BIOCHIP JOURNAL, Vol. 3, No. 4, 344-353, December 2009

Comparative Global Transcription Analysis of Hydrophilic Sapphyrin and Resveratrol on A549 Cell

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Accepted 21 October 2009

Abstract

Anti-cancer agents were studied to use a variety of natural product and chemical synthesis compounds, the effect elucidate cell cycle arrest, growth inhibitor, and death pathway factor expression and so on cancer cell. However molecular mechanisms, by which these anti-cancer agents kill and the extent to which cancer cell, including A549 cell, are resistant remains unclear. In previously DNA microarrays were utilized to analyze the genome-wide transcription changes in A549 cell after anti-cancer compounds (resveratrol, sapphyrin PCI-2050) exposure. In this study, we compared-cell death pathway, cell cycle arrest, growth inhibition and so on-with both gene expression of sapphyrin (PCI-2050) and resveratrol that were treated on A549. And these investigated that resistance to any mechanism to A549 cell. Resveratrol treated A549 cell showed cell death pathway, cell cycle arrest, growth inhibition factor-related gene expression, and sapphyrin treated cell weren't indicated gene expression of cell cycle arrest or growth inhibition. But it exhibited over-expression of c-jun on MAPK (JNK) pathway. Consequently, resveratrol resistance to cancer cell through several apoptotic pathways. But Sapphyrin may estimate to inhibit cancer cell by only certain MAPK (JNK) pathway.

Keywords: A549 cell, Microarray, MAPK (JNK), Apoptotic pathway, Resveratrol, Sapphyrin PCI-2050

Introduction

Resveratrol (3,5,4'-trihydroxy-stilbene) is a phytoalexin found in red wine and a variety of plants, including grapes, peanuts, mulberries, and legumes[1]. Phytoalexins are produced in response to stress, injury, fungal infection, or UV exposure[1,2]. Many studies have been published to date demonstrating the beneficial effects of resveratrol in cellular systems. Epidemiologic studies revealed an inverse correlation between red wine consumption and cardiovascular disease in France (known as the "French Paradox")[3].

During the past 25 years, studies on identifying cancer chemopreventive agents have received considerable attention. Numerous natural and synthetic chemopreventive agents have been established as a result of their efficacy in experimental carcinogenesis models[4].

Reported that resveratrol exerts antitumor properties at all three stages of skin carcinogenesis, including initiation, promotion, and progression. Since then, other studies have confirmed this work, and resveratrol has been shown to have chemopreventive properties in many cancer types, including mammary, prostate, colon, and lung carcinogenesis[5-8].

Its role in prevention and therapy of cancers of several target organs has been extensively reviewed[1,9-12].

Sapphyrins are a class of expanded porphyrins that were first discovered as an unanticipated product during the synthesis of vitamin B12[13]. Subsequently, efficient chemical synthesis of these compounds[14,15] led to the discovery that sapphyrins can function as highly effective anion binding receptors[16] and it was determined that sapphyrins can selectively accumulate in tumors, relative to surrounding tissues, similar to porphyrins and other expanded porphyrin systems such as the texaphyrins[17]. It was also noted that sapphyrins could display a significant degree of inhibitory activity in cellular assays even in the absence of light[18]. Recently, sapphyrin related studies reported the anti-cancer activity of several sapphyrin derivatives in hematologic cell lines and tumors, confirming that sapphyrins possess intrinsic anticancer activity that is independent of their photosensitizing properties[19-21].

This study compares two-resveratrol and sapphyrin PCI-2050-independent gene expression reports. we compared-cell death pathway, cell cycle arrest, growth inhibition and so on-with both gene expression of sapphyrin (PCI-2050) and resveratrol that were treated on A549 and these investigated that resistance to any mechanism to A549 cell.

