

## Original Article

# Pathogenesis and anti-proliferation mechanisms of Crocin in human gastric carcinoma cells

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**Abstract:** Gastric cancer is the fourth most common cause of cancer death globally and the second most common in Asia. Many studies suggest that Crocin has the potential for gastric cancer antineoplastic combined chemotherapy protocols. Here we investigated genomic changes related to the inhibitory effect of Crocin, and elucidated the molecular mechanism of this inhibition in gastric carcinoma cells. We found that, compared with the control group, 216 significantly upregulated and 301 significantly downregulated genes were identified in Crocin-treated AGS cells. Many of these differentially expressed genes in AGS cells are involved in Nrf2-mediated oxidative stress response, p53 signaling, and integrin signaling, which suggested the mechanism of Crocin functions in therapy of gastric cancer. In summary, our study indicates that Crocin has the potential for gastric cancer adjuvant treatment through reducing cell oxidative stress levels.

**Keywords:** Crocin, gastric cancer, IPA, Nrf2, oxidative stress

## Introduction

Saffron is used not only as highly valued natural food coloring and flavoring in European and Asian countries widely, but also used as traditional medicine has a thousand years of use history [1, 2]. Crocin is considered to be the main medicinal ingredient in saffron, which has been reported the antitumor activity both in animal models and cell models of several carcinomas, such as pancreatic, breast, colorectal, ovarian, prostate, hepatic and gastric carcinoma [1-3].

Gastric cancer is the fourth most common cancer globally and the second most common cause of death from cancer in Asia [4]. In the study by Bathaie et al, the beneficial effect of saffron aqueous extract (SAE) on 1-methyl-3-nitro-1-nitrosoguanidine (MNNG)-induced gastric cancer in rats was explored, thus the investigators recommend Crocin as a potential anti-cancer agent [5]. Crocin is also used as a gastric mucosa protectant. There is a gastro-protective effect of Crocin against indomethacin-induced gastric lesions by inhibition of MDA, reduction in iNOS and caspase-3, and inhibition of the

reduction in mucus content induced by indomethacin [6], and a gastro-protective effect of Crocin against ethanol-induced gastric lesions by significant down-regulation of cytochrome c and caspase-3 mRNA expression, significant decrease in caspase-3 activity, and mitigated DNA fragmentation [7]. Cisplatin plays an important role in the treatment of gastric cancer, but often grade 3-4 toxicities occur [8]. In our previous research, Crocin combined with cisplatin presented a potential anticancer drug for the treatment of gastric cancer [8]. Compared with anti-tumor drugs (docetaxel, 5-fluorouracil and platinum drugs) in the treatment of gastric cancer, Crocin has less toxicity and higher pharmaceutically safe dosage [9, 10]. These features suggest that Crocin has the potential for gastric cancer antineoplastic combined chemotherapy protocols.

The anti-proliferation mechanisms of Crocin in gastric cancer remain unclear. In the present study, we evaluated the anti-proliferation effects of Crocin on gastric cancer cell lines AGS and SGC-7901. DNA microarray analysis was selected to reveal the transcriptome's differentially expressed genes (DEGs) between

the Crocin treatment group and untreated AGS cells. Subsequently, the DEGs were analyzed according to gene ontology (GO), KEGG pathway enrichment analysis, co-expression and protein-protein interaction (PPI) analysis. These findings may provide insights on Crocin in the adjuvant treatment of gastric cancer, and provide therapeutic targets for future research.

### Materials and methods

#### *Cell lines and cell culture*

Human gastric carcinoma cell line AGS and SGC-7901 were obtained from the Cell Resource Center of Shanghai Institutes for Biological Sciences and cultured in DMEM (Corning, USA) containing 10% FBS (Ausbian, Australia), 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

#### *Cytotoxicity assay*

Cell viability assay was measured with Cell Counting Kit 8 (CCK-8). Briefly, cells were plated at a density of  $4 \times 10^3$  cells/well on 96-well plates. Crocin (Sigma, USA) and cisplatin (Sigma, USA) were added at different concentrations single dose or in combination. 24 hours after stimulation, the cell viability was measured by CCK-8 from Sigma according to the manufacturer's instructions.

#### *Extraction, quantitation, and quality verification of total RNA*

AGS cells were treated without or with Crocin at 2.4 mg/ml for 72 h. This dose was selected on our preliminary results as well as the previous observation that Crocin at this dose significantly suppressed estrogen-induced proliferation of AGS cells. After being treated with Crocin, total RNA of AGS cells was extracted according to the Isolation Protocols provided in TRIzol™ RNA Isolation Kit. After being completely dissolved, the concentration and quality of the isolated total RNA from AGS cells of Crocin-treated group (DRUG) and control (NC) group were quantified with NanoDrop™ 2000 (Thermo Scientific, USA). The quality and integrity of total RNA were checked with Agilent Bioanalyzer 2100. The values of A260/A280 ratio of all the total RNA samples were in the range of 1.97-2.05, the R<sub>in</sub> values were in the range of 8.1-

9.6, and the values of the 28S/18S ratio were in the range of 1.5-1.8, indicating that total RNA samples isolated from AGS cells of both groups were well qualified for use in gene expression analysis with Gene Expression Array.

