RESEARCH ARTICLE

Expression Profile of Genes Modulated by *Aloe emodin* in Human U87 Glioblastoma Cells

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Abstract

Glioblastoma, the most aggressive and malignant form of glioma, appears to be resistant to various chemotherapeutic agents. Hence, approaches have been intensively investigated to targeti specific molecular pathways involved in glioblastoma development and progression. *Aloe emodin* is believed to modulate the expression of several genes in cancer cells. We aimed to understand the molecular mechanisms underlying the therapeutic effect of *Aloe emodin* on gene expression profiles in the human U87 glioblastoma cell line utilizing microarray technology. The gene expression analysis revealed that a total of 8,226 gene alterations out of 28,869 genes were detected after treatment with 58.6 μg/ml for 24 hours. Out of this total, 34 genes demonstrated statistically significant change (p<0.05) ranging from 1.07 to 1.87 fold. The results revealed that 22 genes were up-regulated and 12 genes were down-regulated in response to *Aloe emodin* treatment. These genes were then grouped into several clusters based on their biological functions, revealing induction of expression of genes involved in apoptosis (programmed cell death) and tissue remodelling in U87 cells (p<0.01). Several genes with significant changes of the expression level e.g. SHARPIN, BCAP31, FIS1, RAC1 and TGM2 from the apoptotic cluster were confirmed by quantitative real-time PCR (qRT-PCR). These results could serve as guidance for further studies in order to discover molecular targets for the cancer therapy based on *Aloe emodin* treatment.

Keywords: *Aloe emodin* - microarray analysis - gene expression - human glioblastoma cells

Introduction

Glioblastoma (WHO grade IV) is the most common and most aggressive type of malignant brain tumour. Although glioblastoma differs from other type of cancer due to the fact that it is rarely metastasize (Rich et al., 2005), the mortality rate is still nearly 100% and survival of patients are still less than 1 year after conventional treatment was used (Caffo et al., 2013). Hence, glioblastoma remains one of the most common fatal diseases. Current treatment for glioblastoma patients includes the use of a combination of surgery, radiotherapy and chemotherapy. Despite these advances in treatment, overall outcome of glioblastoma remains poor in patients (Wintner et al., 2011). Some of these treatments are quite expensive and highly toxic. Therefore, new therapeutic agent with low cost and less toxicity need to be developed.

Progression of glioblastoma is believed to be associated with the accumulation of a variety of genetic alterations and the changes of gene expression pattern (Godoy et al., 2013). Understanding the altered genetic molecular pathways of glioblastoma progression in order to develop promising new treatment targets may help to improve survival in patients (Collins and Workman, 2006). Nowadays, many drugs are found to give side effect and

consequently affect the prognosis of cancer treatment. Approximately 30% of patients are prone to give up due to the severity of modern drug side effects. Thus, many attempts have been made to increase the therapeutic effects and reduce the side effects by developing new therapeutic approaches with new mechanisms of action (Zhao, 2012).

Recently, researchers have shown an increased interest in plant-based compounds as therapeutic agents and some of these natural products are believed to have the ability to prevent cancer and other chronic diseases. Up to now, many innovations of new drugs particularly covering in the areas of cancer, originated from natural sources. As one of the tropical Chinese medicines, *Aloe emodin* has a long history of use throughout the world in treating and preventing human diseases (Cheng et al., 2012). Despite increasing demands for natural compounds as the modern medicine considered expensive, *Aloe emodin* has been extensively studied in the last decades for its anticancer therapeutic effects (Pecere et al., 2000).