The resveratrol is showed that arrested A549 cell were found in the G1 phase of the cell cycle but sapphyrin PCI-2050 were found G2 phase of the cell

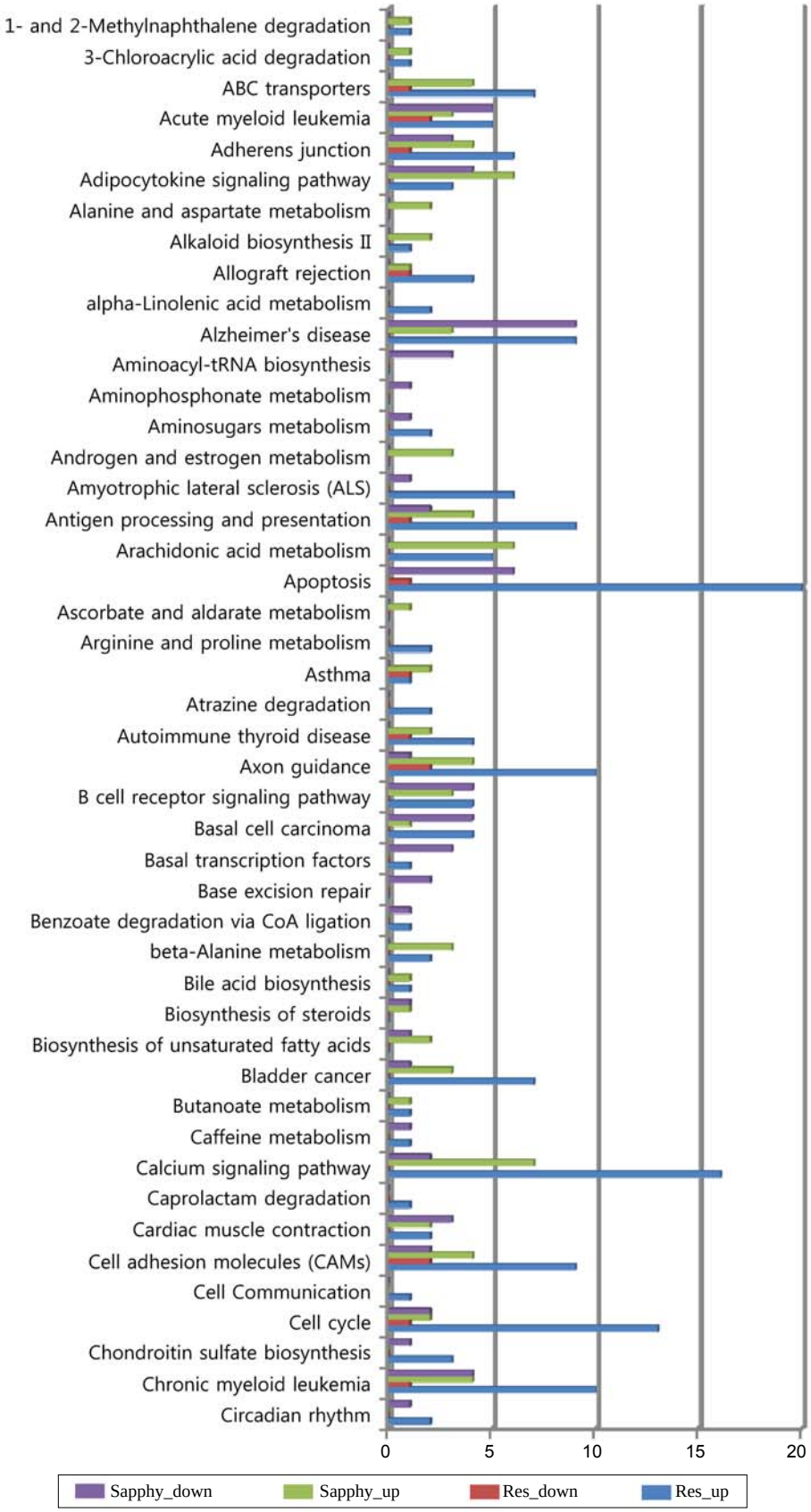

Figure 1. Gene Ontology classifications of genes based on comparison of gene expression between experimental resveratrol-treated and sapphyrin-treated and control (non-treated) in A549 lung cancer cells.

