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ORIGINAL ARTICLE

Gene expression profiling of DMU-212-induced apoptosis and anti-angiogenesis in vascular endothelial cells

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Abstract

Context: *trans*-3,4,5,4′-Tetramethoxystilbene (DMU-212), an derivative of resveratrol, shows strong antiproliferative activities against many cancer cells. In our previous study, we demonstrated that DMU-212 possesses potent proapoptosis and antiangiogenesis effects on vascular endothelial cells (VECs), which made it a promising agent for the treatment of angiogenesis-related diseases.
Objective: We studied the gene expression profile of DMU-212-treated VECs to gain further insight into the mechanisms by which DMU-212 exerts its potent pro-apoptosis and antiangiogenesis effects.
Materials and methods: The potential changes in the gene expression of VECs incubated with DMU-212 were identified and analyzed using the Affymetrix HG-U133 Plus 1.0 array. In addition, the gene expression profile was validated by quantitative real-time PCR (qRT-PCR) analysis for seven of those altered genes.
Results and conclusion: DMU-212 was found to regulate a diverse range of genes, including cytokines (*IL8, selectin E, MPZL2, EGR1, CCL20, ITGB8, CXCL1, VCAM1, KITLG,* and *AREG*), transport proteins (*TRPC4, SLC41A2, SLC17A5,* and *CREB5*), metabolism (*CYP1B1, CYP1A1, PDK4, CSNK1G1, MVK, TCEB3C,* and *CDKN3*), enzymes (*RAB23, SPHK1, CHSY3, PLAU, PLA2G4C,* and *MMP10*), and genes involved in signal transduction (*TMEM217, DUSP8,* and *SPRY4*), chromosome organization (*HIST1H2BH* and *GEM*), cell migration and angiogenesis (*ERRFI1, HBEGF,* and *NEDD9*), and apoptosis (*TNFSF15, TNFRSF9, CD274, BCL2L11, BIRC3, TNFAIP3,* and *TIFA*), as well as other genes with unknown function (*PGM5P2, SNORD1142, LOC151760, KRTAP5-2, C1orf110, SNORA14A, MIR31, C2CD4B, SCARNA4, C2orf66, SC4MOL, LOC644714,* and *LOC283392*). This is the first application of microarray technique to investigate and analyze the profile of genes regulated by DMU-212 in VECs. Our results lead to an increased understanding of the signaling pathways involved in DMU-212-induced apoptosis and antiangiogenesis.

Keywords

Angiogenesis, microarray, *trans*-3,4,5,4′-tetramethoxystilbene

History

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Introduction

Resveratrol (*trans*-3,4′,5-trihydroxystilbene), a naturally polyphenolic phytoalexin found in grapes, cranberries, peanuts, cranberries and other dietary constituents, exerts a variety of pharmacological activities, including antioxidative, antiinflammatory, antitumor, and antiplatelet both *in vitro* and *in vivo* (Cong et al., 2014; Olas et al., 2002; Stakleff et al., 2012; Yun et al., 2014). In recent years, although resveratrol has been reported to regulate angiogenesis, it possesses either pro- or antiangiogenic properties depending on the situation, applied dosage, or cell type (Wang et al., 2010). In addition, its biological effects *in vivo* appear to be strongly limited because of a poor oral adsorption and metabolic stability (Walle et al., 2004). Structural modifications of resveratrol are needed to increase its bioavailability while preserving its beneficial activities.

The trihydroxystilbene scaffold of resveratrol has been the subject of structural alternations to improve the pharmacokinetic properties of this natural compound. The structure–activity studies pointed that the introduction of methoxy groups in place of hydroxy moieties could increase its stability (Piotrowska et al., 2014). An especially auspicious finding concerning these derivatives is the fact that *trans*-3,4,5,4′-tetramethoxystilbene (DMU-212; Figure 1) possesses more favorable bioavailability in mouse liver and plasma than resveratrol (Sale et al., 2004). DMU-212 also possesses stronger antiproliferative activities in a variety of cells, such as transformed fibroblasts, prostate, cervical, ovarian, breast, hepatoma, and colon cancer cells (Piotrowska et al., 2014). DMU-212 exerts superior antitumor activity over resveratrol because this compound induces cell death and antiproliferation events through different mechanisms from those of resveratrol (Ma et al., 2008). In addition to the introduction of G_2/M arrest, DMU-212 down-regulates the expression of