Studies have suggested that *Aloe emodin* possess anti-tumour effect, which regulates or modulates the expression of genes that are involved in the control of cancer cell development and progression (Avecedo-Duncan et al., 2004). *Aloe emodin* exerts broad modulation in various cellular events. It modulates cellular processes

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such as DNA damage and DNA repair genes activity (Chen et al., 2010), induction of the cell cycle arrest and apoptosis (Suboj et al., 2012) and inhibition of cellular invasion, migration and angiogenesis (Suboj et al., 2012). *Aloe emodin* is reported to suppress lung cancer cells proliferation via downregulating expression of ERCC1 and Rad51 and inactivating ERK1/2 signaling pathway (He et al., 2012) and it also capable to inhibit breast cancer cell proliferation by downregulating ERα protein levels and suppressing ERα transcriptional activation (Huang et al., 2013). Recently, there was also information showed that these compounds modulated epigenetic modification of oncogenic gene expression in bladder cancer (Cha et al., 2013). Despite many studies suggesting that *Aloe emodin* as preventive agent in many cancer cell lines, there is not enough molecular modulation information or evidence at this time on the effect of *Aloe emodin* in brain tumor cells. In the present study, we used the high-throughput technique of gene microarray analysis to understand the molecular mechanisms underlying the anti-tumour action of *Aloe emodin* treatment in U87 cells by modulating gene expression.

Materials and Methods

U87 cells culture

U87 is a glioblastoma cell line originated from human brain malignant gliomas used in this study was obtained from American Type Culture Collection (Manassas, VA, USA). U87 cells were cultured in Dulbecco's-modified eagle's medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin (Gibco, Carlsbad, CA, USA) and maintained at 37°C with 5% CO_2 in a humidified atmosphere.

Aloe emodin treatment on U87 cells

Aloe emodin and dimethyl sulfoxide (DMSO) were purchased from Sigma- Aldrich Co. (St. Louis, MO, USA). *Aloe emodin* was dissolved in less than 1% DMSO to a stock concentration at 10 mg/ml. It was further diluted in fresh growth medium to the desired working concentration before use. U87 cells were seeded in 96 well plates at a density of 5 x 10^4 per well. A graph curve was plotted and indicated an exponential curve pattern across 3 days (24, 48 and 72 hours). The cells were then treated with different concentrations of *Aloe emodin* (0, 20, 40, 60 and 80 μg/ml) for various lengths of time (24, 48 and 72 hours).

Cell proliferation and viability assay

The viability of U87 cells treated with and without *Aloe emodin* was analyzed by CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA), also known as MTS assay. MTS is a tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium, inner salt] that undergoes a color change caused by its bioreduction of MTS into a blue formazan product. The 50% Inhibitory concentration (IC50) value was determined as previously described (Freshney, 2000).

Total RNA extraction

Total RNA of untreated and treated cells (24 hours) were extracted using Trizol® reagent (Life technologies, Carlsbad, CA, USA) following the manufacturer's instructions. The quality and the concentration of the RNA were determined using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Dreieich, Germany) and 1% agarose gel. Qualified total RNA was further purified by RNeasy® mini kit (QIAGEN, Valencia, CA, USA). RNA was then stored at -80°C until further analysis.

Microarray experiment

Microarray gene expression experiment was performed using GeneChip® Human Gene 1.0 ST Array (Affymetrix, Santa Clara, CA, USA) that contains approximately 28,869 well annotated genes. RNA was reverse transcribed and amplified into cDNA using Applause™ WT-Amp ST System and Encore™ Biotin Module according to manufacturer's protocol (NuGEN Technologies, San Carlos, CA, USA). Amplified cDNA was then purified using MinElute® Reaction Cleanup Kit (Qiagen, Valencia, CA, USA). The raw data obtained from the Affymetrix Software was then analysed using GeneSpring GX 12.0 Software (Agilent Technologies, Santa Clara, CA, USA) and followed by biological function analysis using Database for Annotation, Visualization and Integrated Discovery (DAVID) Software (http://david.abcc.ncifcrf.gov) was used as previously described (Huang et al., 2009).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

To validate microarray data, the qRT-PCR on selected genes was performed in triplicates using Taqman gene expression assay and analyzed using Stratagene Mx3005P Real-Time PCR Machine (Stratagene, La Jolla, CA, USA). Primer probe sets were purchased from Thermo Scientific Solaris qPCR Gene Expression Assay (Thermo Scientific, Germany) and the assay was performed as described by the manufacturer. Total RNA from the untreated and treated cells previously extracted, was reverse transcribed into cDNA using SuperScript™ III First-Strand Synthesis SuperMix (Life Technologies, Carlsbad, CA, USA). The qRT-PCR assay was conducted with amplification conditions at 95°C for 15 minutes for enzyme activation, 40 cycles of denaturation at 95°C for 15 seconds, 60°C for 1 minute for annealing and extension. β-actin was used as an internal control, and the expression level was estimated by the Relative Expression Software Tool Software (Pfaffl et al., 2001).