#### *Analysis of gene expression profiles induced by Crocin treatment with human gene expression array and data preprocessing*

The microarray platform in this study was Genechip PrimeView™ human (Affymetrix, USA). The gene expression profile was preprocessed using Limma [11] (version 3.83, linear models for microarray data, [www.bioconductor.org/packages/2.8/bioc/html/limma.html](http://www.bioconductor.org/packages/2.8/bioc/html/limma.html)) package in Bioconductor Affymetrix annotation files from Brain Array Lab (version 20, [http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/CDF\\_download.asp](http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/CDF_download.asp)) [12]. The background correction, quantile normalization and probe summarization of the microarray data were performed using the Robust Multi-Array Average algorithm [13] to obtain the gene expression matrix.

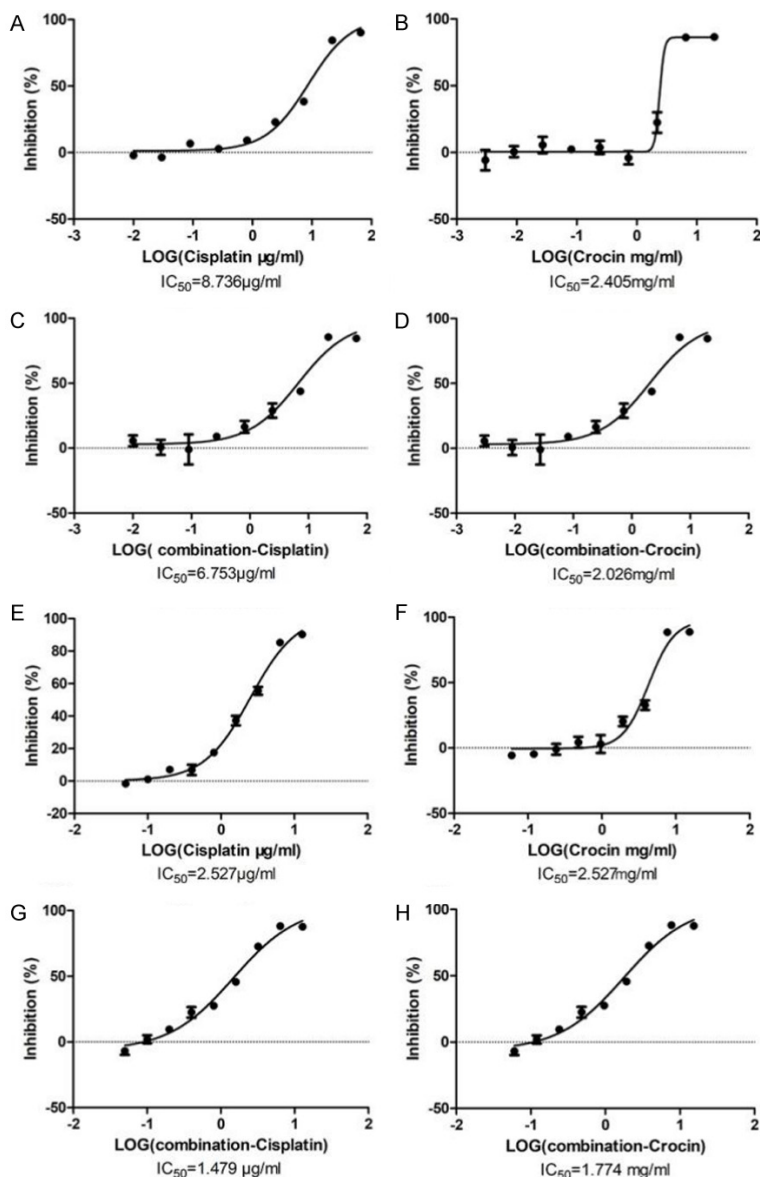
#### *Identification of differentially expressed genes (DEGs)*

The normalized data were calculated with the Limma package [11], and genes with  $P < 0.05$  and  $|\log_2 \text{fold change}| \geq 1.5$  were considered to indicate a statistically significant difference between groups.

#### *Bioinformatics analysis*

IPA of differentially expressed genes (DEGs) was conducted with Qiagen's Ingenuity Pathway Analysis algorithm ([www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity), Qiagen, Redwood City, CA, USA). Canonical pathway analysis, functional analysis, upstream analysis, regulatory effect analysis, and interaction network analysis were performed. The activation z-score and overlapping  $p$ -value were calculated as described in a previous work [14]. Gene set enrichment analysis (GSEA) and Metascape analysis was performed to detect if a series of pre-defined biological processes or gene sets were enriched in the gene rank derived from differentially expressed genes between Crocin-treated AGS cells and control cells.

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**Figure 1.** Dose-response curve of anti-proliferation activity of the drugs in different gastric carcinoma cell lines ( $n = 3$ ). (A) Cisplatin in AGS cells, (B) Crocin in AGS cells, (C) combination of Crocin and Cisplatin in AGS cells (Cisplatin), (D) combination of Crocin and Cisplatin in AGS cells (Crocini), (E) Cisplatin in SGC-7901 cells, (F) Crocin in SGC-7901 cells, (G) combination with Crocin and Cisplatin in SGC-7901 cells (Cisplatin), (H) combination of Crocin and Cisplatin in SGC-7901 cells (Crocini).

