BIOCELL
2018 42(3): 99-104

ISSN 1667-5746 (on-line)
ISSN 0327-9545 (printed)

Gene expression profiling of $HepG_2$ cells after treatment with black tea polyphenols

Jie ZHONG[1,#]; Li DENG[2,#]; Yu JIANG[3]; Lianhong ZOU[3]; Huabing YUAN[4]; Shuang-xiang TAN[1,*]

[1] Scientific Research Department, Hunan Province Institute of Gerontology, Hunan Provincial People's Hospital/The First Affiliated Hospital of Hunan Normal University, Changsha, China

[2] Center of Reproductive Medicine, Hunan Provincial People's Hospital/The First Affiliated Hospital of Hunan Normal University, Changsha, China

[3] Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, Hunan Provincial People's Hospital/The First Affiliated Hospital of Hunan Normal University, Changsha, China

[4] Pharmacy Department, Tianmen First People's Hospital, Tianmen, Hubei, China

Key words: Black tea polyphenols, Microarray, HepG2 cells, Gene expression

Abstract: This study aimed to determine the effects of black tea polyphenols on gene expression in hepatocellular cancer cells. The total RNA from HepG2 hepatocellular cancer cells treated with black tea polyphenols was subjected to Human 14K cDNA microarray analysis. Real-time PCR and Western blot analysis were conducted to verify microarray data. Black tea polyphenols treatment at the dose of 20 mg/L, 40 mg/L or 80 mg/L for one to three days inhibited the growth of HepG2 cells in a dose and time dependent manner. A total of 48 genes showed more than two-fold change after black tea polyphenols treatment, including 17 upregulated genes and 31 downregulated genes, and they were involved in the regulation of cell growth, cell cycle, apoptosis, signaling, angiogenesis, tumor invasion and metastasis. Real-time PCR analysis of the selected genes showed that their mRNA expression changes were consistent with the microarray data. In addition, Western blot analysis of the selected genes showed that their protein expression changes were consistent with mRNA expression. In conclusion, gene expression profiles provide comprehensive molecular mechanisms by which black tea polyphenols exerts growth inhibition effects on cancer cells. The novel molecular targets identified in this study may be further exploited as therapeutic strategies for hepatocellular cancer.

Introduction

Hepatocellular carcinoma (HCC) is the most common cancer and the forth leading cause of cancer related deaths (Ferlay *et al*., 2018). Although various therapies such as surgery, radiotherapy, and chemotherapy are used for HCC, the most effective therapeutic approach is yet to be discovered.

Recently, great attention has been paid to food components with potential anti-cancer effect (Bao *et al*., 2018; Ke *et al*., 2017; Riezzo *et al*., 2005). Identification of effective chemopreventive diets or dietary supplements for human use is of much interest. Polyphenols present in food have been demonstrated to inhibit various types of experimental carcinogenesis (Femia *et al*., 2005; Lewandowska *et al*., 2014; Olech *et al*., 2012). Although most of the studies focused on green tea and its components, a number of studies have reported that polyphenols from black tea are similar effective (Lu *et al*., 1997; Yang *et al*., 2000). It has been demonstrated that black tea polyphenols (BTPs) are capable of inhibiting HCC in animal models, and inducing cell cycle arrest and apoptosis in cell culture models (Jia *et al*., 2005). However, thedetail molecular mechanisms underlying chemo-preventive effect of black tea polyphenols on HCC are still unclear.

The advent of cDNA microarray technology makes it possible to monitor expression levels of a multitude of genes simultaneously (Ataei *et al*., 2017; Liu and Zhang, 2017 Viknesh and Manikandan, 2017). To better understand the precise molecular mechanisms by which BTPs exert anti-cancer efficacy on HCC, in this study we utilized high-throughput gene chip to determine the alteration of gene expression of HepG2 hepatocellular carcinoma cells exposed to BTPs.