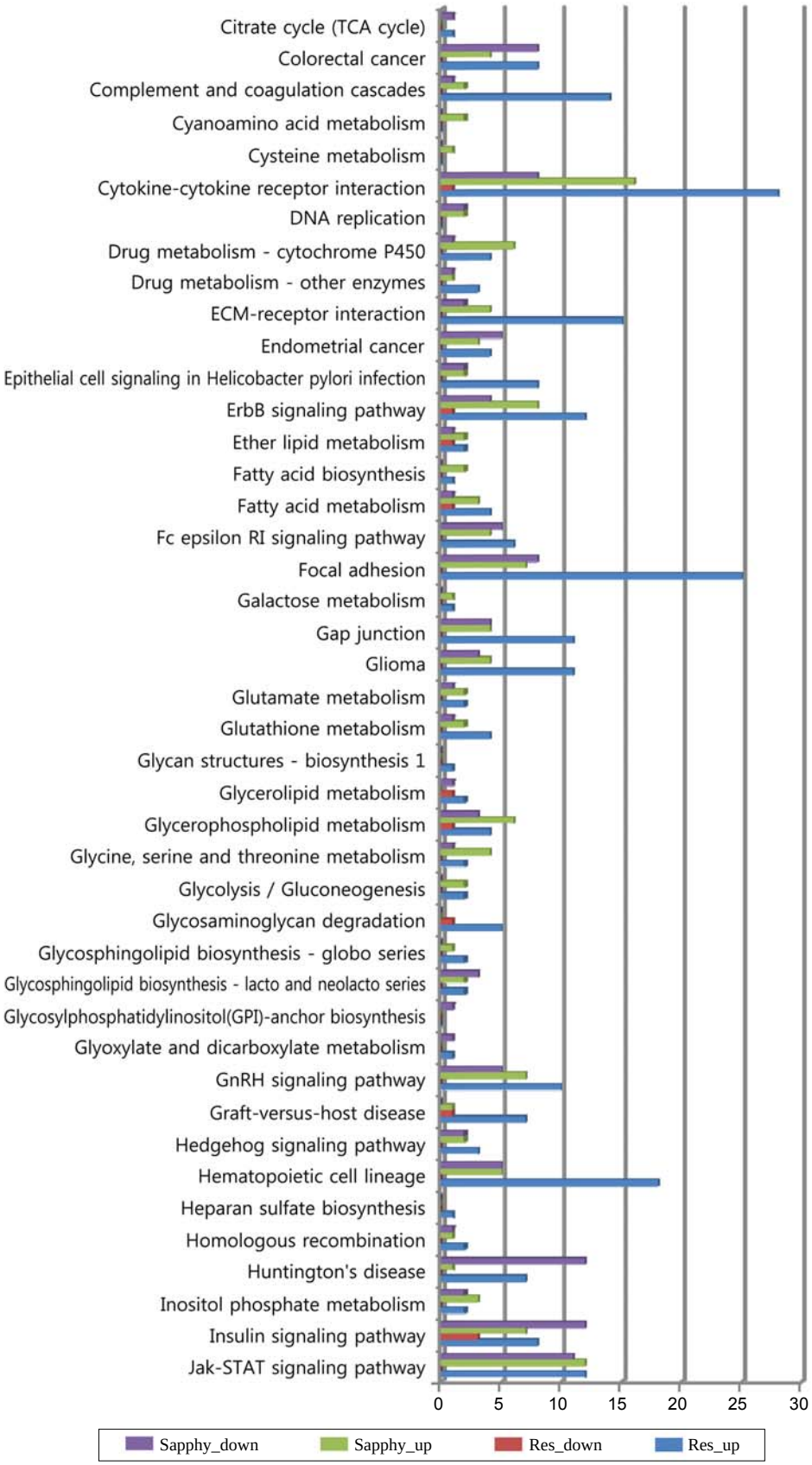

Figure 1. Continued.

