

RESEARCH ARTICLE

# The Natural Flavonoid Fisetin Inhibits Cellular Proliferation of Hepatic, Colorectal, and Pancreatic Cancer Cells through Modulation of Multiple Signaling Pathways

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## Abstract

Digestive cancers are major causes of mortality and morbidity worldwide. Fisetin, a naturally occurring flavonoid, has been previously shown anti-proliferative, anti-cancer, neuroprotective, and antioxidant activities. In our study, the anti-tumor activities in addition to regulatory effects of fisetin on some cancer cell lines were investigated. Data presented here showed that fisetin induces growth inhibition, and apoptosis in hepatic (HepG-2), colorectal (Caco-2) and pancreatic (Suit-2) cancer cell lines. Gene expression results showed that 1307 genes were significantly regulated in their expression in hepatic and pancreatic cell lines. 350 genes were commonly up-regulated and 353 genes were commonly down-regulated. Additionally, 604 genes were oppositely expressed in both tumor cells. *CDK5* signaling, NRF2-mediated oxidative stress response, glucocorticoid signaling, and *ERK/MAPK* signaling were among most prominent signaling pathways modulating the growth inhibitory effects of fisetin on hepatic and pancreatic cancer cells. The present analysis showed, for the first time, that the anti-tumor effect of fisetin was mediated mainly through modulation of multiple signaling pathways and via activation of *CDKN1A*, *SEMA3E*, *GADD45B* and *GADD45A* and down-regulation of *TOP2A*, *KIF20A*, *CCNB2* and *CCNB1* genes.

## Introduction

Digestive cancers are major causes of cancer mortality and morbidity in the world [1]. Gastrointestinal cancers include malignancies arising in the esophagus, gallbladder, pancreas, liver and bile ducts, small intestine, stomach, colon and rectum. The incidence and mortality rates of these tumors differ significantly. Approximately, one-fifth of the cancer incidence and nearly one-fourth of the cancer related deaths in the US were due to gastrointestinal cancers. Colorectal and gastric cancers are the most common gastrointestinal cancers all over the world [2]. The 5-year survival is 90% and 63%, respectively, when these malignancies are detected

early and at localized stage [2,3]. Pancreatic cancer is one of the most lethal human cancers, with almost identical incidence and mortality rates [4]. Early detection of gastrointestinal tumors may significantly reduce deaths, and thereby, identification of novel biomarkers for early detection is an urgent need [3,5].

Recently, attention has been focused on the use of natural products, especially dietary sources, and their semi-synthetic derivatives to overcome human diseases including tumors [6]. Previous reports suggested that consumption of natural products including vegetables and fruits is associated with decreased incidences of many chronic diseases, including tumors. Recently, a study reported that some bioactive compounds in plants can activate or suppress multiple signaling pathways through targeting small molecules in cancer cells indicating the massive impact that natural products may have [7,8].

Fisetin (3,7,3',4'-tetrahydroxyflavone) is a polyphenol, naturally occurring flavonoid and abundantly found in fruits and vegetables such as apple, strawberry, grape, persimmon, cucumber, and onion [9]. Previously, fisetin has shown anti-proliferative, anticancer, neuro-protective, and antioxidant activities [10–12]. In recent years, Fisetin has been reported to inhibit cell proliferation, migration and invasion, and induce apoptosis in several cancer types, such as colon cancer [13], glioma cancer [14], lung cancer [15], nasopharyngeal carcinoma [16], prostate cancer [17], and bladder cancer [18] and cervical carcinoma [19]. It also inhibits micro-ophthalmia associated transcription factor (MITF) in melanoma cells and inhibits invasion of melanoma cells via modulation of the MAPK and NF- $\kappa$ B pathways [20–22]. Recently, it was reported that fisetin causes cell cycle arrest, apoptosis (caspase-dependent), and potentiate the anti-tumor effect of Chemotherapeutics in triple-negative breast cancer cells [23].

Here, the growth inhibitory effect of fisetin on human cancer cell lines representing three different tumor types, hepatic, colorectal and pancreatic tumors, was investigated. Our results showed that fisetin inhibited cellular growth and proliferation, induced apoptosis, through activation of caspases in these tumors. Furthermore, expression analysis results pointed out that fisetin growth inhibitory effect was modulated through multiple signaling pathways including *CDK5* signaling, NRF2-mediated oxidative stress response, glucocorticoid signaling, and *ERK/MAPK* signaling. The present analysis showed, for the first time, that the anti-tumor effect of fisetin was mediated mainly through activation of *CDKN1A*, *SEMA3E*, *GADD45B* and *GADD45A* and down-regulation of *TOP2A*, *KIF20A*, *CCNB2* and *CCNB1* genes.

The present analysis is aimed to be a starting point for generation of hypotheses on significantly regulated candidate genes and for a more detailed functional analysis of individual transcripts for the activity of fisetin in tumor cells.

## Materials and Methods

### Cell lines and materials

The human hepatocellular carcinoma (HepG-2), the colorectal carcinoma (CaCo-2) and the human resistant pancreatic cancer (Suit-2), cells were used in our study. Cells were cultured and treated as previously described [4,24–26]. Briefly, cells were maintained in DMEM (Invitrogen, Carlsbad, CA) medium, supplemented with penicillin, and streptomycin and fetal bovine serum (FCS) (Invitrogen, Carlsbad, CA). All cell lines were tested with Mycoplasma detection kits (Minerva Biolabs, Berlin, Germany) and shown to be free of mycoplasma as previously mentioned [26].

### Effect of fisetin on cellular viability as determined by SRB assay

In order to evaluate the growth inhibitory effect of fisetin on different cancer cell lines, the colorimetric assay SRB (Sulforhodamine B assay) was used as previously described [27,28].

Briefly, cells were cultured with or without gradually increasing doses of fisetin for 48 h. After medium aspiration, the cells were fixed, stained and incubated at room temperature for 30 min. After washing, tris-base solution (10 mM) was added to dissolve retained SRB dyes. Finally, absorbance was measured using a precision microplate reader (Molecular Devices, Sunnyvale, CA).

### Caspase-glo 3/7 assay

The effect of fisetin on apoptosis was investigated by caspase 3/7 activity assay as we previously described [24,28,29]. Cells were either treated with fisetin ( $2 \times IC_{50}$ ;  $IC_{50}$  and  $\frac{1}{2} \times IC_{50}$ ) or untreated (control). After 6 h treatment, caspase reagent was added to each well, mixed and incubated for 1 h at room temperature. Luminescence was then measured and caspase activity was expressed as percentage of the untreated control.

### Quantitative determination of prostaglandin E2

In order to investigate the inhibitory effect of fisetin on prostaglandin E2 (PGE2) production, the PGE2 EIA Kit (Cayman Chemicals, Michigan, USA) was utilized as we previously described [4,30]. Briefly, twenty four hours after fisetin treatment, cell lines were stimulated with arachidonic acid and prostaglandin E2 levels were estimated by a competitive enzyme immunoassay relative to untreated control.

### Expression profiling using array techniques

Cells were either treated with 10  $\mu$ M fisetin or with DMSO (control) for 48 hours. RNA extraction, purification and microarray processing has been carried out as we described previously [4,24,26,29].

