



Michele R. Sassi. *Animal Market Day in Rajasthan, India*. Photograph, 2007.

Several genes have been recently identified as potential prognostic markers of either tumor progression, metastasis, or overall clinical outcome for patients with melanoma

The Impact of Genomics in Understanding Human Melanoma Progression and Metastasis

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Background: Recent technological advances in the analysis of the human genome have opened the door to improving our primitive understanding of the gene expression patterns in cancer. For the first time, we have an overview of the complexities of tumorigenesis and metastatic progression of cancer. The examination of the phenotypic and (epi)genetic changes in cutaneous melanoma has identified several genes deemed central to the development and progression of melanoma.

Methods: A review of the recent literature was performed to determine the role of array-based high-throughput gene expression analysis in understanding the specific genes involved as well as the pathways and the comparative gene expression patterns of primary and metastatic melanoma.

Results: Most studies utilizing gene microarray analysis and other whole genome approaches reveal a wide array of genes and expression patterns in human melanoma. Furthermore, several of the same genes have been found in comparative studies, with some studies attempting correlation with clinical outcome. Several genes have been identified as potential prognostic markers of tumor progression and overall clinical outcome.

Conclusions: High-throughput gene expression analysis has had a major impact in melanoma research. Several gene expression platforms have provided insight into the gene expression patterns in melanoma. Such data will provide the foundations for the future development of prognostic markers and improved targeted therapies for patients with melanoma.

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Abbreviations used in this paper: PCM = primary cutaneous melanoma, MM = metastatic melanoma, NHEM = normal human epidermal melanocyte, RGP = radial growth phase, VGP = vertical growth phase, TSG = tumor suppressor gene.

Introduction

Our simplistic understanding of the genetic basis for human disease has only recently begun to change as a result of the complete decoding of the human genome in 2001, led by the pioneering efforts of several research groups.¹⁻⁴ The unprecedented findings have enlightened and stimulated researchers worldwide to re-think our current paradigms of cancer development and metastatic progression in favor of shifting our approach to the level of gene function and regulation. It is compelling to think of the amazing technological

advances that have occurred over the last 10 years, with such advances highlighting the sheer beauty, complexity, and intricate nature of the human genome and how it relates to the development of human malignant transformation. Indeed, we are on the first word of the first page of a very long novel.

Over the past decade, microarray-based high-throughput gene expression analysis of cancer has come to the forefront of cancer research, allowing us a comprehensive understanding of gene expression patterns involved in cancer initiation and progression. Since the absolute number of cases of primary cutaneous melanoma (PCM) is increasing at an astounding rate, researchers worldwide have refocused their efforts on trying to understand the genetic basis for the malignant transformation of this deadly disease.⁵ The specific genes involved in this transformation process and subsequent progression of melanoma have not been fully described.⁶⁻¹¹

There is ample evidence supportive of a strong correlation between the thickness of the PCM and the metastatic capacity of spreading either via the draining lymphatic channels or hematogenously.^{12,13} Once melanoma has metastasized by either route, the overall survival for patients greatly diminishes.^{14,15} Whereas patients with thin primary tumors are usually cured with the appropriate surgical resection, patients diagnosed with metastatic melanoma (MM) have a poor prognosis, with 6 out of every 7 skin cancer deaths ultimately due to MM.^{5,16,17} Most patients with advanced disease do not respond to currently available therapies, thereby necessitating a greater need for the development of more effective treatments. In order to do so, we will need to vastly improve our current understanding of the molecular, genetic, immunologic, and cellular events that are intertwined with gene function and regulation as they relate to the malignant transformation of normal human epidermal melanocytes (NHEM) to a PCM and subsequently to metastatic disease.

Impact of High-Throughput Gene Expression Analysis in Human Melanoma

Human melanoma is an ideal tumor model in which to improve our understanding of the mechanisms for human tumor progression. The development of the Clark model of melanoma tumorigenesis and progression has provided a basic and relatively straightforward platform for the hypothesized stepwise transformation of NHEM to melanoma, further describing the five distinct stages of progression based on histological criteria.¹⁸ Sequentially, this model describes a benign nevus transforming into a dysplastic nevus, progressing into a radial growth phase (RGP) melanoma, and then becoming a vertical growth phase (VGP) primary melanoma. This process is followed by the final progression of a PCM to that of an MM, having gained the appropriate gene expression patterns to spread elsewhere throughout the body. Thus, this mol-

ecular seed and soil model incorporates many of the prevailing ideas as to the involved mechanisms and specific genes involved in this process.

A global view of the available malignant genome, transcriptome, and proteome has proven invaluable not only for understanding the intricate process of neoplastic transformation, but also — and even more importantly — for the profound downstream benefits of these discoveries. For instance, understanding the genes involved in malignant transformation may lead to improved diagnostics, predictive markers of treatment responses, gene expression profiles of clinical outcome, and the development of targeted therapeutics against select genes. A wide variety of high-throughput DNA and tissue microarray platforms have been applied to identify molecular targets associated with biological and clinical phenotypes by comparing samples representative of distinct pathophysiological states.¹⁹⁻²⁴

DeRisi et al²⁵ were one of the first groups to examine the utility of high-density DNA microarrays to search for differences in gene expression associated with tumor progression in human melanoma cell lines. They started with approximately 1,100 gene elements printed onto a standard glass microscope slide, subsequently confirming the identification of several candidate genes and putative melanoma-associated antigens involved in melanoma tumor progression, such as TRP-1, gp75, MCP-1, and WAF1 (p21).²⁶⁻²⁸ Ryu et al²⁹ analyzed expression signatures associated with melanoma progression using functional annotations and categorized these transcripts into three distinct classes of genes: (1) upregulation of activators of cell cycle progression, DNA replication, and repair (eg, CDCA2, NCAPH, NCAPG, NCAPG2, PBK, NUSAP1, BIRC5, ESCO2, HELLS, MELK, GINS1, GINS4, RAD54L, TYMS, DHFR), (2) loss of genes associated with cellular adhesion and melanocyte differentiation (eg, CDH3, CDH1, c-KIT, PAX3, CITED1/MSG-1, TYR, MELANA, MC1R, OCA2), and (3) upregulation of genes associated with resistance to apoptosis (BIRC5/survivin). While these broad classes of gene transcripts have previously been implicated in the progression of melanoma and other malignancies, the specific genes identified within each class are quite novel. Despite a decade or more of gene expression studies, few true advances have been made that ultimately resulted in the development of reliable diagnostic and prognostic markers.