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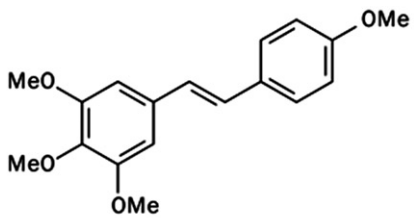

Trans-3,4,5,4′-tetramethoxystilbene (DMU-212)
Chemical formula: $C_{18}H_{20}O_4$
Molecular weight: 300.3490

Figure 1. The chemical structure of *trans*-3,4,5,4′-tetramethoxystilbene (DMU-212). DMU-212 has the molecular formula $C_{18}H_{20}O_4$ and a molecular weight of 300.3490 g/mol.

antiapoptotic proteins, including cyclin D1, BCL2-like 1(BCL2L1), and B cell CLL/lymphoma 2(BCL2), in cancer cells (Ma et al., 2008). Despite these widely described antitumor properties of DMU-212, very little is known concerning its antiangiogenic potential.

Our previous study showed that DMU-212 could induce apoptosis and antiangiogenesis effects in vascular endothelial cells (VECs) both *in vitro* and in *vivo* (Chen et al., 2013). However, the mechanism by which DMU-212 exerts its proapoptosis and antiangiogenesis effects are complex and remains unclear. In the present study, we performed DNA microarrays on cultured human umbilical VECs (HUVECs) treated with DMU-212 to examine the potential changes in the gene expression. A network extension of the signaling pathways that participate in apoptosis and antiangiogenesis mediated by DMU-212 was then created.

Materials and methods

Cell culture

HUVECs were obtained in our laboratory as described (Jaffe et al., 1973). Cells were cultured on gelatin-coated plastic dishes in MCDB31 medium (Sigma, St Louis, MO) supplemented with 20% fetal bovine serum (FBS, HyClone Laboratory, Logan, UT) and 70 ng/mL fibroblast growth factor 2 in a humidified incubator at 37 °C with 5% CO_2. Cells were used at or before passage 8. The identity of HUVECs was confirmed by their cobblestone morphology and strong positive immunoreactivity to CD31.

RNA isolation, microarray experiment, and analysis

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer from the HUVECs treated with DMSO or DMU-212 (20 μM) for 6 h. The quality of the samples was assessed by spectrophotometry (Nanodrop 2000, Thermo Fisher Scientific, Waltham, MA), and samples of high quality were transferred to the Gene Tech (Shanghai, China) Co., Ltd for labeling and hybridization to the Affymetrix Human GeneChip® Gene 1.0 ST Arrays according to the protocol of the manufacturer. The microarray data analysis was also performed by Gene Tech (Shanghai, China) Co., Ltd. Genes with >2-fold changes and both the false discovery rate (FDR, q) and the Wilcoxon Rank-Sum test significance level of $(p) < 0.05$ were considered significantly regulated.

Quantitative real-time PCR (qRT-PCR)