### Quantitative Real-Time RT-PCR Assays

The same RNA samples used for expression analysis experiment were used for RT-PCR (quantitative real-time) experiment for independent verification of the expression differences on some transcripts selected during analysis (e.g. *Top2A*, *CDKN1A*, *GADD45B*, *GADD45A* and *CCNB2* and *SEMA3E*) with QuantiTect SYBR Green Kit (Qiagen) as previously described [24,29,31].

### Identification of networks and signaling pathways

Here, Ingenuity Pathway Analysis software (IPA) (Mountain View, USA) was used to identify list of networks of genes and canonical pathways modulated due to fisetin treatment of liver and pancreatic cancer cell lines ([www.ingenuity.com](http://www.ingenuity.com)). Using Ingenuity Pathway Analysis tool, significantly regulated transcripts were grouped and classified based on their biological importance and pathways involved in their effects.

### Data analysis

Microarray optimization, normalization and cluster analysis were performed as described in our previous studies [24–26,29]. Briefly, microarray quality assessment, correspondence cluster analysis and normalization were performed with the MIAME compatible analysis. Only variations with a p value of less than 5% were taken into account. Cluster analysis was performed using correspondence analysis [24–26,29,31].

## Cytochrome P450 (CYP3A4) inhibition assay

In order to monitor the inhibitory effect of fisetin on recombinant human CYP3A4, CYP450-Glo™ assay (Promega, Mannheim, Germany) was used according to manufacturer instructions [32]. At room temperature, equal volumes of different compound concentrations and a reaction mixture containing CYP3A4 specific substrate (luciferin 6-benzyl ether in phosphate buffer) and CYP3A4 were incubated for ~10 min. The enzyme reaction was initiated after addition of NADPH regeneration system in citrate buffer. Thirty minutes later, 50µl luciferin was added. After 20 min, luminescence was recorded using a Tecan™ Safire II reader. Each experiment has been done at least 3 times (6 replica each). Ketoconazole was used as a positive control.

## Inhibition of Glutathione-S-transferase (GST) assay

In order to evaluate the possible inhibitory effect of fisetin on Glutathione-S-transferase enzyme, GST enzyme activity assay has been carried out. The method has been done as described previously [33]. Using 1-chloro-2,4-dinitrobenzene (CDNB) as GST substrate. Briefly, untreated and treated cell lysates were used for this assay by preparing a sample with a standard assay mixture containing CDNB, reduced glutathione (GSH) and PBS buffer. The reaction was detected spectrophotometry at 340 nm.

## Results

### Fisetin inhibits cellular proliferation and viability of hepatic, colorectal and pancreatic cancer cell lines

In order to investigate the cellular anti-proliferative effect of fisetin on different cancer cell lines, HepG-2, Caco-2 and Suit-2 cells were cultured and treated with increasing concentrations of fisetin for 48h. The rate of cellular viability and growth was assessed using SRB assay. Fisetin showed growth inhibitory effects in a concentration dependent manner (Fig 1). The human liver cancer cells (HepG-2, IC<sub>50</sub>: 3.2µM) were more sensitive to fisetin effect compared to colorectal (Caco-2, IC<sub>50</sub>: 16.4 µM) and pancreatic cancer cells (Suit-2, IC<sub>50</sub>: 8.1µM).

### Apoptotic induction by fisetin is dose-dependent

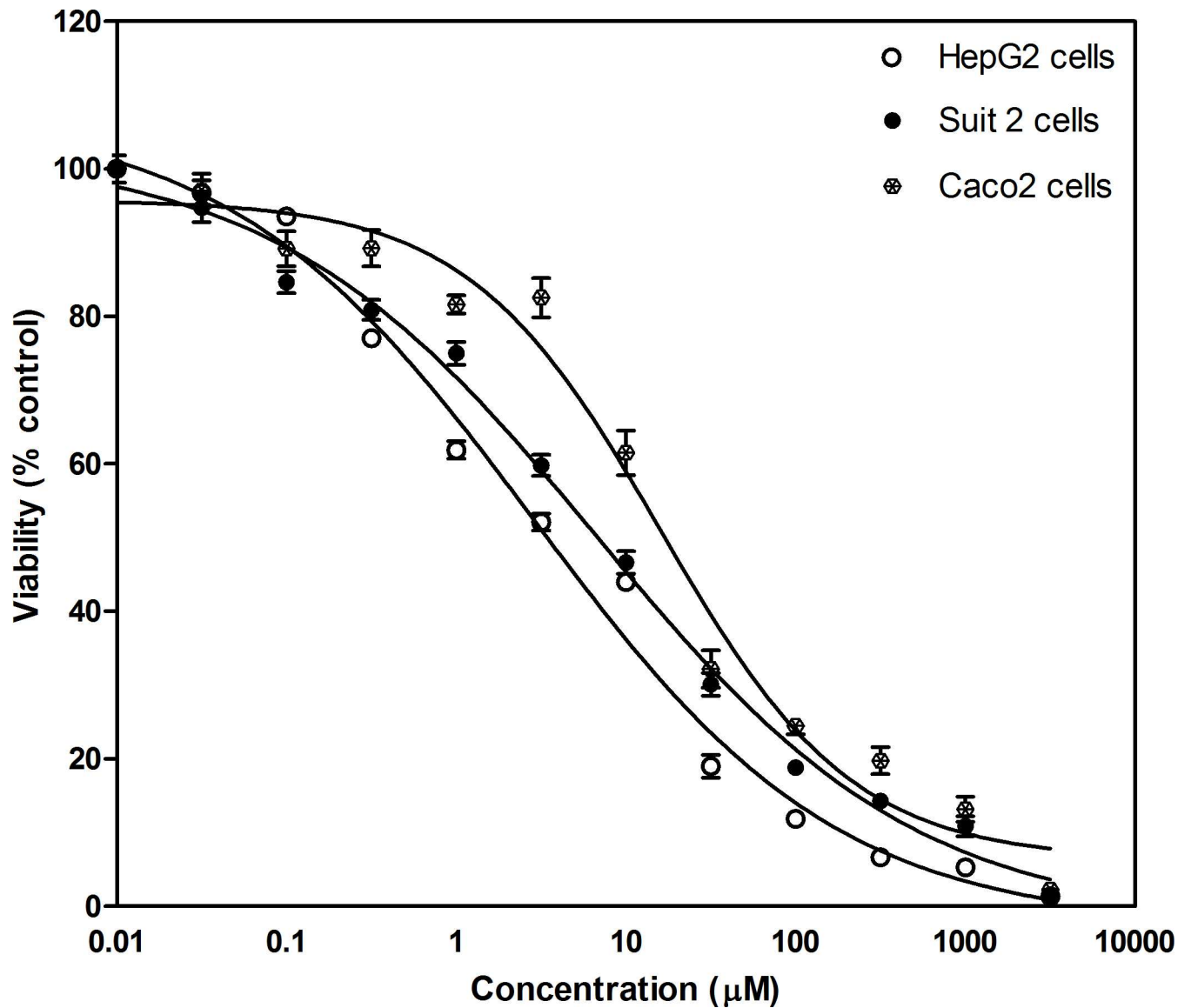
Induction of apoptotic cascade is one of the central mechanisms of chemotherapy-induced cell death [34]. Determining whether the chemo-preventative effect of fisetin demonstrated above was a result of its ability to activate the apoptotic pathway; HepG-2, Caco-2 and Suit-2 cells were treated with or without fisetin for 6 hours then the activity of caspase 3/7 was measured using the Caspase-Glo 3/7 assay kit. Fisetin caused significant increase in activation of caspase 3/7 compared to untreated control and we concluded that fisetin induced apoptosis was mediated through activation of apoptotic cascade (Fig 2).