Utilizing comparative gene microarray analysis of 55 freshly procured human PCM and MM samples, investigators at Moffitt Cancer Center identified a gene expression profile that is capable of differentiating an MM from that of a PCM³⁰ and have also found a striking correlation between the gene expression patterns of melanoma and overall tumor thickness of the primary melanoma, as measured by Breslow's method. Furthermore, we have defined a "transition point" of PCM tumor progression where a distinct set of gene expres-

sion change occurs at the intermediate (Breslow's tumor thickness 1 to 4 mm) to thick (Breslow's tumor thickness greater than 4 mm) groups of PCM, with an average tumor thickness of about 2.4 mm. Since each primary melanoma is derived from different patients, we cannot interpret the comparative analysis as a continuous series of data points. However, it is clear that a distinct transition point occurs where different genes are over- and underexpressed during this time point, representing the fluid and dynamic nature of gene expression change during primary melanoma tumor progression (Figure). Additionally, we show that there are specific genes that are overexpressed (MAGE, GPR19, BCL2A1, MMP19, SOX5, BUB1, RGS20) and underexpressed (SPRR1A/B, KRT16/17, CD24, LOR, GATA3, MUC15, TMPRSS4) during this same time point.

In comparison to similar studies examining PCM, Winnepenninckx et al¹⁰ identified 175 genes that were overexpressed as tumor thickness increased. Likewise, Smith et al⁷ showed a distinct transition point of gene expression during the VGP of melanoma tumors, initially comparing RGP to VGP primary melanomas to normal skin, benign nevi, melanoma in situ, and MM.⁷ We then compared our defined gene sets to those of the most

recent papers describing similar comparisons between PCM alone or with MM.^{6,11} We found a significant overlap between many genes both over- and underexpressed within PCM of varying tumor thicknesses and compared to MM (Appendix 1 and 2). In addition, we identified several putative genes associated with tumor progression and metastasis, such as SPP-1, MITE, CITED-1, GDF-15, c-Met, and several of the HOX loci, some of which are concordant in other studies. Several other genes were identified that are thought to be centrally involved in the suppression of tumor growth, such as PITX-1, CST-6, PDGFRL, DSC-3, POU2F3, and CLCA2, previously found in other nonmelanoma tumor histologies. Similar to our study, other researchers have utilized gene expression microarrays to further delineate the complex processes involved with the malignant transformation and progression of melanoma.^{6,11}

We also examined the gene expression differences found between NHEM and thin PCM and MM samples, attempting to gain a better understanding of those genes primarily involved in malignant transformation rather than tumor progression (Appendix 1 and 2). Interestingly, we found the greatest degree of gene overlap between studies that utilized NHEM as part of their comparative

