REVIEW

Toxicogenomics of A375 human malignant melanoma cells

Sun-Long Cheng[1], Rosa Huang-Liu[2], Jin-Nan Sheu[3], Shui-Tein Chen[4,5], Supachok Sinchaikul[4] & Gregory J Tsay[6†]

[†]Author for correspondence
[1]Chung Shan Medical University, Department of Plastic Surgery, Chung Shan Medical University Hospital, Taichung, 40242, Taiwan
[2]Chung Shan Medical University, School of Nutrition, Taichung, 40242, Taiwan
[3]Chung Shan Medical University, Institute of Medicine, Taichung, 40242, Taiwan
[4]Academia Sinica, Institute of Biological Chemistry and Genomics Research Center, Taipei, 11529, Taiwan
[5]National Taiwan University, Institute of Biochemical Sciences, College of Life Science, Taipei, 10617, Taiwan
[6]Chung Shan Medical University, Institute of Immunology, 110 Sec. 1, Chien-Kuo N. Road, Taichung, 40242, Taiwan
Tel.: +886 424 738 170; Fax: +886 423 248 172; E-mail: gjt@csmu.edu.tw

Toxicogenomics applications are increasingly applied to the evaluation of preclinical drug safety, and to explain toxicities associated with compounds at the mechanism level. In this review, we aim to describe the application of toxicogenomics tools for studying the genotoxic effect of active compounds on the gene-expression profile of A375 human malignant melanoma cells, through the other molecular functions of target genes, regulatory pathways and mechanisms of malignant melanomas. It also includes the current systems biology approaches, which are very useful for analyzing the biological system and understanding the entire mechanisms of malignant melanomas. We believe that this review would be very potent and useful for studying the toxicogenomics of A375 melanoma cells, and for further diagnostic and therapeutic applications.

Keywords: A375 cells, gene expression, genotoxic effect, microarray, toxicogenomics

Malignant melanoma is a serious type of skin cancer that begins in the skin's pigmentation system. It usually derives from the transformation and proliferation of melanocytes that normally reside in the basal cell layer of the epidermis and also develops when the melanocytes no longer respond to normal control mechanisms of cellular growth. They may then invade nearby structures or spread to other organs in the body (metastasis), where again they invade and compromise the function of that organ. To enable a better understanding of the molecular and cellular mechanisms involved in the progression of cutaneous melanoma, human malignant melanoma cells have been widely used as the melanoma skin model for *in vitro* testing. This is because it is a highly reproducible, quantifiable and easily handled cell culture, a structural cell model closely paralleled to the progression of melanoma *in vivo*, and a cost-effective alternative to animal and clinical testing. The anti-apoptotic mechanisms regulating cell death have been implicated in conferring drug resistance to tumor cells [1,2]; therefore, further knowledge on the nature of this resistance and a better understanding of signal transduction leading to tumor cell death could allow the identification of new target molecules to overcome drug resistance and improve the melanoma therapy.

In recent years, toxicogenomics has represented the merging of toxicology with genomics and bioinformatics, and there have been significant practical challenges in pharmacology for both predictive and mechanism-based toxicology in an effort to identify candidate drugs and toxic agents more quickly and economically [3–5]. Owing to the current development of experimental approaches, this field has begun systematic approaches to dissect the biological basis and genotoxic mechanisms of melanoma. The purpose of this review is to describe the toxicogenomics of A375 human malignant melanoma cells, including genotoxic effect of drugs or compounds on gene-expression profile in A375 cells, molecular functions of targeted genes, signaling pathways and regulatory mechanisms of tumorigenesis or related side effects, and a better understanding of the entire biological system of malignancy of melanocytic tumorigenesis through the application of systems biology approaches. Owing to the availability of more data based on A375 as a preliminary model of human skin cells and the original derivation of these cells from skin cancer cells, this review will also discuss the toxicogenomics of the A375 cell line and compared it with other melanoma cells. We believe that this review may provide a very useful guideline and introduce some potent approaches for genome studies of malignant melanomas and for further pharmaceutical applications in cancer therapy.

General pharmacological characterization of malignant melanoma

Characterization of malignant melanomas

Malignant melanomas, characterized by their high capacity for invasion and metastasis, are one of the most frequent forms of skin cancer. Primary cutaneous melanomas have been divided into four groups based on histopathology: superficial spreading melanoma, nodular melanoma, lentigo

maligna melanoma and acral lentiginious melanoma [6]. Superficial spreading melanoma is the most common type of melanoma and grows outwards at first to form an irregular pattern on the skin with an uneven color. It tends to start by spreading out across the surface of the skin, known as the radial growth phase. Nodular melanoma occurs most often on the chest or back. It tends to grow deeper into the skin – quite deeply if it is not removed. This type of melanoma is often raised above the rest of the skin surface and feels like a bump. It may be very dark brown–black or black. Lentigo maligna melanoma is most commonly found on the faces of elderly people. It grows slowly and may take several years to develop. Acral lentiginious melanoma is usually found on the palms of the hands, soles of the feet or around the toenails. Although melanoma is almost always curable in its early stage, it may be late if the melanomas spread to other parts of the body. The early diagnosis of melanoma is therefore very important and necessary to examine carefully for further melanoma therapy.

Effects on melanogenesis & tumor progression of melanomas

Many of the general risk factors for developing melanoma include sun exposure, fair skin that burns easily, blistering sunburn, previous melanoma, previous nonmelanoma skin cancer (basal cell carcinoma, squamous cell carcinoma), family history of melanoma, large numbers of moles and abnormal moles. In melanoma progression, different proteolytic enzyme systems, including the plasminogen-activator system and matrix metalloproteinases, play an important role in the degradation and remodeling of the extracellular matrix and basement membranes [7]. Tyrosinase is the main enzyme regulating melanogenesis, which catalyzes three distinct reactions in the melanogenic pathway: hydroxylation of monophenol (L-tyrosine), dehydrogenation of catechol (L-DOPA) and dehydrogenation of dihydroxyindole. By contrast, catalase is the proteolytic enzyme that regulates H_2O_2 removal, in which H_2O_2 is a potent inhibitor of tyrosinase. Peroxidase also serves to enhance eumelanin polymer formation from monomers in the presence of hydrogen peroxide and metal ions, especially copper ions, which have the greatest enhancing effect on the conversion of monomers to polymers [8]. Thus, changes of enzyme expression level, including modifications in protein- and gene-expression levels, influence melanogenesis in melanomas. Otherwise, the complex regulatory control of the biosynthesis machinery involved in melanogenesis includes receptor-mediated pathways activated by hormones, neurotransmitters, cytokines, growth factors and eicosanoids as well as receptor-independent mechanisms activated or modified by nutrients, micromolecules, microelements, pH, cation and anion concentrations, and the oxidoreductive potential in the physicochemical milieu. Soluble factors can reach their target (melanocyte) from circulation, from release by nerve endings or from local production to act as positive or neative melanogenesis regulators.

Although it is well known that malignant melanoma is a skin cancer and can arise in the skin anywhere on the body, melanoma can be completely cured in the early stage by operation, which is dependent on the vigorous spreading of tumors. Thus, the key to successful treatment is early diagnosis coupled with the right treatments, such as chemotherapy. There are many active compounds that have an effect on the cell cytotoxicity of malignant melanoma cells and may be used as potent drugs for further therapy (Table 1). Although chemotherapy drugs can kill cancer cells, they may damage some normal cells and cause side effects that include effects on other cellular and molecular mechanisms in the melanoma system. These side effects depend on the type of drugs used, the amount taken and the length of treatment. Thus, the use of drugs in chemotherapy should be carefully considered for the stage of tumor and its spreading.

Genomic analysis of gene-expression profiles in A375 cells

Gene-expression profile analysis by microarray

The high-throughput technology of DNA microarray is a powerful tool uniquely selected for genomic research by allowing the study of the function of thousands of genes simultaneously, showing differential gene-expression profiles, and elucidating the exact mechanisms and defects in genetic aberrations. Recently, many microarrays have been used to analyze gene-expression profiles of human melanomas (Table 2). However, only a few microarray analyses of gene-expression profile in A375 cells have been reported. For example, the metastasis of A375 cells has been studied in nude mice by microarray analysis [9]. When the pulmonary metastases of nude mice that were injected intravenously with amelanotic human A375 tumor cells were analyzed by oligonucleotide microarrays (human: 7070 genes;

Table 1. Effect of active compounds on cell cytotoxicity of A375 human malignant melanoma cells and other melanoma cells.

Compound	Description	Action on melanoma cells	Ref.
Apomine	Biphosphonate ester	Induces cell death in A375 cells through a novel membrane-mediated mechanism that is independent of caspase-3 activation	[51]
(Bu2Sn)2TPPS; (Bu3Sn)4TPPS	Diorganotin (i.v) and triorganotin (i.v)-meso-tetra (4-sulfona tophenyl) porphine derivatives	Induces cell death in A375 cells by activating caspase-8 and caspase-9 leading to caspase-3 activation	[52]
		Induces phototoxicity in G361 human melanoma cells	[53]
Camptothecin	Quinoline-based alkaloid found in the barks of the Chinese *Camptotheca* tree and the Asian *Nothapodytes* tree	Induces apoptosis in A375 cells	[54]
		Inhibits proliferation of BRO malignant melanoma cells *in vitro*, induces apoptosis by traversing S–G_2 phase, results in dramatic morphological cellular changes	[55]
		Inhibits migration of B16F10 melanoma cells, inhibits adhesion of B16F10 to fibronectin and laminin, exhibits the antiangiogenesis effect in the chicken chorioallantoic membrane model	[56]
Dimethylfumarate	Fumaric acid ester	Inhibits proliferation of A375 melanoma cells, arrests the cell cycle at the G_2–M boundary, acts as proapoptotic, reduces proliferation rates of tumor cells	[57]
Endothelin-1	Peptide	Induces apoptosis of A375 melanoma cells by inhibiting serum-dependent growth of asynchronized A375 cells	[58]
		Promotes invasive behavior via hypoxia-inducible factor-1α in human melanoma cell lines 1007 and M10 derived from metastatic lesions	[59]
		Inhibits B-16 murine melanoma cell migration by decreasing K^+ currents	[60]
Epicatechin, 4β-(*S*-cysteinyl)catechin 3-*O*-gallate (Cys-ECG); epigallocatechin-3-gallate	Catechin derivatives or antioxidant cysteinyl–flavanol conjugates	Reduces cell viability in A375 and Hs-294T melanoma cells, induces cell-cycle arrest and apoptosis of melanoma cells mediated via modulations in the cki–cyclin–cdk network and Bcl2 family proteins	[61]
		Reduces cell proliferation of human melanoma cell lines (A375 amelanotic malignant melanoma and Hs-294T metastatic melanoma), induces apoptosis of melanoma cells, inhibits the colony-forming ability of melanoma cells	[62]
		Inhibits the cell adhesion of B16 murine melanoma cells	[63]
Erianin	2-methoxy-5-[2-(3,4,5-trimethoxy-phenyl)-ethyl]-phenol	Causes moderate growth delay in xenografted human melanoma cell line A375	[64]
Evodiamine	Quinozole alkaloid constituent of *Evodia rutaecarpa*	Induces A375-S2 cell death dose- and time-dependently	[65]
Ginsenoside Rh_2	Plant glycoside (natural product from *Panax ginseng*)	Suppresses the growth of A375-S2 cells *in vitro* by inducing apoptosis	[66]
		Induces G_1 arrest and concomitantly suppresses the Cdk2 activity in B16 murine melanoma cells and carcinogen-susceptible BALB/c 3T3 A31-1-1 and A31-1-13 cell lines	[67]
Heparin	Anticoagulant	Inhibits metastasis by blocking the P-selectin-based interaction of platelets with A375 cells	[68]
		Inhibits lung colony formation and liver metastasis development in SCID mice treated with HT168-M1 melanoma cells	[69]
		Inhibits melanoma metastasis of B16F1 melanoma cells	[70]

iv.: Intravenous; SCID: Severe combined immunodeficiency; TRAIL: TNF-related apoptosis-inducing ligand.

