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Differentially Expressed Genes (DEGs) Analysis Indicating 6-Shogaol Anticancer Activity against HCT-116 Cells Performed Primarily by Affecting Genes in Common (shared-DEGs) of Apoptotic and p53 Signaling Pathways

(Analisis Pengekspresan Gen Berbeza (DEGs) Menunjukkan Aktiviti Antikanser 6-Shogaol terhadap Sel HCT-116 Dijalankan Terutamanya dengan Mempengaruhi Gen Bersama (shared-DEGs) Tapak Jalan Pengisyaratan Apoptotik dan p53)

ARYO TEDJO[1,2,3,] DIMAS R NOOR[4], KUSMARDI[2,4,5,6,*], NUR AYU RAMADANTI[3] & AMELIA KASSIM[7]

[1]*Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[2]*Drug Development Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[3]*Master's Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, DKI Jakarta, Depok, Indonesia*
[4]*Human Cancer Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[5]*Department of Pathology Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[6]*Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, DKI Jakarta, Depok, Indonesia*
[7]*Faculty of Information Science and Technology, Multimedia University, 75450 Bukit Beruang, Melaka, Malaysia*

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ABSTRACT

Ginger have strong anti-inflammatory and antioxidant properties that can inhibit growth and trigger apoptosis of colorectal cancer cells. Dried ginger containing the main bioactive compound 6-shogaol is widely used for practical reasons in storage. In this study, the mechanism of 6-shogaol apoptosis was tried to be explained by looking at the Differentially Expressed Genes (DEGs) changes in HCT-116 cells. The results of DEGs analysis on Gene Expression Omnibus (GEO) data which contained gene expression data for the HCT-116 cell group treated with 6-shogaol and the control group showed that there were 142 DEGs of the apoptotic pathway with 9 (nine) of them being share-DEGs of the apoptotic pathway with other pathways related to apoptosis such as MAPK signaling pathway, p53 signalling pathway, and protein processing in endoplasmic reticulum pathways. Based on the regulation of the 9 share-DEGs, it was also explained that the apoptotic activity of 6-shogaol mainly occurs through the p53 signalling pathway which involves up-regulation of BAX, BBC3, GADD45G, and TNFRSF10A.

Keywords: Apoptosis; Differentially Expressed Genes; HCT-116; p53 signaling pathway; 6-shogaol

ABSTRAK

Halia mempunyai sifat anti-radang yang kuat dan antioksidan yang boleh menghalang pertumbuhan serta mencetuskan apoptosis sel kanser kolorektal. Halia kering yang mengandungi sebatian bioaktif utama 6-shogaol digunakan secara meluas atas alasan praktikal dalam penyimpanan. Dalam kajian ini, mekanisme apoptosis 6-shogaol cuba diterangkan dengan melihat perubahan Pengekspresan Gen Berbeza (DEG) dalam sel HCT-116. Hasil analisis DEG pada data Pengekspresan Gen Serbaneka (GEO) yang mengandungi data pengekspresan gen bagi kumpulan sel HCT-116 yang dirawat dengan 6-shogaol dan kumpulan kawalan menunjukkan bahawa terdapat 142 DEG dalam tapak jalan apoptosis dengan 9 daripadanya adalah DEG bersama dalam tapak jalan apoptosis dengan tapak jalan lain yang berkaitan seperti tapak jalan pengisyaratan MAPK, tapak jalan pengisyaratan p53 serta tapak jalan pemprosesan protein dalam retikulum endoplasma. Berdasarkan pengawalan 9 DEG bersama itu juga diterangkan bahawa aktiviti apoptosis 6-shogaol utamanya berlaku melalui tapak jalan pengisyaratan p53 yang melibatkan peningkatan pengawalan atas BAX, BBC3, GADD45G dan TNFRSF10A.

Kata kunci: Apoptosis; HCT-116; Pengekspresan Gen Berbeza; tapak jalan pengisyaratan p53; 6-shogaol

Introduction

World Health Organization (WHO 2022) data shows that colorectal cancer (CRC) is cancer with the third incidence and the second leading cause of death among cancers worldwide. The currently available treatments for colorectal cancer are laparoscopic surgery, resection, palliative, neoadjuvant chemotherapy, and radiotherapy. Chemotherapy causes unwanted side effects. Besides being often ineffective, chemotherapy treatments are also relatively expensive (De et al. 2023). The use of phytochemicals as an anticancer alternative is attractive because natural products are considered relatively safer because their consumption by humans is widespread, and cost-effective.