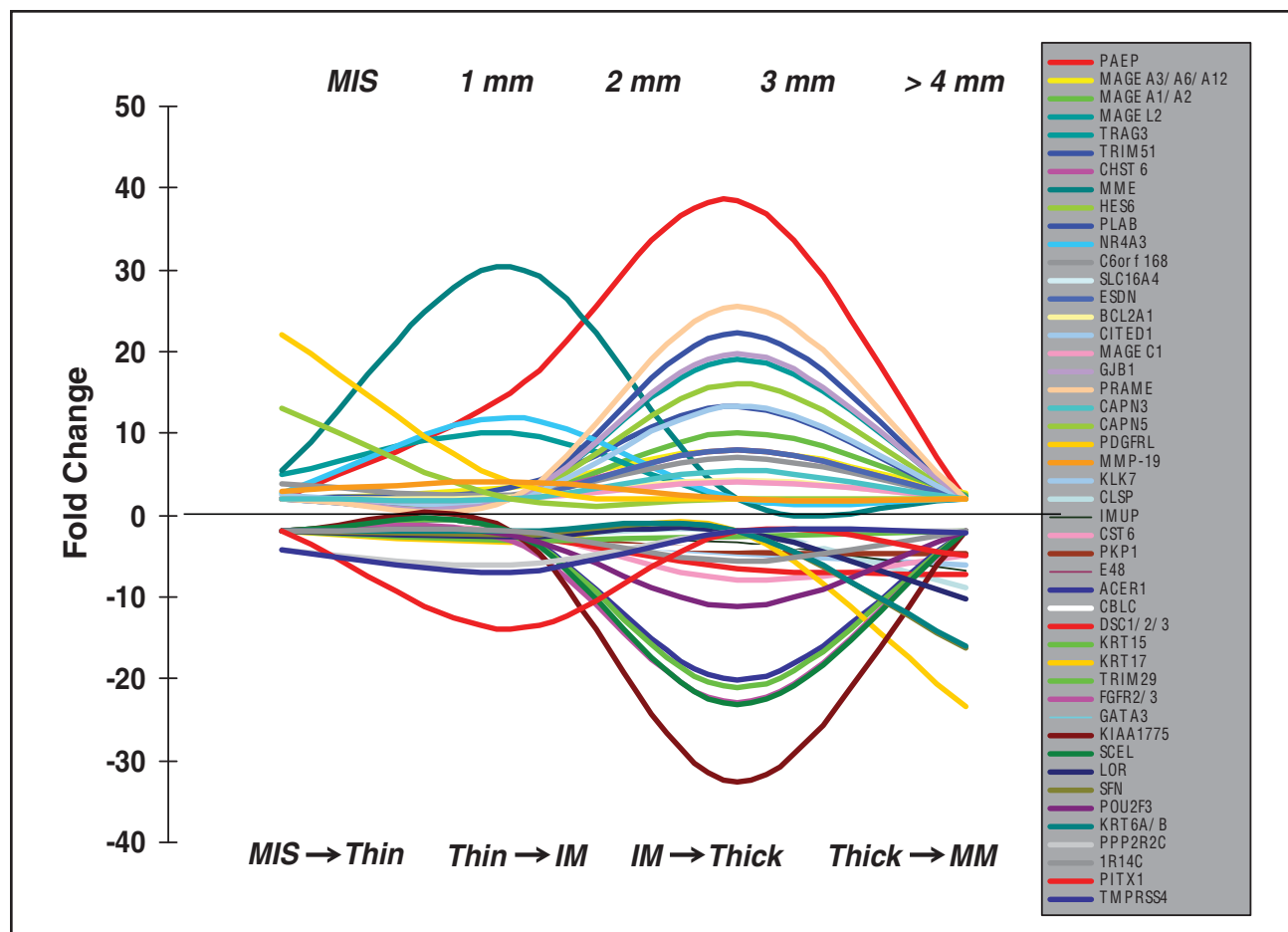


Figure. — Representation of the hypothetical dynamic and fluid nature of gene expression change within primary cutaneous melanomas of different tumor thicknesses. MIS = melanoma in situ, IM = intermediate, MM = metastatic melanoma.

gene analysis.^{6,7} For both over- and underexpressed gene comparisons, a comparison of specific genes yields similar trends in expression, although with differences noted in overall fold change. Some studies were unique in that there were no comparative studies that had performed the same gene expression profiling on samples.^{8,10} For example, Haqq et al⁸ performed several comparisons utilizing skin as the primary internal control for comparative analyses. Although several unique genes were identified when comparing skin to melanocytic nevi and radial vs VGP melanomas, we also identified several overlapping genes when similar comparisons were evaluated. For instance, we grouped the gene expression patterns of normal skin and early melanoma samples and compared them to more advanced primary melanomas and metastatic samples. We found several overexpressed genes, such as CD24, DUSP6, MMP10 and DSC2, to name just a few. Interestingly, they also note that they failed to identify the overexpression of genes in the transition to a VGP melanoma. Instead, this transition was solely accompanied by the loss of gene expression.

Several groups have shown distinct differences in the gene expression patterns along the spectrum of melanoma tumor progression, with many able to show a distinct set or group of over- and underexpressed genes that are validated as having a distinct and key role in this neoplastic process. Some have attempted to develop large gene classifier sets, composed of several hundreds, often thousands, of genes. For instance, the Melanoma Group of the European Organization for Research and Treatment of Cancer (EORTC) recently reported that melanoma patients with an average PCM thickness of 2.0 mm had a favorable prognosis, whereas patients in a second group with a thickness of 5.3 mm had an unfavorable prognosis.¹⁰ They were also able to identify a relatively small set of 254 genes capable of classifying the metastatic potential of a PCM. Comparative analysis with our resultant gene sets again revealed a large degree of concordance between specific genes identified within PCM of different thicknesses (Appendix 3).

Gene expression analysis by microarray has also greatly enhanced our understanding of gene regulation and signaling pathways involved in neoplasia. Nambiar et al³¹ examined the signaling networks in 10 samples of MM utilizing a cDNA microarray platform, finding several genes such as c-met, growth factor receptor-bound protein 10, BRAF, and many mitogen-activated protein kinase (MAPK) genes significantly upregulated in MM and numerous melanoma cell lines. Others have identified the transcription factor NF- κ B as a putative master regulator of melanoma invasion due to its binding sites identified as consistent consensus sequences within the promoter regions of genes associated with the invasive nature of melanoma cells.²⁹ Utilizing a 17,500-element cDNA microarray platform, Mandruzza et al³² examined 43 melanoma samples derived from

patients with stage III and IV melanoma. They identified a set of 70 genes associated with overall survival, from which 45 overexpressed genes were significantly associated with longer survival and 35 genes correlating with shorter survival times.

Thus, the current clinical and histopathological criteria in standard use today to define the prognosis of melanoma patients are largely inadequate for predicting the clinical outcome of melanoma patients. Unfortunately, we have been unsuccessful in utilizing gene expression profiling to accurately predict which group or individual patient should receive adjuvant therapy for stage III and IV disease and, furthermore, to predict which patients will actually respond to therapy based on their unique melanoma gene signature. The development of such a prognostic test is greatly anticipated as this will allow clinicians to base treatment decisions on improved predictors of response, as shown with gene microarray profiling of melanoma.

Epigenetic Mechanisms Involved in Human Melanoma

The irreversible changes that occur within the human DNA sequence, including chromosomal deletions, amplifications, and gene mutations, have all been implicated in the development and progression of melanoma. The biological insight gained from epigenomic profiling of DNA methylation and histone modification patterns in NHEM and melanoma cells are complementary to profiles of gene expression patterns and genetic alterations, providing novel prognostic and diagnostic markers as well as potential therapeutic targets. More importantly, the potential reversibility of epigenetic changes through pharmacological manipulation makes this area of research of particular interest for the future treatment options of cancer patients.