Table 1. Effect of active compounds on cell cytotoxicity of A375 human malignant melanoma cells and other melanoma cells. (cont.)

Compound	Description	Action on melanoma cells	Ref.
Resveratrol	Polyphenol	Inhibits cell growth and induces apoptosis in A375 and SK-Mel28 cells, induces phosphorylation of ERK1/2 in A375 but not in SK-Mel 28 cells	[71]
		Induces G_1 arrest in melanoma cells (SK-Mel and Colo38) that are associated with downregulation of surviving expression and sensitization for TRAIL-induced apoptosis	[72]
		Inhibits cell proliferation of autologous human melanoma Line IV clone 1 and clone 3 cells, which were originally established from a primary malignant melanoma lesion	[73]
Sodium ascorbate	Vitamin C	Induces cell-cycle arrest at G_1/S phase and apoptosis in the A375.S2 cell line in a dose-dependent manner Induction of apoptosis involved an increase in the levels of p53, p21 and cellular Ca, and a decrease in mitochondrial membrane potential and activation of caspase 3, before culminating in apoptosis in sodium ascorbate-treated A375.S2 cells	[74]
		Induces apoptosis in B16 murine melanoma cells via the downregulation of transferin-receptor dependent iron uptake (appear to be initiated by a reduction of total fertility rate expression, resulting in a downregulation of iron uptake followed by an induction of apoptosis) and a caspase-8-independent pathway	[75,76]

iv.: Intravenous; SCID: Severe combined immunodeficiency; TRAIL: TNF-related apoptosis-inducing ligand.

mouse: 6347 genes, with approximately 50% overlap in the genes represented), a pattern of gene expression correlating with progression to a metastatic phenotype was observed, and 15 out of 16 genes continued to show enhanced expression when metastatic A375 cells were grown as a subcutaneous tumor, indicating that the expression of these genes is intrinsic to the metastatic cells. Otherwise, the enhanced expression of several genes, such as RhoC, fibronectin, two collagen subunits, α2(I) and α1(III), matrix Gla protein, fibromodulin, biglycan and thymosin-β4, involved in extracellular matrix assembly and of a second set of genes that regulate the actin-based cytoskeleton. Otherwise, the microarray has been used to assess the global expression profiles in A375 cells exposed to β-carotene and the overall numbers of differentially expressed genes were 140 upregulated genes and 570 downregulated genes [10]. The effect of β-carotene suppressed vital cellular functions that are correlated with the expression of the proapoptotic BCL2-associated X protein (BAX), which was upregulated in A375 cells. However, the results of microarray and real-time PCR indicated that β-carotene-induced expression changes of *BAX* and other *BCL2* pathway genes did not lead to the predicted induction of apoptosis in the A375 cells.

On the other hand, we have used DNA microarrays to investigate the genotoxic effects of skin-whitening agents, such as kojic acid and arbutin, on the differential gene-expression profile in A375 cells through its effect on melanocytic tumorigenesis for cancer therapy and other related side effects (Figure 1). The overall numbers of up- and down-regulated genes were 136 out of 225 and 88 out of 236 for kojic acid-treated A375 cells and arbutin-treated A375 cells, respectively [11,12]. The gene ontology (GO) of differentially expressed genes provided more understanding of the genetic basis of metabolic and cellular responses in tumorigenesis of human skin cancer. Interestingly, seven downregulated genes in kojic acid-treated A375 cells (*APOBEC1*, *ARHGEF16*, *CD22*, *FGFR3*, *GALNT1*, *UNC5C* and *ZNF146*) and four downregulated genes in arbutin-treated A375 cells (*AKT1*, *CLECSF7*, *FGFR3* and *LRP6*) served as candidate genes and correlated to suppress the biological processes in the cell cycle of cancer progression. The differentially expressed genes may become useful markers of skin malignant melanoma for further diagnostic and therapeutic applications. Although DNA microarrays have become important in genomic research of melanomas, and are used to rapidly analyze thousands of expressed genes, they are still effective in

Table 2. Comparison of gene-expression profile analysis of melanomas by microarrays .

Study	Samples (cell type, tissue)	Microarray	Discovery (gene-expression profile & significant genes)	Ref.
DeRisi *et al.* (1996)	Human melanoma cell lines, UACC-903 and UACC-903(+6)	cDNA microarray (1161 probes)	870 different cDNAs and controls with 63 up- and 15 down-regulated genes were identified and were found to be correlated with suppression of tumorigenicity by introduction of chromosome 6 Waf-1 (p21), MARCKS, collagenase, MCAF/MCP-1 and α-1-antichymotrypsin are found to be the key mediators of tumor suppression	[77]
Bittner *et al.* (2000)	31 melanomas and seven controls	cDNA microarray (8150 probes)	Genes expressed in common in the highly invasive uveal melanoma cells (*WNT5A*, *MART-1*, pirin, *HADHB*, *CD63*, *EDNRB*, *PGAM1*, *HXB*, *RXRA*, *ESTs*, integrin β1, i, tropomyosin1, *AXL*, *EphA2*, *GAP43*, *PFKL*, synuclein-α, annexin A2, *CD20* and *RAB2*) were strongly anticorrelated with the same genes from the major cluster of cutaneous melanoma samples Specific genes with reduced expression in the major cluster included integrin β1, integrin β3, integrin α1, syndecan 4 and vinculin In A375 cells, there are 276 significant expressed genes that are correlated with the major cluster	[78]
Clark *et al.* (2000)	Human cell lines (A375P, A375M1, A375M2, A375SM) and mouse B16 cells	HUM 6.8K microarray (Affymetrix, 7070 probes)	Several upregulated genes found in A375 cells are *RhoC*, fibronectin, two collagen subunits (α2(I) and α1(III)), matrix Gla protein, fibromodulin, biglycan and thymosin-β4 RhoC is important in tumor-cell invasion and capable of inducing metastasis in A375 cells	[9]
Brem *et al.* (2001)	Metastatic (NMCL-1) and non-metastatic human melanoma cell lines (530)	Affymetrix HuGeneFL array (6800 probes)	Differentially expressed genes are involved in control of transcription, regulation of the cell cycle, proteolysis, cell adhesion, immune response and signaling The significant genes are *MAGE-2*, cystatin, *p21*, *HLA-DR-β1*, *cbl-b*, p65-phosphoprotein and *p27*, which are involved in the metastasis of cancer	[79]
de Wit *et al.* (2002)	Human melanoma cell lines 1F6 and Me157	Affymetrix Hu6800 (7129 probes) and Hu35K (35000 probes) oligonucleotide arrays	Eight differentially expressed genes are apolipoprotein CII, tyrosinase-related protein 1, TGF-β superfamily, subtilisin-like protein, elongation factor 1 α2, α2-macroglobulin, human cell division cycle 10 and serine/threonine protein kinase (*DYRK1A*) These genes are involved in the malignant progression of melanoma	[80]

BAD: BCL2-antagonist of cell death; BAX: BCL2-associated X protein; ERK: Extracellular-regulated kinase; MARCKS: Myristoylated Ala-rich C-kinase substrate; MCAF: Monocyte-chemotactic and -activating factor; MCP: Monocyte-chemotactic protein; THF: Tetrahydrofolate.

Table 2. Comparison of gene-expression profile analysis of melanomas by microarrays (cont.).

Study	Samples (cell type, tissue)	Microarray	Discovery (gene-expression profile & significant genes)	Ref.
Tschentscher *et al.* (2003)	Tumor and peripheral blood samples from uveal melanomas with disomy 3 and monosomy 3	Affymetrix GeneChip system HG-U95Av2 oligonucleotide arrays containing ~12500 probes	The average difference values of 7902 genes were found to be expressed in more than 10% of the tumors The 42 significant expressed genes contained 33 up- and nine down-regulated genes The major upregulated genes were *IL12RB2*, *SPP1*, *PDE4B*, *M8C*, *TIMP3*, *ATIP1*, *KIAA0828*, *PLXN81* and *COXBA2*, whereas the major downregulated genes were *RARRES2*, *47g10cDNA*, *HTR2B* and *WAR8*. Seven genes (*CHL1*, *fls485*, *H1FX*, *PDE3A*, *POB1*, *HCGIV.9* and Clone 24421) showed complete loss of expression in tumors with monosomy 3 but were expressed in tumors with disomy 3	[81]
Hoek *et al.* (2004)	Normal human melanocytes and six melanoma cell lines (MNT1, WW165, YUMAC, YUSIT1, YUHEIK, YUCAL)	Affymetrix GeneChip system HG-U133A microarray (~14,500 probes) and OHU16K human library oligonucleotide array (16,659 probes)	589 significant genes (315 up- and 274 down-regulated genes) in the Affymetrix U133A dataset and 186 significant genes (99 up- and 87 down-regulated genes) in the OHU16K arrays Major upregulated genes were *NOTCH2*, *HEY1/HERS1*, *ITGA (3,4,6,7)*, *IGF1*, *FGF13/FHF2*, Twist and *TBX3*, whereas the major downregulated genes were *MX1/IF178*, *OAS-1*, *UCHL1*, Necdin, *NBL1*, *CIRBP* and *RRAGA* Some genes were identified; for example, *FGF13* as a new autocrine factor, Twist as a clinically valuable prognostic marker for patients with melanomas, and Necdin as a potent melanoma-growth suppressor	[82]
Bodzioch *et al.* (2005)	Human malignant melanoma cell line A375	U133A microarray (Affymetrix, 18,400 probes)	In response to β-carotene, 710 significant genes contained 140 up- and 570 down-regulated genes In response to THF, 589 significant genes contained 350 up- and 239 down-regulated genes BAX, BAD, BAK1, BCLXS, BCLXL, BCL2a and BCL2b are involved in the metastasis of A375 cells in response to β-carotene, in which BAD showed proapoptotic property in A375 cells at all time points and BCXL and BCL2a showed antiapoptotic property at 24 h of treatment	[10]
Haqq *et al.* (2005)	Skin tissues from 25 melanoma patients and nevus volunteers (primary and metastases)	cDNA array (Research Genetics, 20,862 probes)	Metastatic melanoma is characterized by two different gene-expression signatures with common features compared with the signatures of radial and vertical growth phases of primary melanoma, and the microarray result showed 672 positive and 114 negative significant genes. The major upregulated genes were *SPP1*, *CXCL1* and *RAB32*, whereas the major downregulated genes were *WIF1*, *ECM2* and *SLIT3* Other genes, such as *CDH3*, *MMP10*, *PM09* and *MM14* were also involved in pathogenesis of metastatic melanoma	[83]

