

RESEARCH ARTICLE

Microarray and Quantitative PCR Analysis of Gene Expression Profiles in Response to Treatment with Tomato Leaf Extract in MCF-7 Breast Cancer Cells

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Abstract

We previously found cytotoxic effects of tomato leaf extract (TLE) on the MCF-7 breast cancer cell line. The aim of this study was to ascertain the molecular mechanisms associated with the usage of TLE as an anticancer agent by microarray analysis using mRNA from MCF-7 breast cancer cells after treatment with TLE for 1 hr and 48 hrs. Approximately 991 genes out of the 30,000 genes in the human genome were significantly ($p < 0.05$) changed after the treatment. Within this gene set, 88 were significantly changed between the TLE treated cells and the untreated MCF-7 cells (control cells) with a cut-off fold change > 2.00 . In order to focus on genes that were involved in cancer cell growth, only twenty-nine genes were selected, either down-regulated or up-regulated after treatment with TLE. Microarray assay results were confirmed by analyzing 10 of the most up and down regulated genes related to cancer cells progression using real-time PCR. Treatment with TLE induced significant up-regulation in the expression of the *CRYAB*, *PIM1*, *BTG1*, *CYR61*, *HIF1- α* and *CEBP- β* genes after 1 hr and 48 hrs, whereas the *TXNIP* and *THBS1* genes were up-regulated after 1 hr of treatment but down-regulated after 48 hrs. In addition both the *HMG1L1* and *HIST2H3D* genes were down-regulated after 1 hr and 48 hrs of treatment. These results demonstrate the potent activity of TLE as an anticancer agent.

Keywords: Cancer - cytotoxic - MCF-7 - microarray - real-time PCR - transcriptomics - tomato leaves

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Introduction

Tomato (*Solanum lycopersicon*) is one of the most widely consumed fresh vegetables in the industrialized world. It is also commonly used by various food industries as raw material for production of derived products such as ketchup and puree. Because tomatoes are highly versatile and easy to prepare, they are recommended to be part of a healthy diet. Few studies have showed that by eating tomato the breast (Zhang et al., 2009), head and neck cancers incidents were decrease (Freedman et al., 2008) and tomato may also be strongly protective against neurodegenerative diseases (Rao and Balachandran, 2002). Lycopene from the fruit and α -tomatine from the leaves of tomato showed anti-oxidant and anti-cancer activity to few cancer cell lines *in-vitro* and *in-vivo* (Shieh et al., 2011; Palozza et al., 2011; 2012; Chao et al., 2012; Kelkel et al., 2011; Lee et al., 2012; Teodoro et al., 2012; Tuzcu et al., 2012; Sharoni et al., 2012). In addition, previous studies have reported that tomato leaves synthesize glycoalkaloid dehydrotomatine and α -tomatine, which may have

anticancer properties (Friedman, 1995; Friedman, 2009). Since there are no commercial uses for tomato leaves, they may be readily available as natural sources for new discoveries in the treatment of cancer. In our recent study, we determined that TLE actively decreased the viability of MCF-7 breast cancer cells (Chik, 2010). Further investigation of the molecular mechanisms by which TLE decreases cancer cell viability will contribute to new insights and drug discovery. Furthermore, better understanding of the molecular mechanisms underlying normal and dysfunctional biological processes will be achieved by using recent bioinformatics and high-throughput technologies, such as microarray analysis. Microarray studies and other genomic techniques are also furthering the discovery of new targets for the treatment of disease, which is aiding drug development, and immuno- and gene-therapies (Lock et al., 2002). Moreover, through microarray analysis or gene expression profiling, thousands of genes can be measured from a single RNA sample (Clarke, 2004). In this study, a microarray analysis was conducted to determine the cancer cell growth related

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genes that were altered in response to the treatment with TLE in MCF-7 breast cancer cells. The results of the microarray analysis were then confirmed by real-time PCR analysis.