Recently, there has been a revitalized interest in the epigenetic mechanisms involved in melanoma progression, with evidence that the tumor microenvironment is capable of inducing the malignant transdifferentiation of NHEM through epigenetic mechanisms.³³ Furthermore, Seftor et al³³ showed that the microenvironment of melanoma cells induces marked alterations in the epigenetic and gene expression patterns of NHEM, leading to an aggressive melanoma-like cell phenotype. Thus, it is becoming clearer that epigenetic events remain at the heart of phenotypic variation in health and disease. Therefore, understanding and manipulating the epigenome hold enormous promise for preventing and treating patients with cancer.³⁴

The most common epigenetic phenomenon in malignancy appears to involve histone modification and DNA methylation. Although hypermethylation of human cancer is better described for other non-melanoma malignancies, such as myelodysplastic syndrome, there is growing evidence for the epigenetic

modification of genes in human melanoma.³⁵⁻³⁷ Human melanoma may involve global and gene-specific hypomethylation and hypermethylation, in addition to widespread chromatin modifications.³⁴ The epigenetic inactivation of individual tumor suppressor genes (TSGs) as a result of DNA hypermethylation has been shown in melanoma, identifying genes such as retinoic acid receptor beta-2 (RAR- β 2), found to have a high frequency of gene methylation in over 70% of all melanoma tissues examined.³⁶ Similarly, others have shown comparable levels of hypermethylation of suspected TSGs, such as Ras-association domain family 1 (RASSF1A, 55%), PYCARD (50%), O⁶-methylguanine DNA methyltransferase (MGMT, 34%), DAPK (19%), APC (19%), D-type p16INK4a cyclin-dependent kinase inhibitor (CDKN2A, 10%), and CDKN1B (9%).^{36,38-41}

Several recent advances in technology, such as epigenomic reactivation screening, restriction landmark genomic scanning (RLGS), differential methylation hybridization (DMH), methylated DNA immunoprecipitation (methyl-DIP) for DNA methylation profiling, and chromosomal immunoprecipitation (ChIP) combined with DNA arrays (ChIP-on-chip) for profiling histone modifications, have allowed the field of cancer epigenetics to be studied at a genome-wide level.⁴² We are readily finding more genes that are epigenetically modified in melanoma samples, highlighting the need for more research into the exact mechanisms of hypermethylation and its impact on gene function in melanoma progression.

To specifically identify CpG islands (CGIs) aberrantly methylated in melanoma, Furuta et al³⁷ carried out a genome-wide search using methylation-sensitive representational difference analysis. They found CGIs in putative promoter regions of 34 genes, with evidence of hypermethylation in a minority of cell lines (1 in 13) and freshly procured melanoma (3 in 36), with no evidence of methylation in cultured melanocytes. Among these genes, Peroxiredoxin 2 (PRDX2) gene expression was related to the level promoter methylation, further identifying 12 CGIs as candidate melanoma biomarkers due to their high rate of methylation in greater than 9 of the 13 examined melanoma cell lines. Upon demethylation, PRDX2 gene expression was restored, further showing that the level of PRDX2 gene methylation inversely correlated with the level of protein expression by immunohistochemical analysis of surgical melanoma specimens.

One of the most useful high-throughput approaches to identify epigenetically modulated genes is a reactivation screening strategy that combines treatment of cancer cells *in vitro* with a DNA methyltransferase and histone deacetylase (HDAC) inhibitor. This is followed by global gene microarray expression analysis in order to identify evidence for the reexpression and overexpression of previously inactivated genes. The key advantage to this approach is that it directly identifies genes in

which epigenetic changes lead to altered gene expression. Using this approach, Muthusamy et al³⁵ identified 17 genes (LXN, WFDC1, PCSK1, QPCT, COL1A2, DAL1, BST2, CDKN1C, LRRCC2, HOXB13, CYP1B1, MFAP, GDF15, PTGS2, CDH8, DNAJC15, SYK) that were not previously known to be silenced via hypermethylation of promoter regions in melanoma. Three of these genes, QPCT, CYP1B1, and LXN, were found to be densely methylated in more than 95% of uncultured melanoma tumor samples, thus making them promising markers for their successful induction and reactivation via demethylation for future melanoma clinical trials. Two other methylation-silenced genes, HOXB13 and SYK, demonstrated TSG function in melanoma.

Indeed, from our previous cDNA microarray analysis on clinical melanoma samples, we found several genes that are in common with this list. We are actively examining them further for evidence of epigenetic silencing via hypermethylation of promoter CGIs.⁴³ It is too early to know whether the epigenetic silencing of suspected TSGs is critical to the progression of melanoma. However, increasing data highlight the utility of gene microarray analysis for the identification and downregulation of specific genes involved in melanoma, allowing us to perform an in-depth analysis of key pathways and genes thought to be involved in this process. Hellebrekers et al⁴⁴ utilized gene expression profiling to decipher the potential epigenetic mechanisms of tumor neovascularization, finding that demethylating agents and HDAC inhibitors can directly repress endothelial cell growth and tumor angiogenesis. They performed microarray analysis to identify genes downregulated in tumor-conditioned vs quiescent endothelial cells, re-expressed after treatment with 5-aza-2'-deoxycytidine (DAC) and trichostatin A (TSA), a known HDAC inhibitor. Among the 81 genes identified, 77% harbored a promoter CpG island, with silencing of these genes in tumor-conditioned endothelial cells correlating with promoter histone H3 deacetylation and loss of H3 lysine 4 methylation.

On the whole, epigenetic profiling of melanoma is still in its infancy, posing an enormous challenge for researchers and clinicians. However, it will have tremendous potential for future investigations and clinical studies in melanoma patients. To date, more than 50 genes have been reported as being altered and nonfunctional secondary to aberrant DNA methylation in melanoma. There is also growing evidence suggesting that certain histone modifications and associated altered chromatin remodeling activities can play a key role in melanoma tumor progression.⁴⁵ These epigenetic events are involved in different aspects of tumorigenesis including cell cycle control, apoptosis, cell signaling, tumor cell invasion and metastasis, drug resistance, and immune recognition. These findings suggest a promising future for the utility of drugs such as DAC and HDAC inhibitors, including suberoylanilide hydroxamic acid

(SAHA), in treating patients with evidence of TSG hypermethylation or histone deacetylation.