Materials and Methods

Cell culture

$HepG_2$ cells were grown in the Dulbecco's modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin in a 37℃ incubator with 5% CO_2 and saturated humidity. Black tea polyphenols (BTPs, purity>80%) were purchased from Sigma Aldrich, Inc. and dissolved in DMSO to make stock solution for cell treatment.

*Address correspondence to: Shuang-xiang Tan, tanshuangxiang@hunnu.edu.cn.

# Jie Zhong and Li Deng contributed equally to this article.

Trypan blue dye exclusion growth assay
$HepG_2$ cells were treated with BTPs (20 mg/L, 40 mg/L or 80 mg/L) or 0.01% DMSO as control for one to three days. Cells were harvested by trypsinization and centrifuged and stained with trypan blue. The number of dead and viable cells were counted using a hemato-cytometer.

Microarray analysis
$HepG_2$ cells were treated with 80 mg/L BTPs or 0.1% DMSO (vehicle control) for 6 h, 36 h or 72 h. Total RNA from each sample was isolated by Trizol (Invitrogen) and purified by RNeasy Mini kit (Invitrogen) according to the manufacturer's protocol. The quantity and quality of all RNA preparations were assessed by gel electrophoresis and spectrophotometry. DNA microarray was performed according to the standard protocol. For each sample, Cy3- and Cy5- labeled cDNAs were generated from 50 μg total RNA. Labeled cDNA derived from the control sample was mixed with labeled cDNA from BTPs- treated samples, and co-hybridized to Human 14K cDNA microarray slides (Biochip, Shanghai, China). After hybridization for 16 h at 45°C, the arrays were washed with 1X SSC and 0.2% SDS at 55°C for 15 min, followed by washing with 0.1X SSC and 0.2% SDS at 55°C for 10 min and washing with 0.1X SSC at room temperature for 5 min. Signals of hybridization with Cy3- and Cy5-labeled probes were measured using the Agilent Scanner (General Scanning). Three independent experiments were performed per treatment to verify the reproducibility of results.

Microarray data normalization and analysis
Images were quantified using ImaGene Software (Biodiscovery) that uses a local background subtracted from the signal. Signals not consistently detectable (background corrected signal lower than 2 times of background standard deviation) were eliminated. Data were normalized using the algorithm as described previously (Mercurio and Singla, 2016). Average differences of gene expression between treated and untreated samples that were greater that twofold were compared by *t* test. Clustering and annotation of the gene expression were analyzed by using Cluster and TreeView (Eisen *et al*., 1998), Onto-Express (Khatri *et al*., 2002) and GenMAPP (Dahlquist *et al*., 2002). Genes that were not annotated or not easily classified were excluded from the functional clustering analysis.

Real-time RT-PCR
Total RNA and cDNA were prepared from each sample as described above. The primer sequences employed for real-time RT-PCR were listed in Tab. 1. PCR reactions were carried out in 96 well microtiter plates in a 25 μl reaction volume with SYBR Green Master Mix (PE Biosystems) using ABI Prism 7000 Sequence Detector (Applied Biosystems). Each sample was assayed in duplicate for each gene. Cycle parameters were an initial cycle of 50°C for 2 min followed by a denaturation stage of 95°C for 10 min. Forty rounds of amplification were performed at 95°C for 15 s and 60°C for 1 min. Data were analyzed using the comparative cycle threshold (Ct) method (Livak and Schmittgen, 2001).

Western blot analysis
Cells were collected and lysed in modified RIPA lysis buffer (50 mmol/LTris-HCl pH 8.0, 150 mmol/L NaCl, 0.5% sodium deoxycholate, 1 mmol/LEDTA, 1% NP-40, 0.1 mg/L PMSF) on ice for 30 min. The supernatants were collected after centrifugation at 12,000 rpm for 30 min at 4°C. The protein concentration of the supernatants was measured, and then total cell proteins were separated using 10% SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes (Millipore). The membranes were blocked with 5% nonfat milk at room temperature for 2 h, incubated with antibody for SCCA1 or β-actin (Abcam) at 4°C overnight, and then incubated with horseradish peroxidase-labeled goat anti-rabbit IgG antibody (Santa Cruz Biotech) at room temperature for 1 h. The membranes were washed and incubated with substrates using enhanced chemiluminescence kit (Amersham Biosciences), then exposed to X-ray film and analyzed by plus 5.1 software.