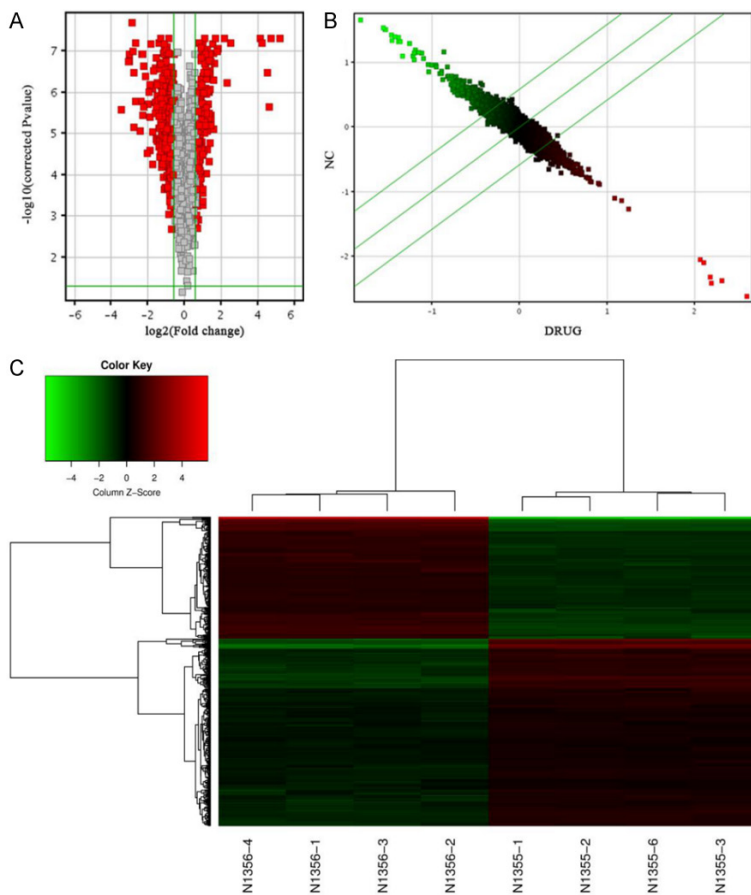
## Results

### Effects of Crocin on cell viability assay

To assess effects on in vitro cell proliferation, we treated human gastric carcinoma cell line AGS and SGC-7901 with Crocin and monitored growth with a Cell Counting Kit-8 (CCK8) and a colony forming assay. As shown in **Figure 1**, the

CCK8 assays found that cells incubated with certain concentrations of Crocin and Cisplatin had significantly reduced cell amounts compared with DMSO-treated control cells. Moreover, the growth response of cells varied in response to stimulation by different Crocin or Cisplatin concentrations. This cytotoxicity effect was verified by gastric carcinoma AGS cells (**Figure 1A, 1B**) and another gastric carcinoma cell line, SGC-7901 (**Figure 1E, 1F**). AGS cells were cultured with Cisplatin (0.01, 0.03, 0.09, 0.27, 0.81, 2.43, 7.29, 21.87, 65.61  $\mu\text{g}\cdot\text{ml}^{-1}$ ) for 48 h. It was observed from the cell growth curve that Cisplatin inhibited cell growth, and the inhibitory effect increased with the increase of drug concentration. AGS cells were cultured with Crocin (0.003, 0.009, 0.027, 0.081, 0.243, 0.729, 2.187, 6.561, 19.68  $\text{mg}\cdot\text{ml}^{-1}$ ) for 48 h. From the cell growth curve, Crocin inhibited the growth of AGS cells, and the inhibition on cell proliferation increased with increasing concentration. When the two drugs were combined, both IC<sub>50</sub>s were down-regulated. Cisplatin AGS 48 h IC<sub>50</sub> was 6.753  $\mu\text{g}\cdot\text{ml}^{-1}$ , and Crocin AGS 48 h IC<sub>50</sub> was 2.026  $\text{mg}\cdot\text{ml}^{-1}$ , both of which were not significantly down-regulated, and the measurement point of the synergism effect level of the two drugs did not

fall on the added line (**Figure 1C, 1D**). More evidence was obtained from SGC-7901 cells. When the two drugs were combined, both IC<sub>50</sub>s were down-regulated. Cisplatin SGC-7901 48 h IC<sub>50</sub> was 1.479  $\mu\text{g}\cdot\text{ml}^{-1}$ , and Crocin SGC-7901 48 h IC<sub>50</sub> was 1.774  $\text{mg}\cdot\text{ml}^{-1}$ , both of which were not significantly down-regulated. The measurement point of the synergism effect level of the two drugs did not fall on the added line



**Figure 2.** Differentially expressed genes between Crocin-treated group (DRUG) and control (NC) group. A. Volcano Plot, demonstrating the distribution of the differentially expressed genes between Crocin-treated group and control group. The X-axis represents the logarithm conversion of the fold difference to base 2 and the Y-axis represents the logarithm conversion of the corrected significant levels to base 10. The red color represents all the probes with fold difference > 1.5 at significance  $P < 0.05$ . B. Scatter plot, which exhibits the distribution of the signals between the TAM-treated group and control group in a Cartesian coordinate plane. The X-axis represents the TAM-treated group, and the Y-axis represents the control group. The ordinate value and the abscissa of each spot represent the expression values of one probe in the Crocin-treated group and control group. The parts above the green lines represent the down-regulated probes in relative to the control group. The parts underneath the green lines represent the up-regulated probes as compared to those of the control group. C. Number of significantly changed genes. G2710-1, G2710-2, G2710-3 and G2710-4 were the Crocin-treated group (DRUG), while G2711-1, G2711-2, G2711-3 and G2711-4 were the control (NC).

