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Identification of commonly regulated genes in HPV18- and HPV16-infected cervical cancer cells treated with the curcumin analogue 1,5-bis(2-hydroxyphenyl)-1,4-pentadiene-3-one

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ABSTRACT

Objective: To identify commonly regulated genes in HPV-infected HeLa and CaSki cervical cancer cells treated with curcumin analogue 1,5-bis(2-hydroxyphenyl)-1,4-pentadiene-3-one (MS17) and to explore potential mechanisms that underlie its cytotoxic, anti-proliferative and apoptotic activity. **Methods**: HeLa and CaSki cells were treated with $2\times EC_{50}$ and $3\times EC_{50}$ doses of MS17 for 24 h and the RNA extracts were subjected to one-colour microarray-based gene expression profiling. Pair-wise significant genes (false discovery rate-corrected, $P<0.05$) were analysed for fold change (FC) compared to control samples. Differentially expressed genes with FC$\geqslant$2.0 (up-regulated; FC$\geqslant$2.0 and down-regulated; FC$\leqslant$-2.0) compared to the control samples were filtered through and analysed to create a global gene expression profile. Mutually regulated genes were ranked by FC and categorised by gene ontology. **Results**: Our data indicated dose-dependent regulation by MS17 and identified top 20 mutually up- and down-regulated genes each in HeLa and CaSki cells. Amongst these 17 were commonly regulated in both cell lines. These include the up-regulation of *CCL26*, *DEFB103B*, *IL1RL1*, *LY96*, *GCNT3*, *MMP10*, *MMP3*, *GADD45G* and *HSPA6*, and the down-regulation of *TENM2*, *NEBL*, *KIFC1*, *CTDSP1*, *IGFBP5*, *LTBP1*, *NREP* and *MXD3*. These genes were associated with key biological functions that were proposed to mediate the anticancer activity of MS17 in cervical cancer cells such as immune response, metabolic processes, proteolysis, programmed cell death, unfolded protein response, cell adhesion, cytoskeletal organisation, phosphatase activity, signal transduction and transcription regulator activity. **Conclusions**: Identification of seventeen common genes modulated by MS17 could be used as potential therapeutic targets in both cervical cancer cell lines and the findings of this study could be used to present an insight into the potential antitumor activity of MS17 in cervical cancer.

1. Introduction

Cervical cancer is the fourth most common cancer affecting women worldwide[1] and ranks as the second most frequent cancer affecting Malaysian women. Curcumin is a widely studied phytochemical.

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The treatment of cervical cancer cells with curcumin activates caspase-3-mediated apoptosis, restores p53 and pRb expression and inhibits AP-1 and NF-κB activity[2–4]. Notably, curcumin demonstrates potential antiviral activity due to its ability to inhibit the expression of E6 and E7 viral oncogenes[5,6] which significantly increases the cytotoxic and apoptotic effects of curcumin treatment on cervical cancer cells[7]. Synthetic 1,5-diaryl-3-oxo-1,4-pentadiene curcumin analogues known as diarylpentanoids reported greater growth suppressive effects in cancer cells over curcumin or other 7-carbon curcumin analogues[8]. Similarly with curcumin, these diarylpentanoids mediated programmed cell death, mitotic arrest and anti-proliferative effects in numerous cancer cell lines via a wide variety of molecular targets including p53, NF-κB, STAT3, c-Myc and PTEN[9–11]. EF24 is the most studied DAP to date due to its ability to collectively target molecules that induce, among others, anti-proliferative, apoptotic, anti-angiogenesis and anti-oxidative effects in cancer cells[12–14]. The treatment of HeLa cervical cancer cells by EF24 demonstrated the inhibition of cell viability via the suppression of NF-κB signaling[14], and studies by two other groups reported the potential anti-proliferative and apoptotic effects of treatment on cervical cancer cells[15,16]. Recently, a PGA-based curcumin nanoparticle formulation has been shown to effectively inhibit growth, induce apoptosis and cause cell cycle arrest in CaSki and SiHa cervical cancer cell lines[17]. Beyond these studies however, the anticancer effect of diarylpentanoids on cervical cancer cell lines have not been extensively studied.