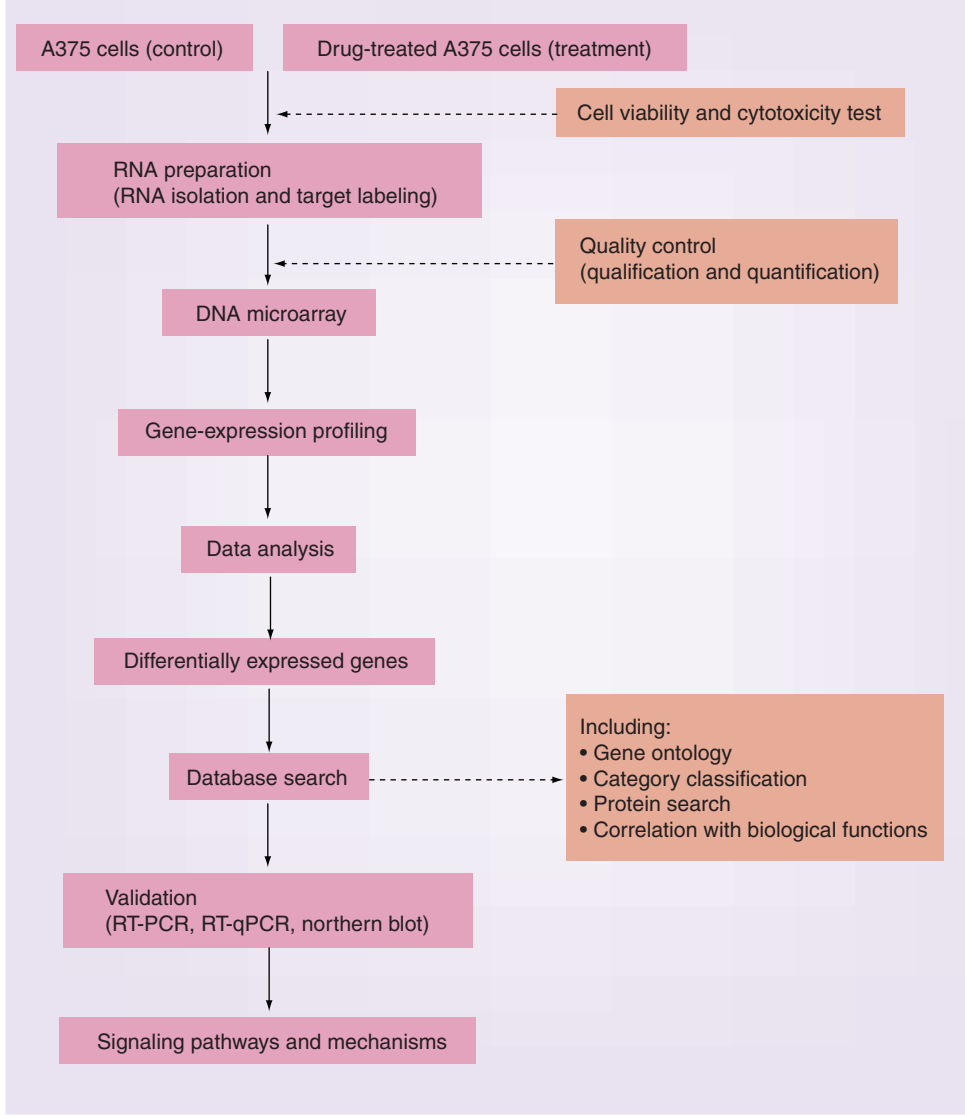
BAD: BCL2-antagonist of cell death; BAX: BCL2-associated X protein; ERK: Extracellular-regulated kinase; MARCKS: Myristoylated Ala-rich C-kinase substrate; MCAF: Monocyte-chemotactic and -activating factor; MCP: Monocyte-chemotactic protein; THF: Tetrahydrofolate.

Table 2. Comparison of gene-expression profile analysis of melanomas by microarrays (cont.).

Study	Samples (cell type, tissue)	Microarray	Discovery (gene-expression profile & significant genes)	Ref.
Nambiar *et al.* (2005)	Human melanoma cell lines (BLM, M13, MV3, SK-Mel-28) and cutaneous melanoma metastases	Atlas human apoptosis cDNA array and Atlas human cancer cDNA array (Clontech, 1.2K)	The MAPK/ERK pathway is activated in most of the cutaneous melanoma metastasis specimens Given the subsets of cutaneous melanoma metastases with phosphorylated Grb10 and transcriptional upregulation of c-met, B-RAF, Raf-1, MAPKK6, MAPKK7 and MEK2 and downregulation of IGF-I receptor, IGF-I and IGF-II, Grb10 may have a critical regulatory function in melanoma metastasis in promoting IGF-I receptor-independent Raf/MAPK signaling to elicit cell-cycle progression, proliferation and metastasis	[84]
Cheng *et al.* (2006)	Human malignant melanoma cell line A375 (kojic acid treatment)	Agilent human 1A oligonucleotide microarray (20,173 probes)	A total of 361 differentially expressed genes were distinctly changed with 136 up- and 225 downregulated genes Seven downregulated genes (*APOBEC1*, *ARHGEF16*, *CD22*, *FGFR3*, *GALNT1*, *UNC5C* and *ZNF16*) were shown to be the tumor suppressor genes in melanoma cancer cells	[11]
Cheng *et al.* (2007)	Human malignant melanoma cell line A375 (arbutin treatment)	Agilent human 1A oligonucleotide microarray (20,173 probes)	A total of 324 differentially expressed genes were distinctly changed with 88 up- and 236 down-regulated genes Four downregulated genes *(AKT1*, *CLECSF7*, *FGFR3* and *LRP6*) were shown to be the tumor suppressor genes in melanoma cancer cells	[12]
Jaeger *et al.* (2007)	19 primary melanomas and 22 cutaneous melanoma metastases from 41 different patients after surgical excision of tumors	Human Genome U133A microarray (Affymetrix, 22,215 probes)	In total, 308 differentially expressed genes were identified with 261 up- and 47 downregulated genes The major upregulated genes were *Cdc6*, *CKS2*, *SPT6*, mitosin, *KIF2C* and *STK6* The major downregulated genes were *CDH1*, *FGFBP*, *KRT10*, *DSC1*, *DSC3*, *DSG1*, stratifin/14–3-3σ, chemokine *CCL27*, *TP73L*, *PAK6*, *FGFR2* and *FGFR3*	[85]

BAD: BCL2-antagonist of cell death; BAX: BCL2-associated X protein; ERK: Extracellular-regulated kinase; MARCKS: Myristoylated Ala-rich C-kinase substrate; MCAF: Monocyte-chemotactic and -activating factor; MCP: Monocyte-chemotactic protein; THF: Tetrahydrofolate.

Figure 1. Genomic analysis platform of A375 human malignant melanoma cells exposed to drugs or skin agents.



combination with the bioinformatic tools and quantitative analysis for validating the gene targets, studying the biological functions through the mechanisms and understanding the pathogenesis of malignant melanoma.

Advanced genomic approach & quantitative analysis of differentially expressed genes

Although several studies have evaluated the use of PCR amplification of tyrosinase mRNA to detect melanoma cells in blood [13–15], the application of this technique for patients with solid malignancies is still under debate because of the inconsistent reports on the detection of melanoma cells in peripheral blood by reverse-transcriptase-PCR have resulted in uncertainty on the prognostic value of circulating melanoma cells. Quantitative PCR or quantitative real-time PCR are further-developed techniques that are both more sensitive and faster methods of measuring gene expression. It allows one to not only quantify the level of expression of melanoma markers but also assess sample quality [16]. The development of quantitative PCR has eliminated the variability traditionally associated with quantitative PCR, thus allowing the routine and reliable quantitation of PCR products. Another potential application of quantitative real-time PCR for

melanoma markers is the assessment of marker expression as an inclusion criterion and during follow-up in studies of gene therapy.

RNAi is a process of sequence-specific post-transcriptional gene silencing initiated by dsRNA homologous with the target gene, resulting in null or hypomorphic phenotypes [17]. The selective and robust effect of RNAi on gene expression makes it a valuable research tool, both in cell culture and in living organisms; synthetic dsRNA introduced into cells can induce suppression of specific genes of interest. RNAi may also be used for large-scale screens that systematically shut down each gene expressed in the cell, which can help to identify the components necessary for a particular cellular process or an event such as cell division. This technique was used to inhibit the growth and invasive ability of ten melanoma cell lines, including A375 melanoma cells, by inactivation of mutated v-raf murine sarcoma viral oncogene homolog B1 (BRAF) with lentivirus-mediated RNAi. The results demonstrated that RNAi inhibited the growth of most melanoma cell lines *in vitro* as well as *in vivo*, which was accompanied by a decrease of both RAF protein and ERK phosphorylation [18]. In addition, the results clarified that the mutated BRAF is essentially involved in the malignant phenotype of melanoma cells through Ras–Raf–MEK–ERK (MAPK) activation and is an attractive molecular target for melanoma treatment. Likewise, the tetracyclin-inducible shRNAi method was directly used to test the antitumorigenic effect *in vivo* of BRAF inhibition on melanoma tumor progression in A375 cells [19]. Using an RNAi approach, oncogenic BRAF was shown to be causally involved in tumor growth, and key questions were addressed regarding the contribution of this oncogene to tumor growth, maintenance and resistance to apoptosis. In addition, RNAi technology was successfully used to knock down the expression of CD147 in A375 melanoma cells in an effort to explore the role and mechanisms of CD147 in the progression of malignant melanoma [20]. Interestingly, CD147-targeting siRNA could significantly downregulate the CD147-mRNA level in melanoma cells and then inhibit their proliferation, invasiveness and metastatic activity *in vitro* and *in vivo*. It is indicated that highly expressed CD147 on the surface of melanoma cells plays an important role in the invasiveness and metastasis of malignant melanoma. Moreover, the delivery of siRNA directed against vascular endothelial growth factor (VEGF) has been studied and shown not only to provide efficient and specific downregulation of VEGF expression, inhibit proliferation of A375 cells and induce apoptosis of A375 cells *in vitro*, but also to suppress growth of malignant melanoma *in vivo* [21]. Therefore, RNAi is a very useful and attractive technique for functional genomic studies of melanoma cells and exploitation in gene therapy.