(Figure 1G, 1H). The results thus confirm that Crocin had a cytotoxicity effect on the growth of human gastric carcinoma cells. Combined with cisplatin, the inhibition of cell proliferation was enhanced, but a synergistic effect was not achieved. These results suggest that Crocin has different sensitivity to proliferation inhibition in different gastric cancer cell lines.

*Effects of Crocin on AGS cell gene expression*

In this study, gene expression was analyzed using Affymetrix GeneChip PrimeView Human Gene Expression Arrays. Pearson's correlation coefficient between the pooled and non-pooled RNA samples was > 0.95, suggesting that the results of the gene chip array were reliable. A heatmap of the microarray created upon comparison of the differential gene expression of all samples suggested that many genes were significantly altered by Crocin (Figure 2C). The volcano plots in Figure 2A show the overall features of the gene sets in AGS cells exposed to Crocin. After data standardization and analysis, 216 significantly up-regulated genes and 301 significantly downregulated genes were identified ( $|fold\ change| > 1.5$  and  $P < 0.05$ ) between the Crocin-treated group (DRUG) and control (NC) group (Figure 2B). The up-regulated genes included AKR1C1/AKR1C2, CYP1A1, MMP1, ETV6, CST1, GDF15, ALDH3A1, SQSTM1, AKR1C3, AKR1B10, ALPP, CYB5B, TIPARP, PIR, FTH1, INSIG1, PHLDA1, IDI1, SOS1 and GPX2 and their expression levels were up-regulated by 36.735 to 2.501 folds by Crocin. The down-regulated genes included CHI3L1, TAGLN, PAGE4, BST2, PLP1, APO-L2, APOL1, CDKN1C, DMBT1, COL4A1, IFI27, TGM2, PSG5, DPYSL3, VTN, REG1A, SYNPO,

NAV2, TCF4, ID2, EPST1, OLR1, OGDHL, FILIP1L, DDX60, MX2, CDH17, FOLR1, MYL7, NPPB, HPGD, PRF1, PRSS23, RARRES3, ESRRG and WSB1 and their mRNA levels were down-regulated by 11.202 to 2.529 folds by Crocin (Table 1). Many of these DEGs are involved in cell death, morbidity or mortality differentiation of cells.

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**Table 1.** Differentially expressed genes between Crocin-treated group (DRUG) and control (NC) group

Gene name	Fold Change	Location	Molecule type
AKR1C1/AKR1C2	36.735	Cytoplasm	Enzyme
CYP1A1	18.246	Cytoplasm	Enzyme
MMP1	5.679	Extracellular Space	Peptidase
ETFB	4.523	Cytoplasm	Transporter
CST1	3.462	Extracellular Space	Other
GDF15	3.172	Extracellular Space	Growth factor
ALDH3A1	2.99	Cytoplasm	Enzyme
SQSTM1	2.967	Cytoplasm	Transcription regulator
AKR1C3	2.891	Cytoplasm	Enzyme
AKR1B10	2.708	Cytoplasm	Enzyme
ALPP	2.692	Plasma Membrane	Phosphatase
CYB5B	2.658	Cytoplasm	Enzyme
TIPARP	2.657	Nucleus	Enzyme
PIR	2.6	Nucleus	Transcription regulator
FTH1	2.596	Cytoplasm	Enzyme
INSIG1	2.596	Cytoplasm	Other
PHLDA1	2.596	Cytoplasm	Other
IDI1	2.511	Cytoplasm	Enzyme
SOS1	2.51	Cytoplasm	Other
GPX2	2.501	Cytoplasm	Enzyme
WSB1	-2.529	Cytoplasm	Other
ESRRG	-2.548	Nucleus	Ligand-dependent nuclear receptor
RARRES3	-2.554	Cytoplasm	Enzyme
PRSS23	-2.586	Extracellular Space	Peptidase
PRF1	-2.603	Cytoplasm	Other
HPGD	-2.612	Cytoplasm	Enzyme
NPPB	-2.631	Extracellular Space	Other
MYL7	-2.663	Cytoplasm	Enzyme
FOLR1	-2.685	Plasma Membrane	Transporter
CDH17	-2.687	Plasma Membrane	Transporter
MX2	-2.695	Nucleus	Enzyme
DDX60	-2.736	Cytoplasm	Enzyme
FILIP1L	-2.746	Nucleus	Other
OGDHL	-2.853	Other	Enzyme
OLR1	-2.854	Plasma Membrane	Transmembrane receptor
EPSTI1	-2.875	Other	Other
ID2	-2.996	Nucleus	Transcription regulator
TCF4	-3.037	Nucleus	Transcription regulator
NAV2	-3.068	Nucleus	Other
SYNPO	-3.382	Cytoplasm	Other
REG1A	-3.387	Extracellular Space	Growth factor
VTN	-3.689	Extracellular Space	Other
DPYSL3	-3.722	Cytoplasm	Enzyme
PSG5	-3.733	Extracellular Space	Other
TGM2	-3.799	Cytoplasm	Enzyme
IFI27	-3.918	Cytoplasm	Other
COL4A1	-4.481	Extracellular Space	Other