Materials and Methods

Preparation of Methanol Extract of Tomato Leaves
Tomato leaves were collected from Malaysian Agricultural Research Development Institute (MARDI), Cameron Highland, Pahang, Malaysia. The leaves were washed using tap water, rinsed with distilled water and were dried at 40°C for one day until the dry weight was stable. The dried leaves were ground and powdered leaves were extracted with 82% methanol in a shake flask at a 1:10 (w/v) ratio. The mixture was agitated at 22°C, 110 rpm for 24 hrs (Chik et al., 2010). The mixture was filtered with Whatman No.1 filter paper to collect the filtrate. Finally, the filtrate was concentrated in a water bath at 40°C.

Partial purification by column chromatography

The extract was subjected to column chromatography in order to collect the fractions with anticancer activity according to the previous report (Chik et al. 2010). The purified fractions were collected separately into tubes. The collected fractions from the TLE were concentrated in a vacuum concentrator and dissolved in 10% dimethyl sulfoxide (DMSO) for further use.

Cell culture and TLE treatment

Frozen MCF-7 breast cancer cells were thawed, inoculated into 5 ml media (Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS), 100 µg/ml streptomycin, 100 U/ml penicillin, cultured in a 95% air, 5% CO₂ atmosphere) in a T-25 flasks, allowed to grow until 60-80% confluent. After three passages, the cells were inoculated into 5 mL of fresh media with each flask containing initial density of 2 x 10⁵ cells/mL. At 8 hours intervals thereafter, one flask containing cells was harvested and counted under phase contrast on a hemocytometer after staining with trypan blue. In a separate batch of each flask was treated with 5.85 µg/ml (IC₅₀) of partially purified TLE as described previously (Chik et al., 2010). Prior to treatment, partially purified extract was dissolved in 10% of DMSO. 1% (v/v) DMSO was also used to treat control cells. The numbers of viable and non-viable cells were recorded to plot a growth curve.

Total RNA extraction

Based on the growth curves, total RNA of MCF-7 cells specifically at 48 hours (mid-log phase) inoculation (control) and treated cells after 1 hour and 48 hours of TLE treatments were harvested prior to RNA extraction. This is to identify the genes that responded very early after the treatment and genes that responsive during the late stationary phase of cancer cell progression. Total RNA of untreated and treated cells from three different batch were extracted using the RNeasy mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The quality and the concentration of the RNA

were determined using spectrophotometer (Eppendorf, NY, USA) and 1.6% gel electrophoresis (Figure 1). RNA was stored at -80°C until further analysis. Figure 1 here

Microarray analysis

Microarray analysis was performed using the GeneChip® Gene 1.0 ST Array System for Human (Affymetrix, Ohio, USA) containing total of 30,000 genes in human genome. The microarray was performed in three batches. The cDNA of each sample was synthesized from 100 ng of the total RNA using the GeneChip® cDNA Synthesis Kit (Invitrogen, NY, USA). The cDNA was then used for expression profiling using GeneChip® Hybridization, Wash and Stain kits (Invitrogen, NY, USA). The probe array was scanned through the Affymetrix GeneChip® Scanner 3,000 7G (Affymetrix, Ohio, USA) and analyzed using NetAffy Analysis Center and GeneChip® Operating Software (GCOS) (Affymetrix, Ohio, USA).

Normalization of the microarray data and differential gene expression analysis

Global scaling normalization was performed and

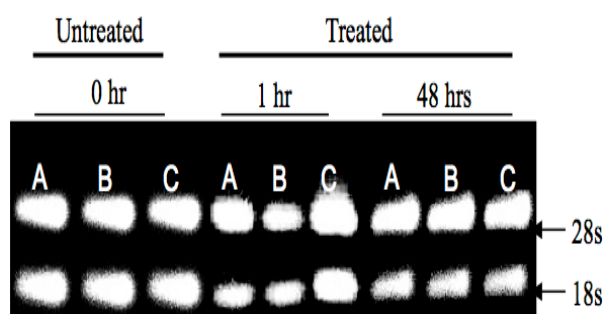


Figure 1. Intact Total RNA from Untreated (0 hour) and Treated MCF-7 Cells (1 and 48 hours) Run on 1.6% Gel Electrophoresis, Samples were Run in Three Batches, Labeled as A, B and C. The 28S rRNA band shows approximately twice as intense as the 18S rRNA band. This 2:1 ratio (28S:18S) is a good indication that the RNA is completely intact and has a good RNA quality