### Fisetin inhibits PGE2 production

Here the colorectal cancer cell line Caco-2 with higher COX-2 expression was used for the assay [29]. In order to evaluate the anti-inflammatory effect of fisetin through inhibition of PGE2 production, the PGE2 levels produced by cancer cells after fisetin treatment were determined. Our results showed that fisetin significantly inhibited PGE2 production in a dose-dependent manner (Fig 3).

### Identification of target molecules and pathways mediating the anti-proliferative effect of fisetin on cancer cells

For the identification of molecular targets, pathways and networks involved in the cytotoxic effect of fisetin, HepG-2, and Suit-2 cells were treated with or without 10  $\mu$ M fisetin for 48h. Gene expression analysis has been carried out as previously described [24,26,29]. Microarray analysis results showed that fisetin commonly regulated 1307 genes in both HepG-2 and Suit-2 cells. 350 genes were commonly up-regulated and 353 genes were commonly down-regulated in both HepG-2 and Suit-2 cell lines. Additionally, 604 genes were oppositely expressed in both tumor cells. Regulated genes were mainly those involved in cell cycle, apoptosis, angiogenesis, and metastasis. The top 40 commonly up- and down-regulated in both cell lines were represented according to expression level in Suit-2 and HepG-2 cells, respectively (Fig 4).



**Fig 1. Fisetin inhibits growth of HepG-2, Caco-2 and Suit-2 cells.** Exponentially growing cells were cultured and treated with fisetin for 2 days. The SRB assay was then performed to assess cell viability and proliferation. Results are represented as mean value (M)  $\pm$  S.E.M. of at least 3 independent experiments (8 replica each).

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### Rt-PCR validation of expression analysis results

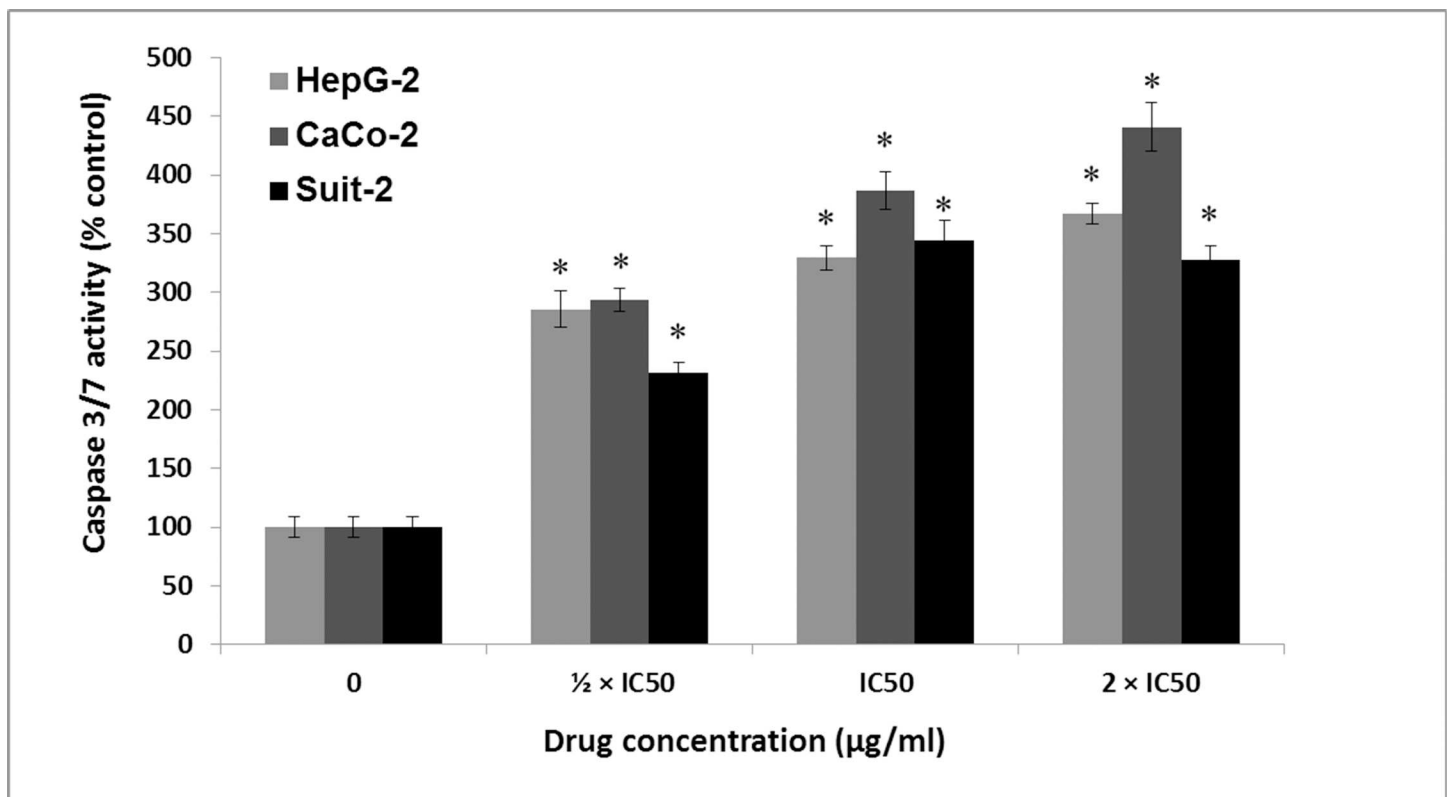
Expression of some significantly regulated genes after data analysis was then confirmed by RT-PCR, and normalized to GAPDH. Array results and Rt-PCR results were significantly matched (Table 1).

### Pathway analysis and classification of significantly regulated genes

In order to get deeper insights into canonical pathways and common mechanisms involved in the growth inhibitory effect of fisetin on hepatic and pancreatic cancer cells growth, the Ingenuity Pathway Analysis (IPA) tool was used [24]. The top five canonical pathways, regulated after fisetin treatment of HepG-2 cells were the mitotic roles of polo-like kinase, estrogen receptor signaling, protein ubiquitination pathway, cell cycle regulation (Fig 5.) and *NRF2*-mediated oxidative stress response. Additionally, the top five canonical pathways, regulated after fisetin treatment of Suit-2 cells were the *CDK5* signaling (Fig 6.), *ERK/MAPK* signaling, 4-1BB signaling in T-Lymphocytes, *NRF2*-mediated oxidative stress response, and *PI3K/AKT* signaling.