Results

Effect of *Aloe emodin* on U87 cell proliferation

In this study, we found *Aloe emodin* significantly decreased proliferation of U87 cells in a time and dose-dependent manner (Figure 1). After treatment with 40, 60 and 80 μg/ml of *Aloe emodin* for all time periods (24, 48 and 72 hours), a significant decrease in the number of viable cells was observed in U87 cells as ($p<0.05$)

compared to untreated cells. In addition, treatment with 60 and 80 μg/ml for 72 hours caused a similar significant reduction in cell viability. The IC50 value of *Aloe emodin* at 24 hours was 58.6μg/ml and this decreased to 25.0μg/ml and 24.4μg/ml at 48 hours and 72 hours, respectively.
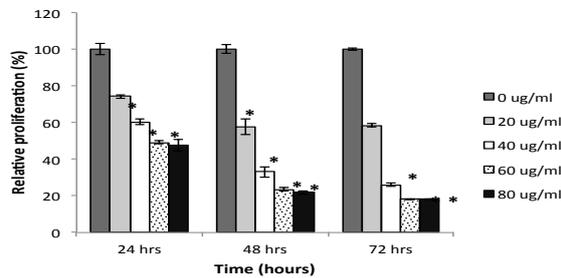

Figure 1. Effect of Aloe Emodin Treatment on the Proliferation of U87 Cells. Cells were treated in the absence or presence of increasing concentration of *Aloe emodin* (0, 20, 40, 60 and 80 μg/ml) for specified time periods (24, 48 and 72 hours). Values were mean percentage of n=6 with error bars. Values of $p<0.05$ were considered statistically significant (*). Statistical analysis using repeated measure of ANOVA (SPSS 19)

Gene expression profile of U87 cells in response to aloe emodin

We investigated the gene expression profile of U87 cells after 24 hours *Aloe emodin* treatment. The identification of differentially expressed genes in *Aloe emodin* treated and untreated groups were carried out using GeneSpring GX 12.0 software. In this study, 8,226 out of 28,869 genes were altered at 24 hours *Aloe emodin* treatment. Out of these genes, there were 34 genes (either up or down-regulated) found to be significant ($p<0.05$) with fold change more than 1. Among them, 22 up-regulated genes and 12 down-regulated genes. The upregulated and downregulated genes are listed in Tables 1A and 1B, respectively.

By using DAVID bioinformatics tools, clusters of genes associated with relevant biological functions annotation termed as gene ontology (GO) were identified. The clusters that had enrichment score more than 1.0 were shown in Table 2. The group of significant terms (GO terms) related to apoptosis revealed the highest enrichment score which was 1.81. This enrichment score was calculated based on the genes that shared or having

Table 1A. Genes that are Up-Regulated in U87 Cells at 24 Hours After *Aloe emodin* Treatment