In addition, PCR amplification and RNAi techniques are not only used for validation of melanoma gene targets from microarray data, but other contemporary scientific methods, such as laser-capture microdissection [22], comparative genomic hybridization [23], SNP analysis [24] and chromatin immunoprecipitation [25], can also be combined with microarray to create entirely new fields of interest in the more global quest to better define the molecular basis of malignancy of melanoma.

Systems biology approach for integrating toxicogenomics in A375 cells

Although gene-expression profile using DNA microarrays or other genomic techniques holds great promise for identifying genes that may play an important role in biological systems, this approach has some significant limitations. The most obvious limitation is not directly affected by the mRNA profiles created by DNA microarray but indirectly by the protein products of these mRNAs. Although changes in mRNA levels may be surrogates for changes in the respective protein levels, studies have shown that protein levels often do not correlate with mRNA level and one gene or one mRNA does not necessarily correlate with one protein [26–28]. Another limitation of gene-expression profile is that the functions of proteins are often regulated by post-translational modifications. Many mRNAs are known to be differentially spliced and generate different protein entities. Therefore, the integration of genomics tools with proteomics and bioinformatics tools is very important and potentially useful for analyzing the systematic changes in melanoma cells, leading to a better understanding of biological systems in developmental toxicology.

Proteomic analysis for emerging approaches in toxicogenomics

At present, proteomics tools produce a global view of complex biological systems by examining complex protein mixtures using large-scale and high-throughput technologies. Proteomics

tools have been used to study the protein-expression profiles in malignant melanoma cells, as summarized in Table 3. However, there is only one report that studied the proteomic analysis in A375 cells [29]. Using 2D electrophoresis (2DE) and MS analyses, 24 proteins in A375 cells have been identified. Some chaperones and heat-shock proteins (HSPs), such as HSP90β, protein disulfide isomerase A6, Hsc70-interactering protein with three tetratricopeptide-repeat domains, FK506-binding protein 4, peptidyl-prolyl-cis-transisomerase A and endoplasmic reticulum protein 29, have been identified and play important roles in tumor biology, and still are holding centre stage of tumor progression. Alterations in the protein expressions of chaperones and HSPs that occur following drug treatment or environmental stresses serve as oncogenes or biomarkers that can provide useful information regarding the mechanism of cell protection under harmful conditions through tumor progression or suppression for cancer therapy. Furthermore, α-enolase, cytokeratin, stathmin, tropomyosin, Cu/Zn superoxide dismutase, nucleoside diphosphate kinase A, galaptin and triosephosphate isomerase showed some post-translational modifications and played important roles in the tumorigenesis of melanoma cells [30]. Recently, we used the 2DE to compare the protein-expression profiles between A375-cell controls and A375 cells treated with kojic acid or arbutin, and the 2DE results revealed varying protein patterns among groups. Although it is a preliminary result and the differentially expressed proteins have not yet been identified, the differential protein expressions can provide information regarding the effect of compounds on protein expression in A375 cells, which may be useful for comparative and integrative analysis with the microarray data. Therefore, the proteomic tools used in protein separation, quantification and identification are not only used for studying protein-expression levels, they are expanding rapidly to integrate genome analysis to help understand the multifaceted nature of biological systems.

Bioinformatic tools for investigating GO & mechanism of genotoxicity

Owing to the numerous data in genome and proteome analyses and the lack of strong data interpretation and organization, the bioinformatic tools of genome and proteome studies are necessary to develop many software and database systems to automate these higher level organizational tasks, which are used for analyzing complex samples and organizing the data in a biological context. According to our experiences in microarray data analysis and other related researches in proteomic analysis, we would like to introduce the useful bioinformatic tools that include databases combining genome and proteome with completed analytical information and used for searching the GO and category classification, protein information, pathways and interactions, and related toxicogenomics (Table 4). Of these databases, Bulk Gene Search System for Java (BGSSJ) is one of the most interesting programs established in our laboratory that is able to classify the expressed genes from DNA microarrays. BGSSJ is a searching system accomplished by open-database connectivity, UniGene database and Gene Ontology knowledgebase. We have used the BGSSJ program to search the GO of the gene numbers from kojic acid- or arbutin-treated A375 cells, which were classified according to cellular component, molecular function and biological process [11,12]. Conversely, Bulk Gene Search System (BGSS) is also a searchable database for protein classification. It is composed of the UniGene, LocusLink and Proteome databases, and can be easily used to find the associated protein functions and related information in worldwide databases by applying only accession numbers. BGSS can be incorporated with the BGSSJ program to search the gene annotation that identified from DNA microarray and their biological functions. The combination of both search-engine programs is very useful and effective for correlating genome and proteome information, allowing greater understanding of the systems biology of melanoma cells.

On the other hand, there are two new toxicogenomics databases: the Comparative Toxicology Database (CTD) and dbZACH (Zacharewski laboratory database). CTD is the database used for identifying interactions between chemicals and genes and facilitating cross-species comparative studies of these genes [31]. CTD provides the major data type of nucleotide and protein sequences, reference publications, curated genes, gene sets, a hierarchical vocabulary of chemicals, GO terms and organism taxonomy. CTD integrates controlled, hierarchical vocabularies for organism taxonomy (National Center for Biotechnology Information), chemicals (National Library of Medicine's Medical Subject Headings and Supplementary Concepts), and GO to ensure

Table 3. Proteomic studies on malignant melanoma cells .

Study	Melanoma cells	Proteomic techniques	Discovery (protein expression & significant genes)	Ref.
Bernard *et al.* (2003)	Five early primary melanoma cell lines, five primary melanoma cell lines, nine metastatic melanoma cell lines	2DE, MALDI-TOF	Approximately 2000 distinct protein spots were detected on each gel of melanoma cell lines and only eight candidate markers were identified as differentially regulated in transformed cells Hepatoma-derived growth factor and nucleophosmin B23 were strongly correlated with melanoma progression	[86]
Sinha *et al.* (2003)	Human melanoma cell line MeWo (etoposide treatment)	2DE, MALDI-TOF	The 2DE of the parental melanoma cancer cell line and its chemoresistant variants yielded approximately 1500 spots each and approximately 130 proteins were identified. Twelve significant proteins (HSP27, HSP27 variant, HSP60, HSP60 variant, HSP90, HSX70 variant, hypothetical protein DKFZp566J20466, nicotinamide *N*-methyltransferase, nucleolar protein B23, peroxiredoxin 1, phosphoglyceromuase and proteasome subunit, β-type) were differentially expressed in chemoresistant cell lines	[87]
Craven *et al.* (2004)	Melanoma cell lines MeWo and MW418 (IFN-α treatment)	2DE, Q-TOF MS/MS	On average, 1535 protein spots were detected for MM418 and 1145 for MeWo, with no significant difference in numbers of proteins detected following drug treatment for either cell line The significant proteins expressed in both cell lines were UCRP, IFP53, gelsolin, PA28α, LAP, cathepsin B, HSP90, TUBB and α-SNAP.	[88]
Chi *et al.* (2006)	Pigmented (MNT-1) and nonpigmented (SK-mel28) human melanoma cells	IMAC melanoma removal, 2DE, LTQ-MS/MS	Comparative profiling and functional characterization of the melanosome proteomes identified approximately 15,000 proteins in melanosomes of all stages, with approximately 600 significant proteins in any given stage Approximately 100 proteins shared by melanosomes from pigmented and nonpigmented melanocytes define the essential melanosome proteome Proteins validated by confirming their intracellular localization include PEDF and SLC24A5, which are involved in biogenesis of melanosomes	[89]

2DE: 2D electrophoresis; CLIM: Carboxyl-terminal LIM-domain protein; CRHSP: Ca^{2+}-responsive heat-stable protein; ETHE: Ethylmalonic encephalopathy; HSP: Heat-shock protein; IFP: Interferon-induced protein; IMAC: Immobilized metal affinity chromatography; LTQ: Linear ion-trap quadropole; MALDI: Matrix-assisted laser desorption/ionization; MS/MS: Tandem mass spectrometry; NSF: Soluble N*-ethylmaleimide-sensitive-factor attachment protein; PA: Proteasome activator; PEDF: Pigment epithelium-derived factor; Q-TOF: Quantitative TOF; SLC24A5: Sodium/potassium/calcium exchanger 5, NCKX5; SNAP: Soluble NSF attachment protein; TOF: Time of flight; TUBB: Tubulin-β; UCRP: Ubiquitin cross-reactive protein.*

Table 3. Proteomic studies on malignant melanoma cells (cont.).

Study	Melanoma cells	Proteomic techniques	Discovery (protein expression & significant genes)	Ref.
Culp *et al.* (2006)	B16-F10 mouse melanoma cells (tumor treatment in C57BL/6 mice)	2DE, MALDI-TOF MS	Comparative proteome analysis performed on B16–F10 derived tumors in C57BL/6 mice at days 3, 5, 7 and 10 showed 53 protein spots ($p < 0.001$) following six predominant kinetic paths of expression as the tumor progressed Proteins involved in glycolysis, inflammation, wounding, superoxide metabolism and chemotaxis increased during tumorigenesis, such as vascular endothelial growth factor and active cathepsin-D Proteins involved in electron transport, protein folding, blood coagulation and transport decreased during tumorigenesis	[90]
de Souza *et al.* (2006)	Murine melnaoma cell lines Tm1 and Tm5, and nontumoral cell melan-α	2DE, MALDI-TOF MS	In total, 71 out of 452 spots (average) were differentially accumulated and 44 spots were identified by MALDI-TOF, 15 with increased and 29 with decreased protein levels Downregulated proteins were glutathione *S*-transferase, superoxide dismutase, aldehyde dehydrogenase, thioredoxin, peroxiredoxin 2 and peroxiedoxin 6 The accumulation of exogenous peroxides was significantly higher in the tumor cells, while the levels of glutathionylation were two-times lower in the tumor cells compared to melan-α Differential accumulation of proteins involved in oncogene/tumor suppressor pathways by inhibiting p53 pathways and activation of Ras and c-myc pathways	[91]
Stockwin *et al.* (2006)	B16F10 malignant melanoma cells (1% O_2 treatment, 24 h)	Differential $^{16}O/^{18}O$ stable isotopic labeling and multidimensional liquid chromatography	MS analysis resulted in the identification and quantitation of 2,433 proteins Aminopeptidase N (CD13), carbonic anhydrase IX, potassium-transporting ATPase, matrix metalloproteinase 9 and SDF-1) were upregulated proteins in hypoxia-adapted malignant melanoma cells	[92]
Zuidervaart *et al.* (2006)	Human uveal melanoma primary cells (Mel270) and two metastatic cells (Omm-1.3 and -1.5)	2DE, MALDI-TOF-TOF	In a total of 1184 protein spots, a set of 24 proteins was differentially expressed in both of the two metastatic cells compared with a cell line derived from the primary tumor Major upregulated proteins (CLIM1, tropomodulin 3, β-hexosaminidase, eIF5A and CRHSP-24) and downregulated proteins (20S proteasome α2 subunit, 26S proteasome regulatory chain 4, enolase 1, ETHE1 and ribosomal protein L12) involved in tumor progression	[93]

2DE: 2D electrophoresis; CLIM: Carboxyl-terminal LIM-domain protein; CRHSP: Ca^{2+}-responsive heat-stable protein; ETHE: Ethylmalonic encephalopathy; HSP: Heat-shock protein; IFP: Interferon-induced protein; IMAC: Immobilized metal affinity chromatography; LTQ: Linear ion-trap quadropole; MALDI: Matrix-assisted laser desorption/ionization; MS/MS: Tandem mass spectrometry; NSF: Soluble N*-ethylmaleimide-sensitive-factor attachment protein; PA: Proteasome activator; PEDF: Pigment epithelium-derived factor; Q-TOF: Quantitative TOF; SLC24A5: Sodium/potassium/calcium exchanger 5, NCKX5; SNAP: Soluble NSF attachment protein; TOF: Time of flight; TUBB: Tubulin-β; UCRP: Ubiquitin cross-reactive protein.*

Table 4. Bioinformatics tools for studying the genome and proteome of malignant melanoma cells.