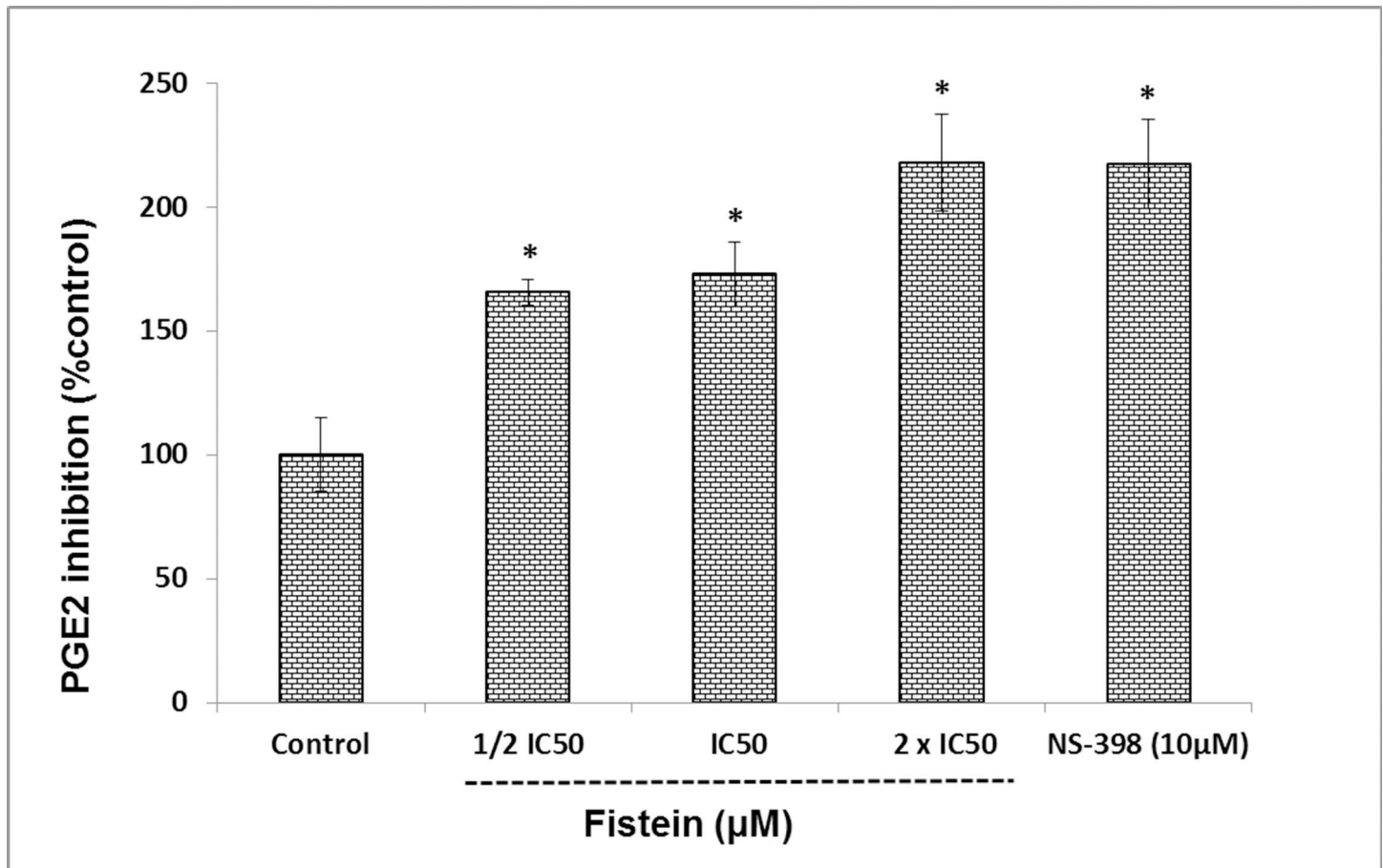
Moreover, the top molecular and cellular functions involved in the anti-proliferative effect of fisetin on HepG-2 cells were cell cycle, cell death and survival, protein synthesis, gene expression, and cellular growth and proliferation, while those for the effect of fisetin on Suit-2 cells were cellular growth and proliferation, cell death and survival, cellular development, cell cycle and cellular movement.

Biomarker analysis results showed that 1307 genes were significantly regulated in both cell lines after fisetin treatment. Common biomarkers significantly up-regulated in both cell lines



**Fig 2. Apoptotic effect of fisetin as shown by measuring caspase 3/7 activation.** Cells were treated with gradient concentrations of fisetin and caspase 3/7 assay was carried out. Results are presented as mean value (M) ± S.E.M. of at least 3 independent experiments (4 replica each), \* significantly different from control group (P < 0.001) using 2 way ANOVA test.

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**Fig 3. Inhibition of prostaglandin E2 after fisetin treatment of Caco-2 cells.** Results are represented as mean value (M) ± S.E.M of at least three independent experiments (4 replica each). \* Significantly different from control group (P< 0. 05) using one way ANOVA test.

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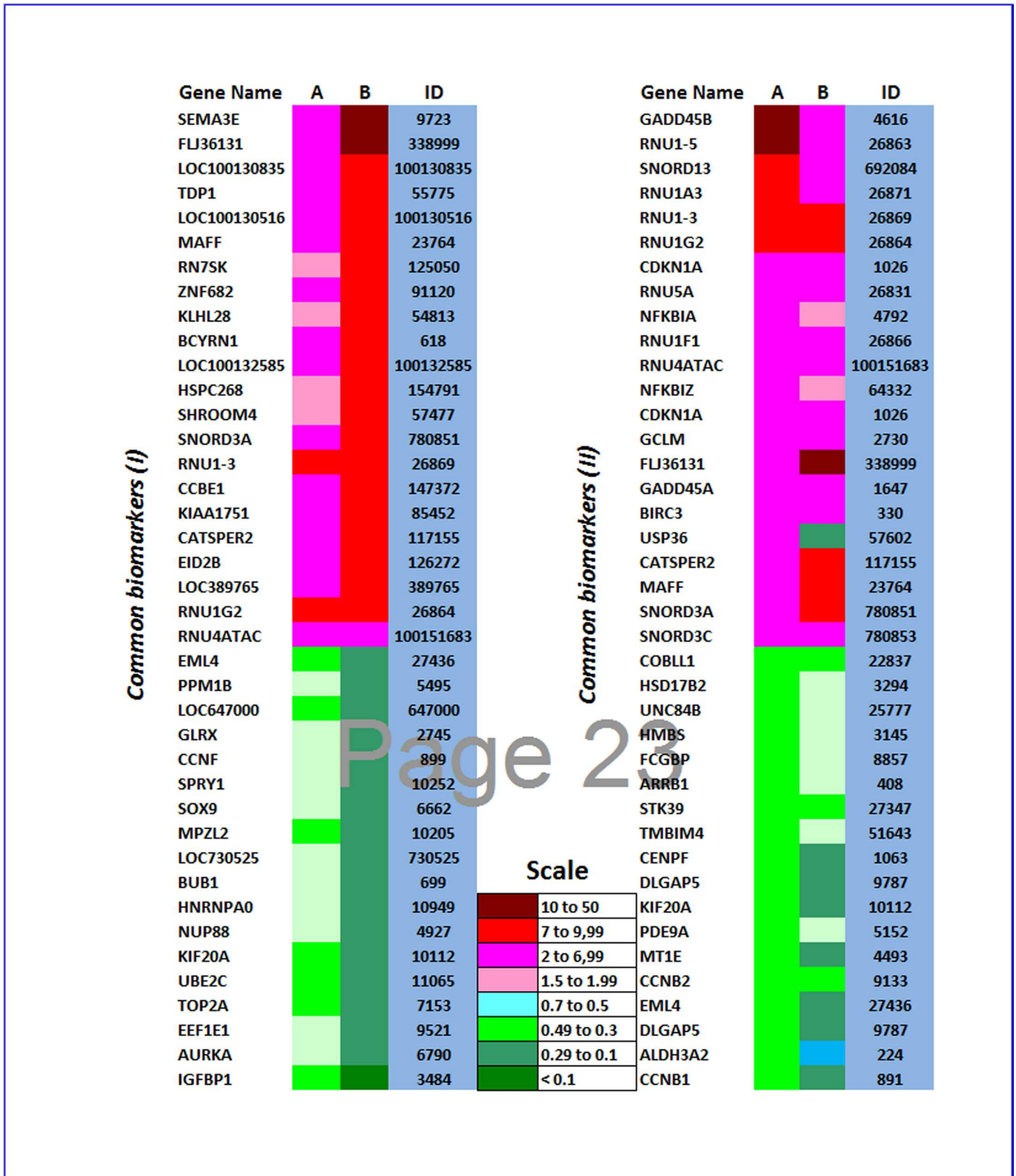
included 350 genes, while 353 were commonly down-regulated in both cell lines indicating a common set of genes modulating the antitumor effect of fisetin. It was obvious that the NRF2-mediated oxidative stress response was specifically regulated after fisetin treatment of both tumor types. Among genes involved in NRF2 pathway and were significantly altered in their expression in our study are *NRF2*, *JUN*, *SOD*, *MRP4*, *ASK1*, *PI3K*, *FRA1*, and *PMA1* genes (data not shown).