GenBank ID	Gene Symbol ID	Gene Description	p value	Fold change
AB016816	MFHAS1	Malignant fibrous histiocytoma amplified sequence 1	0.027	1.06
BC017280	PNPLA2	Patatin-like phospholipase domain containing 2	0.012	1.09
AF151893	FIS1	Fission 1 (mitochondrial outer membrane) homolog (S. cerevisiae)	0.027	1.11
BC028166	C8orf38	Chromosome 8 open reading frame 38	0.001	1.11
BC013018	MRPS26	Mitochondrial ribosomal protein S26	0.021	1.11
DQ005958	C19orf6	Chromosome 19 open reading frame 6	0.049	1.12
AB115770	MTSS1L	Metastasis suppressor 1-like	0.047	1.13
L25879	EPHX1	Epoxide hydrolase 1, microsomal (xenobiotic)	0.002	1.13
BX640646	ZKSCAN1	Zinc finger with KRAB and SCAN domains 1	0.023	1.13
AB006909	MITF	Microphthalmia-associated transcription factor	0.048	1.13
BC075852	STAT6	Signal transducer and activator of transcription 6, interleukin-4 induced	0.002	1.14
BC101548	SGK196	Protein kinase-like protein SgK196	0.031	1.15
AK301885	GOLGA6L5	Golgi autoantigen, golgin subfamily a-like pseudogene	0.043	1.16
AK300311	SCAP	SREBF chaperone	0.044	1.19
BC036655	ST5	Suppression of tumourigenicity 5	0.029	1.2
AA426047	PCNXL3	Pecanex-like 3 (Drosophila)	0.03	1.2
BC065292	BCAP31	B-cell receptor-associated protein 31	0.042	1.21
FJ655995	SHARPIN	SHANK-associated RH domain interactor	0.041	1.26
AK092583	WASH1	Similar to WAS protein family homolog 1	0.035	1.26
AK126924	NDRG1	N-myc downstream regulated 1	0.02	1.37
AJ001258	NIPSNAP1	Nipsnap homolog 1 (C. elegans)	0.019	1.41
BC068450	SIAE	Sialic acid acetylesterase	0.026	1.87

Fold changes in expression were determined relative to *Aloe emodin* untreated (control)

Table 1B. Genes that are Down-Regulated in U87 Cells at 24 Hours After *Aloe emodin* Treatment

GenBank ID	Gene Symbol ID	Gene Description	p value	Fold change
BC067796	C8orf76	Chromosome 8 open reading frame 76	0.041	-1.11
DQ231041	HIVEP2	Human immunodeficiency virus type 1 enhancer binding protein 2	0.019	-1.21
AF064102	CDC14A	CDC14 cell division cycle 14 homolog A (S. cerevisiae)	0.018	-1.23
D67029	SEC14L1	SEC14-like 1 (S. cerevisiae) SEC14-like 1 pseudogene	0.027	-1.27
AL591493	HIST2H2AB	Histone cluster 2, H2ab	0.024	-1.28
AK054918	C6orf141	Chromosome 6 open reading frame 141	0.027	-1.29
BC060853	HN1L	Hematological and neurological expressed 1-like	0.023	-1.3
M55153	TGM2	Transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltranferase)	0.044	-1.3
EU832717	RAC1	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac 1)	0.049	-1.41
AK301677	KIAA0146	KIAA0146; SPIDR scaffolding protein involved in DNA repair	0.02	-1.47
BC032702	GPR3	G protein-coupled receptor 3	0.043	-1.47
AK297904	C15orf29	Chromosome 15 open reading frame 29	0.015	-1.79

Fold changes in expression were determined relative to *Aloe emodin* untreated (control)

Table 2.Summary of Differential Gene Expression Cluster Based on GO Term of Biological Process (combination of up and down-regulated genes)

Annotation cluster 1 Enrichment Score: 1.81		p value
GO:0012501- programmed cell death	FIS1, SHARPIN, BCAP31, RAC1, TGM2	< 0.01
GO:0008219- cell death	FIS1, SHARPIN, BCAP31, RAC1, TGM2	< 0.05
GO:0016265- death	FIS1, SHARPIN, BCAP31, RAC1, TGM2	< 0.05
GO:0006915- apoptosis	FIS1, SHARPIN, RAC1	< 0.05
Annotation cluster 2 Enrichment Score: 1.05		p value
GO:0048771- tissue remodeling	MITF, RAC1, TGM2	< 0.01
GO:0042981- regulation of apoptosis	MITF, RAC1, TGM2	0.291
GO:0043067- regulation of programmed cell death	MITF, RAC1, TGM2	0.295
GO:0010941- regulation of cell death	MITF, RAC1, TGM2	0.296

*The functional annotation clusters were shown as enrichment score. Apoptotic-related cluster showed the highest enrichment score (1.81) with statistical significance value ($p<0.01$). GO terms with significant p value were in bold. (DAVID software)