Database search	Source
Gene ontology	
National Center for Biotechnology Information (NCBI)	www.ncbi.nlm.nih.gov
Ensembl	www.ensembl.org
The Institute for Genome Research	www.tigr.org
GeneCards	www.genecards.org
The GDB Human Genome Database	www.gdb.org/
Ace View	www.ncbi.nlm.nih.gov/IEB/Research/Acembly/index.html
T1DBase	http://t1dbase.org/cgi-bin/dispatcher.cgi/Welcome/ display
EBI Genome	http://www.ebi.ac.uk/genomes/
Category classification of genes	
Swiss-Prot	www.expasy.ch/sprot
Bulk Gene Search System for Java	http://servx8.sinica.edu.tw/bgss-cgi-bin/gene.pl
Protein search	
Swiss-Prot	www.expasy.ch/sprot
Proteome	www.proteome.com/databases/HumanPD/reports
PubMed	www.ncbi.nlm. nih.gov/PubMed
Bulk Gene Search System	http://servx8.sinica.edu.tw/bgss-cgi-bin/protein.pl
Pathway & interaction	
Biocarta	www.biocarta.com
Kyoto Encyclopedia of Genes and Genomes (KEGG)	www.genome.ad.jp/kegg/pathway.html
GeneNetwork	http://genenetwork.sbl.bc.sinica.edu.tw
PubMed	www.ncbi.nlm. nih.gov/PubMed
Protein lounge (pathway and interaction)	www.proteinlounge.com/inter_home.asp
Protein, Signaling, Transcriptional Interactions and Inflammation Networks Gateway	http://pstiing.licr.org/search.jsp
Toxicogenomics database	
Comparative Toxicogenomics Database	http://ctd.mdibl.org/
Zacharewski laboratory database (dbZACH)	http://dbzach.fst.msu.edu/

consistency in data integration, annotation, access and interpretation. Therefore, CTD advances the understanding of the effects of environmental chemicals on human disease. In addition, the toxicogenomic information-management system dbZACH is a modular relational database with associated data insertion, retrieval and mining tools that manages traditional toxicology and complementary toxicogenomic data to facilitate comprehensive data integration, analysis and sharing [32]. It consists of four core subsystems (i.e., clones, genes, sample annotation and protocols), four experimental subsystems (i.e., microarray, Affymetrix, real-time PCR and toxicology) and three computational subsystems (i.e., gene regulation, pathways and orthology) that comply with the minimum information regarding a microarray experiment standard. Therefore, both databases are very useful for the toxicogenomic study of human diseases.

Genotoxic functions & correlated signaling pathways in A375 cells

Genotoxic functions of targeted genes in A375 cells

In the previous study of toxicogenomic effect of kojic acid and arbutin on gene-expression profiles in A375 malignant melanoma cells, seven downregulated genes in kojic acid-treated A375 cells and four downregulated genes in arbutin-treated A375 cells were shown to be highly related to the regulation of tumorigenesis and served as tumor suppressor genes in melanoma cells. For example, the *APOBEC1* gene downregulated by kojic acid led to suppression of cytidine deamination and generated inappropriate polypeptides, dominant–negative proteins or mutations in tumor suppressor genes and thus contributed to the tumor-formation process [33]. Rho guanine exchange factor (ARHGEF) 16 is one of multiple GEF members

that have a crucial role in activating small GTPases by exchanging GDP for GTP and regulating various cellular functions that directly link to the Rho GTPase signaling pathway [34]. The *ARHGEF16* gene is downregulated by kojic acid and may suppress the tumor progression of A375 cells. FGF receptor 3 (FGFR3) is one of four high-affinity tyrosine kinase receptors for the FGF family of ligands. Upon ligand stimulation, it undergoes dimerization and tyrosine autophosphorylation, resulting in cell proliferation or differentiation. This process is passed through the MAPK and phospholipase Cγ signal transduction pathways [35]. The inhibition of FGFR3-induced differentiation and apoptosis in multiple myeloma and served as an oncogene that contributed to tumor progression in multiple myeloma, including breast cancer [36]. Furthermore, we also present the targeted genes/proteins and essential factors that play crucial roles in A375 melanoma cells in Table 5.

Correlated signaling pathways in A375 cells

In melanoma, both the MAPK and the PI3K-AKT (AKT) signaling pathways are constitutively activated through multiple mechanisms, and thus exert several key functions in melanoma development and progression [37]. According to our previous results, we have proposed the hypothetical model of signaling pathways of arbutin-responsive genes (*AKT1*, *FGFR3* and *LRP6*) in A375 cells, which were correlated with the major signaling pathways (AKT, RAS, MAPK and WNT) in cell-cycle processes including proliferation, survival, differentiation, apoptosis and malignant melanogenesis and tumorigenesis (Figure 2). *AKT1* was normally activated via the AKT signaling pathway that correlated with the upstream signaling pathways of RAS, MAPK, MEK and ERK [38,39]. *FGFR3* was correlated with several pathways, such as the MAPK, phospholipase Cγ, RAC1 and AKT signaling pathways [40]. *LRP6* was correlated with WNT and MAPK signaling pathways [41,42]. Thus, the downregulation of these genes may lead to the suppression of cell proliferation, survival, differentiation and apoptosis of the arbutin-treated A375 cells. Therefore, the correlation of arbutin-responsive genes with many signaling pathways, as shown in the hypothetical model, provided a clearer understanding of the arbutin-suppression effect on the malignancy of melanocytic tumorigenesis.

Table 5. Partial targeted genes/proteins and essential factors in A375 melanoma cells.

Target	Type	Genotoxic effect	Ref.
Stat3, Stat5	Transcription factor	Induces A375 cell proliferation, tumor formation and progression	[94,95]
TEL (ETV6)	Signal transducer and activator of Stat3	Inhibits the Stat3 signaling pathway	[94]
A3 adenosine receptor	Receptor agonist	Inhibits cell proliferation via phosphatidylinositol 3-kinase/Akt-dependent inhibition of the extracellular signal-regulated kinase 1/2 phosphorylation in A375 cells	[96]
TGF-β1	Proangiogenic factor	Promotes angiogenesis, growth and metastasis of human melanoma	[97]
IL-1β	Proinflammatory cytokine	Induces endogenous IL-1β production, enhances cleavage of caspase downstream substrates and promotes mitochondria-mediated apoptosis in A375-S2 cells	[98]
IL-2	Proinflammatory cytokine	Induces the proliferation rate of A375 cells, which was associated with an increase in glutathione levels, the enhancement of IL-2Rα expression and the endogenous production of IL-2 in these tumor cells	[99]
PTEN	Tumor suppressor gene	Involved in the inhibition of focal adhesion formation, cell spreading and migration, as well as the inhibition of growth factor-stimulated MAPK signaling	[100]
Angiogenin	Tumor angiogenic factor	Essential for melanoma progression and metastasis of A375 cells	[101]
BRAF	Oncogenic gene	Regulates tumor growth and maintenance in A375 cells	[102]