Statistical analysis
The data were presented as mean ± standard deviation ($\overline{x} \pm \mathrm{sd}$). Data from two samples were compared using *t* test and data from multiple samples were compared using single factor analysis of variance. $P<0.05$ was considered statistically significant.

TABLE 1

The primers used for real-time PCR

Gene	Primer sequence	PCR product
P21	forward: CCTAATCCGCCCACAGGAA reverse: AAGATGTAGAGCGGGCCTTTG	68 bp
MMP1	forward: GGGAGATCATCGGGACAACTC reverse: GGGCCTGGTTGAAAAGCAT	72 bp
MMP9	forward: TGGGGGGCAACTCGGC reverse: GGAATGATCTAAGCCCAG	224 bp
BAK1	forward: GAACAGGAGGCTGAAGGGGT reverse: TCAGGCCATGCTGGTAGACG	307 bp
BCL-XL	forward: GGTCGCATTGTGGCCTTT reverse: TCCGACTCACCAATACCTGCAT	85 bp
BIRC2	forward: TCAGTAACTGGGAACCAAAGGATG reverse: CAACAGCTGTTCAAGAAGATGAGG	401 bp
cyclin B1	forward: AGCTGCTGCCTGGTGAAGAG reverse: GCCATGTTGATCTTCGCCTTA	91 bp
GAPDH	forward: CCTGTTCGACAGTCAGCCG reverse: CGACCAAATCCGTTGACTCC	101 bp

Results

Growth inhibition activity of black tea polyphenols

As shown in Fig. 1, BTPs inhibited the growth of $HepG_2$ cells in a dose- and time-dependent manner.

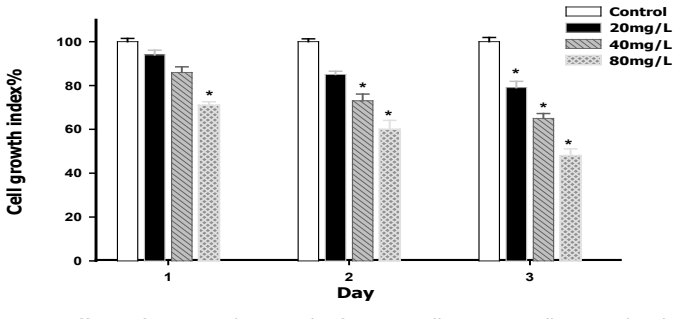


FIGURE 1. BTPs inhibited the growth of HepG2 cells in a dose and time dependent manner. **P*<0.05 *vs.* Control (n=3).

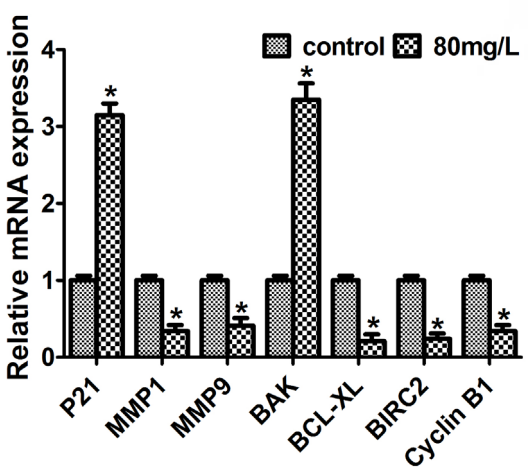
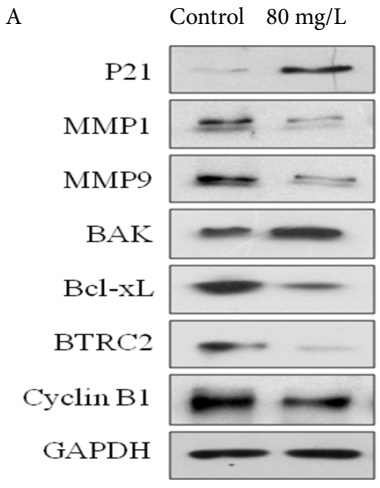


FIGURE 2. Real-time PCR showing the altered expression of specific genes in HepG2 cells treated by BTPs. HepG2 cells were treated with BTPs (80 mg/L) for 6 h. The amount of each product was normalized to GAPDH and expressed as fold of untreated control set as 1. **P*<0.05 *vs.* Control (n=3).