Table 3. Comparison of Fold Change Expression for SHARPIN, BCAP31, FIS1, RAC1 and TGM2 Genes in U87 Cells Upon *Aloe emodin* Treatment at 24 hours

Gene description	Fold-change	
	Microarray	qRT-PCR
SHARPIN	1.26	1.35
BCAP31*	1.21	1.56
FIS1	1.11	1.09
RAC1	-1.41	-1.24
TGM2	-1.3	-1.89

The expression level by fold changes observed with microarray and qRT-PCR analysis are indicated, where a positive value indicates an increase in gene expression and a negative value indicates a decrease in gene expression. (*) gene that showed a significant correlation of $p<0.05$ between microarray and qRT-PCR data
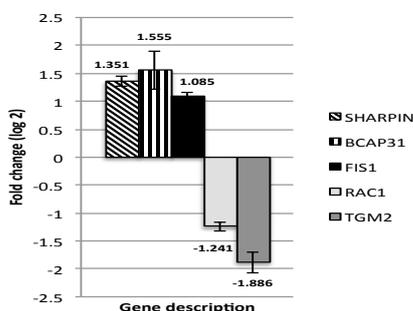

Figure 2. Validation of Relative Expression Level (fold-change) of Selected Genes (SHARPIN, BCAP31, FIS1, RAC1 and TGM2 Genes) in Treated group Relative to Untreated Group Using qRT-PCR. The expression levels of selected genes from qRT-PCR are consistent with microarray results. The gene expression level quantitatively considered up-regulated if more than 1 and down-regulated if less than -1. Error bar represents the mean±SEM of fold change from triplicate (n=3). (REST©-MCS software)

similar biological functions. In this group, a total of five genes were identified. These genes were thought to be an indication of biological relevance specifically in apoptosis that acts as the critical point and plays an important role in this study. It could be suggested that these findings may serve new information of newly candidate genes regulated by *Aloe emodin*. Therefore, these genes have been selected for validation analysis using qRT-PCR.

Validation of microarray data by qRT-PCR

To verify the results from the microarray gene expression profile data, qRT-PCR experiment was performed to assess the changes of gene expression at the mRNA level. The five selected genes of the *Aloe emodin*-regulated (3 genes from up-regulation and 2 genes from down-regulation) were chosen for the qRT-PCR confirmation. The mRNA levels of 5 selected genes (SHARPIN, BCAP31, FIS1, RAC1 and TGM2) were confirmed significantly to be regulated ($p<0.05$) in response to 24 hours treatment with 58.6 μg/ml of *Aloe emodin* (Figure 2). In general, our results showed a significant and consistent correlation between the microarray data and the qRT-PCR data (Table 3).

Discussion

Aloe emodin, a natural bioactive anthraquinone compound present in the leaves of Aloe vera, appears to exert multiple inhibitory effects on cancer cells via modulation of multiple cellular signaling pathways (Lu et al., 2007). Although the antitumor effects of *Aloe emodin* have been investigated extensively in various cancers, its modulatory effects on gene expression have not been studied in brain tumour. In the present study, we found that *Aloe emodin* significantly inhibits U87 cell proliferation in dose-dependent and time-dependent manners. This result is consistent with previous reports on inhibitory effects of *Aloe emodin* treatment in various human cancer cells such as hepatoma cells (Kuo et al., 2002), skin cancers (Wasserman et al., 2002), gastric cancers (Qin et al., 2010), leukaemic cells (Tabolacci et al., 2011) and breast cancer cells (Huang et al., 2013). Our study also demonstrated that the IC$_{50}$ at 24 hours of *Aloe emodin* treatment in U87 cells was much higher as compared to other human cancer cells such as lung squamous cancers (Lee, 2001), liver cancers (Kuo et al., 2002) and tongue squamous cancers (Chiu et al., 2009). It could be suggested that *Aloe emodin* may exert a cell-dependent type effect which is less sensitive against U87 cells than other cells. In addition, our microarray gene expression results revealed that 22 and 12 of the 8,226 expressed genes (out of 28,869 analyzed genes) are significantly ($p<0.05$; fold change of >1) up or down-regulated, respectively, in response to *Aloe emodin* treatment in U87 cells. These findings demonstrated that *Aloe emodin* upregulated and downregulated important genes that belong to apoptosis, lipid metabolism and tissue

remodelling in U87 cells.