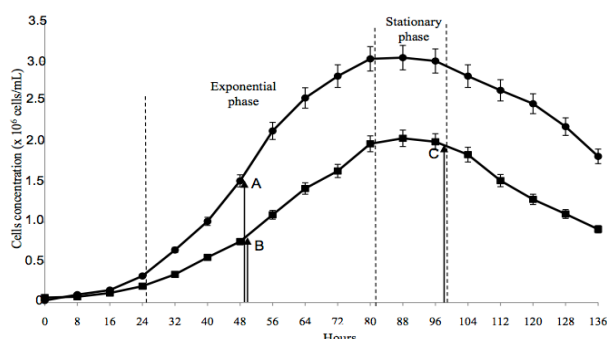


Figure 2. Graph of MCF-7 Growth Curves before and After Treatment with TLE (IC₅₀=5.85 µg/mL) Presented with the means±SD Values Obtained from Three Independent Experiments. (i) Growth of untreated MCF-7 cells (■) shows decrement of cells number after treated with TLE (●). (A) Total RNA was extracted from untreated cells at 48 hours, (C) after 1 hour (at 49 hours) and (D) 48 hours (at 97 hours) of treatment for microarray analysis

the fold changes were calculated based on the relative signal intensities. GeneSpring GX version 7.2 (Agilent Technologies, California, USA) was used to simplify the procedure. A filtering step was performed using one-way analysis of variance (ANOVA) tests, which allowed for determination of the statistical significance of every pairwise gene between the treatment periods. The default multiple testing corrections used was Benjamini and Hochberg False Discovery Rate with a p-value cut-off < 0.05. The testing correction is the least stringent of all corrections and provides a good balance between the 15 discovery of statistically significant genes and the limitation of false positive occurrences by removing all gene spots with a p-value more than 0.05 in all conditions (Zar, 1999).

This narrowed the list of genes to those significantly affected by TLE. Later, the listed genes ($p < 0.05$) were filtered again with a fold-change > 2.00. Finally, the self-organized map (SOM) analysis was utilized to group and display genes with similar expression profiles. Data gained by this technique may help to understand more on *in vitro* studies of cytotoxic drugs. Raw microarray data is available in GEO database (<http://www.ncbi.nlm.nih.gov/geo/info/linking.html>) under the accession number GEO33443.

Real-time PCR analysis of differentially expressed genes

Previously extracted total RNA from the untreated and treated cells was isolated and reverse transcribed into cDNA using SuperScript III Reverse Transcriptase, (Invitrogen, NY, USA). Later, the annealing temperature of selected genes (Table 2) was determined followed by performing quantitative PCR (real-time PCR) using the QuantiTectTM SYBR[®] Green RT-PCR Kit.

Next, the real-time PCR reactions were subjected to 40 cycles of denaturation condition (95°C, 30 s) with annealing and extension temperatures ranges from 50-60 °C, depending on the primers used. The standard curve for each primer was constructed using serial dilutions of cDNA, and the amount of target mRNA in each sample was normalized using GAPDH (housekeeping gene). The reactions were carried out in triplicates using STRATAGENE MX3000P Quantitative Real-Time PCR (STRATAGENE, California, USA).

Results

MCF-7 cancer cells growth profiles

MCF-7 cells were treated separately with TLE ($IC_{50} = 5.85 \mu\text{g/mL}$) and Taxol ($IC_{50} = 0.039 \mu\text{g/mL}$), and every 8 hours intervals the untreated and treated cells were harvested prior to cell quantification. The numbers of viable cells were used to plot the MCF-7 growth profile. The growth behavior of MCF-7 cells and its changes after TLE treatment can be seen at Figure 2. The untreated and treated cells had started to proliferate after 24 hours. This exponential or log phase took place for 56 hours before the cells achieved their stationary phase after 80 hours. The mid- log phase found to be 48 hours after inoculation. The untreated and treated cells achieved its maximum growth within 80-98 hours.