ETV: Ets variant; TEL*: Translocation ets leukemia.*

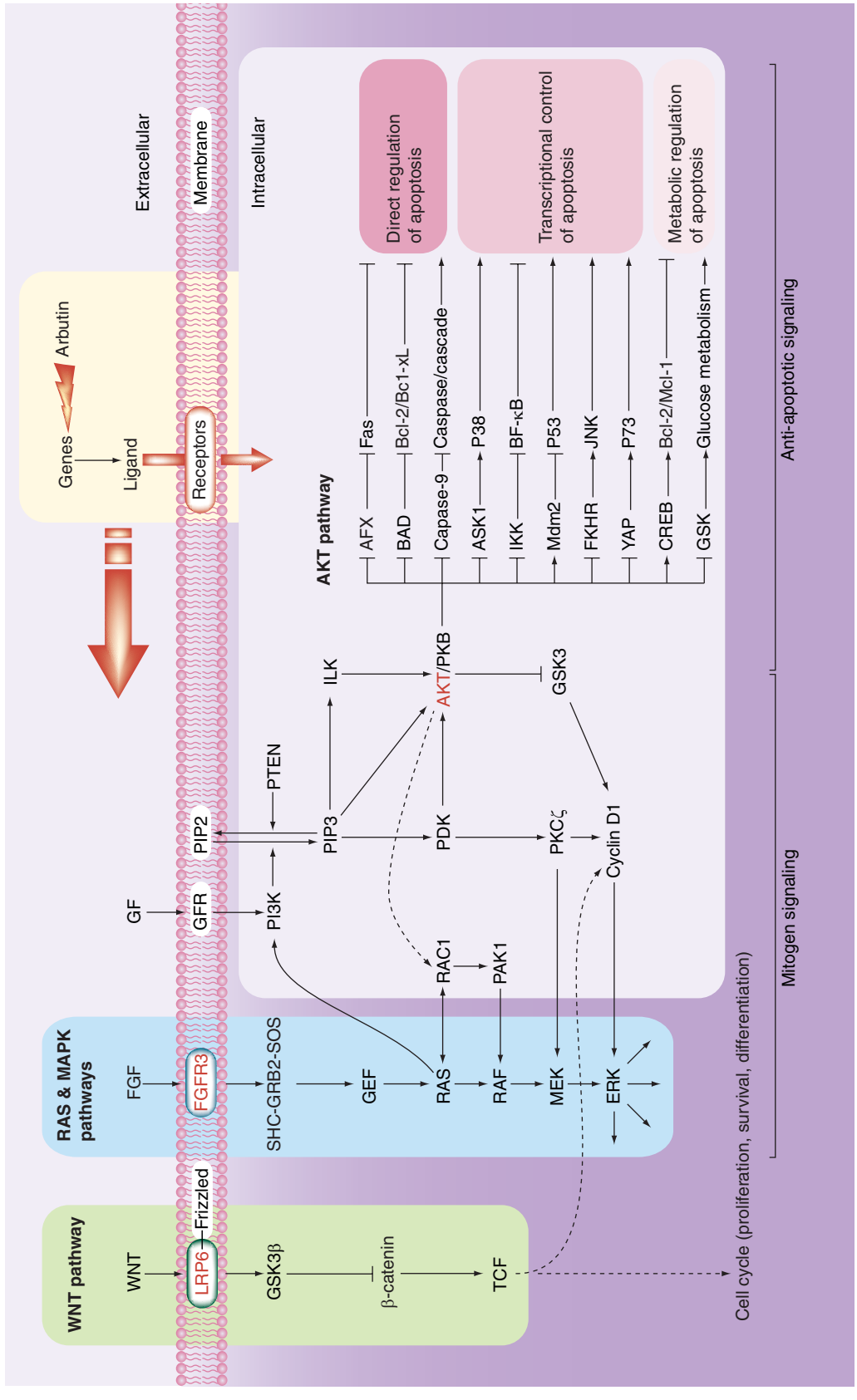


Figure 2. Hypothetical pathway of arbutin-responsive genes (*AKT1*, *FGFR3* and *LRP6*) in A375 cells correlated with AKT, WNT, RAS and MAPK signaling pathways in malignant melanogenesis and tumorigenesis.

This hypothetical pathway demonstrated the correlated arbutin-responsive genes with the major signaling pathways via the cellular mechanisms.
GEF: Guanine nucleotide exchange factor; GSK3: Glycogen synthase kinase 3; ILK: Integrin-linked kinase; MAPK: Mitogen-activated protein kinase 3; PI3K: Phosphoinoside-3 kinase; PIP2: Phosphatidylinositol-4,5-biphosphate; PIP3: Phosphatidylinositol-3,4,5-triphosphate; PDK-1: Phosphainositide-dependent kinase-1; PKC: Protein kinase C; PTEN: Phosphatase and tensin homolog; SON: Son of sevenless homolog 1; TCF: Transcription factor 7.

To understand the regulatory mechanisms of A375 melanoma cells, we thus described the major signaling pathways that correlated with the cellular response of A375 cells treated with arbutin. There are many correlated signaling pathways, such as AKT, RAS, MAPK, ERK and WNT. The AKT signaling pathway regulates the functional and therapeutic significance in malignant melanoma [43]. AKT kinase family members Akt1/PKBα, Akt2/PKBβ and Akt3/PKBγ, share extensive structural similarity and perform common, as well as unique, functions within cells. The AKT signaling pathway initiates at the cell surface when growth factors or other extracellular stimuli activate PI3K. Activated PI3K generates a lipid second messenger, phosphatidylinositol-3,4,5-triphosphate (PIP_3), causing translocation of AKT to the plasma membrane where it becomes phosphorylated and activated. The balance of cellular PIP_3 is regulated primarily by a phosphatase called phosphate and tensin homolog 10 (PTEN) that reduces PIP_3 levels, thereby lowering AKT activity. In melanomas, decreased PTEN activity elevates PIP_3 levels, resulting in AKT activation. Active AKT then phosphorylates downstream cellular proteins that promote melanoma cell proliferation and survival. Recently, Akt3 was discovered to be the predominant isoform activated in sporadic melanomas and its level of activity is increased during melanoma progression, with metastatic melanomas having the highest activity. Targeted reduction of Akt3 activity decreased survival of melanoma tumor cells, leading to inhibition of tumor development, which may be therapeutically effective for shrinking tumors in melanomas.

The RAS signal-transduction pathway is important for the genesis of melanoma. RAS functions downstream of several receptor tyrosine kinases and MAPK pathways. This pathway includes BRAF and contributes to the control of cellular proliferation by RAS, in particular the control of malignant cell proliferation by activated RAS [44]. RAS also controls the PI3K–PTEN–AKT pathway, as RAS directly binds the catalytic subunit of PI3K, resulting in apoptosis. RAS activation contributes not only to growth advantage, transformation and apoptosis, but also to tumor maintenance. Thus, the deactivation of the RAS signaling pathway leads to blockage of the other signal transduction pathways and suppression of the melanoma progression. In addition to inhibition of this pathway, there are numerous inhibitors of the RAS–MAPK signal transduction, such as signal-transduction pathway inhibitors (sorafenib, CCD-779 and PD032591), those conferring resistance to apoptosis (oblimersen sodium), proteasome inhibitors (bortezomib), histone deacetylase inhibitors (MS-275), antiangiogenetic drugs (thalidomide) and integrin-receptor inhibitors (vitaxin) [45]. Those are useful inhibitors and can be used for melanoma therapy.

The MAPK signaling pathway is frequently activated in human cancers, leading to malignant phenotypes such as autonomous cellular proliferation [46]. With mutually exclusive mutations in RAS and BRAF, the MAPK signaling pathway is constitutively activated in over 85% of cases of malignant melanoma, indicating the importance of this pathway in melanoma development [47]. MAPK signaling is also important in the regulation of cytokine signaling pathways. Although the MAPK signaling pathway has not yet been fully accredited as a melanoma target, the inhibition of MAPK signaling may provide a therapeutic strategy for the prevention and treatment of melanoma.

The ERK signaling pathway is downstream of the RAS/RAF/MEK pathways. The high constitutive ERK activity in melanoma is most likely a consequence of mutations in upstream components of the MAPK pathway [48]. There are at least two pathways whereby growth factors can activate ERK in melanoma. The first classical pathway, utilized by receptor tyrosine kinases, involves direct activation of the RAS/RAF pathway. The second pathway, used by G-protein-coupled receptors, involves the prior activation of adenylate cyclase, leading to increased intracellular cAMP and activation of RAS through a novel cAMP-driven exchange factor. The increased activity of ERK is implicated in rapid melanoma cell growth, increased cell survival, and resistance to apoptosis, and may also be a major driving force behind the highly metastatic behavior of melanoma through increased invasiveness of melanoma (matrix degradation and regulation of cell adhesion).

The WNT signaling pathway is involved in various cellular activities, including determination, proliferation, migration and differentiation in melanoma cells [49]. WNT ligands may activate one of three pathways, the canonical pathway, involving β-catenin (WNT/β-catenin pathway), the planar cell-polarity pathway and the WNT/calcium pathway. Aberrant activation of these pathways can lead to the development and progression of melanomas. The

WNT/β-catenin pathway plays the important and more specific role in melanoma because several components of the WNT/β-catenin pathway, such as adenomatous polyposis coli, inhibitor of β-catenin and T cell factor, lymphoid enhancer binding factor 1 and β-catenin, are modified in melanoma tumors and cell lines, leading to activation of this signaling [50]. Indeed, β-catenin is found in approximately 30% of human melanoma nuclei, indicating a potentially specific role for this pathway in tumor progression. It can induce ubiquitous genes (*myc* or cyclinD1), cell lineage-restricted genes (*Brn2*) and melanocyte-specific genes (*Mif-M* and *Dct*). All of these genes play a critical role in melanocyte survival, proliferation and differentiation. Furthermore, Wnt5a also enhances the invasiveness of melanoma cells. Thus, the deactivation of this pathway may suppress the tumorigenesis of melanoma cells.

Expert commentary

Although toxicogenomics is an attempt to use genomics and bioinformatics tools to understand the physiological basis for a toxic event based on an analysis of transcriptional, translational or metabolomic profiles, these studies are still complicated by nontoxic adaptive responses and other influent factors that are proximally related to the toxic event. Many toxicogenomics tools and the interpretation of numerous data are crucial, and must be organized and performed well in order to promote wider acceptance of toxicogenomics approaches and obtain the reliable biological meaning for understanding the genotoxic mechanism and carcinogenesis in melanomas and through further therapeutic applications. The experimental conditions should be well defined and the quality control at each step of experiments should be thoroughly examined for obtaining standardization, and thus providing reproducible and reliable data. Meanwhile, the quantitative analysis of differentially expressed genes or gene markers is also required for validating the targeted genes compared with the corresponding experimental data. In this review, we demonstrated an example of toxicogenomic analysis platform using microarray and bioinformatic tools to explore the genotoxic effect of kojic acid or arbutin on the differential gene-expression profile in A375 malignant melanoma cells through its effect on tumorigenesis and related side effects. Using our toxicogenomics approaches, the GO of differentially expressed genes was classified by which GO provided more understanding of the genetic basis of metabolic and cellular responses in melanoma cells, and those genes also led to an exploration of more valuable data in the regulation of melanoma carcinogenesis. Although this platform can be used as the standard procedure or strategy for other genotoxic studies, the development of experimental approaches capable of differentiating a wide range of genotoxic mechanisms is still required to significantly improve risk assessment and reduce the initial cost and time consumption for individual analysis. The integration of all approaches will have a great impact on biological discovery in the systems biology of malignant melanomas, and has an important role in further melanoma studies and its pharmaceutical therapy. In addition, the integration of systems biology approaches, including proteomics and metabolomics tools, will be helpful for biological discovery in the systems biology of malignant melanomas, and will improve the overall understanding of mechanisms of toxicity and disease etiology. In this regard, it may be argued that only one cell line of A375 cells is not a model for all melanomas. In fact, multiple cell lines used can provide many significant and convincing data; if many cell lines are used the variability of genetics is reduced. Nevertheless, it is dependent on the purpose and direction of studies. Only one cell line with the appropriate purpose and study can give the significant data that may be used as preliminary data for further analysis and validation. Owing to the availability of the A375 cell line as a human skin model and its derivation from a cancer cell line, the A375 cell line is generally used as a preliminary model for studying the genomic effects against potent drugs or compounds, including anticancer compounds and skin-whitening agents. However, future studies on the genotoxic effect of compounds on the biological and molecular mechanisms of human skin cells by *in vivo* test and the assessment of drug response are necessarily required for further therapeutic applications.