After treatment, total RNA was extracted from cells with use of TriZol Reagent (Invitrogen Life Technologies, Waltham, MA). RNA was reverse-transcribed by use of M-MLV reverse transcriptase (Promega, Madison, WI). Forward (F) and reverse (R) primers designed for each genes were as follows: interleukin 8(*IL8*)-F 5′-GGTGCAGTTTTGCCAAGGAG-3′, *IL8*-R 5′-TTCCTTGGGGTCCAGACAGA-3′; early growth response 1 (*EGR1*)-F 5′-AGTCCCATTTACTCAGCGGC-3′, *EGR1*-R 5′-GTTCATCGCTCCTGGCAAAC-3′; ERBB receptor feedback inhibitor 1 (*ERRFI1)*-F 5′-GGGCATGCT TCCAAATCTGC-3′, ERRFI1-R 5′-ATCTTCGGTGGGTC TGAGGT-3′; transient receptor potential cation channel (*TRPC4*)-F 5′-TCTGCAAATATCTCTGGGAAGAATGC-3′, *TRPC4*-R 5′-AAGCTTTGTTCGTGCAAATTTCCATTC-3′; baculoviral IAP repeat-containing 3(*BIRC3*)-F 5′-GGAGA CAGAGTGGCTTGCTT-3′, *BIRC3*-R 5′-TCATCTCCTG GGCTGTCTGA-3′; cytochrome P450, family 1, subfamily B, polypeptide 1(*CYP1B1*)-F 5′-GTCATGAGTGCCGTGT GTTTC-3′, *CYP1B1*-R 5′-TGGTCACCCATACAAGGCAG-3′; mevalonate kinase (*MVK*)-F 5′-GTGTGCGAGGAGATCCC AAA-3′, *MVK*-R 5′-CGAGGGACTTTGGTGTTGGT-3′; glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)-F 5′-CTT TGGTATCGTGGAAGGACTC-3′, *GAPDH*-R 5′-TCTTCCT CTTGTGCTCTTGCTG-3′. The qRT-PCR reactions were performed with the Eppendorf PCR System (Eppendorf AG, Hamburg, Germany). The level of each gene was calculated by comparing the Ct value in the samples to a standard curve generated from serially diluted cDNA from a reference sample and normalization to the level of GAPDH.

Statistical analyses

All experiments were repeated at least three times. The data are expressed as the means ± standard error of the mean (SE). The differences between groups were analyzed by one-way variance (ANOVA), and the means of two groups were compared using Student's *t*-test with SPSS 17.0 (SPSS Inc., Chicago, IL) version. Differences were considered statistically significant at $p < 0.05$.

Results

Microarray analysis of DMU-212-regulated gene expression profile

The differential expressed genes in VECs treated with DMU-212 were detected and analyzed using the Affymetrix HG-U133 Plus 1.0 array. Microarray profiling data revealed a total of 56 altered genes (genes that were up or down-regulated >2-fold), including 44 up-regulated genes and 12 down-regulated genes in DMU-212-treated cells compared with its matched control cells. Although most of these genes were found to encode proteins with known function, the function of a few of genes is as yet unknown. Categorizing these genes based on their molecular functions revealed that they consisted of genes that encode products involved in the

Table 1. Genes whose average expression differed by greater than 2-fold are grouped by functional category: labeled cRNA prepared from HUVECs at 6 h after infection with DMU-212 use Affymetrix Human GeneChip® Gene 1.0 ST Arrays.