### Effect of fisetin on cellular metabolism (CYP3A4 assay)

To estimate the inhibitory effect of fisetin on CYP3A4, CYP450-Glo™ assay was carried out (Fig 7). The assay showed that fisetin caused a significant inhibition of CYP3A4 in a dose dependent manner and we concluded that fisetin induced cell death was, in part, due to its effect on cellular metabolism.

### Effect of fisetin on Glutathione-S-transferase enzyme (GST assay)

In order to evaluate the probable inhibitory effect of fisetin on Glutathione-S-transferase enzyme, GST enzyme activity assay has been carried out. The results showed that fisetin induced enzyme inhibition in a dose dependent manner (Fig 8).



**Fig 4. Top 40 common biomarker genes expressed in both Suit-2 (A) and HepG-2 (B) cells.** Arranged in a descending order according to gene expression levels in Suit-2 (I) and HepG-2 (II), respectively.

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**Table 1. Confirmation of microarray gene expression results with Rt-PCR.**

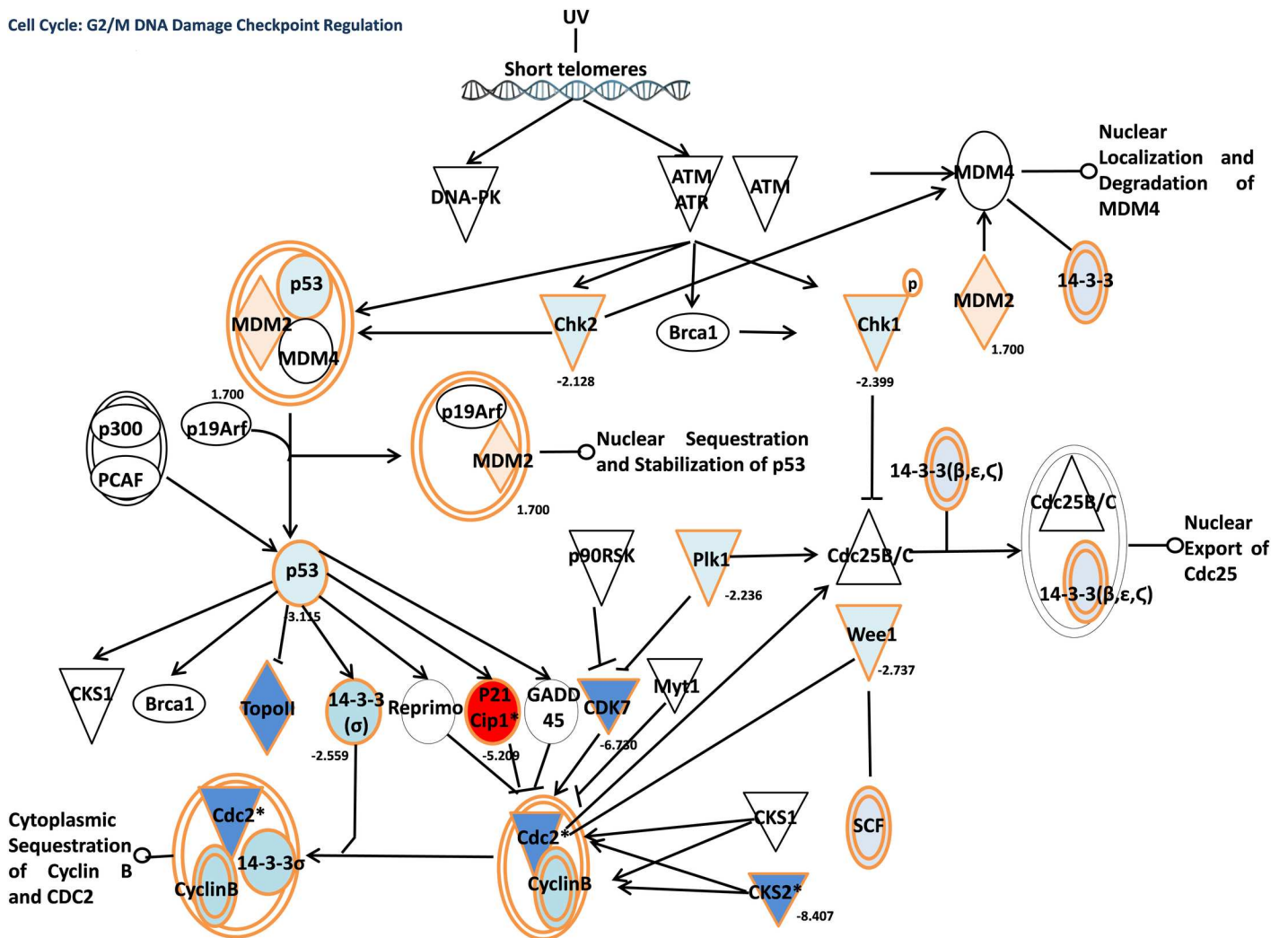
qRT-PCR verification of Microarrays results			
Official gene name	Gene Symbol	Fold change (Microarray)	Fold change (qRT-PCR)
Topoisomerase (DNA) II alpha	TOP2Aa	- 2,17	- 1,87
Cyclin Dependent Kinase Inhibitor 1A	CDKN1A	+ 6,65	+ 4,75
Growth Arrest And DNA Damage Inducible Alpha	GADD45A	+ 4,14	+ 5,50
Growth Arrest And DNA Damage Inducible Beta	GADD45B	+ 13,03	+ 8,95
Cyclin B2	CCNB2	- 2,94	- 3,45
Semaphorin 3E	SEMA3E	+ 2,53	+3,90

doi:10.1371/journal.pone.0169335.t001

## Discussion

The deleterious side effects of present clinically used anticancer agents augment cancer mortality and morbidity and underscore an urgent need for new and safer remedies.