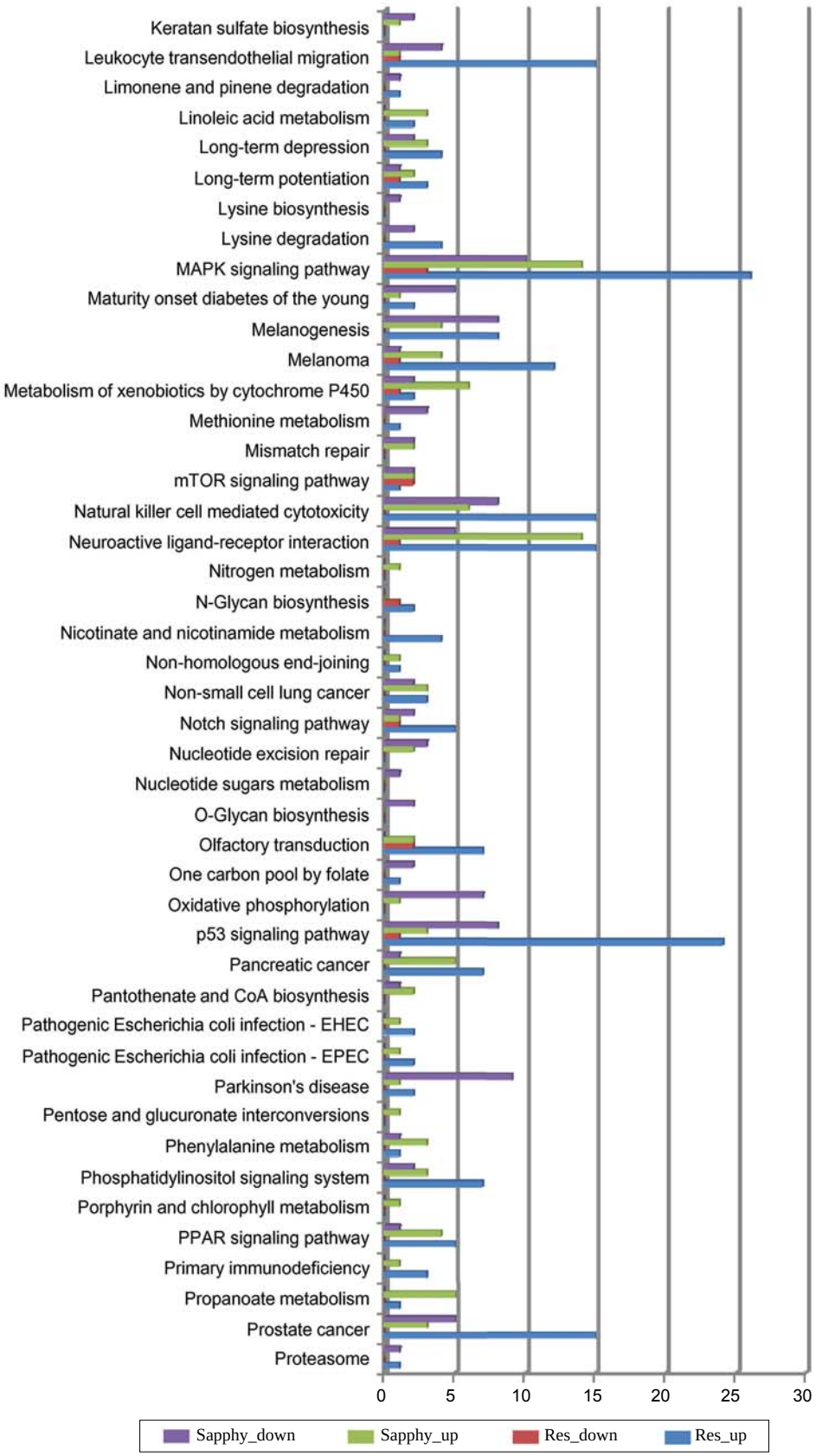

Figure 1. Continued.