We have previously established[18] that treatment of 1,5-bis(2-hydroxyphenyl)-1,4-pentadiene-3-one, known as MS17, on CaSki and HeLa cervical cancer cells demonstrated significant cytotoxic and anti-proliferative activity compared to curcumin. MS17 also demonstrated significant down-regulation of HPV18- and HPV16-associated E6 and E7 oncogene expression following treatment. Furthermore, MS17-exposed cells exhibited significant morphological changes consistent with apoptosis at their respective $2\times EC_{50}$ (HeLa; 6 μM, CaSki; 4 μM) and $3\times EC_{50}$ (HeLa; 9 μM, CaSki; 6 μM) dosages at 24 h of treatment. This suggested that an increase in treatment dose by 1.5-fold was able to effect apoptotic changes within the cells. To the best of our knowledge, this is the first report of its kind on HPV-positive cervical cancer cells. To investigate the mechanisms of action of the MS17 curcumin analogue on cervical cancer cells at a molecular level, microarray-based gene expression profiling of MS17-treated HPV-18 infected HeLa and HPV-16 infected CaSki cells was performed. Dose-dependent changes in gene regulation that occurred in response to treatment were analysed and the regulation of 17 genes that were mutual to both cervical cancer cell lines were identified as potential common targets of MS17. The molecular changes in the regulation of these genes that potentially justifies the cytotoxic, anti-proliferative and apoptotic activity of MS17 on cervical cancer cells have been presented here and discussed.

2. Materials and methods

2.1. Cell culture and preparation of MS17

HPV-infected HeLa and CaSki cervical cancer cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained using MEM and RPMI 1640 (Gibco) culture media respectively and supplemented with 10% Fetal Bovine Serum (FBS, Gibco) and penicillin (100 U/mL)/streptomycin (100 μg/mL) (Gibco). Cells were cultured at 37 ℃ in a humidified atmosphere with 5% CO_2. The curcumin analogue 1,5-bis(2-hydroxyphenyl)-1,4-pentadiene-3-one (MS17) was prepared as previously described[18].

2.2. Treatment of cervical cancer cells and total RNA extraction

Cancer cells were plated at 4×10^5 cells per mL in 1 mL culture medium per well as described earlier[18], exposed to MS17 at their respective doses for 24 h and prepared for RNA extraction. Experiments were performed in triplicate and included a set of untreated control cells (media with DMSO alone). Total RNA was extracted using the Qiagen RNeasy® Mini Kit (Qiagen, Valencia, CA., USA) according to the manufacturer's instructions. RNA samples with an A_{260}/A_{280} and A_{260}/A_{230} ratio of $\geqslant$1.9 and with a RNA integrity number score of approximately $\geqslant$8 were selected for gene expression profiling.

2.3. Microarray analysis

Agilent oligonucleotide microarrays were used for one-colour gene expression analysis and samples were prepared according to the protocols provided by One-Color Low Input Quick Amp labeling kit (Agilent, Valencia, CA, USA). Information from features on the array was extracted using Agilent's Feature Extraction Software 11.5.1 (Agilent Technologies, 2004, USA) and analysed (GeneSpring GX version 13.0 software, Agilent, USA). Normalisation was performed using Agilent Single Color 28004 Technology. Raw signals were log transformed, normalized and baseline transformed to the median of all samples. Values were normalised by shifting to the 75th percentile. Probes were subjected to additional steps of filtering on detected flags and expression value. Filtering on expression removes probes with low raw signal intensities (<20) and entities where at least 100 percent of samples in any 1 out of 4 conditions have values exceeding 20 qualified to proceed to the next filtering criteria.

2.4. Statistical analysis

Statistical analysis was performed using analysis of variance with Benjamini-Hochberg false discovery rate multiple testing correction, followed by a *post hoc* testing using Tukey's Honestly Significance Difference to identify pair-wise significant genes. Genes that passed the significance threshold with a false discovery rate-corrected adjusted *P*-value of $P<0.05$ was analysed for its fold change (FC) compared to control samples. FC measures the relative change in expression between treatment (MS17-treated samples) and control (DMSO-treated) conditions for a particular gene. Differentially expressed genes (DEGs) with FC$\geqslant$2.0 (up-regulated; FC$\geqslant$2.0 and down-regulated; FC$\leqslant$-2.0) compared to the control samples were filtered through and analysed to create a global gene expression profile. Hierarchical cluster analysis was performed on this DEG list using software-recommended parameters.