B

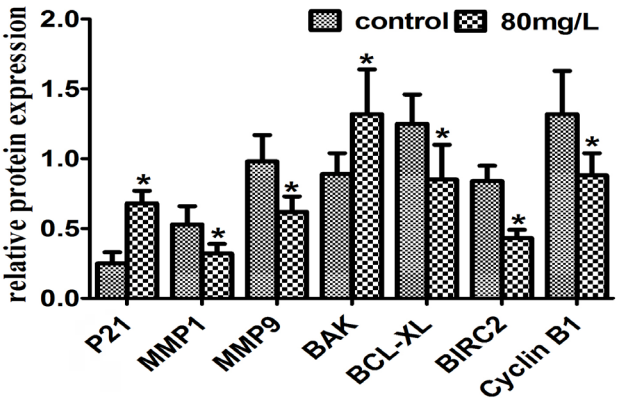


FIGURE 3. Western blot analysis showing the altered expression of specific proteins in HepG2 cells treated by BTPs. HepG2 cells were treated with BTPs (80 mg/L) for 6 h. The amount of each product was normalized to GAPDH and expressed as fold of untreated control. **P*<0.05 *vs.* Control (n=3).

cDNA microarray analysis of black tea polyphenols treated cells

The gene expression profile of $HepG_2$ cells treated with BTPs for 6 h, 36 h, 72 h was analyzed. A cut-off value of two fold or more change between samples (BTPs treatment *vs.* control) was used to define differential expression. Microarray analysis showed that the alterations of gene expression occurred as early as 6 h after BTPs treatment and were consistent over time.

Based on biological functions, we found the up-regulation of 17 genes and down-regulation of 31 genes, which are mainly involved in the process of cell growth, cell cycle, apoptosis, signaling transduction, angiogenesis, tumor cell invasion and metastasis (Tab. 2).

Validation of differential expression by real-time PCR

We performed real-time PCR to confirm the changes in mRNA expression of the selected genes in $HepG_2$ cells treated with BTPs (80 mg/L) for 6 h and found a good correlation with microarray results, although the fold change in the expression level was not exactly same between two methods (Fig. 2).

Validation of differential expression by Western blot analysis

We performed Western blot analysis to confirm the changes in protein expression of the selected genes in $HepG_2$ cells treated with BTPs (80 mg/L) for 6 h. The results showed that protein expression of the selected genes was consistent with mRNA expression (Fig. 3).

Discussion

Tea polyphenols have been demonstrated as chemopreventive agents in a number of experimental models. However, the molecular mechanisms for cancer chemo-prevention by black tea polyphenols have not been fully elucidated. In this study we explored gene expression profile of BTPs treated HepG2 cells and found that BTPs caused alterations in the expression of many genes related to the control of cell growth, cell cycle, apoptosis, signaling, angiogenesis, tumor cell invasion and metastasis.