Gene symbol	Gene ID	Name	Fold change	Category
TNFSF15	9966	Tumor necrosis factor (ligand) superfamily, member 15	2.29	Apoptosis
TNFRSF9	3604	Tumor necrosis factor receptor superfamily, member 9	2.15	
CD274	29126	CD274 molecule	2.59	
BCL2L11	10018	BCL2-like 11	2.15	
BIRC3	330	Baculoviral IAP repeat-containing 3	2.13	
TNFAIP3	7128	Tumor necrosis factor, alpha-induced protein 3	2.09	
TIFA	92610	TRAF-interacting protein with forkhead-associated domain	2.02	
IL8	3576	Interleukin 8	3.98	Cytokines
selectin E	6401	Selectin E	3.86	
MPZL2	10205	Myelin protein zero-like 2	3.19	
EGR1	1958	Early growth response 1	2.98	
CCL20	6364	Chemokine (C-C motif) ligand 20	2.98	
ITGB8	3696	Integrin, beta 8	2.45	
CXCL1	2919	Chemokine (C-X-C motif) ligand 1	2.39	
VCAM1	7412	Vascular cell adhesion molecule 1	2.36	
KITLG	4254	KIT ligand	2.65	
AREG	374	Amphiregulin	2.14	
TMEM217	221468	Transmembrane protein 217	2.77	MAPK pathway
DUSP8	1850	Dual specificity phosphatase 8	2.56	
SPRY4	81848	Sprouty homolog 4	2.20	
RAB23	51715	RAB23, member RAS oncogene family	2.40	Enzyme
SPHK1	8877	Sphingosine kinase 1	2.23	
CHSY3	337876	Chondroitin sulfate synthase 3	2.22	
PLAU	5328	Plasminogen activator, urokinase	2.15	
PLA2G4C	8605	Phospholipase A2, group IVC	2.14	
MMP10	4319	Matrix metallopeptidase 10	2.07	
CYP1B1	1545	Cytochrome P450, family 1, subfamily B, polypeptide 1		Metabolism
CYP1A1	1543	Cytochrome P450, family 1, subfamily A, polypeptide 1	0.27	
PDK4	5166	Pyruvate dehydrogenase kinase, isozyme 4	0.45	
CSNK1G1	53944	Casein kinase 1, gamma 1	0.48	
MVK	4598	Mevalonate kinase	0.49	
TCEB3C	162669	Transcription elongation factor B polypeptide 3C	0.43	
CDKN3	1033	Cyclin-dependent kinase inhibitor 3	0.47	
HIST1H2BH	8345	Histone cluster 1, H2bh	0.45	Chromosome organization
GEM	2669	GTP binding protein overexpressed in skeletal muscle	2.34	
ERRFI1	54206	ERBB receptor feedback inhibitor 1	2.87	Angiogenesis and Migration
HBEGF	1839	Heparin-binding EGF-like growth factor	2.35	
NEDD9	4739	Neural precursor cell expressed, developmentally down-r	2.08	
TRPC4	7223	Transient receptor potential cation channel	2.58	Transport protein
SLC41A2	84102	Solute carrier family 41, member 2	2.54	
SLC17A5	26503	Solute carrier family 17 (anion/sugar transporter), memb	2.14	
CREB5	9586	cAMP responsive element binding protein 5	2.56	
AKAP12	9590	A kinase (PRKA) anchor protein 12	2.54	PKA pathway
PGM5P2	595135	Phosphoglucomutase 5 pseudogene 2	2.66	Unclear
SNORD1142	767578	Small nucleolar RNA, C/D box 114-2	2.59	
LOC151760	151760	Hypothetical LOC151760	2.54	
KRTAP5-2	440021	Keratin-associated protein 5-2	2.31	
C1orf110	339512	Chromosome 1 open reading frame 110	2.30	
SNORA14A	677801	Small nucleolar RNA, H/ACA box 14A	2.29	
MIR31	407035	microRNA 31	2.27	
C2CD4B	388125	C2 calcium-dependent domain containing 4B	2.14	
SCARNA4	677771	Small Cajal body-specific RNA 4	2.01	
C2orf66	401027	Chromosome 2 open reading frame 66	0.42	
SC4MOL	6307	Sterol-C4-methyl oxidase-like	0.44	
LOC644714	644714	Hypothetical LOC644714	0.46	
LOC283392	283392	Hypothetical LOC283392	0.48	

regulation of cell apoptosis, migration, inflammation, signal transduction, and metabolism (Table 1).

Verification of microarray analysis by qRT-PCR

To validate the data of microarray, we performed qRT-PCR analysis for seven randomly selected genes (*IL8*, *EGR1*, *ERRFI1*, *TRPC4*, *BIRC3*, *CYP1B1*, and *MVK*). The changes of all the selected genes were in accordance with corresponding microarray data. All these expression pattern variations were statistically significant (Figure 2).