Ginger, or *Zingiber officinale*, is a spice plant that is widely used as a spice in cooking around the world. Apart from having a distinctive taste and aroma, ginger is also known to have several health benefits, one of which is anti-cancer. Several studies have shown that ginger has strong anti-inflammatory and antioxidant properties and may help combat inflammation and oxidative stress associated with cancer (Citronberg et al. 2013; Habib et al. 2008; Mashhadi et al. 2013; Nile & Park 2015). Ginger is known to have anti-proliferative properties, which can help inhibit cancer cell growth (Habib et al. 2008).

Several *in vitro* studies have shown that ginger and its active components inhibit the growth and proliferation of colorectal cancer cells. Ginger is known to have inhibitory properties on the formation of colorectal cancer by carcinogens such as studies on mice induced by azoxymethane (AOM) and dimethylhydrazine (El-Sayed et al. 2022; Yoshimi et al. 1992). A phase II study of the effects of the ginger root extract in normal people showed that there was a decrease in eicosanoid levels, which are markers of inflammation that trigger colorectal cancer in biopsies, colon mucosa (Zick et al. 2011). Ginger extract also has a synergistic effect with chemotherapeutic compounds. Sarmoko et al. (2020) showed that ginger extract was able to increase the cytotoxic effect of the chemotherapeutic agent 5-Fluorouracil on Widr cells, which are adenocarcinoma cells of the colon.

The known anticancer mechanisms of ginger extract include influencing the expression of MMP-2 and KRAS (Malmir, Ebrahimi & Mahjoubi 2020). Fibroblasts constitutively express matrix metalloproteinase 2 (MMP-2) and KRAS play a role in regulating the steady-state expression of matrix metalloproteinase 2 in fibroblasts (Liao, Wolfman & Wolfman 2003). Fibroblasts are known to help cancer cells become more aggressive through the proteins they produce. 6-gingerol is the compound in ginger that inhibits cell growth and apoptotic activity against colorectal cancer cells through inhibition of the MAPK/AP-1 signaling pathway (Lee, Cekanova & Baek 2008; Radhakrishnan et al. 2014). Meanwhile, 8-gingerol (isomer of 6-gingerol) was known to inhibit proliferation, trigger apoptosis, and inhibit cancer cell migration by regulating the EGFR/STAT/ERK pathway (Hu et al. 2020). Other compounds in ginger such as 6-shogaol are also known to enhance the anticancer effects of 5-fluorouracil, oxaliplatin, and irinotecan by increasing apoptosis and autophagy in colon cancer cells under hypoxic conditions (Woźniak et al. 2020).

Based on the explanation, the mechanism of antiproliferation and apoptosis of ginger extract or compounds contained in ginger can be seen through the pattern of changes in the expression (or regulation) of genes involved in the apoptotic signaling pathway or the proliferation of cancer cells due to administration of extracts/compounds. This suggests that Differentially Expressed Genes (DEGs) analysis can be used to see the effect of a cancer therapy. In this study, changes in DEGs related to apoptosis were taken from the Gene Expression Omnibus (GEO) database in HCT-116 colorectal cancer cells treated with 6-shogaol. 6-gingerol is the most abundant bioactive compound in fresh ginger, while 6-shogaol is abundant in dried ginger (Bischoff-Kont & Fürst 2021). Dried ginger preparations are of course preferred because of their practicality in storage. A comparison of the apoptotic activity of gingerol and 6-shogaol was shown by Bawadood et al. (2021). In the study, MDA-MB-231 cancer cells were known to experience a significant increase in apoptosis before and 24 h after being treated with 6-shogaol compared to gingerol. Thus, the aim of this study was to determine which genes were expressed significantly differently by HCT-116 colorectal cancer cells due to administration of 6-shogaol so that their activity as a pro-apoptotic agent for colorectal cancer could be predicted.

Materials and Methods

The GSE57006 data obtained from the Gene Expression Omnibus (GEO) database (Barrett et al. 2012) was analyzed for DEGs using GEO2R and validated using the machine learning method with the Orange (Demsar et al. 2013). The GSE57006 data contained gene expression data from the HCT-116 cell group treated with 6-shogaol and DMSO (control). The research method that generated the GSE57006 data in the GEO database involved culturing HCT-116 cells in 100 × 20 mm culture plates and allowing them to adhere overnight at 37 °C. The cells were then treated with 20 μM 6-Shogaol or DMSO (control) and incubated for 24 h. Subsequently, the cells were collected, and total RNA was isolated. RNA quantity was measured using