Future perspective

The applications of advanced techniques in genomic research and bioinformatic tools make toxicogenomics in A375 malignant melanoma cells possible, and these approaches will enable us to gain a better understanding of the cellular responses at the gene-expression level corresponding to the drug treatment. Although these approaches provide the new candidate genes and some correlated pathways, the additional

validations of target genes and their biological functions need to be investigated. More prospective studies of correlated pathways and disease mechanisms are necessary. However, the advanced toxicogenomic approaches that integrate with the systems biology approaches and are promising to explore the novel view of systems biology still depend on many risk factors in disease development and progression. Finally, the candidate genes can potentially be genomic biomarkers for skin cancer and may be useful for further diagnostic and therapeutic applications

Bibliography

1. Soengas MS, Capodieci P, Polsky D *et al.*: Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature* 409, 207–211 (2001).
2. Kaufmann SH, Vaux DL: Alterations in the apoptotic machinery and their potential role in anticancer drug resistance. *Oncogene* 22, 7414–7430 (2003).
3. Pennie WD, Tugwood JD, Oliver GJA, Kimber I: The principles and practical of toxicogenomics: applications and opportunity. *Toxicol. Sci.* 54, 277–283 (2000).
4. Waters MD, Fostel JM: Toxicogenomics and systems toxicology: aims and prospects. *Nature* 5, 936–948 (2004).
5. Boverhof DR, Zacharewski TR: Toxicogenomics in risk assessment: applications and needs. *Toxicol. Sci.* 89, 352–360 (2006).
6. de Braud F, Khayat D, Kroon BB, Valdagni R, Bruzzi P, Cascinelli N: Malignant melanoma. *Crit. Rev. Oncol. Hematol.* 47, 35–63 (2003).
7. Slominski A, Tobin DJ, Shibahara S, Wortsman J: Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiol. Rev.* 84, 1155–1228 (2004).
8. Shibata T, Prota G, Mishima Y: Non-melanosomal regulatory factors in melanogenesis. *J. Invest. Dermatol.* 100, S274–S280 (1993).
9. Clark EA, Golub TR, Lander ES, Hynes RO: Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* 406, 532–535 (2000).
10. Bodzioch M, Dembinska-Kiec A, Hartwich J *et al.*: The microarray expression analysis identifies BAX as mediator of β-carotene effects on apoptosis. *Nutr. Cancer* 51, 226–235 (2005).
11. Cheng SL, Huang Liu R, Sheu JN, Chen ST, Sinchaikul S, Tsay GJ: Toxicogenomics of kojic acid on gene expression profiling of A375 human malignant melanoma cells. *Biol. Pharm. Bull.* 29, 655–669 (2006).
12. Cheng SL, Huang Liu R, Sheu JN, Chen ST, Sinchaikul S, Tsay GJ: Toxicogenomics of A375 human malignant melanoma cells treated with arbutin. *J. Biomed. Sci.* 14, 87–105 (2007).
13. Smith B, Selby P, Southgate J, Pittman K, Bradley C, Blair GE: Detection of melanoma cells in peripheral blood by means of reverse transcriptase and polymerase chain reaction. *Lancet* 338, 1227–1229 (1991).
14. Brossart P, Schmier JW, Krüger S *et al.*: A polymerase chain reaction-based semiquantitative assessment of malignant melanoma cells in peripheral blood. *Cancer Res.* 55, 4056–4068 (1995).
15. Battayani Z, Grob JJ, Xerri L *et al.*: Polymerase chain reaction detection of circulating melanocytes as a prognostic marker in patients with melanoma. *Arch. Dermatol.* 131, 443–447 (1995).
16. Keilholz U, Goldin-Lang P, Bechrakis NE *et al.*: Quantitative detection of circulating tumor cells in cutaneous and ocular melanoma and quality assessment by real-time reverse transcriptase-polymerase chain reaction. *Clin. Cancer Res.* 10, 1605–1612 (2004).
17. Hannon GJ: RNA interference. *Nature* 418(6894), 244–251 (2002).
18. Sumimoto H, Miyagishi M, Miyoshi H *et al.*: Inhibition of growth and invasive ability of melanoma by inactivation of mutated BRAF with lentivirus-mediated RNA interference. *Oncogene* 23, 6031–6039 (2004).
19. Hoeflich KP, Gray DC, Eby MT *et al.*: Oncogenic BRAF is required for tumor growth and maintenance in melanoma models. *Cancer Res.* 66, 999–1006 (2006).
20. Chen X, Lin J, Kanekura T, Su J: A small interfering CD147-targeting RNA inhibited the proliferation, invasiveness, and metastatic activity of malignant melanoma. *Cancer Res.* 66, 11323–11330 (2006).
21. Tao J, Tu YT, Huang CZ, *et al.*: Inhibiting the growth of malignant melanoma by blocking the expression of vascular endothelial growth factor using an RNA interference approach. *Br. J. Dermatol.* 153, 715–724 (2005).
22. Yazdi AS, Morstedt K, Puchta U *et al.*: Heterogeneity of T-cell clones infiltrating primary malignant melanomas. *J. Invest. Dermatol.* 126, 393–398 (2006).
23. Tanami H, Imoto I, Hirasawa A *et al.*: Involvement of overexpressed wild-type BRAF in the growth of malignant melanoma cell lines. *Oncogene* 23, 8796–8804 (2004).
24. Soto JL, Cabrera CM, Serrano S, Lopez-Nevot MA: Mutation analysis of genes that control the G_1/S cell cycle in melanoma: *TP53*, *CDKN1A*, *CDKN2A*, and *CDKN2B*. *BMC Cancer* 5, 36 (2005).
25. Cervoni L, Egistelli L, Eufemi M *et al.*: DNA sequences acting as binding sites for NM23/NDPK proteins in melanoma M14 cells. *J. Cell. Biochem.* 98, 421–428 (2006).
26. Anderson L, Seilhamer J: A comparison of selected mRNA and protein abundances in human liver. *Electrophoresis* 18, 533–537 (1997).
27. Gygi SP, Rochon Y, Franza BR, Aebersold R: Correlation between protein and mRNA abundance in yeast. *Mol. Cell. Biol.* 19, 1720–1730 (1999).
28. Chen G, Gharib TG, Huang CC *et al.*: Discordant protein and mRNA expression in lung adenocarcinomas. *Mol. Cell. Proteomics* 1, 304–313 (2002).
29. Myung JK, Afjehi-Sadat L, Felizardo-Cabatic M, Slavc I, Lubec G: Expressional patterns of chaperones in ten human tumor cell lines. *Proteome Sci.* 2, 8 (2004).
30. Clauser KR, Hall SC, Smith DM *et al.*: Rapid mass spectrometric peptide sequencing and mass matching for characterization of human melanoma proteins isolated by two-dimensional PAGE. *Proc. Natl Acad. Sci. USA* 92, 5072–5076 (1995).
31. Mattingly CJ, Rosenstein MC, Colby GT, Forrest JN Jr, Boyer JL: The Comparative Toxicogenomics Database (CTD): a resource for comparative toxicological studies. *J. Exp. Zool.* 305, 689–692 (2006).
32. Burgoon LD, Boutros PC, Dere E, Zacharewski TR: dbZach: A MIAME-compliant toxicogenomic supportive relational database. *Toxicol. Sci.* 90, 558–568 (2006).
33. Cascalho M: Advantages and disadvantages of cytidine deamination. *J. Immunol.* 172, 6513–6518 (2004).

34. Qi H, Fournier A, Grenier J, Fillion C, Labrie Y, Labrie C: Isolation of the novel human guanine nucleotide exchange factor Src homology 3 domain-containing guanine nucleotide exchange factor (SGEF) and of C-terminal SGEF, an N-terminally truncated form of SGEF, the expression of which is regulated by androgen in prostate cancer cells. *Endocrinology* 144, 1742–1752 (2003).
35. Kanai M, Goke M, Tsunekawa S, Podolsky DK: Signal transduction pathway of human fibroblast growth factor receptor 3. Identification of a novel 66-kDa phosphoprotein. *J. Biol. Chem.* 272, 6621–6628 (1997).
36. Chesi M, Brents LA, Ely SA *et al.*: Activated fibroblast growth factor receptor 3 is an oncogene that contributes to tumor progression in multiple myeloma. *Blood* 97, 729–736 (2001).
37. Meier F, Schittek B, Busch S *et al.*: The RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways present molecular targets for the effective treatment of advanced melanoma. *Front. Biosci.* 10, 2986–3001 (2005).
38. Laprise P, Langlois MJ, Boucher MJ, Jobin C, Rivard N: Down-regulation of MEK/ERK signaling by E-cadherin-dependent PI3K/Akt pathway in differentiating intestinal epithelial cells. *J. Cell. Physiol.* 199, 32–39 (2004).
39. Parsa AT, Holland EC: Cooperative translational control of gene expression by Ras and Akt in cancer. *Trends Mol. Med.* 10, 607–613 (2004).
40. Eswarakumar VP, Lax I, Schlessinger J: Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev.* 16, 139–149 (2005).
41. Schneider WJ, Nimpf J: LDL receptor relatives at the crossroad of endocytosis and signaling. *Cell. Mol. Life Sci.* 60, 892–903 (2003).
42. Quaiser T, Anton R, Kuhl M: Kinases and G proteins join the Wnt receptor complex. *Bioessays* 28, 339–343 (2006).
43. Robertson GP: Functional and therapeutic significance of Akt deregulation in malignant melanoma. *Cancer Metastasis Rev.* 24, 273–285 (2005).
44. Haluska FG, Tsao H, Wu H, Haluska FS, Lazar A, Goel V: Genetic alterations in signaling pathways in melanoma. *Clin. Cancer Res.* 12, S2301–S2307 (2006).
45. Becker JC, Kirkwood JM, Agarwala SS, Dummer R, Schrama D, Hauschild A: Molecularly targeted therapy for melanoma: current reality and future options. *Cancer* 107, 2317–2327 (2006).
46. Chin L, Garraway LA, Fisher DE: Malignant melanoma: genetics and therapeutics in the genomic era. *Genes Dev.* 20, 2149–2182 (2006).
47. Alsina J, Gorsk DH, Germino FJ *et al.*: Detection of mutations in the mitogen-activated protein kinase pathway in human melanoma. *Clin. Cancer Res.* 9, 6419–6425 (2003).
48. Smalley KS: A pivotal role for ERK in the oncogenic behaviour of malignant melanoma. *Int. J. Cancer* 104, 527–532 (2003).
49. Weeraratna AT: A Wnt-er wonderland – the complexity of Wnt signaling in melanoma. *Cancer Metastasis Rev.* 24, 237–250 (2005).
50. Larue L, Delmas V: The WNT/β-catenin pathway in melanoma. *Front. Biosci.* 11, 733–742 (2006).
51. Pourpak A, Dorr RT, Meyers RO, Powell MB, Stratton SP: Cytotoxicity activity of apomine is due to a novel membrane-mediated cytolytic mechanism independent of apoptosis in the A375 human melanoma cell line. *Invest. New Drugs* 25, 107–114 (2007).
52. Costa MA, Pellerito L, Izzo V *et al.*: Diorganotin (IV) and triorganotin (IV) complexes of meso-tetra (4-sulfonatophenyl) porphine induce apoptosis in A375 human melanoma cells. *Cancer Lett.* 238, 284–294 (2006).
53. Kolarova H, Mosinger J, Lenobel R, Kejlova K, Jirova D, Strnad M: *In vitro* toxicity testing of supramolecular sensitizers for photodynamic therapy. *Toxicol. In Vitro* 17, 775–778 (2003).
54. Ribble D, Goldstein NB, Norris DA, Shellman YG: A simple technique for quantifying apoptosis in 96-well plates. *BMC Biotechnol.* 5, 12 (2005).
55. Pantazis P, Hinz HR, Mendoza JT *et al.*: Complete inhibition of growth followed by death of human malignant melanoma cells *in vitro* and regression of human melanoma xenografts in immunodeficient mice induced by camptothecins. *Cancer Res.* 52, 3980–3987 (1992).
56. Wang F, Cao Y, Liu HY, Xu SF, Han R: Anti-invasion and anti-angiogenesis effect of taxol and camptothecin on melanoma cells. *J. Asian Nat. Prod. Res.* 5, 121–129 (2003).
57. Loewe R, Valero T, Kremling S *et al.*: Dimethylfumarate impairs melanoma growth and metastasis. *Cancer Res.* 66, 11888–11896 (2006).
58. Okazawa M, Shiraki T, Ninomiya H, Kobayashi S, Masaki T: Endothelin-induced apoptosis of A375 human melanoma cells. *J. Biol. Chem.* 273, 12584–12592 (1998).
59. Spinella F, Rosano L, Di Castro V *et al.*: Endothelin-1 and endothelin-3 promote invasive behavior via hypoxia-inducible factor-1α in human melanoma cells. *Cancer Res.* 67, 1725–1734 (2007).
60. Liu LY, Hu CL, Ma LJ, Zhang ZH, Mei YA: ET-1 inhibits B-16 murine melanoma cell migration by decreasing K^+ currents. *Cell Motil. Cytoskeleton* 58, 127–136 (2004).
61. Lozano C, Torres JL, Julia L, Jimenez A, Centelles JJ, Cascante M: Effect of new antioxidant cysteinyl-flavanol conjugates on skin cancer cells. *FEBS Lett.* 579, 4219–4225 (2005).
62. Nihal M, Ahmad N, Mukhtar H, Wood GS: Anti-proliferative and proapoptotic effects of (-)-epigallocatechin-3-gallate on human melanoma: possible implications for the chemoprevention of melanoma. *Int. J. Cancer* 114, 513–521 (2005).
63. Suzuki Y, Isemura M: Inhibitory effect of epigallocatechin gallate on adhesion of murine melanoma cells to laminin. *Cancer Lett.* 173, 15–20 (2001).
64. Gong YQ, Fan Y, Wu DZ, Yang H, Hu ZB, Wang ZT: *In vivo* and *in vitro* evaluation of erianin, a novel anti-angiogenic agent. *Eur. J. Cancer* 40, 1554–1565 (2004).
65. Zhang Y, Wu LJ, Tashiro SI, Onodera S, Ikejima T: Evodiamine induces tumor cell death through different pathways: apoptosis and necrosis. *Acta Pharmacol. Sin.* 25, 83–89 (2004).
66. Fei XF, Wang BX, Tashiro S, Li TJ, Ma JS, Ikejima T: Apoptotic effects of ginsenoside RH_2 on human malignant melanoma. *Acta Pharmacol. Sin.* 23, 315–322 (2002).
67. Ota T, Maeda M, Odashima S, Ninomiya-Tsuji J, Tatsuka M: G_1 phase-specific suppression of the Cdk2 activity by ginsenoside Rh_2 in cultured murine cells. *Life Sci.* 60, 39–44 (1997).
68. Wei M, Gao Y, Tian M, Li N, Hao S, Zeng X: Selectively desulfated heparin inhibits P-selectin-mediated adhesion of human melanoma cells. *Cancer Lett.* 229, 123–126 (2005).
69. Bereczky B, Gilly R, Raso E, Vago A, Timar J, Tovari J: Selective antimetastatic effect of heparins in preclinical human melanoma models is based on inhibition of migration and microvascular arrest. *Clin. Exp. Metastasis* 22, 69–76 (2005).
70. Stevenson JL, Choi SH, Varki A: Differential metastasis inhibition by clinically relevant levels of heparins – correlation with selectin inhibition, not antithromotic activity. *Clin. Cancer Res.* 11, 7003–7011 (2005).