2.5. Data analysis

For an analysis on the dose-dependent regulation of genes by MS17, downstream analysis focused on selected mutually regulated DEGs in MS17-treated HeLa (6 μM and 9 μM) and CaSki (4 μM and 6 μM) cells. The top twenty DEGs that were mutually up-regulated and down-regulated each in both cell lines were selected to obtain a list of top ranked genes. Ontological analysis of mutually regulated genes was performed using the Database for Annotation, Visualisation and Integrated Discovery (DAVID)[19] which is freely accessed at http://david.abcc.ncifcrf.gov. Gene symbols of mutually expressed genes from each cell line were submitted to DAVID. Annotations were limited to 'Homo sapiens' and gene ontological (GO) analysis was performed using the functional annotation tool and further analysed using the GO functional categories which provide a description of associated biological processes and molecular functions for the selected genes. Genes were grouped into functional categories based on the exported GO terms from DAVID.

2.6. Validation of microarray results by quantitative real-time PCR

To assess the robustness of the microarray analysis, quantitative real-time PCR analysis was performed to validate a selected panel of DEGs (*CCL26*, *MMP10*, *MMP3*, *CTDSP1*, *IGFBP* and *MXD3*) and normalised against the endogenous gene, human glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). Complementary DNA was generated from the RNA isolated from MS17-treated HeLa and CaSki cells that were used for microarray profiling following the previously described protocol[18]. Transcript levels of the selected DEGs and *GAPDH* were measured by TaqMan® Gene Expression Assays specific to each gene. The primers and TaqMan probe sequences used for the selected genes corresponded to assay IDs (CCL26, Hs00171146_m1; MMP10, Hs00233987_m1, MMP3, Hs00968308_m1; CTDSP1, Hs01105503_m1; IGFBP5, Hs01052295_m1; MXD3, Hs00361007_m1; GAPDH, Hs99999905_m1) acquired from the TaqMan Gene Expression Assay database (Applied Biosystems, USA). Samples were prepared according to manufacturer's instructions and loaded into the StepOnePlus™ real-time PCR system (Applied Biosystems), cycled using the Fast run method and relative mRNA expression levels of the target genes were calculated using the comparative Ct method. Each reaction was run in duplicates and fold change expression of mRNA results from quantitative real-time PCR was converted to log2 fold change and represented in bar graphs alongside log2 fold change data from microarray analyses. The bar graph representing the results was constructed using GraphPad Prism version 6.00 for Windows, GraphPad Software (San Diego, CA., USA).

3. Results

Based on dosages that were previously established to induce apoptosis, HeLa was exposed to 6 μM and 9 μM, while CaSki was exposed to 4 μM and 6 μM of MS17 for 24 h. Only significant entities that demonstrated a *P*-value of $P<0.05$ and were differentially expressed compared to the control by a fold change greater than or equal to 2.0 (FC$\geqslant$2.0) were considered for analysis. Amongst these genes, 1 167 and 1 836 genes in HeLa were differentially expressed by 6 μM and 9 μM treatment respectively while in CaSki, 1 069 and 1 091 genes were differentially expressed by 4 μM and 6 μM treatment. DEGs were subjected to a hierarchical cluster analysis from which a dose-dependent effect of MS17 treatment on both HeLa and CaSki gene regulation was suggested as 826 genes in HeLa and 491 genes in CaSki composed DEGs that were mutually expressed by both treatment doses. Of these DEGs, we have identified top 20 mutually up- and down-regulated genes each in HeLa and CaSki cells.

Ontological classification was performed on the top 20 ranking genes that were differentially regulated by MS17 treatment and mutually expressed by both treatment doses in HeLa (Table 1) and CaSki (Table 2) cells. These genes were subsequently grouped according to their respective biological functions. Amongst the top 20 genes, 17 genes were commonly regulated by MS17 in both cell lines (denoted as asterisks in Table 1 and Table 2). These included the up-regulation of *CCL26*, *DEFB103B*, *IL1RL1*, *LY96*, *GCNT3*, *MMP10*, *MMP3*, *GADD45G* and *HSPA6* which were associated with immune response, metabolic processes, proteolysis, programmed cell death and unfolded protein response, and the down-regulation of *TENM2*, *NEBL*, *KIFC1*, *CTDSP1*, *IGFBP5*, *LTBP1*, *NREP* and *MXD3* which were associated with cell adhesion, cytoskeletal organisation, phosphatase activity, signal transduction and transcription regulator activity. Of these 17 genes, 3 up- and 3 down-regulated genes were selected and validated, and the expression patterns determined from real-time PCR were consistent with the microarray results (Figure 1).

