKR1C1 overexpression counteracts the suppressive effect of glycyrrhizic acid on gastric cancer

Glycyrrhizic acid treatment reduced AKR1C1 protein expression in a dose-dependent manner (Figure 4). However, when the glycyrrhizic acid-induced AGS cells were subsequently transfected with pcDNA-AKR1C1, AKR1C1 protein levels were restored (Figure 5 A). AKR1C1 overexpression in AGS cells attenuated the glycyrrhizic acid-induced decrease in cell viability (Figure 5 B) and increase in cell apoptosis (Figure 5 C). Moreover, the suppressive effects of glycyrrhizic acid on AGC cell migration (Figure 6 D) and invasion (Figure 6 E) were also reversed by AKR1C1 overexpression. These results indicate that the glycyrrhizic acid-mediated suppression of gastric cancer progression occurs through AKR1C1 downregulation.

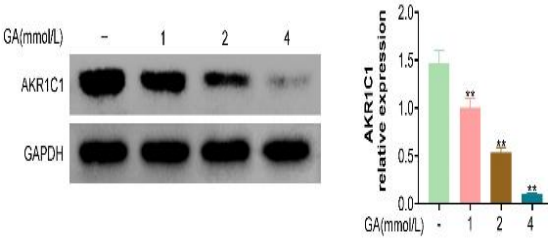

Figure 4: Glycyrrhizic acid treatment reduces AKR1C1 expression in gastric cancer. AKR1C1 protein expression was reduced in AGS cells after glycyrrhizic acid treatment in a dose-dependent manner; ***p* < 0.01

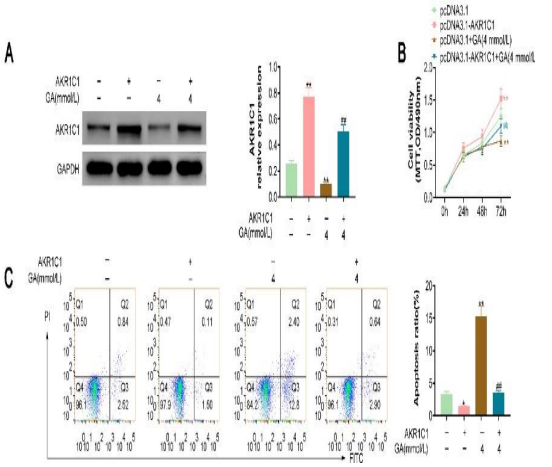

Figure 5: AKR1C1 overexpression counteracts the suppressive effect of glycyrrhizic acid (GA) on gastric cancer. (A) Transfection with pcDNA-AKR1C1 attenuated the glycyrrhizic acid-induced decrease in AKR1C1 in AGS cells. (B) Transfection with pcDNA-AKR1C1 attenuated the glycyrrhizic acid-induced decrease in AGS cell viability. (C) Transfection with pcDNA-AKR1C1 attenuated the glycyrrhizic acid-induced increase of AGS cell apoptosis. *P* < 0.01

DISCUSSION

The AKR1C members regulate progesterone metabolism and participate in tumorigenesis [14].

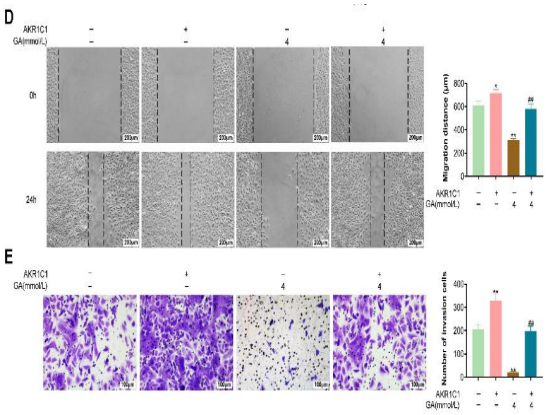

Figure 6: AKR1C1 overexpression counteracts the suppressive effect of glycyrrhizic acid (GA) on gastric cancer. (D) Transfection with pcDNA-AKR1C1 attenuated the glycyrrhizic acid-induced decrease of AGS cell migration. (E) Transfection with pcDNA-AKR1C1 attenuated the glycyrrhizic acid-induced decrease of AGS cell invasion. ** vs. 0 mM GA, $p < 0.01$. ## vs. 4 mM GA+pcDNA. $P < 0.01$

Moreover, AKR1Cs are coactivators or E3 ubiquitin ligase system regulators of tumor cell growth, apoptosis, metastasis, and sensitivity [14]. AKR1C1 inhibitors are used in the treatment of colorectal, breast, or endometrial cancers [14]. This study investigated the functional role of AKR1C1 in gastric cancer.