a spectrophotometer, while its quality was determined through gel electrophoresis and Bioanalyzer. Microarray analysis was used to identify gene expression differences between the 6-Shogaol group and the control. A detailed explanation of the *in vitro* methods leading to the generation of the gene expression data used in this study can be found in the article presented by Chen et al. (2014). In this study, the value of genes with significantly different expressions (DEG) was determined if the probability/ significance value (p)<0.05 or -log10(P value)>1.3. Validation was carried out to obtain sensitivity, specificity, and area under curve (AUC) values as a measure of the ability of the resulting DEGs to differentiate HCT-116 cells treated with 6-shogaol compared to controls. Validation is also carried out by Principal Component Analysis (PCA) to evaluate the clustering that occurs. DEG analysis was performed on apoptosis-related genes obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al. 2023). The validated apoptosis-related DEGs were then used to look at known and predicted apoptotic protein-protein interactions using the STRING database (https://string-db.org/). These interactions include direct (physical) and indirect (functional) associations**.**

Results and Discussion

The results of DEGs analysis related to apoptosis in HCT-116 cells treated with 6-shogaol can be seen in Table 1. There were a total of 142 DEGs (p<0.05 or -log10(Pvalue)>1.3) divided into 9 (nine) lanes. In Table 1 it can be seen that several genes whose expression changes are involved in more than one pathway so that they can become links between pathways. The cross-pathway interactions of the nine pathways can be seen in Figure 1. It can also be seen that the genes in HCT-116 cells whose expression is significantly affected (DEG) by 6-shogaol in the apoptotic pathway (hsa04210) are MAP2K1, BBC3, TNFRSF10A, BAK1, CTSD, BAX, IKBKB, GADD45G, CTSS, DDIT3, ATF4, and BIRC5. Some of these genes are also expressed differently in other signaling pathways as shown in Table 2. These genes are the links between the apoptotic pathway (hsa04210) and other signaling pathways.

Figure 1 shows that the apoptotic pathway (has04210) which is influenced by 6-shogaol was connected to other signaling pathways such as MAPK signaling pathway (hsa04010), NFkB signaling pathway (hsa04064), p53 signaling pathway (hsa04115), Protein processing in endoplasmic reticulum (hsa04141), PI3K-Akt signaling pathway (hsa04151), Natural killer cell mediated cytotoxicity (hsa04650), and TNF signaling pathway_hsa04668. From Table 2 it can be seen that 5 (five) genes with values |log2(fc)| the highest were DDIT3 and ATF4 (down) as well as GADD45G, IKBKB, and BAX (up) where 4 of the 5 genes (DDIT3, ATF4, GADD45G, IKBKB) are involved in the MAPK signaling pathway. In addition, there are 5 out of 9 genes involved in the MAPK signaling pathway (DDIT3, ATF4, GADD45G, IKBKB, and MAP2K1). As for the p53 signaling pathway and the protein processing in the endoplasmic reticulum pathway, there are 4 genes each that also experience changes in expression in the apoptotic pathway due to the administration of 6-shogaol. Thus, the effect of 6-shogaol on changes in apoptotic gene expression in HCT-116 cells seems to be also influenced by the MAPK signaling pathway, the p53 signaling pathway, and the protein processing in the endoplasmic reticulum pathway. However, the different expression of DDIT3 and ATF4 (down-regulation) compared to 7 other pro-apoptotic genes (up-regulation) seems to affect the activity and mechanism of pro-apoptotic 6-shogaol.

To validate the expression of the genes involved in the signaling pathways mentioned played a role in differentiating or grouping HCT-116 cells with and without the addition of 6-shogaol (control), principal component analysis (PCA) and machine learning were used. The results of Principal Component Analysis (PCA) for grouping HCT-116 cells with and without the addition of 6-shogaol (control) based on Differentially Expressed Genes (DEGs) can be seen in Figure 2. In Figure 2 it can be seen that 142 DEGs are associated with apoptosis in HCT-116 cells. 116 (Table 1) were able to separate the HCT-Cells/6S group (6-shogaol treatment) from the HCT-Cells/DMSO (control). Likewise, if PCA analysis was carried out at 9 DEGs of apoptosis with other pathways/ signaling related to apoptosis (Table 2), there was a grouping between HCT-Cells/6S (6-shogaol treatment) and HCT-Cells/DMSO (control).

Validation is also carried out using machine learning methods. The results of the validation can be seen in Table 3. Based on Table 3, the machine learning model used, HCT-Cells/6S (6-shogaol treatment) and HCT-Cells/DMSO (control) can be predicted with accurate classification (classification accuracy, CA = 1,000 and area under the curve, AUC = 1,000). The machine learning model used (logistic regression, SVM, and neural network) has an F1-score = 1,000 (precision = 1,000 and recall = 1,000) where the F1-Score is the harmonic mean of precision and recall. High accuracy (CA) and F1-Score values (close to 1,000) indicate high sensitivity and specificity, whereas if accuracy (CA) = 1, the sensitivity and specificity are also 1,000. These results indicate that these genes can be used as biomarkers to evaluate the anti-cancer activity of 6-shogaol in HCT-116 cells.