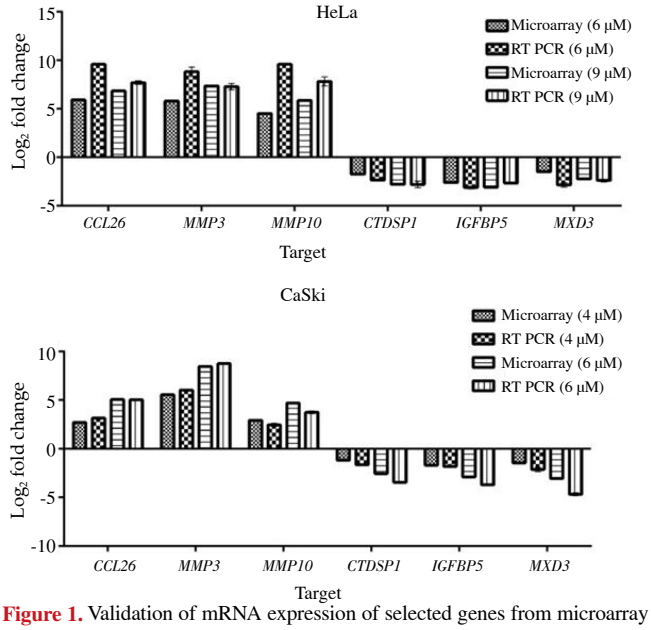

Figure 1. Validation of mRNA expression of selected genes from microarray data by quantitative real time PCR.

Complementary DNA from MS17-treated (A) HeLa and (B) CaSki cells were used for validation of the gene expression obtained from microarray analysis in two independent experiments. The error bars represent the standard deviation of duplicate values. All data were normalized to the reference gene, GAPDH. Data was presented as $\log_2$ fold change in MS17-treated cells compared to untreated cells. The expressions of *CCL26*, *MMP3* and *MMP10* were all up-regulated while those of *CTDSP1*, *IGFBP5* and *MXD3* were down-regulated. The expression patterns determined from real-time PCR were consistent with the microarray results.

4. Discussion

Treatment of HeLa and CaSki cells with MS17 reveals an expression profile that contained common gene targets. This is interesting because although HeLa and CaSki are both infected with high risk subtypes that cause cervical cancer, both cell lines are derived from different sources such that CaSki is of squamous origin, while HeLa is an adenocarcinoma. Our results suggest that the 17 commonly regulated genes are important to specific biological functions and may be implicated in the anticancer activity of this curcumin analogue. Identification of common gene targets in cervical cancer cells despite being infected with different

Table 1

Selected genes mutually induced and repressed in HeLa cells following 6 μM and 9 μM treatment of MS17 at 24 h.