Among the upregulated genes, SHARPIN, BCAP31, FIS1 and STAT6 play an important role in apoptosis. SHARPIN gene (SHANK-associated RH domain interactor) is also known as shank-interacting protein–like 1 (SIPL1). SHARPIN gene encodes for phosphoproteins that are conserved mainly in the cytoplasm (Lim et al., 2001). Up-regulation of this gene in multiple human cancer types have been addressed for tumour-associated role in carcinogenesis (Jung et al., 2010). However, this gene has been identified to regulate intrinsic caspase-dependent mitochondria pathway leading to keratinocyte apoptosis (Liang and Sundberg, 2011). BCAP31 (B cells-associated 31) gene is also known as BAP31, encodes for integral membrane protein that regulates protein processing and transmission from endoplasmic reticulum (ER) to Golgi apparatus (Wakana et al., 2008). Interestingly, overexpression of BCAP31 has been suggested to correlate with better prognosis in colorectal cancer. In contrast, deregulation of this gene leads to more aggressive invasion of the colorectal cancer cells (Ling et al., 2011). This gene also plays important role in apoptotic event (Iwasawa et al., 2011). Fission 1-fission of mitochondrial outer membrane (FIS1) gene encodes for protein involves in regulation of mitochondrial fission. The activation of mitochondrial fission activities is important in the late stage of apoptosis in cancer cells (Zhang and Chan, 2007). The increase of this gene confers to apoptotic stimuli (Mai et al., 2010). Previous study has postulated that FIS1 plays a critical role in the release of caspase-9 and cytochrome c which leads to apoptotic event via caspase-3 activation. Moreover, FIS1 can also induce fragmentation of the mitochondria network leading to apoptosis (Lee et al., 2004). STAT6 is a member of the STAT family of transcription factors. STAT family members are activated by extracellular signalling proteins including cytokines, growth factors and certain peptides and accumulated in the nucleus to promote transcription (Levy and Darnell, 2002). The up-regulation of this gene is in agreement with previous study where activation of STAT6 mediates growth inhibition and apoptosis in human breast cancer cells following interleukin-4 (IL-4) treatment (Gooch et al., 2002). As reviewed by Goenka and Kapla (2011), STAT6 mediated the cytokine signalling pathway and regulated the transcription (Goenka and Kaplan, 2011).

Aloe emodin also upregulated some of the genes involved in lipid metabolism (PNPLA2 and SCAP) as well as tissue remodeling (MITF). PNPLA2 encodes for patatin-motif containing lipases localized in chromosome 11 (also known as ATGL or adipose triglyceride lipase). There are eight types of patatin-like protein phospholipases. PNPLA2 plays major role in the lipid metabolism (Holmes, 2012). Dysregulation of this gene leads to autosomal recessive disorder of neutral lipid storage disease with myopathy (NLSD-M) (Tavian et al., 2012). As brain also metabolizes fatty acid as a source of energy (Marin-Valencia et al., 2013), the overexpression of PNPLA2 induced by *Aloe emodin* could plays a role in positive regulation of lipid metabolism in glioblastoma patients. SCAP is a regulatory element binding protein

(SREBP) cleavage-activating protein (SCAP). SCAP gene plays important role as a sterol sensor (Espenshade, 2006) as well as a transcriptional regulator of lipid metabolism and cellular growth (Williams et al., 2013). Previous research has shown that androgen (therapeutics agent used for advanced prostate cancer) has increased the expression of SCAP suggesting lipogenic effect of this agent in prostate cancer cells (Heemers et al., 2001). Later, a study in line with Heemers et al (2001) findings, showed that the SCAP gene has down-regulated by androgen after its removal from prostate cancer cells (Ettinger et al., 2004).