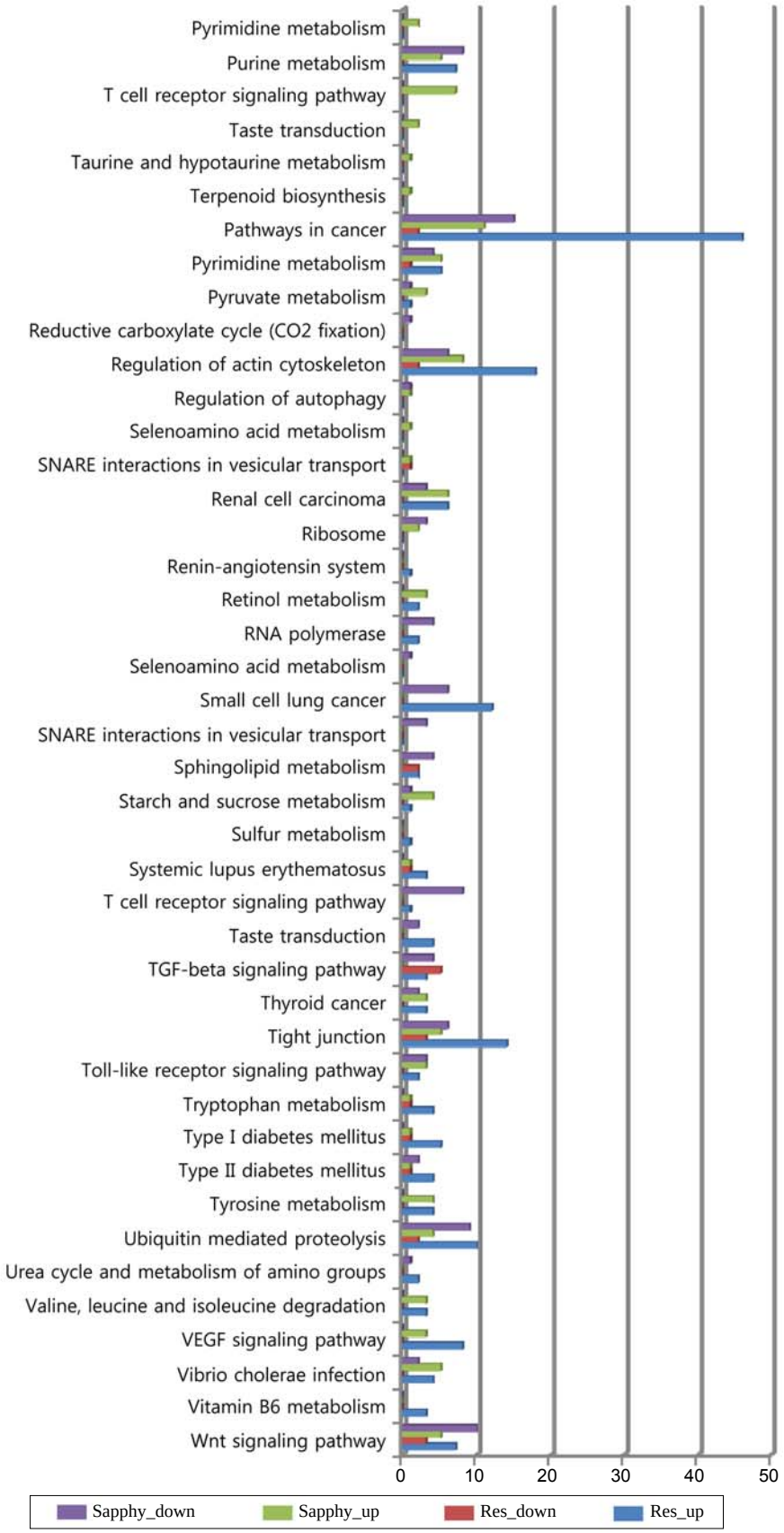

Figure 1. Continued.

Table 1. List of significantly Up-regulated genes based on comparison between experimental (sapphyrin-treated for 4 h) and control (non-treated) in A549 lung cancer cells.