GO term	Gene symbol	Genbank accession	Description	Fold change (6 μM)	Fold change (9 μM)	*P* value
Up-regulated						
Chromatin organization	*NAP1L2*	NM_021963	Nucleosome assembly protein 1-like 2	22.8	56.6	0.004 3
Epidermis development	*SPRR2A*	NM_005988	Small proline-rich protein 2A	27.6	82.7	0.001 6
Immune response	**CCL26*	NM_006072	Chemokine (C-C motif) ligand 26	60.7	115.2	0.002 5
	**DEFB103B*	NM_018661	Defensin, beta 103B	35.4	78.4	0.001 3
	**IL1RL1*	NM_016232	Interleukin 1 receptor-like 1	27.2	74.9	0.001 9
	**LY96*	NM_015364	Lymphocyte antigen 96	31.1	67.1	0.003 8
	REG3G	NM_001008387	Regenerating islet-derived 3 gamma	14.0	36.3	0.005 7
Metabolic process	**GCNT3*	NM_004751	Glucosaminyl (N-acetyl) transferase 3, mucin type	40.0	92.6	0.001 7
	AGMO	NM_001004320	Alkylglycerol monooxygenase	18.5	55.8	0.006 3
Programmed cell death	**GADD45G*	NM_006705	Growth arrest and DNA-damage-inducible, gamma	40.7	146.0	0.013 8
	IL24	NM_001185156	Interleukin 24	25.5	56.1	0.014 1
Protein ubiquitination	*NEURL3*	NM_001285486	Neuralized E3 ubiquitin protein ligase 3	31.5	69.8	0.015 9
Proteolysis	**MMP10*	NM_002425	Matrix metallopeptidase 10 (stromelysin 2)	55.9	164.9	0.000 6
	**MMP3*	NM_002422	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	22.8	57.8	0.004 8
Receptor activity	*PAQR9*	NM_198504	Progestin and adipoQ receptor family member IX	11.6	32.7	0.037 8
Signal transduction	*PNOC*	NM_006228	Prepronociceptin	11.3	40.0	0.003 5
	RGS9	NM_003835	Regulator of G-protein signaling 9	13.3	35.3	0.016 4
Transcription regulator activity	*MYCT1*	NM_025107	Myc target 1	29.7	82.6	0.004 8
	RFPL4AL1	NM_001277397	Ret finger protein-like 4A-like 1	29.0	80.6	0.004 9
Unfolded protein response	**HSPA6*	NM_002155	Heat shock 70kDa protein 6 (HSP70B')	247.7	702.6	0.003 8
Down-regulated						
Cell adhesion	**TENM2*	NM_001122679	Teneurin transmembrane protein 2	-3.4	-10.2	0.008 0
Cell cycle	*FOS*	NM_005252	FBJ murine osteosarcoma viral oncogene homolog	-8.2	-7.1	0.010 7
Cytoskeleton organization	**NEBL*	NM_006393	Nebulette	-5.9	-6.2	0.013 1
	MTUS2	NM_001033602	Microtubule associated tumor suppressor candidate 2	-2.9	-6.1	0.004 8
	**KIFC1*	NM_002263	Kinesin family member C1	-3.7	-5.3	0.003 2
	RDX	NM_001260492	Radixin	-2.9	-4.5	0.028 8
Metabolic process	*LGSN*	NM_016571	Lengsin, lens protein with glutamine synthetase domain	-6.6	-8.6	0.042 1
	CYP24A1	NM_000782	Cytochrome P450, family 24, subfamily A, polypeptide 1	-5.5	-6.8	0.012 3
Phosphatase activity	**CTDSP1*	NM_021198	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	-3.3	-6.9	0.048 4
Signal transduction	*PRKCB*	NM_002738	Protein kinase C, beta	-9.8	-16.6	0.001 9
	PDGFRB	NM_002609	Platelet-derived growth factor receptor, beta polypeptide	-7.2	-14.4	0.0023
	PLCXD3	NM_001005473	Phosphatidylinositol-specific phospholipase C, X domain containing 3	-5.5	-14.2	0.042 0
	**IGFBP5*	NM_000599	Insulin-like growth factor binding protein 5	-6.1	-8.5	0.005 0
	LTBP2	NM_000428	Latent transforming growth factor beta binding protein 2	-4.0	-7.4	0.008 4
	**NREP*	NM_001142483	Neuronal regeneration related protein	-4.6	-6.2	0.008 9
	RAB26	NM_014353	RAB26, member RAS oncogene family	-2.8	-5.5	0.013 0
	**LTBP1*	NM_206943	Latent transforming growth factor beta binding protein 1	-4.4	-5.2	0.036 9
	MAP2K6	NM_002758	Mitogen-activated protein kinase kinase 6	-2.7	-4.8	0.004 9
Transcription regulator activity	*BARX1*	NM_021570	BARX homeobox 1	-3.6	-5.0	0.011 1
	**MXD3*	NM_031300	MAX dimerization protein 3	-2.8	-4.7	0.002 5

Selected DEGs are listed according to biological function as proposed by DAVID Functional Annotation Tables. *P* value denotes the *P* corrected value for this gene list. *Indicates commonly regulated DEGs in treated HeLa and CaSki cell lines.

Table 2

Selected genes mutually induced and repressed in CaSki cells following 4 μM and 6 μM treatment of MS17 at 24 h.