One striking example of the rapid development of technology focused on epigenetic mechanisms of cancer has been the utility of DNA-based markers for clinical application. Compared to total RNA, DNA is robust, survives without much overall degradation, and can be readily amplified by powerful polymerase chain reaction (PCR)-based techniques.⁴⁶ This DNA-based approach forms the basis of a sensitive, specific, robust, and informative test for cancer diagnostics. Indeed, as more genes are being identified, several studies have investigated the potential utility of quantitative, methylation-specific PCR (q-mSPCR) for gene methylation analysis of clinical samples.^{47,48} We recently examined a panel of previously identified genes found to be hypermethylated in melanoma for utility as potential prognostic markers of tumor progression (RASSF1A, RAR- β 2, MGMT, SOCS1, DR4, and CDKN2A). We found that the detection of hypermethylated DNA derived from TSGs in melanoma cell lines derived from patients may contribute to a more sensitive classification system for melanoma.⁴³ These preliminary studies suggest that the development of a methylation-specific qRT-PCR or microarray assay may be useful not only to identify potential gene targets for therapy, but also to improve prognostic information based on the level of TSG hypermethylation in melanoma samples.

Epigenetic alterations in cancer cells affect virtually every cellular pathway involved in cell cycle progression, apoptosis, and cell survival, and it is therefore not surprising that “epigenetic drugs” display pleiotropic activities.⁴⁹ At present, two DNA methylation inhibitors have been approved for human use in the United States for the treatment of patients with myelodysplastic syndrome, while there is a single HDAC inhibitor approved for patients with cutaneous T-cell lymphoma. As we identify new genes and improve our current understanding of the epigenetic mechanisms involved in melanoma, we foresee the development of an array-based DNA assay able to identify a panel of suspected hypermethylated genes within a freshly procured melanoma sample. In doing so, we can then modulate these suspected genes for treatment, possibly treating the patient with a demethylating agent, an HDAC inhibitor, or both.⁵⁰⁻⁵²

The Role of MicroRNAs in Melanoma

MicroRNAs (miRNAs) are naturally occurring single-stranded, noncoding sequences of RNA that mediate gene expression at the posttranscriptional and translational level in both plants and animals.^{53,54} Due to their central role in gene regulation and expression, miRNAs have been shown to play key roles in tumor cell development, differentiation, proliferation, survival, and apoptosis.⁵⁵⁻⁵⁸ It has been suggested that certain miRNAs may assert their function as oncogenes or TSGs. How-

ever, the matter is far more complex than originally thought due to the finding that any single miRNA is capable of mediating the expression of several hundred messenger RNA transcripts. Thus, we speculate that to a large extent, the primary function of miRNAs is to “fine tune” gene expression in response to acute changes in growth conditions rather than function as a classic tumor suppressor gene or oncogene.

Over the past few years, posttranscriptional and translational controls regulated by naturally occurring noncoding RNAs have emerged as an exciting and vibrant field of research, especially in cancer. Translational control mediated by miRNAs provides the cell with a more precise, immediate, and energy-efficient method of controlling the gene expression and transcription of proteins. Rapid changes in protein synthesis can occur with miRNAs without the need for transcriptional activation and subsequent mRNA processing steps. Additionally, miRNA-induced translational control provides for a greater flexibility in cellular response to various cytotoxic stresses. The main function of miRNAs is to repress gene expression at the translational level via binding to the 3'-untranslated region (3'-UTR) of the messenger RNA. Although the exact function for most of the newly discovered miRNAs is still relatively unknown, their ability to regulate cellular proliferation and death has recently been shown.⁵⁹

The first evidence that miRNAs may function as TSGs came from a study by Calin et al⁶⁰ showing that patients with chronic lymphocytic leukemia (CLL) have frequent gene deletions with associated downregulation of two miRNA genes, hsa-miR-15a and hsa-miR-16-1. Cimmino et al⁶¹ and others^{56,62,63} showed that the antiapoptotic gene BCL2 was negatively regulated by hsa-miR-15a and hsa-miR-16-1, suggesting that their deletion or downregulation resulted in an elevated level of BCL2, thus promoting leukemogenesis and lymphomagenesis in hematopoietic cells. Xi et al⁶⁴ were the first to report that miRNAs are a part of the p53 tumor suppressor network, with several recent studies⁶⁵⁻⁶⁷ also confirming the central role of miRNAs in several interrelated pathways of the p53 TSG.

Currently, there is limited information as to the exact role of miRNAs in the pathogenesis of melanoma. One of the first studies of miRNA in melanoma is from Zhang et al,⁶⁸ who utilized a high resolution gene array-based platform to analyze 283 known human miRNA genes in 227 human cancer specimens, including 45 primary cultured melanoma cell lines. They found that 86% of primary melanoma cell lines had DNA copy number alterations in genomic loci containing miRNA genes. They also identified 243 miRNA genes (83 with gains vs 160 with losses) that were unique to melanoma, as well as 41 miRNA genes (26 with gains vs 15 with losses) with gene copy number changes that were shared among breast cancer, ovarian cancer, and melanoma.