Pathway analysis of DMU-212-induced apoptosis and antiangiogenesis in VECs

Functional analysis of the Gene Ontology (GO) terms associated with the differential expressed genes showed significant enrichment for metabolic process, immune

system process, apoptotic process, and various kinds of cellular process (Figure 3). BCL2 is an important antiapoptosis factor. Two *BCL2* inhibitors, BCL2-like 11(*BCL2L11*) and CD274 molecule (*CD274*), were up-regulated by DMU-212, suggesting that these proapoptotic factors may be involved in DMU-212-induced apoptosis in VECs. In addition, tumor necrosis factor (ligand) superfamily, member 15 (*TNFSF15*) and tumor necrosis factor receptor superfamily, member 9 (*TNFRSF9*), members of tumor necrosis factor (TNF) superfamily, may contribute to VEC apoptosis and antiangiogenesis induced by DMU-212. Notably, several kinds of proinflammatory cytokines, such as interleukin-8 (*IL-8)*, selectin E (*SELE*), chemokine (C-X-C motif) ligand 1 (*CXCL1*), and vascular cell adhesion molecule 1 (*VCAM1*) may also participate in DMU-212-induced endothelial dysfunction and apoptosis. Except for the induction of apoptosis in VECs, genes associated with cell proliferation, such as *ERRFI1*, were altered by DMU-212. Furthermore, we used the KEGG orthology-based annotation system (KOBAS) (http://kobas.cbi.pku.edu.cn), which predicted the biochemical pathways in which molecules are involved, to analyze the signaling pathways linked to DMU-212-induced apoptosis and antiangiogenesis in VECs (Table 2). The results showed that the alter genes enriched were related with TNF signaling pathway and NF-κB signaling pathway, which was similar with gene ontology (GO) analysis. Collectively, the differentially expressed genes were collated into a comprehensive signaling network associated with apoptosis and angiogenesis in the present study (Figure 4).

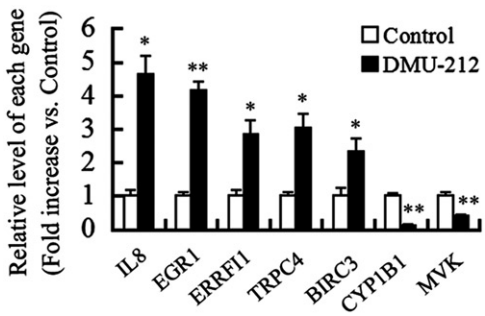

Figure 2. Verification of microarray analysis by qRT-PCR. Histogram showed the mRNA levels of genes as indicated. Data are expressed as the mean (±SE) of three independent experiments. $*p<0.05$, $**p<0.01$ versus control.

Discussion

In the present study, we described the genome-wide transcriptional profiling of DMU-212-regulated genes in VECs. Pathway analysis revealed that TNF signaling pathway and NF-κB signaling pathway are remarkably regulated. TNFSF15, a member of the TNF superfamily, has the ability to enforce growth arrest of VECs in G_0 and G_1 phases of the cell cycle, while inducing apoptosis of proliferating VEC, leading to inhibition of angiogenesis (Yu et al., 2001). More specially, the up-regulation of TNFSF15 stimulated by TNF-α is partly but significantly responsible for TNF-α-induced VEC apoptosis (Xu et al., 2014). TNFRSF9, a member of the TNF receptor family, could enhance apoptosis by elevating TNF-α production via activation of p38 mitogen-activated protein kinase (MAPK) pathway (Nagila et al., 2013). In addition, DMU-212 elevated the gene expression of transmembrane protein 217 (*TMEM217*), dual specificity phosphatase 8 (*DUSP8*), and sprouty homolog 4 (Drosophila) (*SPRY4*), which are participated in p38MAPK signaling pathway. Based on these, we speculated that both TNF signaling and p38MAPK signaling might be participated in DMU-212-induced apoptosis in VECs.

NF-κB also plays an important role in inflammation and apoptosis in VECs. It can be activated by many proinflammatory cytokines, including TNF-α. Data from microarray revealed that sphingosine kinase 1 (*SPHK1*) was up-regulated by DMU-212. It has been demonstrated that SPHK1 is essential for TNF-α induced NF-κB activation (Alvarez et al., 2010; Donnahoo et al., 1999; Hait et al., 2009). Furthermore, NF-κB regulates a variety of genes, such as p38MAPK,

