

# IDENTIFYING MELANOMA RELATED GENES USING PUBLISHED MICROARRAY GENE EXPRESSION DATA



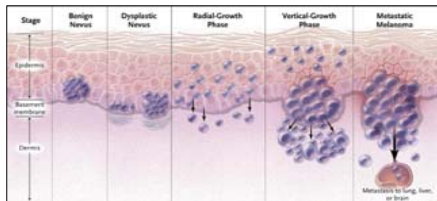
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## INTRODUCTION

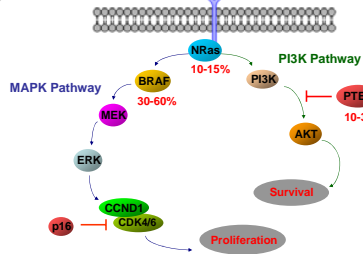
Melanoma is the deadliest form of skin cancer. Though it accounts for only 4% of all dermatological neoplasms, it is responsible for more than 75% of all skin cancer-associated deaths<sup>1</sup>. Given its poor advanced-stage prognosis, and considering that there are more than 53,000 annual melanoma diagnoses in the United States alone<sup>2</sup>, there is still much about this disease that needs to be elucidated. It is known, however, that melanoma follows a distinct step-wise progression that ultimately allows for the metastasis of the tumor cells.

Figure 1. Clark model of melanoma progression<sup>3</sup>



Of the mechanisms involved in cellular function, the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K) signaling pathways are the two most important pathways that are significantly altered in melanoma. Of the key genetic lesions that have come to characterize melanoma onset, the activating V600E mutation in BRAF is the earliest and most prevalent, with 30-60% of all melanoma samples containing the genetic change. With this, however, recent literature has shown that BRAF mutations trigger only initial proliferation and is insufficient for full malignant conversion. A BRAF mutation alone results in senescence, a state in which the tumor cells no longer respond to growth factors and do not divide.

Figure 2. Pathways involved in melanoma progression



This has led to the hypothesis that other genetic alterations must be responsible for further penetrance and metastasis of melanoma. It is with this idea that research into the inactivation of the PTEN tumor suppressor has become so crucial. A mutation in PTEN would result in the increase of tumor cell survival and possibly further progression of the melanoma past its initial proliferative stages.

## OBJECTIVES

- Detect genetic signatures in cell lines that result from the activation of both the MAPK and PI3K pathways.
- Use melanoma microarray data to determine the differentially regulated genes downstream of the PI3K pathway that may be candidate therapeutic targets for preventing melanoma progression.

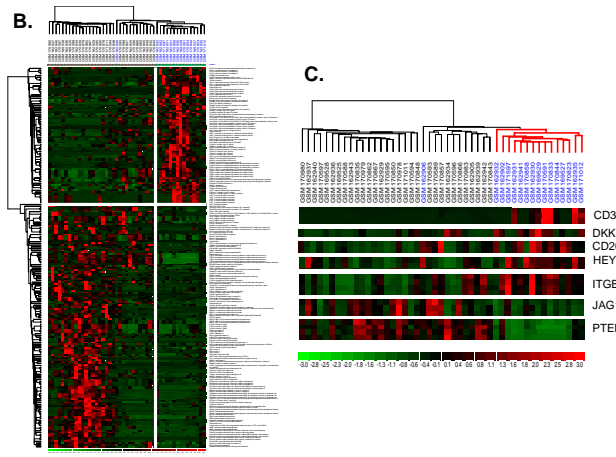
## SAMPLE CHARACTERISTICS

MAPK/PI3K Pathway Mutation	Sample Number	Phenotype
none	8	wildtype
BRAF	31	MAPK pathway upregulated
NRAS	8	MAPK and PI3K pathways upregulated
BRAF/PTEN	16	MAPK and PI3K pathways upregulated
<b>63 TOTAL SAMPLES</b>		

## RESULTS

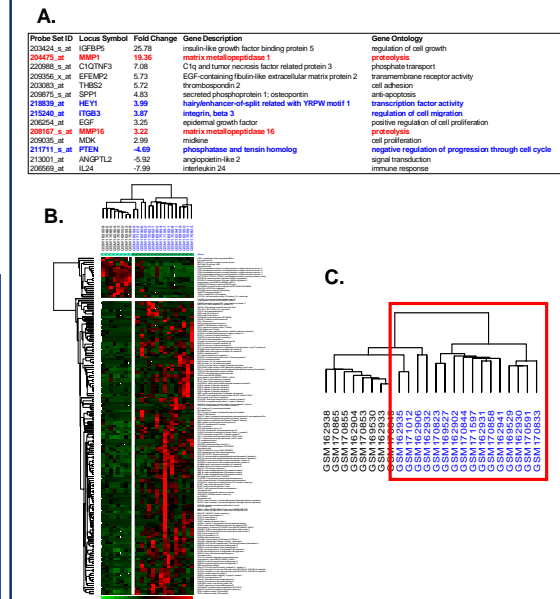
Figure 3. CD36, DKK1, CD200, ITGB3, HEY1, and JAG1 are downstream of the PI3K pathway