TABLE 2

Fold change of genes in $HepG_2$ cells exposed to BTPs

Gene	Fold Change		
	6 h	36 h	72 h
Cell cycle, Cell growth and Apoptosis			
NM_078467 cyclin-dependent kinase inhibitor 1A (p21, Cip1)	4.32	4.10	3.95
NM_005255 cyclin G associated kinase (GAK)	1.75	2.00	2.13
NM_003674 cyclin-dependent kinase 10 (CDK10)	1.31	1.87	2.18
NM_031966 cyclin B1 (CCNB1)	NC	-1.91	-3.04
NM_004701 cyclin B2 (CCNB2)	-2.31	-2.34	-2.67
NM_004060 cyclin G1 (CCNG1)	NC	-2.41	-4.04
NM_001786 cell division cycle 2 (CDC2)	-2.57	-3.00	-3.11
NM_005190 cyclin C (CCNC)	NC	-2.01	-1.98
NM_001237 cyclin A2 (CCNA2)	-4.41	-3.98	-4.10
NM_001188 BCL2-antagonist/killer 1 (BAK1)	3.67	4.55	4.81
NM_003682 MAP-kinase activating death domain (MADD)	2.11	2.98	3.14
NM_003824 Fas (TNFRSF6)-associated via death domain (FADD)	2.13	2.81	2.81
NM_021138 TNF receptor-associated factor 2 (TRAF2)	2.30	2.53	2.53
NM_001166 baculoviral IAP repeat-containing 2 (BIRC2)	-2.00	-3.01	-3.14
NM_001167 baculoviral IAP repeat-containing 4 (BIRC4)	-1.79	-2.18	-2.20
NM_138578 BCL2-like 1 (BCL-xL)	-2.68	-3.00	-4.12
NM_002608 platelet-derived growth factor beta (PDGFB)	NC	-2.01	-2.83
NM_006763 BTG family, member 2 (BTG2)	NC	1.75	2.13
NM_001963 epidermal growth factor (EGF)	NC	-2.50	-1.90
NM_006472.1 upregulated by 1,25-dihydroxyvitamin D-3 (VDUP1)	2.00	2.15	3.10
Transcription and Translation			
NM_012068 activating transcription factor 5 (ATF5)	NC	-2.03	-2.81
NM_003907 eukaryotic translation initiation factor 2B (EIF2B)	NC	-1.23	-2.30
NM_001674 activating transcription factor 3 (ATF3)	-1.40	-1.71	-2.50
NM_005225 E2F transcription factor 1 (E2F1)	-1.11	-2.09	-2.11
NM_013354 CCR4-NOT transcription complex, subunit 7 (CNOT7)	NC	-2.31	-3.14
NM_021953 forkhead box M1 (FOXM1)	-1.22	-2.81	-4.53
NM_003201 transcription factor A, mitochondrial (TFAM)	NC	-1.18	-2.09
Invasion ,Metastasis and Angiogenesis			
NM_002421 matrix metalloproteinase 1 (MMP1)	-2.31	-2.41	-2.10
NM_004994 matrix metalloproteinase 9(MMP9)	-1.3	-1.61	-2.00
NM_003254 tissue inhibitor of metalloproteinase 1 (TIMP1)	-1.30	-2.31	-3.51
NM_005429 vascular endothelial growth factor C (VEGFC)	-4.31	-5.11	-3.13
NM_001901 connective tissue growth factor (CTGF)	1.23	1.52	2.31
NM_000660 transforming growth factor, beta 1 (TGFB1)	NC	-2.09	NC
NM_000089 collagen, type I, alpha 2 (COL1A2)	-1.33	-1.71	-2.07
NM_003253 T-cell lymphoma invasion and metastasis 1 (TIAM1)	NC	-1.43	-2.11
NM_002019 fms-related tyrosine kinase 1	-1.30	-1.54	-2.15
NM_005711 EGF-like repeats and discoidin I-like domains 3	NC	-1.91	-2.03

Table 2 (Continued)

Gene	Fold Change		
	6 h	36 h	72 h
Kinase, Signal transduction and Others			
NM_145110 mitogen-activated protein kinase kinase 3 (MAP2K3)	2.31	3.20	4.11
NM_153831 PTK2 protein tyrosine kinase 2 (PTK2)	NC	-2.22	-2.14
NM_080685 protein tyrosine phosphatase 13 (PTPN13)	-1.73	-2.01	-2.29
NM_139276signal transducer and activator of transcription 3(STAT3)	NC	1.71	2.13
NM_003979 retinoic acid induced 3 (RAI3)	1.3	2.21	1.98
NM_003481 ubiquitin specific protease 5 (USP5)	NC	2.31	2.41
NM_052815 immediate early response 3 (IER3)	1.91	2.54	1.33
NM_198433 serine/threonine kinase 6 (STK6)	1.70	2.34	2.11
NM_004418 dual specificity phosphatase 2 (DUSP2)	1.31	1.79	2.14
NM_000392 ATP-binding cassette, sub-family C , member 2 (ABCC2)	NC	-1.39	-2.04
NM_033151 ATP-binding cassette, sub-family C , member 11 (ABCC11)	-1.70	-2.13	-2.61