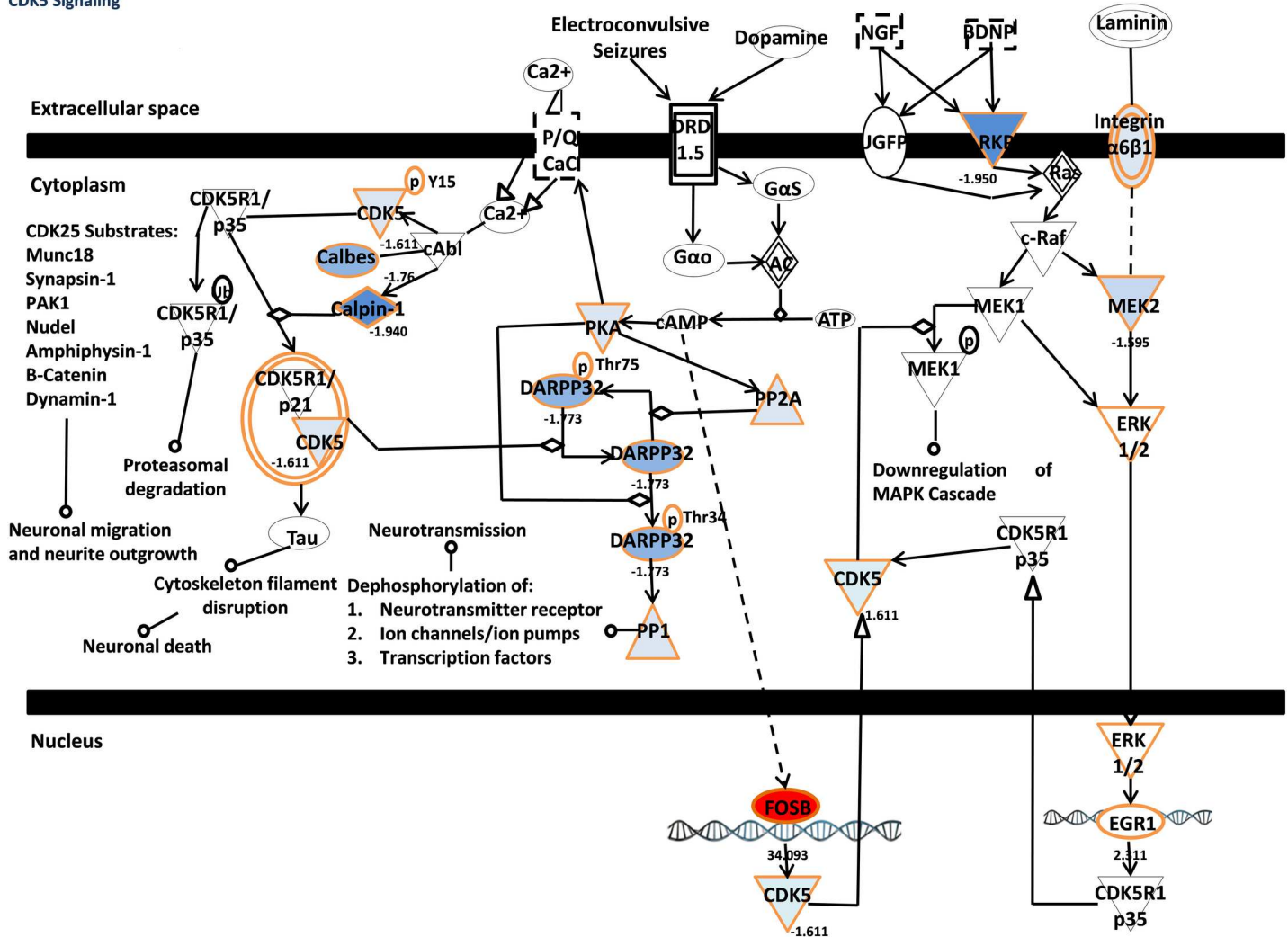
Cell Cycle: G2/M DNA Damage Checkpoint Regulation



**Fig 5. Cell cycle: G2/M DNA damage checkpoint regulation after treating HepG-2 cells with fisetin.**

doi:10.1371/journal.pone.0169335.g005

CDK5 Signaling



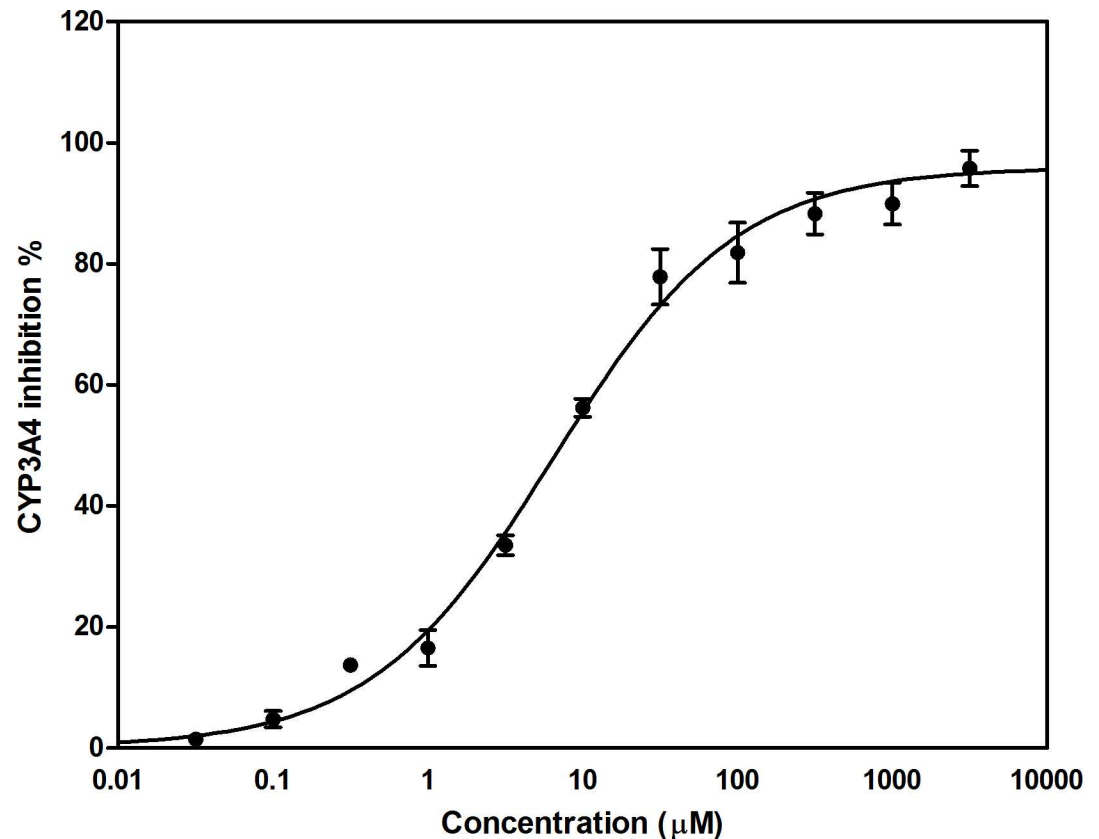
**Fig 6. CDK5 signaling pathway after treating Suit-2 cells with fisetin.**