Microarray analysis

To design successful therapeutic strategies involving compounds either alone or in combination, first, it is necessary to understand their mechanism of action. Microarray analysis is a technique that has been shown to be useful for the simultaneous profiling of global gene expression and the discovery of new genes or new functions of known genes (Thornton et al., 2002). For these reasons, we have conducted a microarray analysis of the global gene expressions profiles in MCF-7 cells following treatment with TLE. The t-test showed that from the 30,000 genes in the human genome, approximately 991 genes were significantly ($p < 0.05$) changed after the treatments. Within this gene set, 88 genes were significantly changed between the treated cells and the control cells with cut-off expression fold change > 2.00. A total of 29 genes that is associated with the growth of cancer cells were shown in Figure 3 and only 10 genes that significantly related to cancer cells proliferation were tabulated according its related pathway in Table 1.

Validation by quantitative real time-PCR

Quantitative real-time PCR was performed to confirm the microarray data for several genes selected as indicators of cancer cell growth (Figure 4). In this real-time PCR analysis, expression of the *CRYAB*, *PIMI*, *BTG1*, *CYR61*, *HIF1- α* and *CEBP- β* genes was up-regulated after 1 hr and

Table 1. List of 10 Up and Down Regulated Genes in MCF-7 Cells That Significantly Related to Cancer Progression After Treated with TLE by Microarray Analysis

Pathway	Symbol	Gene name	Fold Change			
			1 hr	48 hrs	p-value	References
Cell cycle regulation	CYR61	cysteine-rich, angiogenic inducer, 61	2.132	2.387	0.023	(Xu et al. 2008)
	BTG1	B-cell translocation gene 1; anti-proliferative	2.096	2.474	0.05	(Rouault et al. 1992)
	CRYAB	crystallin, alpha B	2.041	2.288	0.006	(Lim et al. 2011)
	HIST2H3D	histone cluster 2, H3D	-2.214	-2.209	0.006	(Osley 1991)
Regulation of transcriptional activator, DNA binding	CEBP β	CCAAT/enhancer binding protein (C/EBP), β	2.178	2.662	0.034	(Shuman et al. 2004)
	TXNIP	Thioredoxin interacting protein	2.186	-2.233	0.006	(Zhang et al. 2010)
	HMG1L1	high-mobility group protein 1-like 1	-2.028	-2.019	0.019	(Rajeswari and Jain 2001)
p53 signaling	THBS1	Thrombospondin 1	2.297	-2.244	0.01	(Dameron et al. 1994)
mTOR signaling	HIF1A	hypoxia-inducible factor 1, alpha subunit	2.152	2.847	0.015	(Arsha et al., 2003)
JAK-STAT pathway	PIM1	Pim-1 oncogene	2.076	2.391	0.007	(Shirogane et al. 1999)

*The 2.0-fold differentially expressed genes were mapped to their relevant pathways provided by the KEGG database

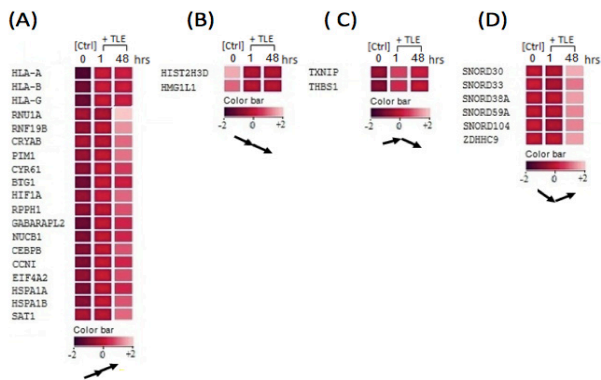


Figure 3. Gene Expression Profiles of MCF-7 Breast Cancer Cells Before (0 hour) and After Treatment (1 hour and 48 hours) with Tomato Leaf Extract (TLE). Each sample in the microarray analysis was performed in triplicates (3 chips). (A) Clustering of genes shows >2-fold of genes that increase in expression from 0 hour to 48 hours upon the extract treatment, (B) shows >2-fold of genes that decrease in expression from 0 hour to 48 hours, (C) shows >2-fold of genes that increase in expression from 0 hour to 1 hour and decrease towards 48 hours and (D) shows >2-fold of genes that decrease in expression from 0 hour to 1 hour and increase towards 48 hours. The data shown represent the mean values of the fold changes in expression level obtained from three independent experiments