The genes in this list showed a>2 fold change in expression in at least one time point in cells exposed to BTPs. NC: No change; Negative value: Decrease; Positive value: Increase.

Cell cycle control is a highly regulated process that involves a complex cascade of events. Modulation of the expression and function of cell cycle regulatory proteins provides an important approach for the inhibition of tumor growth (Cai and Liu, 2017). Our results showed that Cyclins (cyclin A2, B1, B2), CDK2, CDC2 were down-regulated in BTPs treated HepG2 cells, while CDK inhibitor p21WAF1 and other growth inhibitors BTG2 and VDUP1 were upregulated, suggesting that BTPs inhibit the growth of $HepG_2$ cells through the arrest of cell cycle and the inhibition of cell proliferation.

Induction of apoptosis by chemotherapeutic agent also leads to the inhibition of cancer cell growth. It has been reported that BTPs induce apoptosis in many cell types (Banerjee *et al*., 2005; Kundu *et al*., 2005). According to gene expression profile, we found that BTPs increased the levels of TNF receptors, such as TRAF2, and downstream molecules, such as MAP-kinase activating death domain (MADD), Fas-associated via death domain (FADD), which lead to caspase activation and the induction of apoptosis. We also found that BTPs inhibited the expression of anti-apoptotic protein XIAP. BTPs also altered the expression of BCL-2 family members, leading to the upregulation of pro-apoptotic BAK1 and the downregulation of anti-apoptotic BCL-xL. These results suggest that BTPs may induce apoptotic cell death via both the mitochondrial and the death receptor pathways.

It is important to note that BTPs also affected the expression of several genes involved in tumor invasion, metastasis and angiogenesis. In this study we found that BTPs down-regulated the expression of MMP1, MMP9, VEGF, TGF-β, while up-regulated the expression of TIMP1 in $HepG_2$ cells, suggesting that BTPs inhibit HCC angiogenesis, invasion and metastasis of via the modulation of these genes.

In summary, BTPs cause changes in the expression of many genes that are crucially involved in the control of cell growth, cell cycle, apoptosis, signaling, angiogenesis, tumor cell invasion and metastasis. These findings provide insight into the mechanisms by which BTPs exert anti-tumor effects on HCC. The novel molecular targets identified in this study may be further exploited as chemopreventive and/or therapeutic strategies for HCC.

Conflict of Interest

No.

Acknowledgement

This work was supported by grants from the Science and Technology Foundation of Health and Family Planning Commission of Hunan Province (No. C2019048) and Science and Technology Bureau of Changsha (No. ZD1702024).

References

Ataei R, Gholamhoseini M, Kamalizadeh M (2017). Genetic analysis for quantitative traits in bread wheat exposed to irrigated and drought stress conditions. *Phyton-International Journal of Experimental Botany* **86**: 228-235.

Banerjee S, Manna S, Saha P, Panda CK, Das S (2005). Black tea polyphenols suppress cell proliferation and induce apoptosis during benzo(a)pyrene-induced lung carcinogenesis. *European Journal of Cancer Prevention* **14**: 215-221.

Bao T, Ke Y, Wang Y, Wang W, Li Y, Wang Y, Kui X, Zhou Q, Zhou H, Zhang C *et al.* (2018). Taraxasterol suppresses the growth of human liver cancer by upregulating Hint1 expression. *Journal of Molecular Medicine* **96**: 661-672.