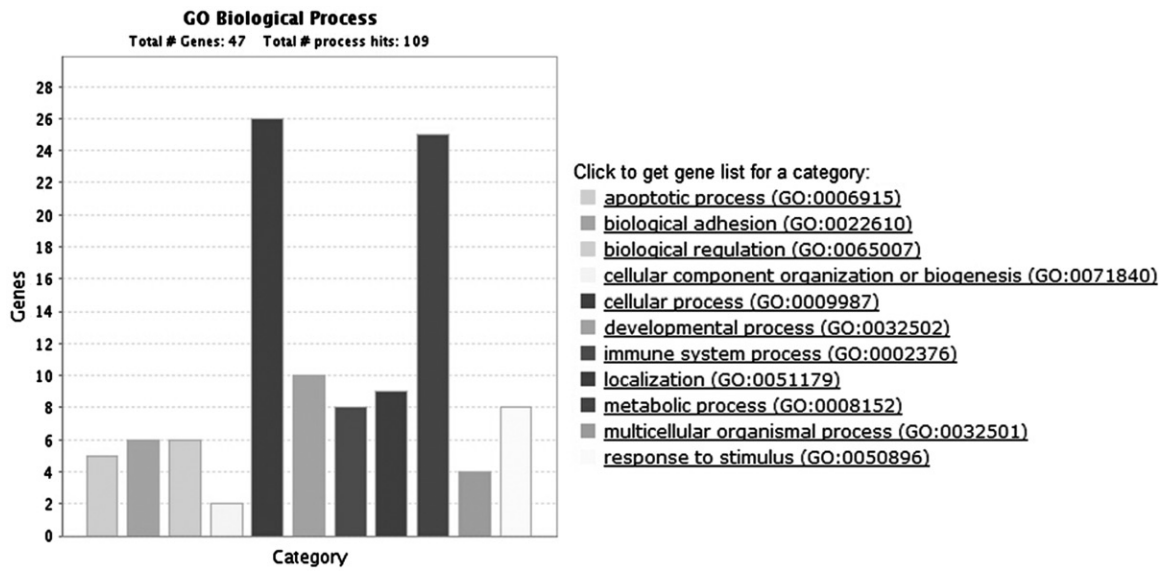

Figure 3. Biological process classification of the differential expressed genes using the GENEONTOLOGY (The Gene Ontology Consortium, Cambridge, UK) and PANTHER (Evolutionary Systems Biology Group, Menlo Park, CA) database.

responsible for the generation of TNF-α, thereby providing a positive regulatory loop (Vallabhapurapu & Karin, 2009; Valladares et al., 2000). Collectively, we speculated that the activation of NF-κB and TNF signaling pathways in response to DMU-212 might lead to a coordinated increase in a wide variety of proinflammatory factors which in turn lead to amplification of the inflammatory response, and subsequently led to excessive cell death in VECs.

As we speculated above, DMU-212 induced inflammation characterized by the upregulation of several key inflammatory mediators, including interleukin-8 (*IL-8*), *SELE*, vascular cell adhesion molecule 1 (*VCAM-1*), and so on, which might consequently result in endothelial dysfunction and cytotoxicity. It is noticed that these cytokines also exerted prosurvival and proangiogenic effects. IL-8 has been reported to increase proliferation and survival of VECs and promote angiogenic

Table 2. Pathway identification analysis using KOBAS.

Term	Database	ID	Input number	Background number	*p* Value	Corrected *p* value
TNF signaling pathway	KEGG PATHWAY	hsa04668	7	110	0.00019984132779	0.0409075197987
NF-kappa B signaling pathway	KEGG PATHWAY	hsa04064	5	92	0.000633080871748	0.0891071290368
Chemokine receptors bind chemokines	Reactome		3	38	0.000864523956997	0.101370672561
NOD-like receptor signaling pathway	KEGG PATHWAY	hsa04621	4	57	0.000917591814748	0.101370672561
Malaria	KEGG PATHWAY	hsa05144	3	49	0.00594722488585	0.239888032495
ATF-2 transcription factor network	PID	atf2 pathway	3	32	0.0061690699712	0.239888032495
Ovarian steroidogenesis	KEGG PATHWAY	hsa04913	3	51	0.00660672532974	0.239888032495
NOD1/2 signaling pathway	Reactome		2	25	0.00667730483376	0.239888032495
Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) signaling pathways	Reactome		2	33	0.0110534296757	0.263054587183
Cytokine-cytokine receptor interaction	KEGG PATHWAY	hsa04060	6	267	0.0132252383208	0.263054587183
Epithelial cell signaling in *Helicobacter pylori* infection	KEGG PATHWAY	hsa05120	3	68	0.0139557639905	0.263054587183