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DMBT1	-4.753	Plasma Membrane	Transmembrane receptor
CDKN1C	-4.958	Nucleus	Other
APOL1	-5.121	Extracellular Space	Transporter
APOL2	-5.408	Cytoplasm	Other
PLP1	-6.564	Plasma Membrane	Other
BST2	-6.813	Plasma Membrane	Other
PAGE4	-7.452	Other	Other
TAGLN	-8.562	Cytoplasm	Other
CHI3L1	-11.202	Extracellular Space	Enzyme

**Table 2.** The most enriched metabolic signaling pathways

Ingenuity Canonical Pathways	$-\log(p\text{-value})$	z-score	Molecules
Nrf2-mediated Oxidative Stress Response	4.01	3.162	MGST1, NQO1, GCLC, DNAJC15, MAP3K5, TXNRD1, FGFR3, HMOX1, FOS, GPX2, GCLM, SQSTM1, EPHX1, FTH1
p53 Signaling	3.78	2.121	FGFR3, TP53INP1, GADD45B, CDKN1A, TIGAR, MDM2, BAX, TNFRSF10A, FAS, TP53I3
Type I Diabetes Mellitus Signaling	2.53	2	SOCS3, PRF1, GAD1, HLA-B, SOCS2, MAP3K5, HLA-F, FAS
Integrin Signaling	1.64	-2.333	ARHGAP5, FGFR3, MYL9, DOCK1, TLN2, SOS1, RHOU, PPP1CB, VCL, MYL7

Significantly changed canonical pathways and a representative pathway. Four significantly altered canonical pathways. The chosen thresholds were a  $|\text{fold change}| > 2$  and a  $P < 0.05$ . A z-score  $> 2$  indicates significant activation of the pathway, and a z-score  $< -2$  indicates significant inhibition.

### Canonical pathways of DEGs in AGS cells exposed to Crocin

IPA was used to further explore potential signaling pathways, and 378 pathways were identified. A total of 4 pathways were significantly altered (with at least a  $|\text{fold change}| > 2$  and a  $P < 0.05$ ) (**Table 1**), with 1 significantly inhibited and 3 activated. Of note, Nrf2-mediated Oxidative Stress Response, p53 Signaling and Integrin Signaling pathway are considered to be closely related to cancer. The ratio of the number of DEGs to the total number of genes in the pathways ranged from 4.6% to 11.8%. Based on an IPA prediction algorithm for molecular activation, the Nrf2-mediated Oxidative Stress Response pathway was found to be the top canonical pathway and was mapped by IPA, as shown in **Table 2**. The Nrf2-mediated Oxidative Stress Response pathway controls the expression of genes whose protein products are involved in the detoxication and elimination of reactive oxidants and electrophilic agents through conjugative reactions and by enhancing cellular antioxidant capacity by MGST1, NQO1, GCLC, DNAJC15, MAP3K5, TXNRD1, FGFR3, HMOX1, FOS, GPX2, GCLM, SQSTM1, EPHX1, FTH1 and other proteins. Additionally, the p53 signaling pathway was significantly changed and was mapped by IPA. Significantly downregulated genes included