Cai Z, Liu Q (2017). Cell Cycle Regulation in Treatment of Breast Cancer. *Advances in Experimental Medicine and Biology* **1026**: 251-270.

Dahlquist KD, Salomonis N, Vranizan K, Lawlor SC, Conklin BR (2002). GenMAPP, a new tool for viewing and analyzing microarray data on biological pathways. *Nature Genetics* **31**: 19-20.

Eisen MB, Spellman PT, Brown PO, Botstein D (1998). Cluster analysis and display of genome-wide expression patterns. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 14863-14868.

Femia AP, Caderni G, Vignali F, Salvadori M, Giannini A, Biggeri A, Gee J, Przybylska K, Cheynier V, Dolara P (2005). Effect of polyphenolic extracts from red wine and 4-OH-coumaric acid on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. *European Journal of Nutrition* **44**: 79-84.

Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, Znaor A, Bray F (2018). Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International Journal of Cancer*. Doi. org/10.1002/ijc.31937. [Epub ahead of print].

Jia XD, Han C, Chen JS (2005). Tea pigments induce cell-cycle arrest and apoptosis in HepG2 cells. *World Journal of Gastroenterology* **11**: 5273-5276.

Ke Y, Bao T, Wu X, Tang H, Wang Y, Ge J, Fu B, Meng X, Chen L, Zhang C *et al.* (2017) Scutellarin suppresses migration and invasion of human hepatocellular carcinoma by inhibiting the STAT3/Girdin/Akt activity. *Biochemical and Biophysical Research Communications* **483**: 509-515.

Khatri P, Draghici S, Ostermeier GC, Krawetz SA (2002). Profiling gene expression using onto-express. *Genomics* **79**: 266-270.

Kundu T, Dey S, Roy M, Siddiqi M, Bhattacharya RK (2005). Induction of apoptosis in human leukemia cells by black tea and its polyphenol theaflavin. *Cancer Letters* **230**: 111-121.

Lewandowska U, Owczarek K, Szewczyk K, Sosnowska D, Koziołkiewicz M, Hrabec E (2014). Differentiated impact of procyanidins from evening primrose on human breast cancer cells. *Open Life Sciences* **9**: 647-658.

Liu M, Zhang X (2017). An integrated analysis of mRNA-miRNA transcriptome data revealed hub regulatory networks in three genitourinary cancers. *Biocell* **41**: 19-26.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**: 402-408.

Lu YP, Lou YR, Xie JG, Yen P, Huang MT, Conney AH (1997). Inhibitory effect of black tea on the growth of established skin tumors in mice: Effects on tumor size, apoptosis, mitosis and bromodeoxyuridine incorporation into DNA. *Carcinogenesis* **18**: 2163-2169.

Mercurio M and Singla P (2016). A tree-based approach for efficient and accurate conjunction analysis. *Computer Modeling in Engineering & Sciences* 111: 229-256.

Olech M, Nowak R, Los R, Rzymowska J, Malm A, Chrusciel K (2012). Biological activity and composition of teas and tinctures prepared from Rosa rugosa Thunb. *Central European Journal of Biology* **7**: 172-182.

Riezzo G, Chiloiro M, Russo F (2005). Functional foods: Salient features and clinical applications. *Current Drug Targets-Immune, Endocrine & Metabolic Disorders* **5**: 331-337.

van de Peppel J, Kemmeren P, van Bakel H, Radonjic M, van Leenen D, Holstege FC (2003). Monitoring global messenger RNA changes in externally controlled microarray experiments. *EMBO Reports* **4**: 387-393.

Viknesh TV, Manikandan V (2017). Modeling of canonical switching cell converter using genetic algorithm. *Computer Modeling in Engineering & Sciences* **113**:109-116.

Yang GY, Liao J, Li C, Chung J, Yurkow EJ, Ho CT, Yang CS (2000). Effect of black and green tea polyphenols on c-jun phosphorylation and H(2)O(2) production in transformed and non-transformed human bronchial cell lines: Possible mechanisms of cell growth inhibition and apoptosis induction. *Carcinogenesis* **21**: 2035-2039.