GO term	Gene symbol	Genbank accession	Description	Fold change (4 μM)	Fold change (6 μM)	*P* value
Up-regulated						
Cytoskeleton organization	*ARC*	NM_015193	Activity-regulated cytoskeleton-associated protein	2.8	36.1	0.004 1
ER calcium homeostasis	*TMTC1*	NM_175861	Transmembrane and tetratricopeptide repeat containing 1	21.4	61.9	0.001 7
Immune response	**DEFB103B*	NM_018661	Defensin, beta 103B	30.5	89.9	0.000 5
	**CCL26*	NM_006072	Chemokine (C-C motif) ligand 26	6.5	33.4	0.001 4
	**IL1RL1*	NM_016232	Interleukin 1 receptor-like 1	6.2	22.1	0.000 9
	**LY96*	NM_015364	Lymphocyte antigen 96	5.0	14.8	0.003 5
Metabolic process	**GCNT3*	NM_004751	Glucosaminyl (N-acetyl) transferase 3, mucin type	6.3	25.0	0.000 9
	CRYM	NM_001888	Crystallin, mu	5.4	18.9	0.000 6
	PNLIPRP3	NM_001011709	Pancreatic lipase-related protein 3	7.2	18.0	0.000 8
	HKDC1	NM_025130	Hexokinase domain containing 1	3.8	7.6	0.002 2
Programmed cell death	**GADD45G*	NM_006705	Growth arrest and DNA-damage-inducible, gamma	2.8	18.5	0.002 7
Proteolysis	**MMP10*	NM_002425	Matrix metallopeptidase 10 (stromelysin 2)	46.6	347.9	0.001 4
	**MMP3*	NM_002422	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	7.6	26.3	0.002 2
Signal transduction	*F2RL2*	NM_004101	Coagulation factor II (thrombin) receptor-like 2	17.4	52.4	0.000 9
	GNGT2	NM_031498	Guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2	14.5	41.3	0.004 9
	RGS4	NM_005613	Regulator of G-protein signaling 4	7.9	21.1	0.001 4
Transcription regulator activity	*MAFB*	NM_005461	V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B	2.0	18.1	0.001 2
	ZFAND2A	NM_182491	Zinc finger, AN1-type domain 2A	2.5	12.6	0.001 7
	EID3	NM_001008394	EP300 interacting inhibitor of differentiation 3	3.2	10.9	0.004 6
Unfolded protein response	**HSPA6*	NM_002155	Heat shock 70kDa protein 6 (HSP70B')	22.6	309.1	0.001 1
Down-regulated						
Cell adhesion	*WISP2*	NM_003881	WNT1 inducible signaling pathway protein 2	-3.8	-9.7	0.003 8
	**TENM2*	NM_001122679	Teneurin transmembrane protein 2	-2.4	-5.2	0.013 5
	FAT2	NM_001447	FAT atypical cadherin 2	-2.5	-5.1	0.022 4
Cell cycle	*AURKB*	NM_004217	Aurora kinase B	-2.3	-5.9	0.001 4
	PLK1	NM_005030	Polo-like kinase 1	-2.4	-4.7	0.010 3
Cell proliferation	*MKI67*	NM_002417	Marker of proliferation Ki-67	-2.7	-6.1	0.000 5
Chromosome segregation	*CENPF*	NM_016343	Centromere protein F, 350/400kDa	-2.6	-4.7	0.004 4
	NEK2	NM_002497	NIMA-related kinase 2	-2.3	-4.5	0.004 4
Cytoskeleton organization	*KIF20A*	NM_005733	Kinesin family member 20A	-2.8	-7.4	0.003 2
	**KIFC1*	NM_002263	Kinesin family member C1	-2.0	-4.8	0.000 8
	KIF4A	NM_012310	Kinesin family member 4A	-2.7	-4.6	0.007 2
	**NEBL*	NM_006393	Nebulette	-2.3	-4.5	0.002 9
DNA replication	*TOP2A*	NM_001067	Topoisomerase (DNA) II alpha 170kDa	-2.0	-5.8	0.001 4
Phosphatase activity	**CTDSP1*	NM_021198	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	-2.2	-5.9	0.016 9
Signal transduction	*WNT4*	NM_030761	Wingless-type MMTV integration site family, member 4	-3.9	-7.7	0.004 1
	**IGFBP5*	NM_000599	Insulin-like growth factor binding protein 5	-3.2	-7.5	0.000 8
	ARHGEF39	NM_032818	Rho guanine nucleotide exchange factor (GEF) 39	-3.3	-6.7	0.005 2
	**LTBP1*	NM_206943	Latent transforming growth factor beta binding protein 1	-4.6	-5.1	0.001 7
	**NREP*	NM_001142483	Neuronal regeneration related protein	-2.6	-4.0	0.002 5
Transcription regulator activity	**MXD3*	NM_031300	MAX dimerization protein 3	-2.7	-8.3	0.002 8