Probe ID	Fold change	Gene_symbol	Gene_title
Sapphyrin vs control			
MAPK pathway			
243334_at	2.6	CACNA1D	calcium channel, voltage-dependent, L type, alpha 1D subunit
214793_at	6.0	DUSP7	dual specificity phosphatase 7
206987_x_at	8.7	FGF18	fibroblast growth factor 18
231382_at	3.8	FGF18	Fibroblast growth factor 18, mRNA (cDNA clone MGC:10529 IMAGE:3948893)
207574_s_at	1.9	GADD45B	growth arrest and DNA-damage-inducible, beta
201466_s_at	6.2	JUN	jun oncogene
1559203_s_at	9.2	KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
1559204_x_at	2.6	KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
216765_at	3.4	MAP2K5	CDNA: FLJ21113 fis, clone CAS05470, highly similar to HSU71087 Human MAP kinase kinase MEK5b mRNA
206296_x_at	3.9	MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1
243476_at	2.8	NF1	neurofibromin 1
214369_s_at	2.1	RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)
216310_at	3.7	TAOK1	TAO kinase 1
Cell cycle			
232764_at	5.7	CCNB2	Cyclin B2, mRNA (cDNA clone MGC:132772 IMAGE:8144115)
207574_s_at	1.9	GADD45B	growth arrest and DNA-damage-inducible, beta
p53 pathway			
232764_at	5.7	CCNB2	Cyclin B2, mRNA (cDNA clone MGC:132772 IMAGE:8144115)
207574_s_at	1.9	GADD45B	growth arrest and DNA-damage-inducible, beta
221640_s_at	2.9	LRDD	leucine-rich repeats and death domain containing

cycle. Resveratrol were found up-regulation of Fas of MAPK and sapphyrin PCI-2050 were showed up-regulation of c-jun of MAPK.

We believe this study and further comparative reports will help elucidate the mechanisms by which anti-cancer agent kill cancer cells and facilitate the design of more effective anti-cancer agents.

Results and Discussion

Transcriptome Changes in Response to Resveratrol and Sapphyrin

Gene-expression profiling of A549 lung cancer cells exposed to 25 μM resveratrol and 2.5 μM sapphyrin PCI-2050 for 48 h and 4 h, respectively, were performed by GeneChip® oligonucleotide expression arrays. Of the 22,283 probe sets analyzed and identified for resveratrol exposure of 48 h[22]. To identify genes responsive to sapphyrin treatment in A549 lung cancer cells, they carried out global-scale DNA microarray analysis of cells cultured at 4 h after sapphyrin treatment[21]. These results indicate that the number of expressed probe sets was approximately constant among the different samples. Complete lists of probe sets from all samples are available on the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE9008 & http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6400).

Functional Classifications Analysis

A comparative global transcription analysis between treatment with resveratrol and sapphyrin that induces MAPK pathway in A549 lung cancer cells revealed that functional classifications of the responding genes are provided in Figure 1. Functional classes are taken from Gene Ontology category in GenPlex v3.0 software.

Metabolic Pathway Analysis

The pathways from the Kyoto Encyclopaedia of Genes and Genomes (KEGG)[23] were downloaded and imported in to the GenPlex v3.0 software and visually inspected for changes based on a 1,726 genes from the t-test analysis. These metabolic pathways were compiled into tables to organize the data based on metabolic pathways. Striking features were revealed by inspection. First, the p53 dependent pathway genes were compiled and organized in to Table 1. All genes were significantly upregulated and downregulated.

Table 2. List of significantly Up-regulated genes based on comparison between experimental (resveratrol-treated for 48 h) and control (non-treated) in A549 lung cancer cells.