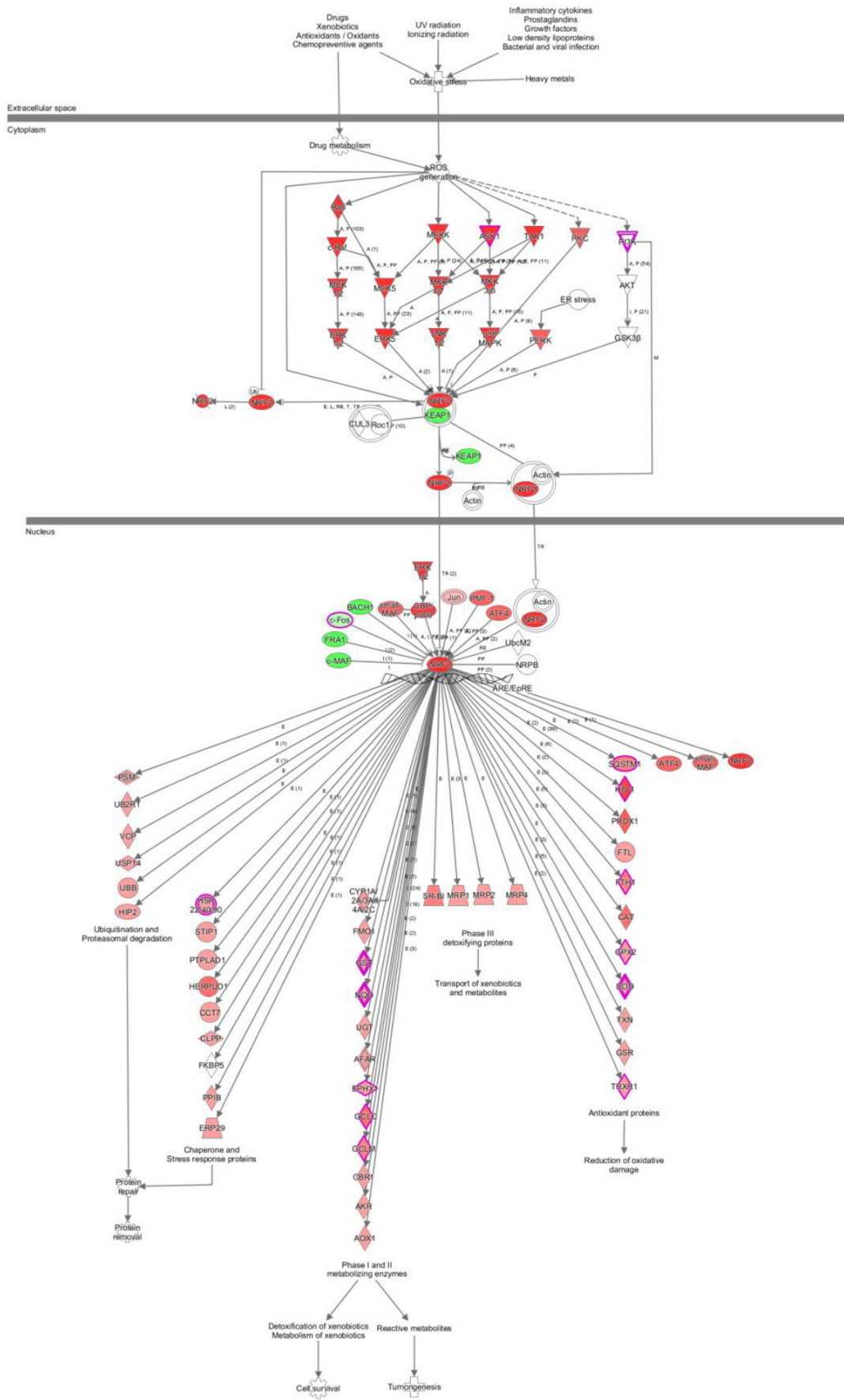
FGFR3, TP53INP1, GADD45B, CDKN1A, TIGAR, MDM2, BAX, TNFRSF10A, FAS, TP53I3 (**Figure 3**).

Metascape analysis was performed to identify the biological pathways and protein complexes between Crocin-treated AGS cells and control cells. The DGEs compared between these two groups are enriched in biologic processes, such as cofactor metabolic process, cholesterol biosynthesis, and monocarboxylic acid metabolic process (**Figure 4**). We also performed a GSEA analysis using the microarray dataset to gain further insight into biological processes in which Crocin may be involved. This analysis was performed to enrich gene sets from DGEs between Crocin-treated AGS cells and control cells. GSEA revealed that genes associated with telomere, protein DNA complex subunit organization, oxidoreductase activity, and nucleosome organization were very enriched in Crocin-treated AGS cells (**Figure 5**), suggesting that Crocin may be involved in these biologic processes of cancer progression.

### Discussion

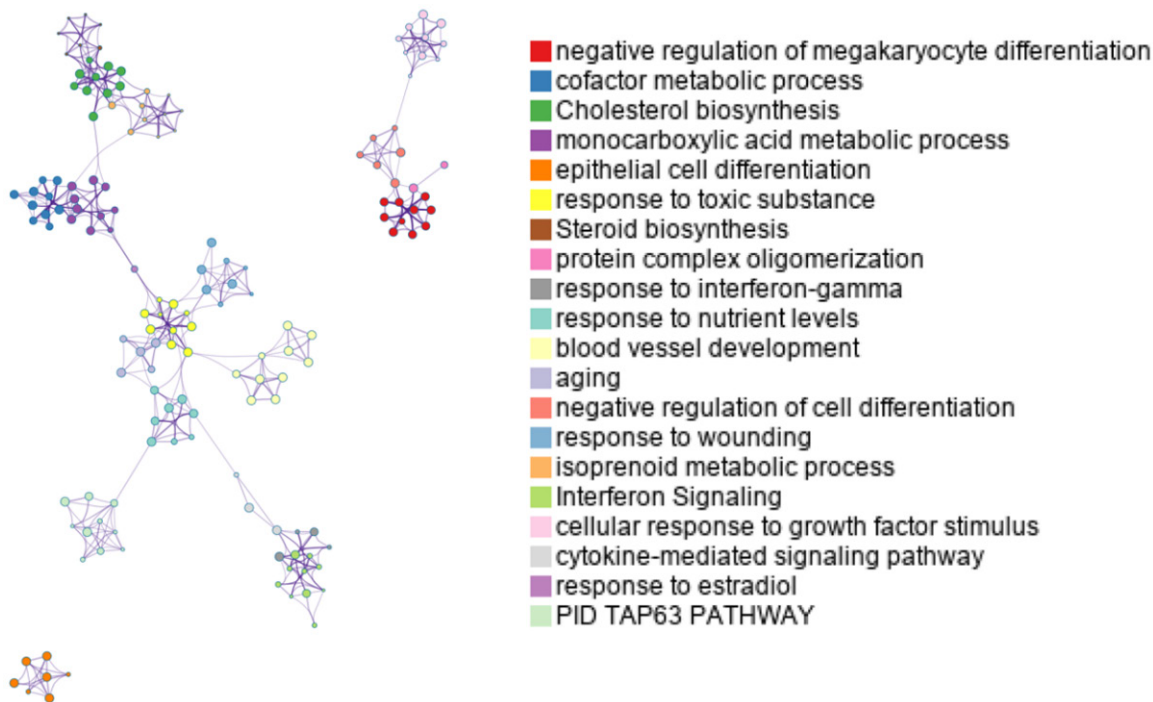
Saffron, a traditional coloration and medical reagent source, has received interest because of its beneficial biologic activities, including its anticancer activity [8-10]. Its anticancer effect