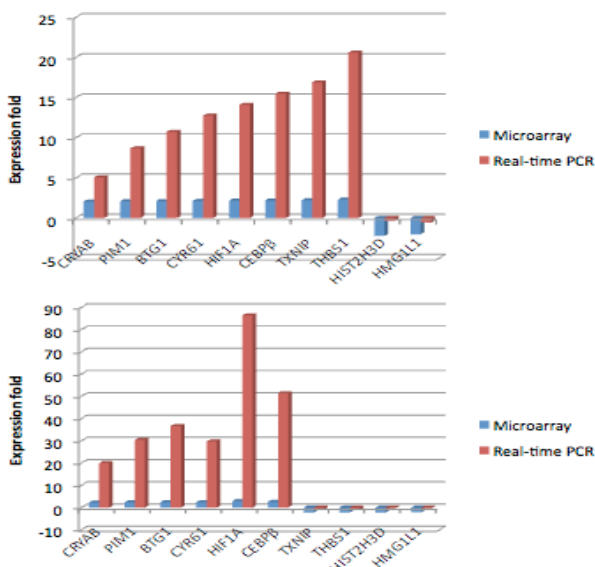


Figure 4. Microarray Identified Gene Validated by Real-time PCR Analysis. List of up and down regulated genes after 1 hour (A) and 48 hours (B) treated with TLE ($IC_{50}=5.85 \mu\text{g/ml}$) by microarray and RT-PCR ($p\text{-value}<0.05$). Fold change was calculated based on GAPDH (housekeeping gene)

48 hrs of treatment with TLE. However, the *TXNIP* and *THBS1* genes were up-regulated after 1 hr of treatment but were down-regulated after 48 hrs of treatment. In contrast, the *HMG1L1* and *HIST2H3D* genes, which were down-regulated in the microarray analysis ($p<22, 0.05$), exhibited only a marginal reduction at the mRNA level in the real-time PCR analysis. The gene expression levels were constantly decreased after 1 hr and 48 hrs of the treatment. These results are consistent with those of the microarray assay (Figure 4).

Discussion

Advances in cell biology and medical research are driving the growing need for technologies that enable the examination of new drugs effect towards dynamic molecular and physiological processes in individual living cells over extended periods of time. Several factors have been analyzed to describe TLE effects on MCF-7 cells by associating the cells growth with the genes expression levels. Hence, the effects of this treatment was studied by treating the MCF-7 cells with TLE ($IC_{50}=5.85 \mu\text{g/ml}$). Based on MCF-7 growth profiles (Figure 2) the exponential phase of the cells growth for both untreated and treated cells can be seen at the first after 24 hours. It shows that TLE exert their major killing effect on cells that are actively dividing at this phase during cell cycle. Furthermore, in our previous experiment, 1% of final concentration of DMSO in cultured cells will not give significant effect to MCF-7 cells growth (Chik et al., 2010).

In associates with MCF-7 growth profiles, cells were treated at 48 hours (mid-log phase) and after 1 hour (at 49 hours) total RNA of the treated cells were collected for microarray analysis in order to identify genes that responded early by the TLE treatment. The total RNA of the treated cells were also collected after 48 hours (at 97 hours) which at stationary phase. During this phase, the cells may be metabolically active even though growth is not occurring (Juliano 2002). The analysis was carried out together with the total RNA of untreated cells at 48 hours as a control (denoted as 0 hour in this experiment). The genes that were differentially expressed in MCF-7 breast cancer cells following 1 hr and 48 hrs of TLE treatment were listed in Figure 3. For the genes that are prominently related to cancer cell proliferation, we validated the results with transcript levels using real-time PCR analysis. The genes studied by real-time PCR were *CRYAB*, *PIM1*, *BTG1*, *CYR61*, *HIF1- α* , *CEBP- β* , *TXNIP*, *THBS1*, *HIST2H3D* and *HMG1L1*.