Probe ID	Fold change	Gene_symbol	Gene_title
Resveratrol vs control			
MAPK pathway			
244256_at	5.9	CACNA1E	Voltage-operated calcium channel, alpha-1 subunit
203367_at	2.1	DUSP14	dual specificity phosphatase 14
209457_at	3.2	DUSP5	dual specificity phosphatase 5
214793_at	16.2	DUSP7	dual specificity phosphatase 7
210984_x_at	4.6	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
216252_x_at	5.7	FAS	Fas (TNF receptor superfamily, member 6)
215719_x_at	4.9	FAS	Fas (TNF receptor superfamily, member 6)
204781_s_at	3.6	FAS	Fas (TNF receptor superfamily, member 6)
204780_s_at	3.0	FAS	Fas (TNF receptor superfamily, member 6)
204421_s_at	2.0	FGF2	fibroblast growth factor 2 (basic)
208417_at	2.4	FGF6	fibroblast growth factor 6
213418_at	4.2	HSPA6	heat shock 70 kDa protein 6 (HSP70B′)
210118_s_at	4.0	IL1A	interleukin 1, alpha
60528_at	2.1	JMJD7 /// JMJD7-PLA2G4B /// PLA2G4B	jumonji domain containing 7 /// JMJD7-PLA2G4B readthrough transcript /// phospholipase A2, group IVB (cytosolic)
203514_at	2.3	MAP3K3	mitogen-activated protein kinase kinase kinase 3
222548_s_at	2.6	MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4
207535_s_at	2.6	NFKB2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
216867_s_at	2.2	PDGFA	platelet-derived growth factor alpha polypeptide
216061_x_at	2.0	PDGFB	platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)
1554828_at	3.6	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide
220423_at	3.1	PLA2G2D	phospholipase A2, group IID
216234_s_at	6.6	PRKACA	protein kinase, cAMP-dependent, catalytic, alpha
209685_s_at	2.7	PRKCB	protein kinase C, beta
204852_s_at	6.2	PTPN7	protein tyrosine phosphatase, non-receptor type 7
214367_at	5.1	RASGRP2	F25B3.3 kinase like protein from C.elegans
240862_at	2.9	RASGRP4	RAS guanyl releasing protein 4
Cell cycle			
1554631_at	2.0	ATM	ataxia telangiectasia mutated
213523_at	2.6	CCNE1	cyclin E1
211814_s_at	5.4	CCNE2	cyclin E2
205034_at	5.1	CCNE2	cyclin E2
202284_s_at	3.9	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
214557_at	5.0	PTTG2	pituitary tumor-transforming 2
209260_at	4.6	SFN	stratifin
33323_r_at	3.7	SFN	stratifin
33322_i_at	3.0	SFN	stratifin
p53 pathway			
1554631_at	2.0	ATM	ataxia telangiectasia mutated
208478_s_at	2.3	BAX	BCL2-associated X protein
211833_s_at	2.1	BAX	BCL2-associated X protein
213523_at	2.6	CCNE1	cyclin E1
211814_s_at	5.4	CCNE2	cyclin E2
205034_at	5.1	CCNE2	cyclin E2
202284_s_at	3.9	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
216252_x_at	5.7	FAS	Fas (TNF receptor superfamily, member 6)
215719_x_at	4.9	FAS	Fas (TNF receptor superfamily, member 6)
204781_s_at	3.6	FAS	Fas (TNF receptor superfamily, member 6)
204780_s_at	3.0	FAS	Fas (TNF receptor superfamily, member 6)
202628_s_at	4.8	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1

Table 2. Continued.

Probe ID	Fold change	Gene_symbol	Gene_title
1568765_at	4.1	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
202627_s_at	4.0	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
218346_s_at	2.9	SESN1	sestrin 1
209260_at	4.6	SFN	stratifin
33323_r_at	3.7	SFN	stratifin
33322_i_at	3.0	SFN	stratifin
210405_x_at	2.2	TNFRSF10B	tumor necrosis factor receptor superfamily, member 10b
210609_s_at	2.7	TP53I3	tumor protein p53 inducible protein 3

Second, the cell cycle-related genes were organized in to Table 2. Third, MAPK-related genes were compiled and organized in to supplementary Table 1.

Although many studies have focused on anticarcinogenic properties of resveratrol, molecular mechanisms by which they selectively induce apoptosis are incompletely characterized. In mouse and rat experiments, anti-cancer, anti-inflammatory, blood-sugar-lowering and other beneficial cardiovascular effects of resveratrol have been reported. Most of these results have yet to be replicated in humans. In the only positive human trial, extremely high doses of resveratrol in a proprietary formulation have been necessary to significantly lower blood sugar[24].

As expected, sapphyrin are pentapyrrolic metal-free expanded porphyrins with potential medical use as anticancer agents. Sapphyrin has been proposed and shown to elicit similar responses to resveratrol through calcium signaling pathway, cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction, and MAPK signaling pathway which were presumed to account for the major anticancer (Figure 1). In Table 1, resveratrol significantly induced the BCL2-associated X protein (*BAX*), cyclin E1 and E2 (*CCNE*1 and *CCNE*2), Fas (tumour necrosis factor (TNF) receptor superfamily member 6 (*FAS*), serpin peptidase inhibitor, clade E (*SERPINE*1) and 14-3-3 protein sigma, stratifin (*SFN*)[25] were all upregulated. Cyclin E2 gene, *CCNE*2 was strongly increased (5.4-fold). TNF receptor superfamily member 6 (*FAS*) was induced (5.7-fold). We examined the role of influence of p53 status during apoptosis induced by resveratrol in A549 lung cancer cells. Resveratrol inhibited cell growth and promoted apoptosis. Increased apoptosis after treatment uniformly stimulated p53 and Bax expression in A549 lung cancer cells. We observed FAS activation, suggesting that these compounds activate both the mitochondrial and death receptor pathways working together to induce apoptosis.