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**Figure 3.** The sumoylation pathway was the top significantly altered signaling pathway and was mapped by IPA. Green: downregulated. Red: upregulated. Reddish-purple border: genes significantly changed by APR.



**Figure 4.** Identification of Crocin-associated biologic processes by GSEA. The analysis showed genes related to telomere capping, telomere organization, protein DNA complex subunit organization, oxidoreductase activity, oxidation reduction process, and nucleosome organization were significantly enriched in Crocin-treated AGS cells.

stems from its constituent carotenoids, including Crocin and crocetin. It was previously shown that Crocin exerts cytotoxic activities. MTT assay showed that the IC<sub>50</sub> values of Crocin on the AGS cells were 2.7 mg/mL after 48 h; caspase-dependent apoptosis played an important role in Crocin-induced AGS growth inhibition [15]. In this study, we evaluated the anti-proliferation effects of Crocin on gastric cancer cell lines AGS and SGC-7901. Crocin revealed a dose-dependent cytotoxic effect against an AGS and SGC-7901 cell line, as determined by CCK-8 assay. Crocin had a cytotoxic effect on the growth of human gastric carcinoma cells. Combined with cisplatin, the inhibition of cell proliferation was enhanced, and combinational treatment of Crocin and cisplatin was more effective than each of them individually. Mollaie's research showed that treatment of cervical cancer cells with Crocin and cisplatin could reduce the expression level of Sox2 and Nanog and also increase the percentage of apoptotic cells and cytotoxicity of cisplatin in these cells [16]. These results suggest that

Crocin causes different sensitivity to proliferation inhibition of different gastric cancer cell lines because of the heterogeneity of tumor cell lines.

In this study, gene expression profiling was used to investigate the molecular anti-proliferation mechanisms of Crocin on gastric cancer cell lines AGS by microarray and IPA analysis. A set of 216 upregulated and 301 downregulated DEGs were identified between Crocin-treated group (DRUG) and control (NC) group. Analysis of the PPI sub-network demonstrated that 56 DEGs were obtained, and AKR1C1/AKR1C2, CYP1A1 and CHI3L1 had > 10 degrees significant differences in expression. Pathway enrichment analysis revealed that these four genes were enriched in the Nrf2-mediated Oxidative Stress Response. p53 Signaling and Integrin Signaling pathway are considered to be closely related to gastric cancer.

Chitinase 3 like 1 (CHI3L1) has been previously reported to induce angiogenesis in cervical