doi:10.1371/journal.pone.0169335.g006

Dietary alterations can lead to widespread differences in the risks and incidences of several types of cancers. Additionally, the long term consumption of natural products present in fruits and spices, with proven safety, favoring their use in cancer chemoprevention [35]. The approach of tumor prevention using safe and nontoxic novel plant derived agents has been fortified by many scientists. To date, natural products and their synthetic or semisynthetic derivatives comprising huge number of approved anticancer drug candidates [24,36,37].

Plentiful natural products have been investigated for their potential use as anticancer agents. Among these agents, fisetin is a highly promising drug candidate. Fisetin is a bioactive flavonol molecule found in fruits and vegetables such as apple, grape, persimmon, strawberry, onion and cucumber [6].

Previous results showed that fisetin exhibits broad activity, including neurotrophic, anti-angiogenic, neuroprotective, radioprotective [15,38], and antioxidant effects [39]. In addition, several studies show that fisetin protects against several cancer types, including colorectal, prostate, breast, cervical, bladder, lung, and breast cancer [38,40,41]. Moreover, other reports showed that fisetin induces cell cycle arrest, apoptosis and suppress the growth of human



**Fig 7. Effect of fisetin on CYP3A4 enzyme activity.** Fisetin inhibits CYP3A4 activity in a dose dependent manner. Results are presented as mean value (M)  $\pm$  S.E.M. of at least 3 independent experiments (6 replica each).

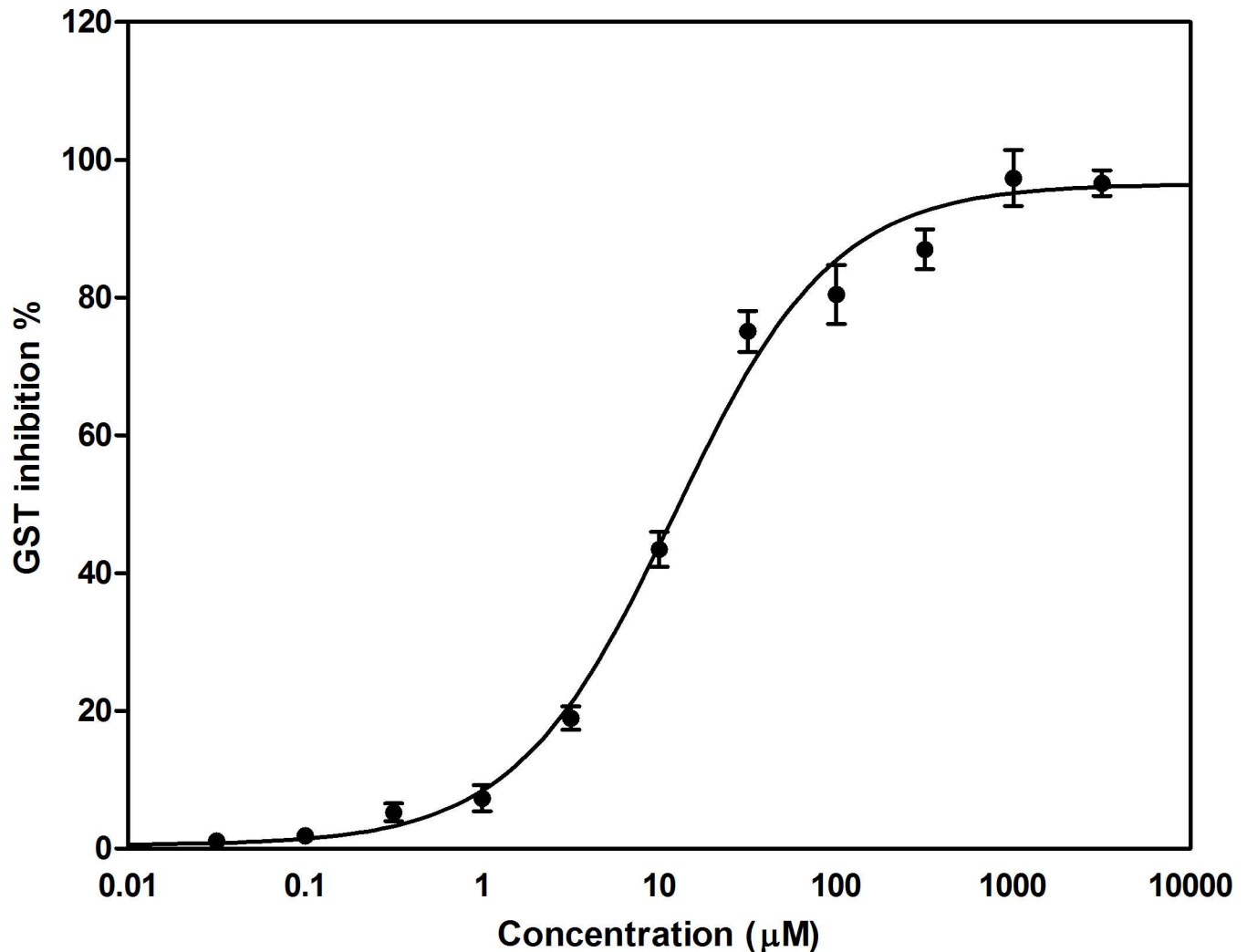
doi:10.1371/journal.pone.0169335.g007

colon cancer cells by inhibiting Wnt/EGFR/NF- $\kappa$ B and COX-2 signaling pathways [42]. Suppression of androgen receptor signaling in addition to apoptotic induction and cell cycle arrest in hepatocellular and prostatic carcinoma cells via the induction of p53 and p21(Waf/Cip-1) were also reported after fisetin treatment [43–45]. Fisetin induces autophagy through affecting TORC1 and mTORC2 pathways [46]. These results suggest that fisetin may be a useful anti-cancer or chemotherapeutic agent [15].

In spite of great efforts that have been previously done, the exact mode of action of fisetin on tumor cell growth is still unknown and needs to be further clarified. Here, the growth inhibitory effect of fisetin on three cell lines representing different tumor types namely hepatic, colorectal and pancreatic were investigated. Cellular viability was monitored by SRB cell proliferation assay. Apoptotic induction was investigated by measuring the amount of caspase 3/7 produced after treatment. Effect of fisetin on metabolic enzymes (Cytochrome P450 3A4 and Glutathione-S- transferase) and prostaglandin production (PGE2) was also evaluated.

Additionally, a transcript expression profiling study was carried out using microarray analysis technique to point out global genes, molecular targets, pathways that mediate the effect of fisetin on hepatic (HepG-2) and pancreatic cancer cell lines (Suit-2).

