Investigating Tumor Suppressor Function and Regulatory Control of NISCH in Breast Cancer

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Introduction

The incidence rate of breast cancer (BRCA) rises with each year, and it is currently the most common non–skin cancer malignant neoplasm in women in the United States. Identifying novel tumor suppressors may be key in enhancing therapeutic targeting and prognostic accuracy of BRCA.

Nischarin (NISCH) is a cytosolic protein involved in a wide range of cellular processes and is known to interact with integrin α5β1 as well as other key regulatory molecules. NISCH is encoded on chromosome 3p21, a locus frequently implicated in various malignancies. Although its role in BRCA remains relatively understudied, emerging evidence indicates that NISCH functions as a tumor suppressor gene.

This study aimed to investigate patterns between NISCH expression and breast cancer prognosis, and explore potential regulatory mechanisms by integrating NISCH expression, promoter methylation, and copy number alteration data.

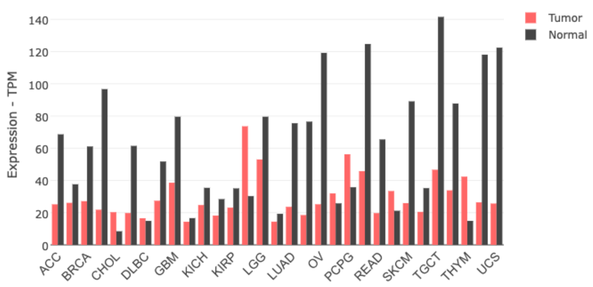
Methods

Expression levels of NISCH and three DNA methyltransferases (DNMTs), promoter methylation beta values, copy-number values, demographic variables, and clinical data were acquired from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) (n = 2509) and The Cancer Genome Atlas (TCGA) (n = 1084) via UCSC Xena and cBioPortal. Data from the GEPIA2 database was used to assess NISCH expression across multiple cancer types. Survival plots for BRCA and NISCH expression were obtained from KMplot.com. All retrospective analyses were performed using statistical software on GraphPad Prism, with significance defined as $p < 0.05$.

To analyze promoter methylation, thirteen CpG islands were found to be negatively correlated with NISCH expression. To focus on candidate regulatory regions, SMARTApp identified three CpG islands located proximal to the NISCH promoter that may play a role in transcriptional silencing. Lastly, copy number values from TCGA were analyzed in relation to NISCH expression to determine whether genomic alterations might contribute to changes in expression levels.

Results

NISCH Expression in Several Cancers

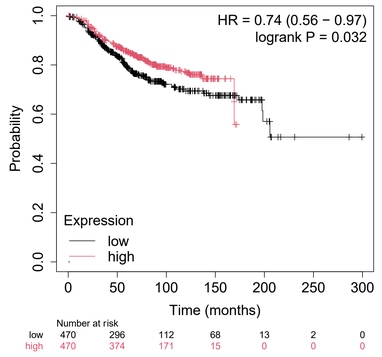


In BRCA, **NISCH expression is significantly higher** in normal breast tissue when compared to tumor breast tissue.