Appendix 1. — Genes Overexpressed in the Comparative Analysis of Human Melanoma

Skin → Nevus ⁹	Skin/Nevi → PCM ⁹ and Nervi → PCM ⁸	Skin/Nevi/MIS → VGP/MM ^{1,7}	NHEM → Thin ¹	ARHGFB ³	GAS7 ⁶	NRP1 ⁶	STK (6, 17A) ⁶	C16orf34 ⁴	MERTK ¹¹	PCM → MM ^{1,11}
BM039 ⁹	HEY1 ⁹	RFC3 ⁸	PEG10 ⁷	ICEBERG ⁷						
BUB1 ⁸	HOXB7 ⁹	RGS1 ⁸	PHACTR1 ⁷	KRT(6A, 6B, 14, 15, DAP) ⁷	GJ (A1, B6) ⁷	OA48-18 ⁶	SULF1 ⁶	C6orf168 ⁷	MET ¹¹	TRIM51 ⁷
C10orf3 ⁸	HSP (105B ⁹ , H1 ⁹)	RNF2 ⁸	PHLDA1 ⁷	LOR ⁷	GPM6A ⁶	PCDH7 ⁶	THBS1 ⁶	CDC (6 ¹¹ , 45L ¹)	MME ^{1,11}	TSPY ⁷
C1QB ⁸	IF16 ⁸	RPA3 ⁸	PRAME ⁷	PKP1 ⁷	GPR (56, 126) ⁶	PDGFC ⁶	TM4SF (1 ⁶ , 10 ⁷ , 13/NET-6 ⁶)	CDH (2, 19) ¹¹	MMP (1, 16) ¹¹ , (8, 12, 14, 19) ⁷	
C6orf173 ⁸	IL (13RA2 ⁸ , 16 ⁹)	S100A12 ⁸	CITED1 ^{7,7}	PPL ⁷	HEY1 ⁶	PEG10 ⁶	TNC ⁶	CDK2 ⁷	MYOZ2 ⁷	
C7orf (30 ⁸ , 32 ⁹)	ISG20 ⁸	SAMSN1 ⁸	RGS20 ⁷	S100 (A7, A9) ⁷	HLA-D (MA, OB1, RA ⁷ , RB1 ⁷ , RB4) ⁶ , (QA1, OA2, RB3) ⁷	PHLDA1 ⁶		CEACAM1 ¹¹	NCBP2 ¹¹	
CA14 ⁸	ITGA3 ⁹	SEMA3B ⁹	SCG2 ⁷	SCEL ⁷	HMGA2 ⁶	PKP4 ⁶	TPM 1 ⁶ , TRAG3 ^{3,6} , TRIM (2,9) ⁶	CENPF ¹¹	NR4A3 ⁷	
CAPG ⁹	KIF23 ⁸	SERPINH1 ⁸	SLC16A4 ⁷	SERPINB3 ⁷	HSPG2 ⁶	PLAT ⁶		CHST6 ⁷	NUDT9 ¹¹	
CAPN8 ⁸	KIT ⁹	SLC (7A5, 12A4) ⁸	SORT1 ⁷	CEACAM1 ⁶	ID1 ⁶	PLAUR ⁶		CITED1 ⁷	PAEP ⁷	
CCL (2, 3, 3L1) ⁸	KPNA2 ⁸	SNRPG ⁸	SOX5 ⁷	CEBPD ⁶	IGF1 ⁶	PLOD2 ⁶	TUBB ⁶	CKMT1 ¹¹	PCDH7 ¹¹	
CCNB2 ⁹	KRT18 ⁸	SORL1 ⁸	SPP1 ^{7,7} STAR D3NL ⁷	CHST6 ⁶	IGFBP (2, 3, 5, 6) ⁶	PRAME ^{7,6}		CKS2 ¹¹ CLEC3B ¹¹	PDGFR ⁷	
CD81 ⁸	KYNU ⁸	SPP1 ^{8,9}	EDN3 ⁷	CITED2 ⁶	IGSF4 ⁶	PTGS2 ⁶		PEG10 ⁷		
CDC (2, 2RPK ⁹ , 25B, A2) ⁸	LGALS1 ⁹	SSR1 ⁸	ESDN ⁷	CKMT1 ⁶	IL1 (B, RAP) ⁶	PTPR (F, Z1) ⁶		COL1A1 ¹¹	PHLDA1 ⁷	
CDH3 ⁸	LPXN ⁸	TBC1D7 ⁸	FCGR1A ⁷	CLCA2 ⁷	IL18 ⁷	PYGB ⁶		CSAG2 ^{7,11}	PLOD2 ¹¹	
CENPE ⁸	LYPD1 ⁸	TMEM (45B, 49, 158) ⁸	FKBP10 ⁷	COL (4A5, 5A1, 6A1, 6A2, 6A3 ⁷ , 9A3, 13A1, 16A1) ⁶	INHBA ⁶	RGS (4 ⁷ , 5 ⁶)	VEGF ⁶	CST1 ⁷	PRAME ⁷	
CENPF ⁸	MAD2L1 ⁸	TNC ⁸	G1P (2, 3) ⁷	CST7 ⁶	ISG20 ⁶	RNF128 ⁶	ZIC1 ⁷	CTAG (1, 2) ⁷	PTPRF ¹¹	

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Appendix 1. — Genes Overexpressed in the Comparative Analysis of Human Melanoma

Skin → Nevus ⁸	Skin/Nevi → PCM ⁹ and Nevi → PCM ⁸		Skin/Nevi/MIS → VGP/MM ^{10,11,12}	NHEM → Thin ⁷	NHEM → MM ^{7,8}		PCM → MM ¹¹	
CHST6 ⁹	MAP3K12 ⁹	TOP2A ⁸	GAGE (4, 8, B1, D2) ⁷	CTAG (1A, 1B, 2) ⁷	ITGA (4, 6, 7) ⁶	CTNNA1 ¹¹	RGS20 ¹¹	
CITED1 ^{8,9}	MCM4 ⁸ MMP	TOPK ⁸	GDF15 ^{10,11,12}	CTSH ⁶	ITGB (3, L1) ⁶	DDAH1 ¹¹	RRM2 ¹¹	
CKS2 ⁸	(10 ⁸ , 11 ⁹) MTHF	TRIM19 ⁸	GJB1 ⁷	CXCL (1 ⁶ , 14) ⁷	ITPR (1, 3) ⁶	DUSP (4, 6) ⁷	S100A1 ¹¹	
COX5A ⁹	(D2, S) ⁸ NEK2 ⁸	TRIP13 ⁸	GPR (19 ⁷ , 158) ⁷	DDAH1 ⁶	KCNK4 ⁶	EDN3 ⁷	SCHIP1 ¹¹	
CRYBB2 ⁹		TYR ⁸	HEY1 ⁷ HOX	DLG1 ⁶	KLF2 ⁶	ENPP1 ¹¹	SEMA3F ¹¹	
CTNNA2 ⁹	NTRK3 ⁹	UBE2 (C, W) ⁸	(A3, A10, B7, B13, D13) ¹⁰	DSCR1 ⁶	KRT (14, 18 ⁶ , DAP) ⁷	ESDN ⁷	SERPINE2 ¹¹	
CTS (B, H) ⁹ , Z ⁸	OA1 ⁹	UBPH ⁸	KDEL3 ⁷	DSP ⁶	KYNU ⁶	ETV1 ¹¹	SMARCA3 ¹¹	
CXCL (1, 9, 10) ⁸	PAEP ⁸	UPP1 ⁸	KHDRBS3 ⁷	DUSP6 ⁶	LAM (A4, P2) ⁶	FKBP10 ⁷	SMC4L1 ¹¹	
D4S234E ⁸	PHACTR1 ⁹	WFDC1 ⁹	KIF (23) ⁷ , (C1) ⁷	EFEEMP1 ⁶	LPXN ⁶	FN1 ¹¹	SNAP25 ¹¹	
DHFR ⁸	PLAT ⁸	WIPI (1 ⁹ , 49 ⁹)	LY96 ⁷	EHF ⁷	LUM ⁶	GAGE (4, 8, B1, D2) ⁷	SOX5 ⁷	
DUSP4 ⁹	PLAUR ⁸	ZIC1 ⁸	MAD2L1 ⁷	ENPP1 ⁶	MADH7/ SMAD7 ⁶	GAS1 ¹¹	SPPT ¹¹	
ETV6- NTRK3 ⁹	PLOD3 ⁹	ZWINT ⁸	MAGE [A (1, 2, 3, 5, 6, 12), C1, L2] ⁷	EPHA3 ^{7,6}	MAFF ⁶	GDF15 ⁷	SPRR1A ¹¹	
FAM26F ⁸	PLP1 ⁹		MGC29643 ⁷	ESDN ⁶	MAGE [A (3, 4, 6, 9), C1 ⁶ , C2] ⁷	H2AFY1 ¹¹	SPRY2 ¹¹	