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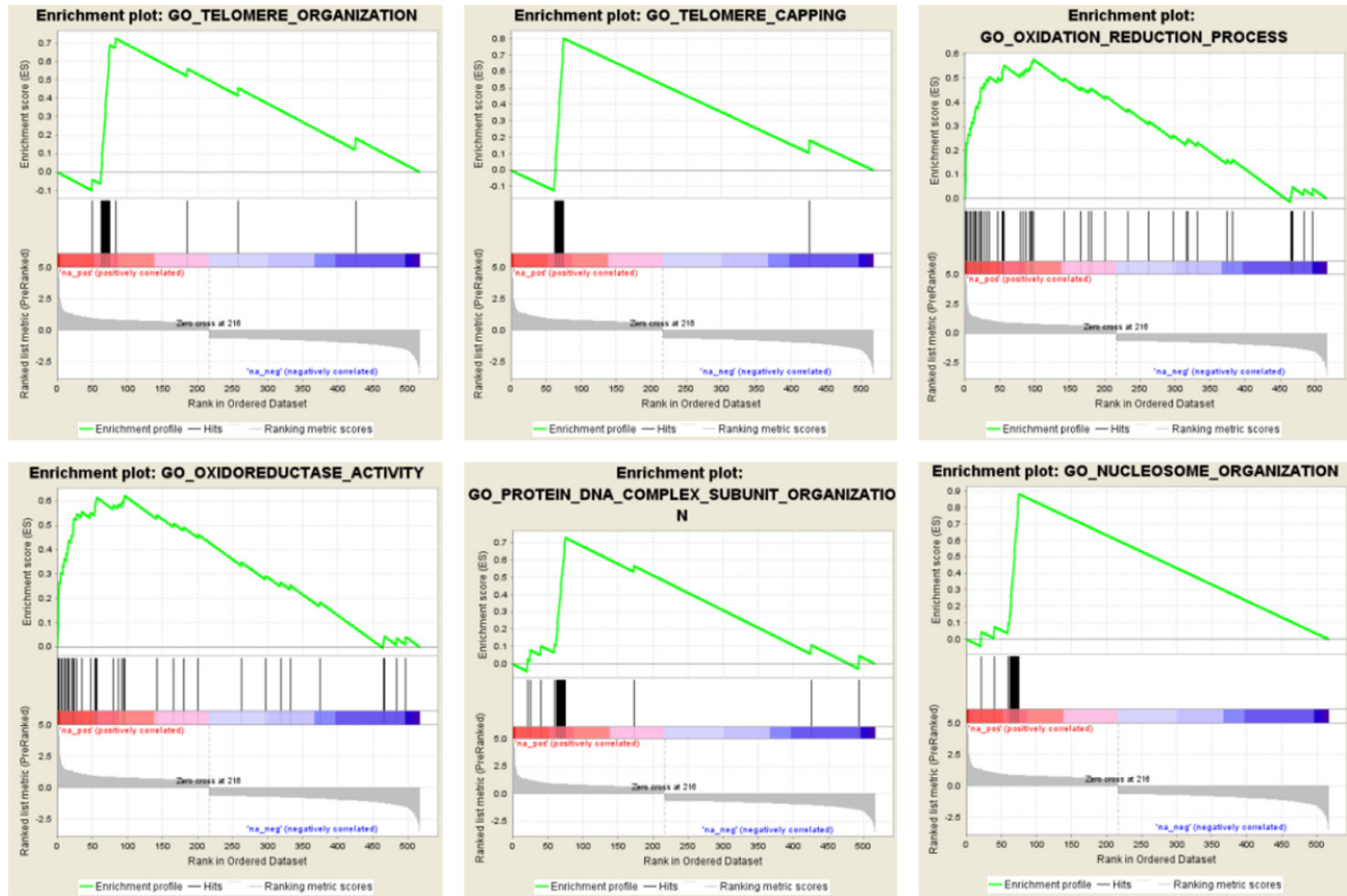


Figure 5. Metascape analysis of differentially expressed genes (DEGs) in Crocin-treated AGS cells, compared with control.

cancer [17]. High expression of CHI3L1 is an unfavorable prognostic factor in urothelial carcinoma of upper urinary tract and urinary bladder [18] and CHI3L1 promotes tumor progression by activating TGF- $\beta$  signaling pathway in hepatocellular carcinoma [19]. CHI3L1 triggered the activation of the mitogen-activated protein kinase signaling pathway, leading to the upregulated expression of matrix metalloproteinase genes, which promoted gastric tumor metastasis [20]. Therefore, high expression of CHI3L1 has been found to be associated with tumorigenesis and progression. Targeting CHI3L1 may be a valuable strategy for gastric tumor treatment. It is interesting to note that Crocin-treated AGS cells showed a significant downregulation of CHI3L1 (11.202 Fold Change).

AKR1C1 and AKR1C2 proteins are members of the AKR superfamily of enzymes, which are involved in the maintenance of steroid hormone homeostasis and in the regulation of prostaglandin metabolism, and have been implicated in biosynthesis, intermediary metabolism, and detoxification. Recent studies have provided evidence of strong correlation between the expression levels of these family members and malignant transformation as well as resistance to cancer therapy [21]. AKR1Cs (AKR1C1/AKR1C2) are classical antioxidant response element (ARE) genes that can be transcriptionally upregulated by nuclear factor erythroid 2-related factor 2 (Nrf2) [22]. The Nrf2 not only protects normal cells from transforming into cancer cells, but may also facilitate cancer cell proliferation and prolong survival [23]. Nrf2 expression in gastric cancer may be useful for evaluation of biologic malignant potential, which may be mediated in part by Nrf2 enhancement of the antioxidant ability of gastric cancer cells. Antioxidant therapy might be a promising approach for the treatment of Nrf2 positive gastric cancer patients [24]. In this study, Crocin as a highly effective antioxidant carotenoid component, showed a significant upregulation of AKR1C1/AKR1C2 (36.735 Fold Change) and significant activation of Nrf2 pathway in AGS cells.

However, the present study has a number of limitations. The studies should also be conducted using real-time quantitative PCR (RT-qPCR). Western blotting and RNA interference (RNAi) were used to elucidate the signal-

ing pathways, upstream regulation, regulatory effects and gene interaction. These limitations need to be addressed in a further study.

In summary, our results demonstrate an anti-cancer effect of Crocin, that is a combination of its effects to inhibit the tumor cell growth and induce tumor cell apoptosis by upregulating AKR1Cs and downregulating CHI3L1 expression. Crocin has the potential for gastric cancer adjuvant treatment through activation of the Nrf2 pathway reducing cell oxidative stress levels. These results provide a theoretical basis for a subsequent experimental study, and may contribute to an improved understanding of molecular mechanisms in gastric cancer.

### Acknowledgements

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### Disclosure of conflict of interest

None.

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