Wnt signaling in age-related Transcriptome Changes in Sentinel Lymph Node and Their Association with Recurrence in Node-Positive Melanoma Patients

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Abstract

Background: Age is an important risk factor for melanoma. Older patients have a greater risk of melanoma mortality, yet a lower incidence of sentinel lymph node (SLN) metastasis. This highlights the needs for further studies into aged-related SLN biology. Understanding the gene expression profile of the SLN may be the first step to direct more effective treatment strategies in aged melanoma patients.

Objective: Our objective was to identify changes in SLN gene expression related to age and melanoma recurrence.

Methods: Three independent sets of RNA samples from patients with melanoma metastasic to the SLN were used in this study. The first dataset included 97 melanoma patients selected from the Sunbelt Melanoma Trial. Total RNA from these SLN samples was used for microarray experiments. A multivariate linear regression model was fitted for each gene of each sample about age (<60 years vs. ≥60), disease status (recurrence vs. no recurrence), and interaction of age and disease status. Differential expressed genes (DEGs) with a *p*-value of less than 0.05 were identified on age, recurrence status and the interaction of age and recurrence status. The second dataset of RNA samples includes 12 patients chosen form the Brown Cancer Center Bio-Repository at the University of Louisville. NanoString was performed to identify the immune genes and pathways that differentiate the recurrence and non-recurrence in the younger and older groups. nSolver software was used to differentiate the genes with a p<0.05. The third dataset of 36 samples from the Brown Cancer Center Bio-Repository was used for RT-PCR analysis to validate the results from microarray and NanoString data.

Results: We showed that FOS, NR4A2, PTGS2, and LINC00518 were all upregulated in recurrence vs. the non-recurrence group. However, the older group had a much higher fold change (FC) than that of the younger group (FC>4 vs. FC>2, $p<0.05$). IL1B was downregulated in the younger group, but was upregulated in the older group when comparing recurrence vs. non-recurrence. The expression of one of the Wnt pathway genes, Wnt10b, was upregulated 4 fold in the younger group, but was upregulated only 1 fold in the older group when comparing recurrence versus non-recurrence ($p<0.05$). The expressions of all those genes were confirmed by RT-PCR. All of these significant genes converged at Wnt signaling pathway.

Conclusions: The Wnt pathway, specifically Wnt10b, is a major pathway associated with melanoma recurrence in older patients. Further research is ongoing to define the mechanisms by which these differentially expressed genes may predispose older patients to nodal metastasis. These findings may lead to development of new therapeutic targets and strategies.

Introduction

- Age: an important risk factor for melanoma
- Older patients: a greater risk of melanoma mortality, yet a lower incidence of SLN metastasis.
- Understanding the gene expression profile of the SLN may be the first step to direct more effective treatment strategies in aged melanoma patients.
- Objective: To identify changes in SLN gene expression related to age and melanoma recurrence.

Methods

RNAs isolated from SLNs from node(+) melanoma patients

→ Microarray

→ Nanostring

Age	Group (Disease Status)	Sample No. from Sunbelt Melanoma Trial	Sample No. from BCC Biorepository
<60 years old	Recur	28	3
	Non-recur	51	3
≥60 years old	Recur	11	3
	Non-recur	7	3

multivariate linear regression model (Gene expression =$\alpha+\beta_1$Age+ β_2Group+ β_3Age*Group)

nSolver software analysis

Significant genes

PCR confirmation

Age	Group (Disease Status)	Sample No. from BCC Biorepository
<60 years old	Recur	9
	Non-recur	9
≥60 years old	Recur	5
	Non-recur	13

Results

Significant genes by multivariate linear regression model from microarray ($p<0.05$)

Gene name	Fold change (FC)
FOS	-1.9
NR4A2	-1.9
IL1B	-1.9
PTGS2	-1.7
LINC00518	1.4

NanoString Immune Panel

Recurrence vs Nonrecurrence >60

Immune Panel Genes in Recurrence vs NonRecurrence by NanoString

	<60 Years Old		≥ 60 Years Old	
Gene name	Fold Change	P-Value	Fold Change	P-value
S100B	-5.64	0.00859	-3.23	0.0468
CDH1	-2.63	0.0235	-2.05	0.0788
BIRC5	-2.4	0.0335	-0.65	0.338
ICAM4	2.09	0.0365	-0.256	0.505
MST1R	2.52	0.00947	-0.0931	0.859
RORC	2.55	0.0205	-0.713	0.268
GATA3	2.74	0.0258	0.372	0.177
IL2	2.81	0.0337	-0.17	0.816
MAPK11	2.84	0.00968	0.903	0.349
AIRE	3.13	0.0223	1.23	0.144
CT45A1	3.33	0.0414	-0.79	0.346
CCL16	3.46	0.0168	0.401	0.743
BAGE	3.58	0.0136	0.697	0.448
IL23R	3.64	0.00545	0.325	0.33
C6	4.28	0.00745	1.35	0.278

Pathway Panel Genes in Recurrence vs NonRecurrence by NanoString

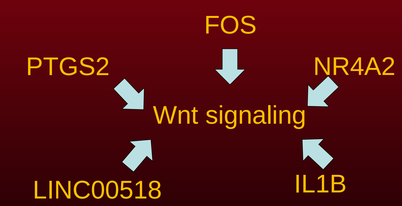
	<60 Years Old		≥ 60 Years Old	
Gene name	Fold Change	P-Value	Fold Change	P-value
ITGB8-	-3.33	0.0362	-0.37	0.624
PLAT	-3.28	0.0236	-0.552	0.461
FN1	-2.91	0.0462	0.708	0.517
CDH1	-2.75	0.0157	-1.9	0.102
DUSP4	-2.5	0.0425	-0.29	0.678
FGF1	-2.15	0.0317	-0.0406	0.965
CDK2	-2.09	0.0313	-1.96	0.0869
CCNB1	-2.07	0.0268	-0.56	0.387
PKMYT1	-2.05	0.0145	0.387	0.662
LAMA5	2.04	0.0358	0.467	0.0018
NKD1	2.13	0.00565	0.537	0.488
IRAK3	2.15	0.00552	1.17	0.0493
HSPA1A	2.2	0.0684	2.04	0.0283
Wnt10b	0.426	0.816	2.27	0.027
FOS	0.952	0.299	1.96	0.0219

NanoString Pathway Panel

Recurrence vs Nonrecurrence ≥ 60

RT-PCR Validation (Recurrence vs. Nonreccurence)

Gene name	Age <60 (Fold Change)	Age ≥ 60 (Fold Change)
FOS	+1.2	+12.6
NR4A2	+1.4	+4.2
PTGS2	+1.8	+3.5
LINC00518	+3.2	+9.0
IL1B	-1.2	+1.1
Wnt10b	+4.0	+1.0



Conclusions

The Wnt pathway, specifically Wnt10b, is a major pathway associated with melanoma recurrence in older patients. These findings may lead to development of new therapeutic targets and strategies.

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