TABLE 1. DEGs regulation related to apoptosis of HCT-116 cells with 6-shogaol treatment

Pathway	Regulation	
	Down	Up
Apoptosis _hsa04210	CTSS;DDIT3;ATF4;BIRC5	MAP2K1;BBC3;TNFRSF10A;BAK1;CTSD; BAX;IKBKB;GADD45G
MAPK signaling pathway_hsa04010	DDIT3;ANGPT2;PRKACB;EREG;A TF4;NR4A1;KITLG;AREG;BDNF; CACNA2D4;NTF4;RPS6KA5;DUSP6; PPP3CA; PTPN5;RASGRF1	MAP2K3;MAPK11;DUSP16;MAP3K7; CD14;CACNG6;MAP2K1;ELK4; RAPGEF2;HSPA1B;DUSP3;PRKCB; TAOK2;SOS1;FGF8;IKBKB;CACNB1; GADD45G; ANGPT1;EGF; HSPA6
Calcium signaling pathway_hsa04020	CAMK2A;CHRM1;PRKACB;HRH1; CXCR4; PLCE1;TNNC1;ADRA1B; CALML4;SLC8A2;PPP3CA;CAMK4; CASQ2; HTR7;PHKA2	PHKG1;GNAQ;AVPR1B;PHKA1;NOS3; TRHR; PRKCB;MYLK2;GRIN2C;FGF8; P2RX6;EGF;GDNF;CCKBR;SLC8A1; AGTR1
NF-kappa B signaling pathway_hsa04064	PTGS2	LYN;CXCL1;MAP3K7;CD14;PRKCB; TNFSF13B; IKBKB; GADD45G
p53 signaling pathway _hsa04115	CCNE2;RRM2;CCNB1;CDK1; SESN3	CCNE1;DDB2;STEAP3;EI24;BBC3; TNFRSF10A; SFN;TP53I3;BAX; SESN1; CDKN1A;GADD45G
Protein processing in endoplasmic reticulum _hsa04141	DDIT3;ATF4;BAG2;MAN1A1	UBE2D4;DAD1;DNAJB11;SEC61A2; SEC31A;SYVN1;PDIA4;WFS1;PREB; EDEM3;CANX;STUB1;HSPA1B;EDEM2; BCAP31;SEC24D;UGGT1;CKAP4;MOGS;B AK1; FBXO2;BAX;HSPA6
PI3K-Akt signaling pathway _hsa04151	ANGPT2;CHRM1;CCNE2; ITGA10; TCL1B;LAMA2;EREG; IL2RB; ATF4; GNG2;NR4A1; KITLG;AREG;BDNF; NTF4;IRS1;DDIT4;BRCA1;CD19; COL4A6	CCNE1;PPP2CB;GH1;LAMA3; MA P2K1;PPP2R2D;GSK3B;TCL1A; NOS3;LAMB2;CREB3L2;ITGB7; IL6R;SOS1;IFNA2;FGF8;IKBKB; SPP1;TN C;CDKN1A;ANGPT1;EGF;THBS2
Natural killer cell mediated cytotoxicity _hsa04650	ULBP1;SHC4;PPP3CA	MAP2K1;TNFRSF10A;PRKCB; SHC1; VAV3;SOS1;IFNA2; SH2D1A;LCP2
TNF signaling pathway _hsa04668	ATF4;ITCH;RPS6KA5;MMP9; PTGS2	MAP2K3;MAPK11;MLKL;CXCL1; MAP3K7;MAP2K1;CREB3L2; IKBKB