The results of the present study suggest that AKR1C1 is upregulated in gastric cancer tissues and cells. A previous study found that low AKR1C1 expression predicts a favorable prognosis for patients with advanced nasopharyngeal carcinoma [15]. The relationship between AKR1C1 expression and clinical characteristics of gastric cancer patients should be assessed to clarify the diagnostic and prognostic roles of AKR1C1 in gastric cancer. Functional assays in this study identified the oncogenic role of AKR1C1 in gastric cancer. Our findings indicate that loss of AKR1C1 in gastric cancer cells promotes cell apoptosis and reduces cell proliferation, migration, and invasion. Additionally, a previous study determined that loss of AKR1C1 promotes chemosensitivity of nasopharyngeal carcinoma cells to cisplatin [15]. Future research should investigate the effect of AKR1C1 on gastric cancer cell chemosensitivity.

The traditional Chinese herb, licorice, exerts antitumor effects [16]. Recent findings show that glycyrrhizic acid, a bioactive triterpene glycoside in licorice, suppresses gastric cancer cell proliferation and metastasis [10,17]. Our results indicate that glycyrrhizic acid treatment reduces gastric cancer cell viability, migration, and invasion and stimulates gastric cancer cell apoptosis. Moreover, liquiritin, the main flavonoid of licorice, is a natural AKR1C1 inhibitor that also interferes with progesterone metabolism [18]. This study shows that AKR1C1 protein expression is downregulated in gastric cancer cells after glycyrrhizic acid treatment. Furthermore, our findings indicate AKR1C1 overexpression counteracts the suppressive effects of glycyrrhizic acid on gastric cancer cell proliferation and metastasis. The phosphatidylinositol 3-kinase (PI3K)/serine-threonine kinase (AKT) pathway is involved in the glycyrrhizic acid-mediated suppression of gastric cancer [17], and AKR1C1 regulates signal transducer and activator of transcription (STAT) signaling to mediate tumor progression and drug resistance [4, 19]. Therefore, the signaling involved in glycyrrhizic acid-AKR1C1-mediated gastric cancer progression should be further investigated.

In summary, the results of this study suggest that AKR1C1 is upregulated in gastric cancer and functions as a gastric cancer oncogene by repressing cell apoptosis and promoting cell proliferation and metastasis. Furthermore, these findings indicate that AKR1C1 attenuates the glycyrrhizic acid-mediated suppression of gastric cancer. A xenografted animal model should be used to investigate the *in vivo* effect of AKR1C1 on gastric cancer growth.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and that all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Lian Qiao and Cheng Shi designed the study and supervised the data collection. Junxiao Gao and Yalei Liu analyzed the data and interpreted the data. Qian Zheng prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved this manuscript. Lian Qiao and Cheng Shi contributed equally to the work and should be considered co-first authors.