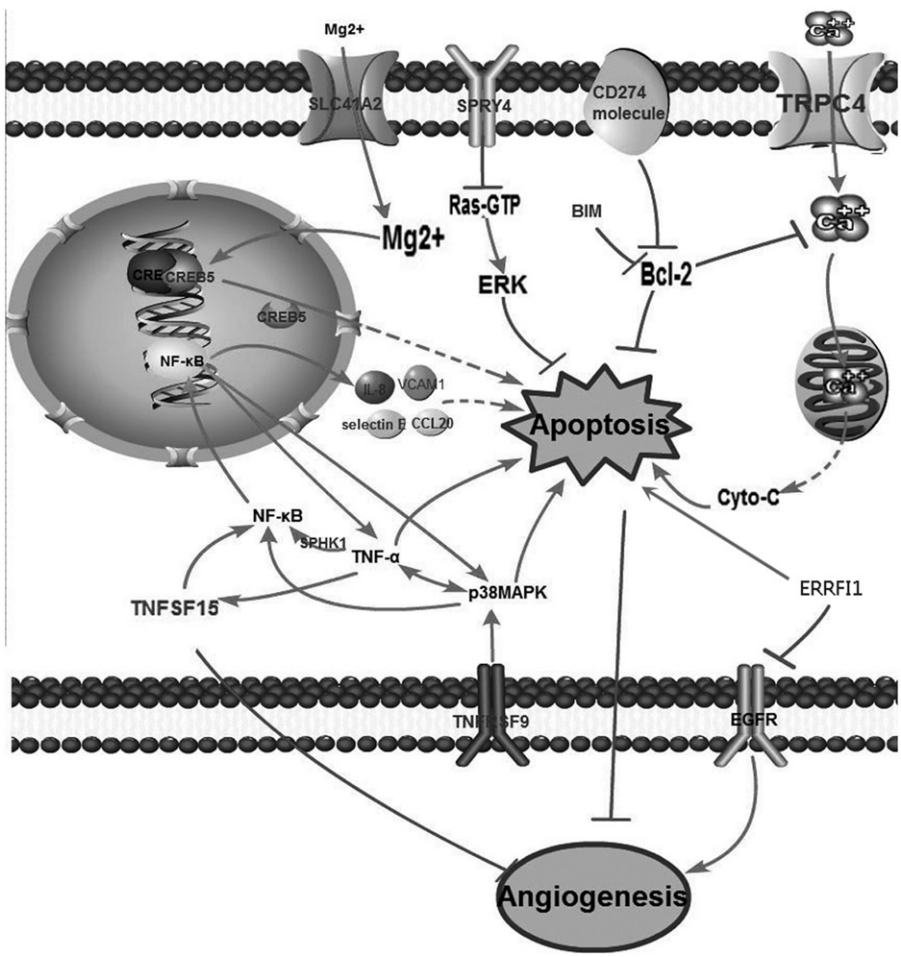

Figure 4. Network extension of the comprehensive signaling pathway collated by the differentially expressed genes that were regulated by DMU-212.

responses (Lee et al., 2014). Down-regulation of vascular selectin E by siRNA profoundly inhibited tumor angiogenesis (Liu et al., 2011). In addition, Kang et al. (2014) reported that VCAM-1 was involved in Curculigoside A-induced angiogenesis. Based on these observations, the role of these elevated cytokines on DMU-212-treated HUVECs needs to be addressed in more detail in our future study.