Probe Set ID	Locus Symbol	Fold Change	Gene Description	Gene Ontology
209169_s_at	CD36	27.12	CD36 molecule (thrombospondin receptor)	receptor activity
225218_s_at	COL1A2	8	collagen, type I, alpha 2	transmembrane receptor protein tyrosine kinase signaling pathway
234242_s_at	EPHB1	4.74	EPH receptor B1	transmembrane receptor protein tyrosine kinase signaling pathway
204602_s_at	DKK1	2.85	Dickkopf homolog 1	signal transducer activity
223434_s_at	GBP3	2.73	guanylate binding protein 3	immune response
221577_s_at	GBP1	2.68	guanylate binding protein 1	immune response
209582_s_at	CD200	2.56	CD200 molecule	integral to plasma membrane
204626_s_at	ITGB3	2.34	integrin, beta 3	regulation of cell migration
44783_s_at	HEY1	2.3	hairy/enhancer-of-split related with YRPW motif 1	transcription factor activity
218874_s_at	SLC12A8	2.26	solute carrier family 12 (potassium/chloride transporter), member 8	integral to plasma membrane
203208_s_at	KIF18A	2.02	kinase-like growth factor 1 receptor	positive regulation of cell proliferation
206376_s_at	SLC6A15	-2.16	solute carrier family 6, member 15	ionogenesis, cell fate determination
214268_s_at	JAG1	0.2	Jagged 1	transmembrane receptor protein tyrosine kinase signaling pathway
203269_s_at	LCP2	-2.87	lymphocyte cytosolic protein 2	transmembrane receptor protein tyrosine kinase signaling pathway
211326_s_at	PTPRN1	-2.94	protein tyrosine phosphatase, receptor type U	transmembrane receptor protein tyrosine phosphatase signaling pathway
307038_s_at	SLC15A6	-3.56	solute carrier family 15, member 6	integral to plasma membrane
203435_s_at	MME	-4.23	membrane metallo-endopeptidase	cell-cell signaling
204003_s_at	PTEN	-4.38	phosphatase and tensin homolog	negative regulation of progression through cell cycle
210083_s_at	NRTN	-4.98	neurturin	neurotrophin development
203028_s_at	MERTK	-5.22	mer tyrosine kinase	cell-cell signaling
209881_s_at	LAT	-5.37	linker for activation of T cells	immune response
205051_s_at	KIT	-9.35	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	signal transduction



Figures 3 and 4 represent analyzed microarray data comparing melanoma cell lines with BRAF/PTEN double mutations to those with only BRAF or NRAS mutations, respectively. (A) Select genes that are differentially regulated between the compared groups. The fold change represents gene expression in the double mutant cell lines compared to that in the single mutant cell lines. Genes highlighted in red have been directly linked to melanoma in previous literature, while genes in blue are common among the two lists. (B) Hierarchical clustering of the melanoma samples based on similarities in gene expression. (C) Tight clustering of BRAF/PTEN double mutants.

Figure 4. Differential gene expression patterns distinguish BRAF/PTEN mutants from NRAS mutants



## CONCLUSIONS

- 131 genes are differentially regulated in melanoma cell lines with BRAF/PTEN double mutations when compared to those with BRAF mutations alone.

46 genes are upregulated  
85 genes are downregulated

- 107 genes are differentially regulated in BRAF/PTEN double mutants when compared to NRAS mutants.

95 genes are upregulated  
12 genes are downregulated

- CD36, DKK1, CD200, ITGB3, HEY1, and JAG1 are downstream of the PI3K pathway and may synergize with BRAF activating mutations to promote melanoma progression

- The genetic basis of melanoma is not linear

- Potential therapy cannot target a single pathway



Figure 5. Melanoma Signaling Network<sup>4</sup>

## FUTURE DIRECTIONS

- Validate microarray data with RT-PCR

- Visualize gene expression at the protein level with western blots and immunohistochemistry

- Use specific drug inhibitors or shRNAs to inhibit the PI3K pathway and observe the resulting gene expression patterns

## REFERENCES

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