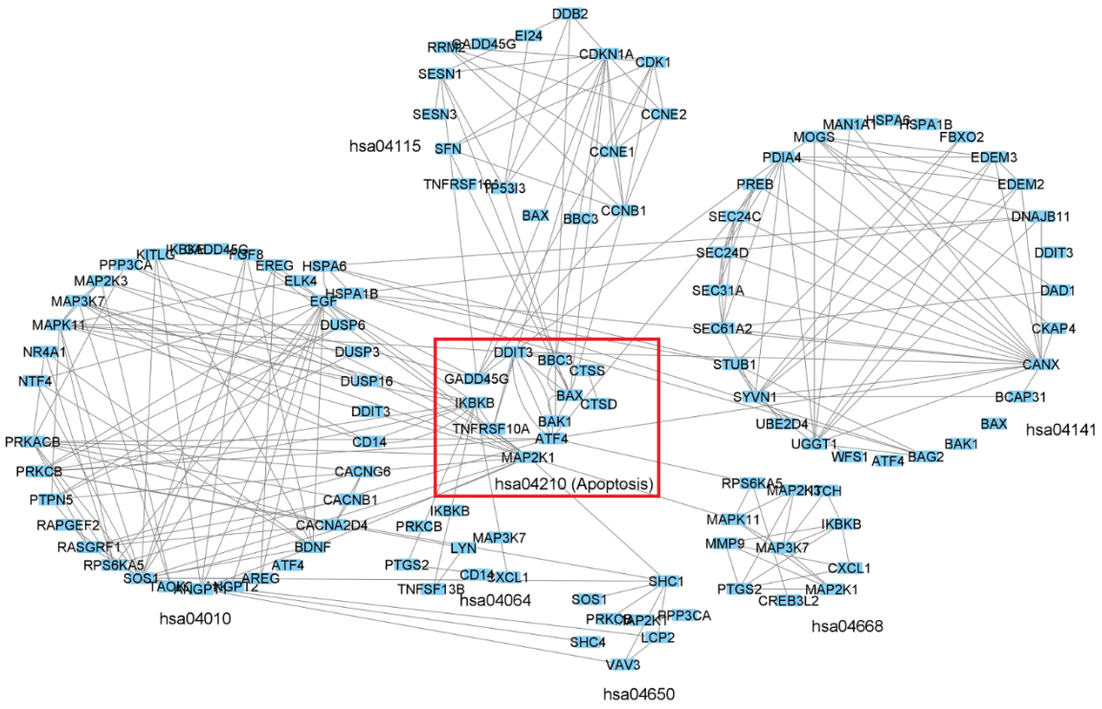

FIGURE 1. Interaction of apoptosis protein pathway (Hsa04210) with apoptosis-related signaling pathway proteins influenced by 6-shogaol. Gene expression networks were constructed using STRING and Cytoscape (Shannon et al. 2003)

TABLE 2. Shared-DEGs apoptotic pathways (Hsa04210) and other signaling pathways related to apoptosis in HCT-116 cells due to treatment with 6-shogaol

Gene symbol	Genes Description	log2(fc*)	-log10 (Pvalue)	Pathways
DDIT3	DNA damage inducible transcript 3	-1.378	4.259	Apoptosis, MAPK signaling pathway, Protein processing in endoplasmic reticulum
ATF4	activating transcription factor 4	-0.583	2.946	Apoptosis, MAPK signaling pathway, Protein processing in endoplasmic reticulum, PI3K-Akt signaling pathway, TNF signaling pathway
MAP2K1	mitogen-activated protein kinase kinase 1	0.407	1.585	Apoptosis, MAPK signaling pathway, PI3K-Akt signaling pathway, Natural killer cell mediated cytotoxicity, TNF signaling pathway
BBC3	BCL2 binding component 3	0.418	2.218	Apoptosis, p53 signaling pathway,
TNFRSF10A	TNF receptor superfamily member 10a	0.433	1.870	Apoptosis, p53 signaling pathway, Natural killer cell mediated cytotoxicity
BAK1	BCL2 antagonist/killer 1	0.520	2.679	Apoptosis, Protein processing in endoplasmic reticulum
BAX	BCL2 associated X, apoptosis regulator	0.670	2.875	Apoptosis, p53 signaling pathway, Protein processing in endoplasmic reticulum,
IKBKB	inhibitor of nuclear factor kappa B kinase subunit beta	0.801	2.096	Apoptosis, MAPK signaling pathway, NF-kappa B signaling pathway, PI3K-Akt signaling pathway, TNF signaling pathway
GADD45G	growth arrest and DNA damage inducible gamma	0.977	2.046	Apoptosis, MAPK signaling pathway, NF-kappa B signaling pathway, p53 signaling pathway

*fc= fold change

TABLE 3. Validation of HCT-116/6s and HCT-116/DMSO based on DEGs

Model	AUC	CA	F1	Precision	Recall
142 DEGs					
Logistic Regression	1.000	1.000	1.000	1.000	1.000
Neural Network	1.000	1.000	1.000	1.000	1.000
SVM	1.000	1.000	1.000	1.000	1.000
9 DEGs					
Logistic Regression	1.000	1.000	1.000	1.000	1.000
Neural Network	1.000	1.000	1.000	1.000	1.000
SVM	1.000	1.000	1.000	1.000	1.000