By real-time PCR analysis, the *Thrombospondin-1* (*THBS1*) gene was found to be significantly affected by TLE treatment, with the gene up-regulated (20.59-fold by real-time PCR, 2.29 by microarray) after 1 hr of treatment and down-regulated (1.13-fold by real-time PCR, 2.24 by microarray) 48 hrs later. The trend was consistent with the microarray data. Previous research has demonstrated *THBS1* as an extracellular matrix glycoprotein that influences cell adhesion, motility and growth (Robert, 1996). Based on its effects on tumor and endothelial cell behavior, this member of the *thrombospondin* gene family has attracted interest as a potential regulator of tumor growth and metastasis (Robert, 1996). His finding suggested that *THBS1* caused the cell cycle arrest, cellular senescence or apoptosis and the molecular interactions of *THBS1* observed *in vitro* may lead to novel therapeutic strategies for controlling cancer progression and metastasis (Robert, 1996). While Jia and Waxman (Jia and Waxman, 2012) found that *THBS1* together with Pigment Epithelium-derived Factor (PEDF) enhance responsiveness of KM12 colon cancer.

Thioredoxin interacting protein (TXNIP) is a tumor suppressor gene, and it has been suggested as a therapeutic target for cancer therapy (Urig and Becker, 2006; Powis and Kirkpatrick, 2007). Based on our microarray (Table 1) and real-time PCR analysis (Figure 4), the gene was shown to have antitumor activity in MCF-7 breast cancer cells. The gene expression was up-regulated (16.88-fold by real-time PCR) after the first hour and down-regulated (0.80-fold by real-time PCR) 48 hrs later. This gene has been evaluated as a therapeutic target, and previous reports have described that the expression of *TXNIP* was reduced after treatment with doxycycline in MCF-7 cells (Cadenas et al. 2010). Moreover, the *TXNIP* gene plays crucial roles in maintaining cellular redox homeostasis and cell survival and it is highly expressed in many cancers.

CEBP- β gene is involved in the regulation of a transcriptional activator and DNA binding element. The gene was up-regulated (15.47-fold by real-time PCR) after the first 1 hour of treatment and continued to increase (51.41-fold) for another 48 hours. Recent studies had reported that this gene is the *CCAAT/enhancer-binding protein-beta* in human (Cao, 1991; Szpirer et al., 1992), and it is a widely expressed transcription factor whose activity is regulated by oncogenic Ha-RasV12 signaling (Shuman et al. 2004). Explaining its response to TLE treatment at the first 1 hr of treatment. Moreover, the involvement of *CEBP- β* in human cancers has been further supported by a recent study showing that *CEBP- β* expression is highly correlated with cyclin D1-dependent tumors and that *CEBP- β* physically associates with cyclin D1 (Lamb et al., 2003). This may mediate cyclin D1-dependent activation of a set of target genes that are important for cell proliferation and tumorigenesis (Zhu et al., 2002).

In our study, transcriptional profiling has showed a down-regulation of the expression of *HMGIL1* during 1 hour and 48 hours of TLE treatment. The trend is consistent with the real-time PCR analysis, showed a marginal difference between 1 hour (0.56-fold) and 48 hours (0.23-fold) of treatment. *HMGIL1* is toxic, which may support its use as a potential target for therapeutic intervention (Wang et al., 1999). A previous report had identified *HMGIL1* as a cytokine that mediates local and systemic responses to necrotic cell death, cancer, invasion by pathogens, trauma, and sepsis (Lotze and Tracey, 2005).

Similarly, expression of *B-cell translocation gene 1 (BTG1)* was significantly affected by TLE. In our microarray dataset and by validation by real-time PCR analysis, an up-regulated trend was observed after 1 hour through 48 hrs of treatment. The gene was up-regulated (10.73-fold by real-time PCR) for the first 1 hour and the up-regulation was maintained (36.66-fold by real-time PCR) for another 48 hours. A previous report postulated that *BTG1* is a member of a new family of anti proliferative genes and negatively regulates cell proliferation (Rouault et al., 1992). This report strongly supports the regulation of the gene during TLE treatment where increased *BTG1* levels will decrease cell proliferation.

HIST2H3D gene was down-regulated (0.34-fold) for the first 1 hour of treatment and continued to decrease (0.24-fold) for another 48 hours. According to previous

reports, the expression of histone genes is restricted to the late G1 and S phases in somatic cells (Osley, 1991). The fact that histone gene expression is rigorously controlled implies that excess production or imbalanced synthesis of histone proteins might in some way harm the cell (Verdone et al., 2006). It is clear that the down-regulation of *HIST2H3D* during TLE treatment might lowering the total cells numbers caused by changes in the cell cycle process (Figure 2).