This appendix provides a list of identified genes that are common between our data (Riker et al^{10,11}) and recent published literature (Hoek et al,⁶ Smith et al,⁷ Haqq et al,⁸ Talantov et al,⁹ and Jaeger et al¹¹). These genes were found to be overexpressed throughout all comparisons. The common gene list was compiled by cross-referencing data sets from each group, showing only those genes with expression changes of > 2-fold, as measured by cDNA microarray analysis.

** represents a comparison of thin PCM (MIS and thin) to MM. PCM encompasses the full spectrum of primary samples (thin to thick). A single, advanced, intermediate-thickness primary tumor (Hoek et al⁶) was incorporated into the comparative data set of NHEM to MM, with all other samples representative of MM.

Appendix 2. — Genes Underexpressed in the Comparative Analysis of Human Melanoma

Skin/Nevi/MIS/RGP → VGP/MM ^{†,*,8,7}	NHEM → MM ^{†,6}		Nevus → PCM ⁸		PCM → MM ^{†,8,11}		PCM → MMI ^{†,8}		RGP → VGP ⁸
	NHEM → Thin [†]								
IVL [*]									
KCNK7	FCGR2 ⁶	SERPIN (F1, G1, I1) ⁶ SLC (5A4, 7A8, 12A7, 22A1L) ⁶	PI16 ⁶	COL (4A6, 7A1, 17A18) ¹¹	LAD ¹¹	SPINT2 ¹¹		UBPH ⁶	
KLK7	FKBP1B ⁶	SNAP (25, C3) ⁶	PLEKHA1 ⁶	CST (A, 6) ¹¹	LAM (A3, B3, C2) ¹¹	SPRR1 (A, B) [*]			
KRT (1B, 6IRS, 6L, 15) ⁷ , (6A, 6B, 16, 17) [*] LGALS7 [*]	FSTL1 ⁶		PTPR (B, F) ⁹	CTNN (BIP1, D1) ¹¹	LGALS7 ¹¹	STAR [*]			
	G1P (2, 3) ⁶	SIPA17 ⁶	PYGL ⁹	CTSG ¹¹	LOR ¹¹	TACSTD2 ¹¹			
	GMMP ⁶	SSX (2, 4) ⁶	RANBP5 ⁸	CXCL14 ¹¹	LTB4R [*]	THBD ¹¹			
	GST (P1, Z1) ⁶	STAT1 ⁶	SEMA3G ⁸	CYP26B1 ¹¹	LY6 (D ¹¹ , G6C ⁹) LYPD3 ¹¹	TM4SF1 ¹¹			
	HLA-E ⁶	TBC1D16 ⁶	SLC12A2 ⁸	DPP6 ¹¹	MAF ¹¹	TMEM45A ¹¹			
	HMGCL ⁶	TPM (2, 4) ⁶	TMEM47 ⁸	DSC (1, 2, 3) ¹¹	MAL ¹¹	TP73 ^{†,8,11}			
	HOX	TRIM (7) ⁶	TRIM9 ⁸	DSG (1, 3) ¹¹	MAP7 ¹¹	TRIM (21, 29) ^{8,11}			
	(B2, B7, B13, D1) ⁶	(14, 22, 34) ⁶	WIF1 ⁸	DSP ^{8,11}	MAPK13 ¹¹	TUBA1 ¹¹			
	HPS1 ⁶	TRPM (1 ⁶ , 4)		DST ^{8,11}	MET [*]	TXNIP ¹¹			
	HSPA1A ⁶	TSPYL5 ⁶		DUSP7 ¹¹	MMP28 ¹¹	TYRP1 ¹¹			
	ID2 ⁶	TYR ⁶		EGFR ¹¹	MYO6 ¹¹	WFDC (1, 5) [*]			
	IFI (27, 35, 44, T1, T2, T4, TM2) ⁶	UBE (1L, 2L6) ⁶		EHF ¹¹	NFIB ¹¹	ZNF185 ¹¹			
	IL (1R1, 24, KAP) ⁶	VAMP (5, 8) ⁶		ELOVL4 [*]	NRCAM ¹¹				
	KLF8 [*]	WARS ⁶		ENPP2 ¹¹	NTRK2 ¹¹				
	MAFB ⁶	WFDC1 ⁶		EPHB6 ¹¹	PCDH21 ¹¹				
	MAP4 [*]			EPPK1 ¹¹					
	MERTK ⁶								

This appendix provides a list of identified genes that are common between our data (Riker et al.^{9,10}) and recent published literature (Hoek et al.⁶ Smith et al.⁷ Haqq et al.⁸ and Jaeger et al.¹¹). These genes were found to be underexpressed throughout all comparisons. The common gene list was compiled in a similar manner as in Appendix 1.