To get an idea of what DEGs are and how they regulate gene expression in HCT-116 cells due to the addition of 6-shogaol, it can be seen the heatmap as shown in Figure 3. Figure 3(a) is a heatmap that relates the expression value of 142 DEGs to the HCT-Cells/group. 6S (6-shogaol treatment) and HCT-Cells/DMSO (control). As explained in Table 1, the 142 DEGs are genes originating from the apoptotic pathway and 8 other pathways related to apoptosis are derived from the KEGG database. Figure 3(b) is a heatmap consisting of 9 shared-DEGs (co-expressed) in the apoptotic pathway and other pathways related to apoptosis as described before. If the 9 shared-DEGs are networked with STRING (Figure 3(c)), then, the 8 DEGs appear to be interconnected (ATF4, BAK1, BBC3, BAX, DDIT3, GADD45G, IKBKB, and TNFRSF10A). Meanwhile, MAP2K1 is not connected to the other 8 DEGs (interaction score: 0.400 or medium). In the shared-DEG network, it is also seen that DDIT3 is a central gene, where the gene is present in the apoptotic pathway, MAPK signaling pathway, and protein processing in the endoplasmic reticulum (Table 2). BAX, BBC3, GADD45G, and TNFRSF10A are involved in the apoptotic pathway and p53 signaling, and BAK1, BAX, ATF4, and DDIT3 are involved in the apoptotic pathway and protein processing in the endoplasmic reticulum.

DISCUSSION

Colon cancer is a type of cancer that occurs in the colon or rectum, which can be caused by various factors such as genetics, environment, and lifestyle. The mitogen-activated protein kinase (MAPK) and apoptosis (programmed cell death) signaling pathways play important roles in cancer development. MAPK signaling pathway is an intracellular signaling pathway that is involved in various cellular processes such as proliferation, differentiation, and apoptosis. Proteins in the MAPK signalling pathway and apoptosis include ATF4 and DDIT3 (CHOP). DDIT3 along with ATF4 is also expressed by the protein processing in the endoplasmic reticulum pathway. Proteins in this pathway are known to play a role in regulating protein folding in the endoplasmic reticulum where misfolding will trigger an unfolded protein response (UPR) that causes endoplasmic reticulum (ER) stress. Cells exposed to ER stress will experience oxidation and failure to restore homeostasis which then induces apoptosis (Marciniak et al 2004; Yadav et al. 2014). MAPKs are activated primarily after exposure of cells to stress elicited by various physical, chemical, and biological stressors Currently, many drugs have been designed to induce irreparable ER stress by cancer cells by targeting key signaling pathways involved in ER stress response, such as ATF4-DDIT3 (Tunçer, Solel & Banerjee 2020).

Based on DEG analysis on GSE57006 data, it is known that 6-shogaol downregulates ATF4-DDIT3. This fact seems to indicate that the role of the two genes/proteins is less significant in the pro-apoptotic activity of 6-shogaol against HCT-116 cells. The hypothesis that can be put forward to explain this is that ER stress triggered by 6-shogaol causes an increase in the expression of

Vascular Endothelial Growth Factor (VEGF). VEGF secreted by tumor cells is known to function in an autocrine manner and promote dedifferentiation and epithelial-mesenchymal transition phenotypes, with consequent increased survival of tumor cells (Goel & Mercurio 2013). This is similar to a study conducted by Tunçer, Solel and Banerjee (2020) who found the fact that tunicamycin, an ER stress inducing agent, increases VEGF expression in HCT-116 cells causing resistance to treatment.