Selected DEGs are listed according to biological function as proposed by DAVID Functional Annotation Tables. *P* value denotes the *P* -corrected value for this gene list. *Indicates commonly regulated DEGs in treated HeLa and CaSki cell lines.

HPV subtypes may suggest that targeting these genes could have therapeutic implications.

The largest proportion of differentially up-regulated genes is associated with immune response. Many members in this cluster encode cytokines which are involved in immune system regulation and inflammation processes. Four highly up-regulated immune response-associated transcripts that were common to both cell lines included *CCL26*, followed by *DEFB103B*, *IL1RL1* and *LY96*. *CCL26* encodes dendritic-cell specific chemokines that attract naive lymphocytes and neutrophils; *DEFB103B* codifies human β-defensin-3; *IL1RL1* expresses the receptor for interleukin-1 and *LY96* responds to toll-like receptor 4 on dendritic cells. They are

respectively involved in the repression of cancer cell migration[20], growth[21,22] and the mediation of anticancer immunity[23]. The findings suggest that induction of an immune response, inhibition of cell migration and growth could be potential mechanisms by which MS17 exhibits anticancer activity on HeLa and CaSki cells.

The metabolism-associated *GCNT3* encodes a beta-6-*N*-acetylglucosamine-transferase which catalyses O-glycan branching on mucin-type glycoproteins. Alterations in the O-glycosylation of mucins often accompany cancer development and the over-expression of *GCNT3* reportedly suppresses cell adhesion, motility and invasion of colorectal cancer cells[24] and may contribute towards anti-tumorigenic activity in treated HeLa and CaSki cells. MS17 treatment positively modulated the increased expression of proteolytic matrix metalloproteinases, specifically of *MMP10* and *MMP3* which degrade the extracellular matrix[25]. *MMP10* is commonly implicated in inflammatory diseases and may be secreted by T-lymphocytes to assist in extracellular matrix degradation[26] during inflammation or an immune response. Cross-talk between matrix metalloproteinases suggests that they may possess pro- and anti-tumorigenic effects. Squamous cell carcinoma tumours in MMP3-null mice exhibited enhanced tumour progression and were inversely correlated with leukocyte infiltration[27]. Therefore the up-regulation of matrix metalloproteinases by MS17 indicates the potential modulation over the proteolytic extracellular matrix degradation of cervical cancer cells to exert its anti-tumorigenic effects. The exact mechanism by which this occurs however needs to be investigated further.

The up-regulation of programmed cell death associated gene *GADD45G* demonstrated that increasing the treatment dose by 1.5-fold showed almost 3.6-fold up-regulation in MS17 treated HeLa cells and 6.6-fold up-regulation in CaSki cells. *GADD45G* codifies the expression of DNA damage inducible proteins which is activated by heat shock or UV irradiation stimuli, a response which is abolished in approximately 50% of cervical cancer tumours[28,29]. Notably, its expression is up-regulated following exposure to curcumin treatment, DNA damage and genotoxic stress and induces cell cycle arrest, apoptosis and inhibits cell growth[30,31] and its induction could contribute to the apoptotic cell death mechanism of MS17 in treated HeLa and CaSki cells.