Our study showed that fisetin inhibited cellular growth and proliferation and induced apoptosis of all studied cell lines. Inhibition of cellular growth was more prominent with HepG-2 cells while the apoptotic effect was more prominent with Caco-2 cells. Fisetin also was able to inhibit cytochrome P450 (CYP450 3A4) and glutathione -S-transferase activity and the effect



**Fig 8. Effect of fisetin on GST enzyme activity.** Fisetin inhibits GST activity in a dose dependent manner. Results are presented as mean value (M)  $\pm$  S.E.M. of at least 3 independent experiments (6 replicas each).

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was dose dependent. Moreover, the anti-inflammatory effect of fisetin was confirmed through inhibition of PGE2 production in Caco-2 cells.

It was reported that a molecule, that is able to target multiple signaling pathways in cancer cells, could be a drug of choice to overcome multiple mechanisms and signaling pathways used by tumor cells to escape various defense mechanisms [47]. Here, using expression analysis technique, we proved that fisetin inhibited cellular growth through affecting multiple signaling pathways proposing its valuable role as safe antitumor agent.

Among the top 40 significantly up-regulated genes after fisetin treatment of both hepatic and pancreatic cancer cell lines is Axon guidance protein Semaphorin 3E (*SEMA3E*). It was previously reported that semaphorins (family of conserved membrane associated proteins) are secreted and use plexin proteins as their primary receptors for signal transduction. In cancers, de-regulation of semaphorins and their receptors is commonly observed [48]. Recent studies showed that *SEMA3E* expression is inversely correlated with tumor prognosis in pancreatic and colorectal cancer [49,50]. *SEMA3E* was among the common biomarkers significantly up-

regulated in their expression after treatment of both cell lines with fisetin that may explain in part the growth inhibitory effect of fisetin on tumor growth.

The growth arrest and DNA damage inducible genes (*GADD45*) are principal players that play an important role in suppressing multistep carcinogenesis. They are mainly up-regulated during cellular stress. *GADD45* plays an important role in suppressing multistep carcinogenesis. Over-expression of *GADD45* results in several processes of growth arrest, survival, apoptosis, or DNA repair [51]. Cell cycle arrest induced by *GADD45* was reported to be through interaction with *PCNA*, *P21*, and cyclin *B1* [52–54]. In addition to be involved in G1 or G2 cell cycle arrest, *GADD45B* has been implicated in and has been linked to the progression of HCC [55,56]. The fact that *GADD45B* and *GADD45A* were significantly overexpressed in both cell lines after fisetin treatment could explain that induction of cell cycle arrest was among mechanisms modulating the anti-tumor activity of fisetin on cancer cell lines.

Additionally, our gene expression results reported significant down-regulation of *CNB1* and *CNB2* in both tumor types after fisetin treatment, confirming cell cycle arrest induced by fisetin through up-regulation of *GADD45A* and *GADD45B* shown above and as indicated by IPA analysis too. It is well known that cyclin *B1* is involved in G2-to-M transition and cyclin *B2* is a G2/mitotic-specific factor. They both play a key role in the S-to-G2/M phases [57].

Moreover, Array results showed that fisetin inhibited significantly *KIF20A* expression in both hepatic and pancreatic cancer cells. Kinesin, present in all eukaryotes, is a microtubule-dependent molecular motor protein family, plays several roles in cellular functions, including chromosome alignment, cytokinesis, and chromosome segregation [58]. *KIF20A* (kinesin family protein member) is involved in tumor growth and progression [59]. Recent study reported that *KIF20A* played an important role in anti-tumor activity of genistein, and introduced *KIF20A* as a potential target for drug intervention of gastric cancer [59]. It was also reported that microinjection of anti-*KIF20A* antibody caused multinucleation of Hela cells [60]. In addition, knock-down of *KIF20A* gene in pancreatic cancer cells (PDAC) by siRNAs, inhibited their growth [61], implicating a vital role in cytokinesis and maintaining cellular viability. Here, we demonstrated that down-regulation of *KIF20A*, thereby disruption of the normal process of cytokinesis and induction of mitotic arrest, is among mechanisms of action of fisetin on cancer cells.

Our results showed that fisetin is able to induce apoptosis in cancer cells, is in complete agreement with previous studies in human cancer cells [15,42,62,63]

It is well known that topoisomerase inhibitors, including doxorubicin and etoposide are widely used antitumor agents [64,65]. Previous results reported that fisetin is a powerful DNA topoisomerase II poison [66]. Here, we showed that fisetin inhibited the transcription of topoisomerase II A (*TOP2A*) enzyme in human HepG-2 and Suit-2 cancer cells and that could be responsible, in part, for the anti-tumor effect of fisetin on those cells.

Drug-drug interaction in cancer patients can lead to either overdosing or under-treatment resulting in unexpected and/or severe clinical consequences. Additionally, herbal medicines, which are orally administered, could affect bioavailability of co-administered drugs. In addition, several herbs can give rise to the potential of harmful interactions with targeted agents [67,68]. In our study, we aimed to test the inhibitory effects of fisetin on CYP3A4 involved in the hepatic metabolism of most drugs. Fisetin was found to exhibit a significant and dose-dependent inhibitory activity against CYP3A4 enzyme indicating a critical sign and/or alarm during development of fisetin-based antitumor strategies and/or combination remedies.

Overexpression of glutathione S-transferases (GST) and efflux pumps in cancer cells may reduce the antitumor activity of various chemotherapeutic agents. Recently, it has become evident that glutathione S-transferases are also involved in the control of apoptosis through involvement of the JNK signaling pathway [69]. Here, we reported that fisetin was able to inhibit GST in a dose-dependent manner indicating a rational for combination-based therapies with fisetin.

Our transcript profile analysis results could be used as starting point for the generation of theories on candidate genes and for a more detailed segmentation of the role of individual transcripts for the activity of fisetin in hepatic and pancreatic cancer cells. Additionally, we provide new insights into fisetin-related signaling pathways and networks that may facilitate the development of fisetin-based antitumor strategies and/or combination remedies.

## Conclusion

The present analysis clearly demonstrated that fisetin inhibited hepatic, colorectal and pancreatic cancer cellular growth and proliferation through affecting important and multiple signaling pathways involved in tumor cell growth and differentiations. The inhibitory effect of fisetin was mainly mediated through activation of *CDKN1A*, *SEMA3E*, *GADD45B* and *GADD45A* and down-regulation of *TOP2A*, *KIF20A*, *CCNB2* and *CCNB1* genes. Our results and the expression analyses data reported here could be used to as a tool for further studies for the development of fisetin-based antitumor strategies and/or combination therapies.

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## Author Contributions

**Conceptualization:** MY WH.

**Data curation:** MY WH.

**Formal analysis:** MY.

**Funding acquisition:** MY WH.

**Investigation:** MY WH.

**Methodology:** MY WH.

**Project administration:** MY WH.

**Resources:** MY WH.

**Software:** MY WH.

**Supervision:** MY WH.

**Validation:** MY WH.

**Visualization:** MY WH.

**Writing – original draft:** MY WH.

**Writing – review & editing:** MY WH.

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