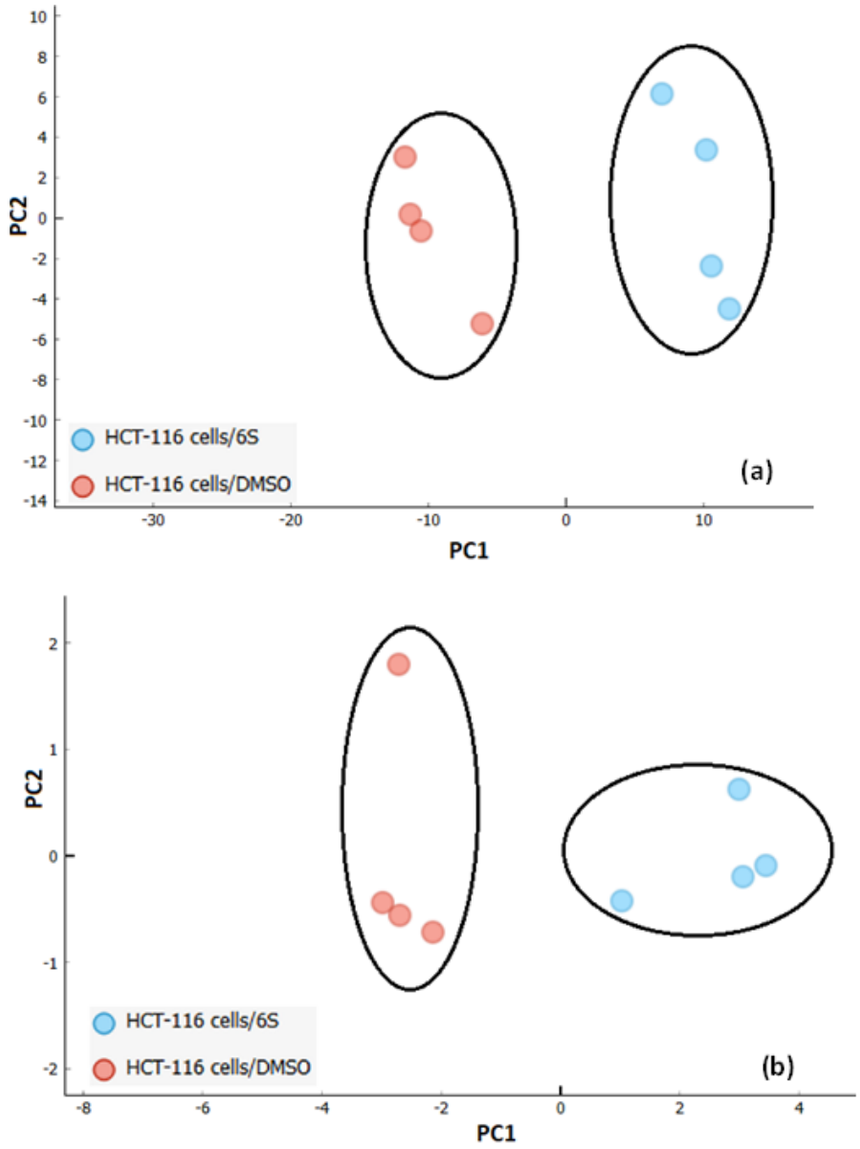

FIGURE 2. Results of PCA analysis for grouping HCT-116 cells with and without the addition of 6-shogaol (6S) based on DEGs: (a) 142 DEGs; (b) 9 DEGs