The heat shock gene *HSPA6* which encodes the stress-inducible Hsp70 protein, is the topmost mutually up-regulated transcript in HeLa cells and demonstrates 13.7-fold up-regulation in response to increasing treatment dose in CaSki cells. Heat shock proteins act as protein chaperones to prevent protein misfolding and Hsp70 expression is commonly up-regulated during cellular oxidative stress which promotes tumour cell growth and contributes to genomic instability and DNA damage in cancer cells. However, the overexpression of oxidative stress also induces cancer cell death[32] and the induction of heat shock protein expression in response to anticancer compounds is not uncommon. Treatment with elescomol triggered apoptosis in melanoma cancer cells by inducing a transcriptional gene profile characteristic of an oxidative stress response, which also included *HSPA6* as its top most up-regulated transcript[32]. Notably treatment of HeLa and leukaemia cells with curcumin and exposure of melanoma cells with curcumin analogue D6 induced the up-regulation of *HSPA6*, correlating strongly with cellular toxicity and was suggested to cause extreme endoplasmic reticulum stress resulting in apoptotic cell death[33-35]. This may thus reflect a similar mechanism by which MS17 up-regulates *HSPA6* expression to modulate oxidative stress responses and trigger apoptosis induction in treated cells thus contributing to anti-tumorigenesis.

The expression of cell adhesion-associated *TENM2* is down-regulated in both cell lines following MS17 treatment. *TENM2* encodes a transmembrane protein that enhances proliferation, promotes cell adhesion, facilitates microtubule organization[36] and is up-regulated by more than 250-fold in vincristine-resistant ovarian cancer cells[37]. The suppression of *TENM2* by MS17 could disrupt adhesive contact between cells and mediate anti-proliferative activity in treated HeLa and CaSki cells.

Cytoskeleton organizing genes *NEBL* and *KIFC1* are also down-regulated by MS17 treatment in both cell lines. NEBL codifies nebulette, an actin protein involved in cytoskeletal organization. The up-regulation of *NEBL* stimulates cell migration[38] while silencing of a related family member, *Lasp1*, in cell lines abrogates metastasis[39]. *KIFC1* encodes microtubule motor proteins that function during spindle formation. The down-regulation of *KIFC1* may destabilize spindle assembly, trigger cell cycle arrest and reduce cell viability[40]. Thus the repression of cytoskeletal organisation genes indicates the role of MS17 in modulating an anchorage network that is important for chromosome stability in mitosis.

The nuclear phosphatase expressed by *CTDSP1* regulates gene expression by selective dephosphorylation of phosphoserine 5 within RNA polymerase Ⅱ and is commonly associated with the silencing of neuronal genes[41]. Its down-regulation by MS17 in cervical cancer cells suggests the potential modulation of transcriptional regulation to inhibit cell survival. *IGFBP5*, *LTBP1* and *NREP* are grouped under signal transduction ontologies. *IGFBP5* encodes IGF-binding proteins which are highly expressed in CIN tissues[42] and regulates the survival of cervical cancer cells by conferring it with anti-apoptotic properties[43]. The down-regulation of *IGFBP5* expression by MS17 suggests the reactivation of apoptosis in HeLa and CaSki cells which potentially contributes to cell death. *LTBP1* encodes a latent TGFβ binding protein which contributes towards glioma cell malignancy while silencing is shown to reduce TGFβ activity[44] suggesting an important modulatory effect of *LTBP1* over malignant tumour phenotypes. On the other hand, *NREP* encodes a neural regeneration protein but demonstrates RAC1-mediated involvement in glioma cell motility[45]. Hence the suppression of NREP by MS17 targets the disruption of actin cytoskeletal organization of HeLa and CaSki cells and impairs malignant cell proliferation and motility. Furthermore, *MXD3* encodes a MAD family transcription

factor which contributes to cell proliferation upon up-regulation but demonstrates anti-proliferative activity following MDX3-specific silencing[46] which may result in the suppression of cervical cancer proliferation following MS17 treatment.

In conclusion, the dose-dependent modulation of high ranking genes demonstrates the notable regulation of 17 genes that are common to both cell lines, suggesting the possibility that MS17 exerts antitumor effects on pathways linked to immune response, metabolism, proteolysis, apoptosis, unfolded protein response, cell adhesion, cytoskeletal organization, phosphatase activity, signal transduction and transcription regulator activity. Despite the differing biological properties of HPV-18 infected HeLa and HPV-16 infected CaSki cells, the common regulation of these genes indicates shared therapeutic targets and mechanism of action by MS17 in cervical cancer cells. Thus the findings of this study present an insight into the potential antitumor activity of MS17 in cervical cancer and identify key biological targets that serve as a basis for future investigation.

Conflict of interest statement

The authors declare no conflict of interest.

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