[†] MMI is type I MMI described by Haqq et al.⁸ with genes revealing contrasting expression patterns between two separate data sets on the same tissue samples (↓[†]); ** represents a comparison of thin PCM (MIS and thin) to MM. PCM encompasses the full spectrum of primary samples (thin to thick).

Appendix 3. — Comparative Gene Expression in PCM

Genes Overexpressed					
MIS → Thin*	MIS → IM*	Thin → IM*		IM → Thick* ¹¹	
C16orf34*	CITED1*	MAGE (A2, A3, A6, L2)*	AKT3*	CSAG2*	PEG10*
CAPN5*	PDGFRL*	MME*	BCL2A1*	DUSP4*	PRAME*
GPR19*			BUB1*	ESDN*	RGS20*
MAGE L2*		MMP19*	C6orf168*	GDF15*	SLC16A4*
MME*		NR4A3*	CAPN3*	GJB1*	SOX5*
MMP19*		PAEP*	CDC45L*	HEY1*	TRAG3*
PDGFRL*		SPP1*	CDK2*	KIFC1*	TRIM51*
				MAGE (A1, A2, A3, A6, A12, C1)*	TYMS*
			CHST6*	PAEP*	UCHL1 ¹¹
			CITED1*		
MIS/Thin → Thick* and Thin → Thick ¹⁰					
ATAD2 ¹⁰	CENP (A, F) ¹⁰	H2AFV ¹⁰	NEK2 ¹⁰	RAD64L ¹⁰	TOP2A ¹⁰
BCL2A1*	CHST6*	HCAP-G ¹⁰	NR4A3*	RAN ¹⁰	TPM4 ¹⁰
BIRC5 ¹⁰	CITED1*	HSP (A4, A5, D1) ¹⁰	NUDT4 ¹⁰	RANBP (1, 7) ¹⁰	TRAG3*
BM039 ¹⁰	CKS (1B, 2) ¹⁰	KDELRL2 ¹⁰	PAEP*	RFC (4, 5) ¹⁰	TRIM51*
BUB1 ¹⁰	CREM ¹⁰	KIF (2C, 11) ¹⁰	PARK2 ¹⁰	RPA3 ¹⁰	TYMS ¹⁰
C10orf3 ¹⁰	DHFR ¹⁰	KPNA2 ¹⁰	PCDH17 ¹⁰	RRM2 ¹⁰	UBPH ¹⁰
C6orf168*	DLG7 ¹⁰	MAGE [A (1, 2, 3, 6, 12), (C1, L2)]*	PDGFB ¹⁰	SLC16A4*	ZNF (367, 587) ¹⁰
CCNB (1, 2) ¹⁰	ESDN*	MAP4K4 ¹⁰	PLEKHC1 ¹⁰	SMARCA5 ¹⁰	ZWINT ¹⁰
CCT (4, 5, 7) ¹⁰	FAM33A ¹⁰	MCM (4, 6) ¹⁰	PLOD2 ¹⁰	SMC2L1 ¹⁰	
CDC (2, 6, 45L) ¹⁰	GDF15*	MME*	PRAME*	SNRPG ¹⁰	
CDC4 (1, 5, 8) ¹⁰	GJ (A7 ¹⁰ , B1*)	MRPL (30, 32) ¹⁰	PRSS25 ¹⁰	SPAG5 ¹⁰	
CDKN3 ¹⁰	GMPS ¹⁰	MRPS (5, 10, 17) ¹⁰	PTTG (1, 2) ¹⁰	STK6 ¹⁰	
Genes Underexpressed					
MIS → Thin*	Thin → IM*	IM → Thick*		Thick → MM*	
LTB4R*	PPP2R2C*	ASAH3*	KLK7*	C19orf33*	KRT (6A, 6B, 16, 17)* LGALS7*
PPP2R2C*		C19orf33*	KRT15*	CD24*	LOR*
STAR*		CST6*	PKP1*	CST6*	PKP1*
		DSC (1,3)*	POU2F3*	DSC2*	RAB25*
		ELOVL4*	PPP1R14C*	FLG*	
		FGFR (2, 3)*	RORA*	ICEBERG*	SFN*
		GATA3*	SCEL*	IVL*	SPRR1 (A, B)*
		ICEBERG*	TP73L*	KLK7*	
MIS/Thin → Thick* and Thin → Thick ¹⁰					
ALDH3A2 ¹⁰	CRY2 ¹⁰	F10 ¹⁰	KLK7*	PKP1*	SPINT2 ¹⁰
CA5 ¹⁰	CST (3, 5) ¹⁰ , (6)*	FGFR (2, 3)*	KRT (15*, AP19-1 ¹⁰)	POU2F3*	TRIM29*
CD1 (A, C, E) ¹⁰	CTNNBIP1 ¹⁰	GATA3*	LGALS2 ¹⁰	PPP (2R2C, 1R14C)*	TXNIP ¹⁰
CEBPA ¹⁰	CXCL14 ¹⁰	GPR105 ¹⁰	LOR*	PTGDS ¹⁰	
CLEC SF14 ¹⁰	DHRS1 ¹⁰	HLA-DQB1 ¹⁰	LTB ¹⁰	RNF125 ¹⁰	
CLIC3 ¹⁰	DSC (1, 3)*	HOXA9 ¹⁰	PI16 ¹⁰	SCEL*	

This appendix represents a comparison of PCM samples only (no MM samples), highlighting the gene expression differences between PCM of varying Breslow's tumor thickness (Riker et al^{30*}, Winnepenninckx et al¹⁰, Jaeger et al¹¹). The 254-gene classifier set of Winnepenninckx was used. Genes in bold type are described more than once and exhibit the greatest fold change between that comparative group, with a > 5-fold change relative to all other listings of the gene in this appendix.

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