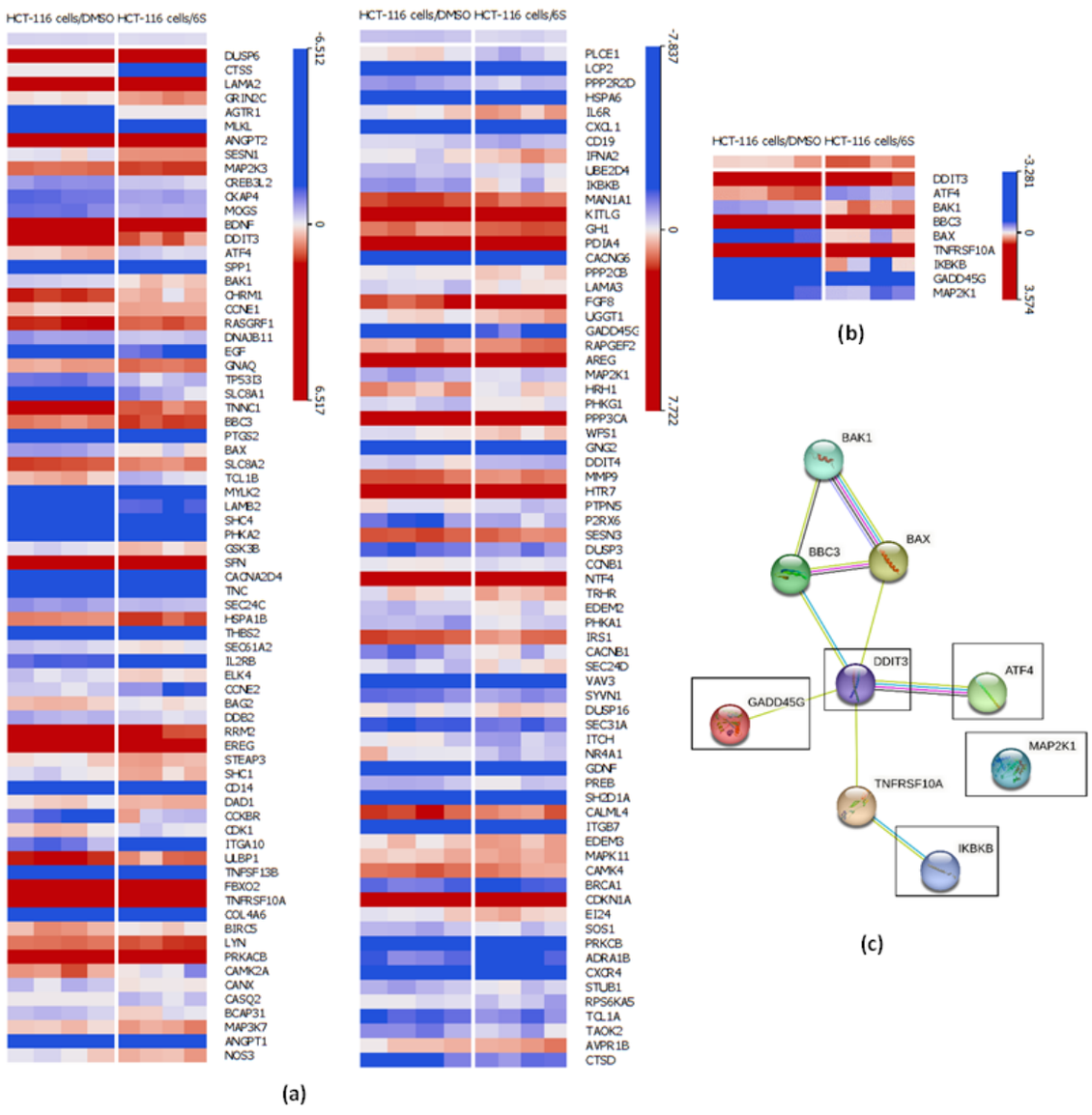

FIGURE 3. Heatmap of the Relationship between DEG and Expression Values: (a) 142 DEG, (b) 9 DEG. Whereas (c) is a 9 DEG interaction made using STRING. The boxed (DEG) genes are genes involved in the apoptotic pathway and the MAPK signaling pathway

In this study, 7 of the 9 Shared-DEG apoptotic pathways with other signaling pathways related to apoptosis namely ATF4, BAK1, BBC3, BAX, DDIT3, GADD45G, IKBKB, TNFRSF10A, and MAP2K1 (besides DDIT3 and ATF4) experienced increased expression. This seems to cause 6-shogaol to retain pro-apoptotic activity against HCT-116 cells, although it may experience slight resistance for reasons as described above. Qi et al. (2015) showed that 6-shogaol inhibited the proliferation of HCT-116 and SW-480 colorectal cancer cells through the main p53/p21 pathway, among others, by upregulating p53 and GADD45G. Meanwhile, Clarke

et al. (2019) showed that BAX and BBC3 are required for the induction of apoptosis based on a decrease in caspase activity in isogenic knockout HCT-116 BAX−/− and HCT-116 BBC3−/−. p53 directly stimulates the transcription of TNFRSF10A, also known as the TRAIL (TNF-related apoptosis-inducing ligand) (Orlic-Milacic 2023). TNFRSF10A is also known to mediate endoplasmic reticulum stress-triggered apoptosis in human lung cancer cells whose expression is regulated by DDIT3 (Li et al. 2015). In general, caspases are activated via the extrinsic pathway or the intrinsic pathway. The extrinsic pathway is usually induced by certain ligands or compounds that activate cell apoptotic signal receptors on the surface and the intrinsic pathway is induced by the release of proteins from the mitochondrial outer membrane due to a family of proteins that mediates mitochondrial permeabilization, namely the pro-apoptotic protein Bcl-2. The release of cytochrome c from the mitochondrial outer membrane is an important step in the intrinsic apoptotic pathway. Several Bcl-2 family proteins, both pro- and anti-apoptotic groups, are under the control of the JNK or MAPK cascades (Sun et al. 2015). However, as has been shown in this study, up-regulation of TNFRSF10A in HCT-116 cells 6-shogaol administration appears to be more influenced by the p53 signaling pathway than the MAPK signaling pathway or ER stress via DDIT3 and ATF4. This seems to strengthen the evidence that the pro-apoptotic activity of 6-shogaol in HCT-116 cells is mainly carried out through the p53 signaling pathway.

Conclusions

Differentially Expressed Genes (DEG) analysis showed that the apoptotic activity of 6-shogaol mainly via the p53 signaling pathway. The genes in this pathway, namely BAX, BBC3, GADD45G, and TNFRSF10A, experienced up-regulation in HCT-116 cells treated with 6-shogaol. DEG analysis also showed that the pro-apoptotic activity of 6-shogaol via the MAPK pathway and protein processing in the endoplasmic reticulum that trigger ER stress appears to be limited by down-regulation of DDIT3 and ATF4. This is thought to occur through increased expression of VEGF, so further research is needed regarding this hypothesis.

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*Corresponding author; email: kkusmardis@gmail.com