71. Niles RM, McFarland M, Weimer MB, Redkar A, Fu YM, Meadows GG: Resveratrol is a potent inducer of apoptosis in human melanoma cells. *Cancer Lett.* 190, 157–163 (2003).
72. Fulda S, Debatin KM: Sensitization for tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by the chemopreventive agent resveratrol. *Cancer Res.* 64, 337–346 (2004).
73. Hsieh TC, Wang Z, Hamby CV, Wu JM: Inhibition of melanoma cell proliferation by resveratrol is correlated with upregulation of quinine reductase 2 and p53. *Biochem. Biophys. Res. Commun.* 334, 223–230 (2005).
74. Lin SY, Lai WW, Chou CC *et al.*: Sodium ascorbate inhibits growth via the induction of cell cycle arrest and apoptosis in human malignant melanoma A375.S2 cells. *Melanoma Res.* 16, 509–519 (2006).
75. Kang JS, Cho D, Kim YI *et al.*: Sodium ascorbate (vitamin C) induces apoptosis in melanoma cells via the down-regulation of transferrin receptor dependent iron uptake. *J. Cell. Physiol.* 204, 192–197 (2005).
76. Kang JS, Cho D, Kim YI *et al.*: L-ascorbic acid (vitamin C) induces the apoptosis of B16 murine melanoma cells via a caspase-8-independent pathway. *Cancer Immunol. Immunother.* 52, 693–698 (2003).
77. DeRisi J, Penland L, Brown PO *et al.*: Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat. Genet.* 14, 457–460 (1996).
78. Bittner M, Meltzer P, Chen Y *et al.*: Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* 406, 536–540 (2000).
79. Brem R, Hildebrandt T, Jarsch M, van Muijen GN, Weidle UH: Identification of metastasis-associated genes by transcriptional profiling of a metastasizing versus a non-metastasizing human melanoma cell line. *Anticancer Res.* 21, 1731–1740 (2001).
80. de Wit NJ, Burtscher HJ, Weidle UH, Ruiter DJ, van Muigen GN: Differentially expressed genes identified in human melanoma cell lines with different metastatic behavior using high-density oligonucleotide arrays. *Melanoma Res.* 12, 57–69 (2002).
81. Tschentscher F, Husing J, Holter T *et al.*: Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res.* 63, 2578–2584 (2003).
82. Hoek K, Rimm DL, Williams KR *et al.*: Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. *Cancer Res.* 64, 5270–5282 (2004).
83. Haqq C, Nosrati M, Sudilovsky D *et al.*: The gene expression signatures of melanoma progression. *Proc. Natl Acad. Sci. USA* 102, 6092–6097 (2005).
84. Nambiar S, Mirmohammadsadegh A, Doroudi R *et al.*: Signaling networks in cutaneous melanoma metastasis identified by complementary DNA microarrays. *Arch. Dermatol.* 141, 165–173 (2005).
85. Jaeger J, Koczan D, Thiesen HJ *et al.*: Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. *Clin. Cancer Res.* 13, 806–815 (2007).
86. Bernard K, Litman E, Fitzpatrick JL *et al.*: Functional proteomic analysis of melanoma progression. *Cancer Res.* 63, 6716–6725 (2003).
87. Sinha P, Poland J, Kohl S *et al.*: Study of the development of chemoresistance in melanoma cell lines using proteome analysis. *Electrophoresis* 24, 2386–2404 (2003).
88. Craven RA, Stanley AJ, Hanrahan S *et al.*: Identification of proteins regulated by interferon-α in resistant and sensitive malignant melanoma cell lines. *Proteomics* 4, 3998–4009 (2004).
89. Chi A, Valencia JC, Hu ZZ *et al.*: Proteomic and bioinformatic characterization of the biogenesis and function of melanosomes. *J. Proteome Res.* 5, 3135–3144 (2006).
90. Culp WD, Neal R, Massey R, Egevad L, Pisa P, Garland D: Proteomic analysis of tumor establishment and growth in the B16–F10 mouse melanoma model. *J. Proteome Res.* 5, 1332–1343 (2006).
91. de Souza GA, Godoy LM, Teixeira VR *et al.*: Proteomic and SAGE profiling of murine melanoma progression indicates the reduction of proteins responsible for ROS degradation. *Proteomics* 6, 1460–1470 (2006).
92. Stockwin LH, Blonder J, Bumke MA *et al.*: Proteomic analysis of plasma membrane from hypoxia-adapted malignant melanoma. *J. Proteome Res.* 5, 2996–3007 (2006).
93. Zuidervaart W, Hensbergen PJ, Wong MC *et al.*: Proteomic analysis of uveal melanoma reveals novel potential markers involved in tumor progression. *Invest. Ophthalmol. Vis. Sci.* 47, 786–793 (2006).
94. Schick N, Oakeley EJ, Hynes NE, Badache A: TEL/ETV6 is a signal transducer and activator of transcription 3 (Stat3)-induced repressor of Stat3 activity. *J. Biol. Chem.* 279, 38787–38796 (2004).
95. Mirmohammadsadegh A, Hassan M, Bardenheuer W *et al.*: STAT5 phosphorylation in malignant melanoma is important for survival and is mediated through SRC and JAK1 kinases. *J. Invest. Dermatol.* 126, 2272–2280 (2006).
96. Merighi S, Benini A, Mirandola P *et al.*: A3 adenosine receptor activation inhibits cell proliferation via phosphatidylinositol 3-kinase/Akt-dependent inhibition of the extracellular signal-regulated kinase 1/2 phosphorylation in A375 human melanoma cells. *J. Biol. Chem.* 280, 19516–19526 (2005).
97. Liu G, Zhang F, Lee J, Dong Z: Selective induction of interleukin-8 expression in metastatic melanoma cells by transforming growth factor-β 1. *Cytokine* 31, 241–249 (2005).
98. Wang C, Wang MW, Tashiro S, Onodera S, Ikejima T: IL-1β acts in synergy with endogenous IL-1β in A375-S2 human melanoma cell apoptosis through mitochondrial pathway. *J. Korean Med. Sci.* 20, 555–561 (2005).
99. del Olmo M, Alonso-Varona A, Castro B, Bilbao P, Palomares T: Cytomodulation of interleukin-2 effect by L-2-oxothiazolidine-4-carboxylate on human malignant melanoma. *Cancer Immunol. Immunother.* 55, 948–957 (2006).
100. Wu H, Goel V, Haluska FG: PTEN signaling pathways in melanoma. *Oncogene* 22, 3113–3122 (2003).
101. Song J, Wang J, Yang J, Jiang C, Shen W, Wang L: Influence of angiogenin on the growth of A375 human melanoma cells and the expression of basic fibroblast growth factor. *Melanoma Res.* 16, 119–126 (2006).
102. Hoeflich KP, Gray DC, Eby MT *et al.*: Oncogenic BRAF is required for tumor growth and maintenance in melanoma models. *Cancer Res.* 66, 999–1006 (2006).