We also found MITF and SIAE are upregulated on *Aloe emodin* treatment. MITF gene encodes for transcription factor with a basic-helix-loop-helix-zipper (bHLH-Zip) DNA-binding protein (Tachibana et al., 1996). It plays a pivotal role in melanocyte progression, differentiation and metastasis (Tachibana et al., 1996). In addition, MITF is a sensitive and specific marker for melanoma, a highly chemotherapy-resistance neoplasm (McGill et al., 2002). MITF was demonstrated to promote the occupancy of osteoclast target promoters (activated form of MITF) and regulation of co-recruitment of chromatin-remodelling complex (Sharma et al., 2007). The up-regulation of MITF gene suggests that *Aloe emodin* may affect tissue remodelling of U87 cells. SIAE (sialic acid acetylesterase) gene expression showed the highest fold change level. The SIAE encodes for enzyme which removes 9-O-acetylation modifications from sialic acid and is largely found in the cytosol or lysosome. Decrement of SIAE gene level was observed in lymphoblasts of childhood acute lymphoblastic leukemia. It suggests that overexpression of this gene may act as a therapeutic agent in chemotherapy (Mandal et al., 2012). Hence, *Aloe emodin* might be a potential anti-resistance agent to chemotherapy based on increased level of SIAE in this study.

Interestingly, microarray analysis also resulted in increased expression of metastasis suppressor 1-like gene (MTSS1L) suggesting that *Aloe emodin* could be a new anti-metastatic therapeutic agent for anticancer therapy. The present findings is in agreement with a study conducted by Lu et al. (2007) where *Aloe emodin* also induced other type of metastatic suppressor, known as NM23 in human hepatocellular carcinoma cell line (Lu et al., 2007).

Aloe emodin-treated U87 cells downregulated the genes RAC1 and TGM2. RAC1 gene is a member of the monomeric G-protein, Rho GTPases family member. It plays crucial roles in signal transduction pathway that control proliferation, adhesion, and migration of cells during embryonic development, invasiveness of tumour cells (Jin et al., 2007) as well as apoptosis (Sun et al., 2006). The expression of RAC1 is important for cell growth, motility and morphology in glioma cells (Hu et al., 2009; Moniz et al., 2013). Blockade of RAC1 signalling in tissue remodelling promotes the accumulation of type I collagen due to decreased collagenase activity (Igata et al., 2010). In addition, reduction of RAC1 was observed in breast cancer cells induced by a pharmacologic inhibitor, NSC27366 which resulted in tumour cell toxicity and apoptosis (Yoshida et al., 2010). TGM2 is conserved in the cytoplasm, nucleus and plasma membrane (Milakovic

et al., 2004). TGM2 encodes for protein of extracellular matrix (ECM) molecules that are capable of catalysing protein cross-links between tumour cells and their microenvironments mediated by cell surface receptors (Fu et al., 2013). The reduced TGM2 level is a potential target in the treatment of highly metastatic cancer cells (Mehta et al., 2004). In contrast, activation of TGM2 has been suggested as a mediator for anti-apoptotic event in hypoxic tumour cells by inhibiting both caspase-3 activation and apoptosis induction (Jang et al., 2010). The increased expression of TGM2 in glioblastoma suggests a highly malignant glioma (Fu et al., 2013).

For validation analysis of pooled microarray data, a total of five significant genes identified in the apoptotic-related cluster e.g. SHARPIN, BCAP31, FIS1, RAC1 and TGM2 genes were successfully reconfirmed using qRT-PCR. All genes showed similar pattern of gene expression (either up-regulation or down-regulation) as the gene expression detected in microarray.

In conclusion, our findings revealed that *Aloe emodin* decreased cell viability of U87 cells and also modulated the expression of some genes that involved in various cellular functions. *Aloe emodin* inhibits the proliferation of U87 cell by disruption of genes that belong to different signaling pathways specifically participated in the apoptosis. Therefore, these findings give the way for further clarifying pathways affected by *Aloe emodin* on brain cancer cells, in order to identify and validate the new molecular targets for drug development.

Acknowledgements

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