Open Access

REFERENCES

1. *Ye X, Liu H, Zeng Z. Long non-coding RNA KCNQ1 opposite transcript 1 (KCNQ1OT1) regulates cell proliferation and invasion via targeting miR-124-3p in gastric carcinoma. Trop J Pharm Res 2021; 20(1): 1-9.*
2. *Suzuki H, Oda I, Abe S, Sekiguchi M, Mori G, Nonaka S, Yoshinaga S, Saito Y. High rate of 5-year survival among patients with early gastric cancer undergoing curative endoscopic submucosal dissection. Gastric Cancer 2016; 19(1): 198-205.*
3. *Jin Y, Duan L, Chen M, Penning TM, Kloosterboer HJ. Metabolism of the synthetic progestogen norethynodrel by human ketosteroid reductases of the aldo-keto reductase superfamily. J Steroid Biochem Mol Biol 2012; 129(3-5): 139-144.*
4. *Zhu H, Chang L-L, Yan F-J, Hu Y, Zeng C-M, Zhou T-Y, Yuan T, Ying M-D, Cao J, He Q-J, et al. AKR1C1 Activates STAT3 to Promote the Metastasis of Non-Small Cell Lung Cancer. Theranostics 2018; 8(3): 676-692.*
5. *Wei X, Wei Z, Li Y, Tan Z, Lin C. AKR1C1 Contributes to Cervical Cancer Progression via Regulating TWIST1 Expression. Biochem Genet 2021; 59(2): 516-530.*
6. *Selga E, Noé V, Ciudad C. Transcriptional regulation of aldo-keto reductase 1C1 in HT29 human colon cancer cells resistant to methotrexate: Role in the cell cycle and apoptosis. Biochem Pharmacol 2008; 75: 414-426.*
7. *Matsumoto R, Tsuda M, Yoshida K, Tanino M, Kimura T, Nishihara H, Abe T, Shinohara N, Nonomura K, Tanaka S. Aldo-keto reductase 1C1 induced by interleukin-1β mediates the invasive potential and drug resistance of metastatic bladder cancer cells. Sci Rep 2016; 6: 34625-34625.*
8. *Chang H-C, Chen Y-L, Chan C-P, Yeh K-T, Kuo S-J, Ko C-J, Fang H-Y. Overexpression of dihydrodiol dehydrogenase as a prognostic marker in resected gastric cancer patients. Digest Disand Sci 2009; 54(2): 342-347.*
9. *Hussain H, Ali I, Wang D, Hakkim L, Westermann B, Ahmed I, Ashour A, Khan A, Hussain A, Green I, et al. Glycyrrhetinic acid: A Promising Scaffold for the Discovery of Anticancer Agents. Expert Opin Drug Dis 2021; 16.*
10. *Mehdinejadiani K, Shirzad H, Fakhari S, Jalili A. An Evaluation the Effect of Glycyrrhetinic and Glycyrrhizic Acids Derived from Licorice Extract on Gastric Cancer Cell Lines. J Babol Univy Med Sci 2015; 17: 53-59.*
11. *Chen L, Hu C, Hood M, Kan J, Gan X, Zhang X, Zhang Y, Du J. An Integrated Approach Exploring the Synergistic Mechanism of Herbal Pairs in a Botanical Dietary Supplement: A Case Study of a Liver Protection Health Food. Int J Genomics 2020; 2020: 9054192-9054192.*
12. *Wang H-W, Lin C-P, Chiu j-c, Chow K-C, Kuo K-T, Lin C-S, Wang L-S. Reversal of inflammation-associated dihydrodiol dehydrogenases (AKR1C1 and AKR1C2) overexpression and drug resistance in non-small cell lung cancer cells by wogonin and chrysin. Int J Cancer 2007; 120: 2019-2027.*
13. *Association WM. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization 2001; 79(4): 373.*
14. *Zeng C-M, Chang L-L, Ying M-D, Cao J, He Q-J, Zhu H, Yang B. Aldo-Keto Reductase AKR1C1-AKR1C4: Functions, Regulation, and Intervention for Anti-cancer Therapy. Front Pharmacol 2017; 8: 119-119.*
15. *Zhou C, Shen G, Yang F, Duan J, Wu Z, Yang M, Liu Y, Du X, Zhang X, Xiao S. Loss of AKR1C1 is a good prognostic factor in advanced NPC cases and increases chemosensitivity to cisplatin in NPC cells. J Cell Mol Med 2020; 24(11): 6438-6447.*
16. *Nazari S, Rameshrad M, Hosseinzadeh H. Toxicological Effects of Glycyrrhiza glabra (Licorice): A Review. Phytother Res 2017; 31(11): 1635-1650.*
17. *Wang H, Ge X, Qu H, Wang N, Zhou J, Xu W, Xie J, Zhou Y, Shi L, Qin Z, et al. Glycyrrhizic Acid Inhibits Proliferation of Gastric Cancer Cells by Inducing Cell Cycle Arrest and Apoptosis. Cancer Manag Res 2020; 12: 2853-2861.*
18. *Zeng C, Zhu D, You J, Dong X, Yang B, Zhu H, He Q. Liquiritin, as a Natural Inhibitor of AKR1C1, Could Interfere With the Progesterone Metabolism. Front Physiol 2019; 10(833).*
19. *Chang W-M, Chang Y-C, Yang Y-C, Lin S-K, Chang PM-H, Hsiao M. AKR1C1 controls cisplatin-resistance in head and neck squamous cell carcinoma through cross-talk with the STAT1/3 signaling pathway. J Exp Clin Canc Res 2019; 38(1): 245-245.*