SPRY4, a glycoprotein can block the binding of Ras-GTP (Sasaki et al., 2003), is also up-regulated by DMU-212. Ras-GTP activates ERK pathway through receptor-mediated activation of the small G-protein (Macdonald et al., 1993). ERK1/2-mediated signaling plays a significant role in cell survival (Xia et al., 1995). In our previous study, we detected that the level of phosphorylation ERK1/2 is decreased by DMU-212 (Chen et al., 2013). From these observations, we speculated that the inactivation of ERK might be involved in DMU-212-induced VEC apoptosis.

BCL2 is an important anti-apoptotic protein. Our result showed that the mRNA levels of BCL2L11 and CD274 molecule, two BCL2 inhibitors, were elevated. BCL2L11 protein containing BCL2 homology domain 3 (BH3), which could interact with the members of BCL2 family, including BCL2, BCL2L1, and myeloid cell leukemia 1 (*MCL1*), acts as an apoptotic activator (Huang & Strasser, 2000; Wilson-Annan et al., 2003; Zong et al., 2001). CD274 is a type 1 transmembrane protein that has been speculated to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease, and other diseases (Francisco et al., 2010). In addition, it has been reported that this molecule could control the accumulation of foreign antigen specific T cells in the lymph nodes through regulating BCL2 and apoptosis (Chemnitz et al., 2004). cAMP responsive element binding protein 5 (*CREB5*) belongs to the CRE-binding protein family. It binds to certain DNA sequences called cAMP responsive element (CRE), which leads to the activation of CREB. CREB activation regulates the transcription of several downstream apoptotic-associated genes, and subsequently, apoptosis occurred (Craig et al., 2001; Zhu et al., 2012). More interestingly, Mg^{2+} is essential for the binding of CREB and CRE (Lin et al., 2009). Data from genechip showed that the mRNA level of solute carrier family 41, member 2 (*SLC41A2*), a Mg^{2+} channel, was elevated by DMU-212. These results promoted us to speculate that DMU-212 might increase intracellular Mg^{2+} through *SLC41A2*, which participated in CREB5 activation and VEC apoptosis.

Besides Mg^{2+} channel, one of the transient receptor potential canonical channels (TRPCs), TRPC4 was also up-regulated. TRPC4 is widely expressed in the vasculature and has been suggested as a key determinant of endothelial functions, such as endothelial permeability (Tiruppathi et al., 2002), nitric oxide (NO)-dependent vasorelaxation (Freichel et al., 2001), barrier stability (Graziani et al., 2010), endothelial proliferation (Abdullaev et al., 2008), and tube formation (Antigny et al., 2012). Although there is no direct evidence that TRPC4 is involved in VEC apoptosis, this channel is important for intracellular Ca^{2+} maintenance in VECs (Bair et al., 2009). The high level of intracellular Ca^{2+} can affect the permeability of mitochondria membrane and cause the release of cytochrome C, leading to mitochondria-initiated apoptosis (Andreyev & Fiskum, 1999; Schild et al., 2001).

Enhanced mRNA levels of ERRFI1 also attracted our attention. ERRFI1 has been identified as an immediate early response gene encoding a scaffolding adaptor protein and a negative regulatory protein that inhibits the kinase domains of epidermal growth factor receptor (EGFR) (Yoon et al., 2012). Activated EGFR pathway may promote cell proliferation, differentiation, angiogenesis, and antiapoptosis (Wang et al., 2015). ERRFI1 might exert its antiapoptosis and antiangiogenic effects through inhibiting EGFR signaling in DMU-212-treated VECs.

Conclusions

In summary, a combination of microarray data and pathway analysis first introduces many possible biological pathways to explain the mechanisms of DMU-212-mediated VEC apoptosis and antiangiogenesis. Although the corresponding mechanisms are complex, the activation of NF-κB and TNF signaling pathways, which led to an amplification of proapoptotic events and proinflammatory responses, might be the main reason for the excessive cell death in DMU-212-treated VECs. These findings help us to further understand the function of DMU-212 and might provide several novel potential molecular targets for antiangiogenesis therapy. Further studies will focus particularly on the roles of SPRY4, CREB5, SLC41A2, TRPC4, and ERRFI1 as potentially important regulators of VEC apoptosis and angiogenesis.

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Declaration of interest

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