Furthermore, it has been reported that *CRYAB* is associated with DNA damage repair and cell cycle checkpoints (Xu et al., 2008). In our study, this gene was up-regulated during 1 hour and 48 hours of treatment. An expression of this gene was observed by real-time PCR analysis at 1 hour (5.05-fold) and 48 hours (19.99-fold). These results suggest that the expression of *CRYAB* in MCF-7 breast cancer cells is up-regulated during TLE treatment. Based on recent studies, *CRYAB* is up-regulated by p53 expression (Watanabe et al., 2009). The report determined that *CRYAB* was up-regulated by both tetracycline-inducible exogenous p53 and stress-inducible endogenous p53. The *CRYAB* gene has been identified as a novel p53-target gene.

The treatment with TLE also affected *proto-oncogene serine/threonine-protein kinase (Pim-1)* expression. Up-regulated expression of this gene was observed by real-time PCR analysis during 1 hour (8.66-fold) and 48 hours (30.46-fold) of TLE treatment. *Pim-1* is an enzyme that is encoded by the *Pim-1* gene in human (Luft, 2010). This gene has been implicated in multiple human cancers, including prostate cancer, acute myeloid leukemia and other hematopoietic malignancies (Schenone, 2010). It is mainly involved in cell cycle progression, apoptosis and transcriptional activation, as well as more general signal transduction pathways (Iadevaia et al., 2010). Besides, the expression of *Pim-1* has been shown to be regulated by the JAK/STAT pathway which has been reported to be associated with many different processes including cell survival, differentiation, and proliferation (de Groot, 1998).

This study has also identified the *HIF1- α* gene that has also been implicated in signaling through the mammalian target of rapamycin (mTOR) kinase. By real-time PCR analysis, this gene was up-regulated during 1 hour (14.08-fold) and 48 hours (86.36-fold) of TLE treatment. It was reported previously, that the up-regulated expression of *HIF1- α* inhibits signaling downstream of mTOR (Arsham, 2003) and mRNA translation initiation through multiple independent mechanisms (Liu et al., 2006). Signaling through this pathway appears to influence both tumor progression and hypoxia tolerance in cancers. The consistent results of the microarray and real-time PCR analyses (Table 1 and Figure 4) strongly support recent reports that have identified *HIF1- α* as caused which influenced common downstream pathways that affect gene expression, metabolism, cell survival, tumorigenesis and tumor growth (Wouters and Koritzinsky, 2008). Targeting tumor metabolism systems will be one of the new approaches to enforce necrotic cell death and tumor regression.

The effects of TLE treatment can also be observed in

the up-regulated expression of *Cyr61* by real-time PCR analysis after 1 hour (12.75-fold) and 48 hours (29.77-fold). *Cysteine-rich 61 (Cyr61)*, a member of the *CCN (Cystein-rich61, Cyr61/ connective tissue growth factor, CTGF/ Nephroblastoma overexpressed, NOV)* protein family, has been implicated in diverse biological processes including cell adhesion, proliferation, angiogenesis, and tumorigenesis (Lv, 2009). Levels of *Cyr61* expression are associated with the status of the tumor suppressor gene p53 in breast cancer cell lines (Mendez et al., 2003). The report showed that over-expression of dominant negative p53 or knock-down of endogenous wild-type p53 resulted in up-regulation of *Cyr61* expression, suggesting a functional link between *Cyr61* and p53 in cancers. Similarly, *Cyr61* was shown to behave as a tumor suppressor gene in promoting growth arrest and up-regulation of p53 as well as attenuating tumor growth *in vivo* (Tong et al., 2001).

In conclusion, microarray and real-time PCR analysis have confirmed that TLE involved with the cytotoxicity or cell death mechanism in TLE treated-MCF-7 cells by showing that several genes related to cancer cell progression were differentially expressed in MCF-7 cells following 1 hour and 48 hours of the extract treatment. The close agreement between the microarray results and the real-time-PCR results validated the specificity and accuracy of the experiments. These results demonstrate the molecular mechanisms associated with the activity of TLE as an inhibitor of cancer cell progression.

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