Fibroblast growth factor (*FGF*) signals play fundamental roles in development and tumorigenesis. Thyroid cancer is an example of a tumor with nonoverlapping genetic mutations that up-regulate mitogen-activated protein kinase (MAPK). Here, we show that FGF receptors (FGF18, 8.7-fold in sapphyrin treatment and FGF2, 2.0-fold; FGF6, 2.4-fold in resveratrol treatment), which are expressed mainly in A549 lung cancer cells, propagates MAPK activation and promotes tumor progression. These data unmask an epigenetically controlled FGFR signal that imposes precisely on the intragenically modified BRAF/MAPK pathway to modulate A549 lung cancer cells. Interestingly, our data showed that genes associated with the cell cycle, cytokine-cytokine receptor interaction, Jak-STAT signaling pathway, MAPK signaling pathway, and p53 signaling pathway were highly upregulated in resveratrol-treated than sapphyrin-treated A549 lung cancer cells (Figure 1, Table 1 and 2). The genes encoding enzymes responsible for the KRAS and leucine-rich repeat and death domain containing (LRDD), growth arrest and DNA-damage-inducible, beta (GADD45B), and RAS guanyl releasing protein 2 (RASGRP2) exhibited the highly upregulated genes after sapphyrin-exposure than resveratrol-treated.

Conclusions

Taken together, these results indicate that resveratrol may have potential efficacy for the treatment of anticancer and apoptosis. However, it may possible for proliferation and differentiation by activating FGF signaling and MAPK pathway. A comparative global transcription analysis between treatment with resveratrol and sapphyrin that induces apoptosis in A549 lung cancer cells revealed that resveratrol resistance Sapphyrin may estimate to inhibit cancer cell by only certain MAPK (JNK) pathway. Elucidation of the mechanisms by which resveratrol conducts its anticancer activities through comparative analysis of the gene expression is necessary to provide a solid foundation for its use as an agent in prevention and treat-

ment strategies.

Materials and Methods

Data Analysis

The MAS5 algorithm was used to evaluate the expression signals generated by the Affymetrix Human Genome U133 Plus 2.0 Array. Global scaling normalization was then performed and the normalized data were log-transformed with base 2. Next, fold change was applied to select the differentially expressed genes (DEGs) using a fold change threshold of 2.0-fold to indicate significance. Each probe set used in the Affymetrix GeneChip produces a detection call, with P (present call) indicating good quality, M (marginal call) indicating intermediate quality and A (absent call) indicating relatively low reliability. Therefore, probe sets that resulted in A calls in the compared groups were removed to filter false positives. The 2.0-fold DEGs were clustered using the GenPlex™ v3.0 software (ISTECH Inc., Korea) using hierarchical clustering with Pearson correlation as a similarity measure and complete linkage as the linkage method. In addition, gene ontology significance analysis was conducted to investigate the functional relationships among the 2.0-fold DEGs using high-throughput GoMiner. The 2.0-fold DEGs were then mapped to relevant pathways using GenPlex™ v3.0 software (ISTECH Inc., Korea). The pathway resources were provided by the KEGG database.

Data analysis was also performed with the GeneSpring GX v. X (Agilent Technologies). The following parameters were employed for GCOS expression analysis: $\alpha_1=0.04$, $\alpha_2=0.06$, and $\tau=0.015$; target signal was scaled to 150. "Fold-change" was calculated as the ratio between the signal averages of four untreated and four treated cultures. Genes with a 2.0-fold or more induction or repression were used in this analysis.

Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No. 2009-0063466).

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