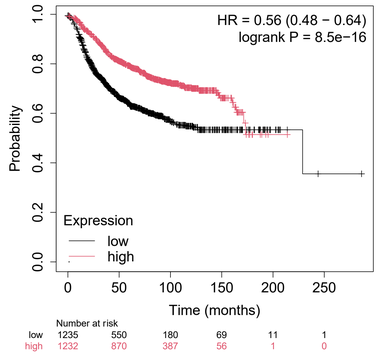
Results

BRCA Prognosis and NISCH Expression

Overall Survival

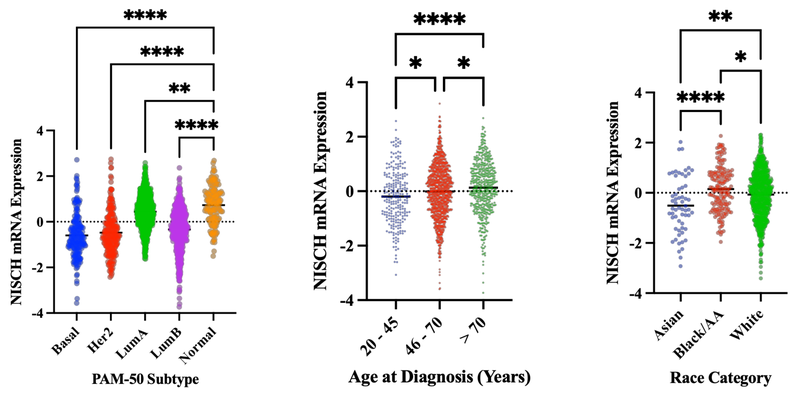


Relapse-Free Survival



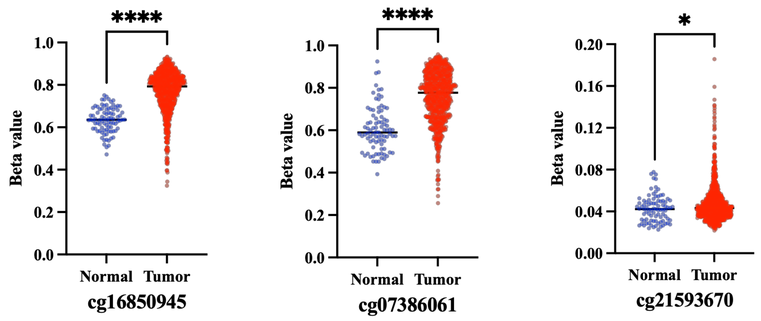
High NISCH expression is associated with better survival outcomes across three metrics.

NISCH Expression is Variable

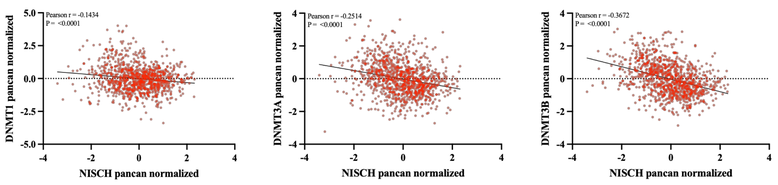


A lower expression of NISCH was observed in patients **diagnosed at younger ages**, within the **Basal PAM-50 subtype**, and among the **Asian race**.

Regulation of NISCH



Three CpG islands were identified as proximal to the promoter region of NISCH and exhibited **increased methylation in tumor breast tissue** as compared to normal breast tissue.

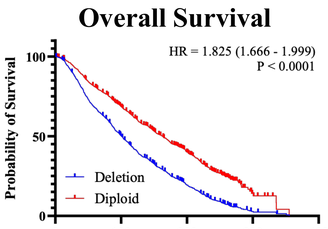


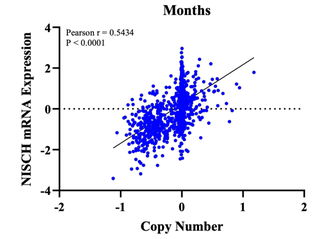
Three DNMTs also displayed **negative co-expression** with NISCH, indicating putative involvement in the silencing of the gene.

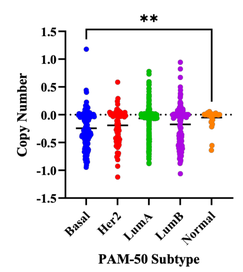
*The highest mean in each category is highlighted.		Mean mRNA Expression		
		DNMT1	DNMT3A	DNMT3B
Age at Diagnosis	20 - 45	**0.09803**	**0.3026**	**0.1089**
	46 - 60	0.03784	-0.003781	0.03039
	> 70	-0.2061	-0.3079	-0.231
Race Category	White	-0.06549	-0.09638	-0.109
	Black/AA	**0.1732**	0.274	0.2711
	Asian	0.1089	**0.4023**	**0.3927**
PAM-50 Subtype	Basal-like	**0.8146**	**0.8031**	0.869
	HER2	0.1036	0.597	**0.9391**
	Luminal A	-0.3491	-0.3717	-0.5584
	Luminal B	0.2218	0.07071	0.2823
	Normal	-0.4048	-0.486	-0.0123

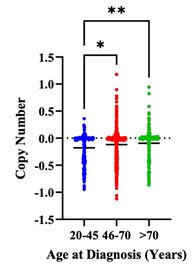
A high expression of all three DNMTs was identified in patients **diagnosed at younger ages**, within the **Basal PAM-50 subtype**, and among the **Asian race**.

Overall Survival









Shallow deletions at the NISCH locus correlated with reduced mRNA expression and were **linked to poorer survival outcomes**.

Conclusions

This study identifies **NISCH as a tumor suppressor in breast cancer**, with low expression levels linked to poor outcomes across multiple survival metrics. Aggressive subsets of BRCA—the **Basal PAM-50 subtype and younger patients**—exhibited reduced NISCH expression, indicating its potential as a marker of impaired prognosis.

Both promoter methylation and shallow deletions resulted in decreased NISCH expression, suggesting that NISCH is regulated through multiple epigenetic and genomic mechanisms. Three DNMTs that were inversely co-expressed with NISCH also showed high expression in similar aggressive subsets of BRCA, providing additional evidence for a **methylation-driven silencing process**.

These findings support further investigation into NISCH's regulatory landscape and its **potential as a prognostic biomarker or therapeutic target in breast cancer.**