Deciphering the Interplay Between SPDEF Expression and Breast Cancer Subtype Heterogeneity

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Introduction

- Breast Cancer (BRCA) is the most commonly diagnosed cancer and second leading cause of cancer-related deaths in women in the United States. Despite leaps in tumor-specific therapy, there is significant heterogeneity in treatment response and disease course.
- Molecular profiling has isolated tumor subtypes – Luminal A, Luminal B, HER2-enriched, Basal – each with unique prognostic and therapeutic implications. Basal tumors, which largely overlap with triple negative BRCA, exhibit aggressive behavior and inferior treatment susceptibility.
- Prostate-Derived Ets Factor/Sam Pointed Domain Ets Factor (SPDEF) is a transcription factor associated with gene regulation in epithelial and secretory tissues. The prognostic significance of SPDEF regulation remains unclear within BRCA and its various subtypes.
- Enhanced understanding of regulatory mechanisms that underlie SPDEF expression may offer novel therapeutic targets.

The objective of this study was to assess the prognostic significance of SPDEF, identify molecular and demographic correlates across tumor subtypes, and investigate SPDEF regulation and co-expression patterns.

Methods

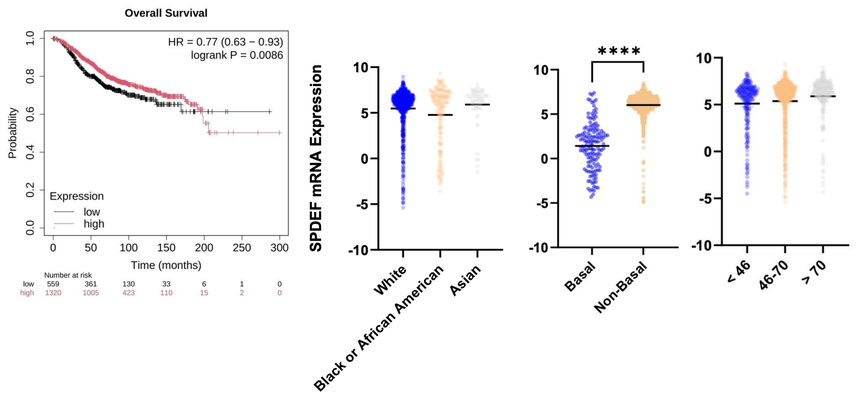
- Tumor genome and clinical data were obtained from The Cancer Genome Atlas BRCA registry (n = 1,247) via UCSC Xena Browser and cBio portal. Analysis was conducted with KMPlotter and GraphPad Prism. $P < 0.05$ was considered statistically significant.
- Prognosis was assessed with log-rank testing. Chi-square, ANOVA, and Student's t-tests were performed to evaluate cohort differences. Pearson correlation analysis was computed to classify linear relationships.
- To investigate epigenetic modifications, the 12 CpG islands along the SPDEF promoter and 4 DNA methyltransferase (DNMT) genes were studied. Beta values at CpG islands correspond with degree of methylation.
- A panel of genes from functional categories associated with BRCA tumorigenesis was selected for SPDEF co-expression analysis. This enabled contextualization of SPDEF expression within relevant biology. Selected genes and their respective categories are depicted below in **Figure 1**.

Gene Panel	
Luminal markers	FOXA1, GATA3, ESR1, PGR, AR, XBP1
Basal/myoepithelial markers	KRT5, KRT14, KRT17, EGFR, TP63, MIA
EMT/stemness regulators	ZEB1, ZEB2, SNAI1, SNAI2, TWIST1, TWIST2, VIM, CDH2, CD44, ALDH1A1
Epithelial integrity markers	CDH1, CLDN3, CLDN4, CLDN7, MUC1
Proliferation genes	MYC, CCND1, CDK4, E2F1, MKI67
DNA repair genes	BRCA1, BRCA2, RAD51, ATM, CHEK1, CHEK2
Immune checkpoint & signaling genes	CD274, CTLA4, LAG3, TIGIT, CXCL9, CXCL10, STAT1

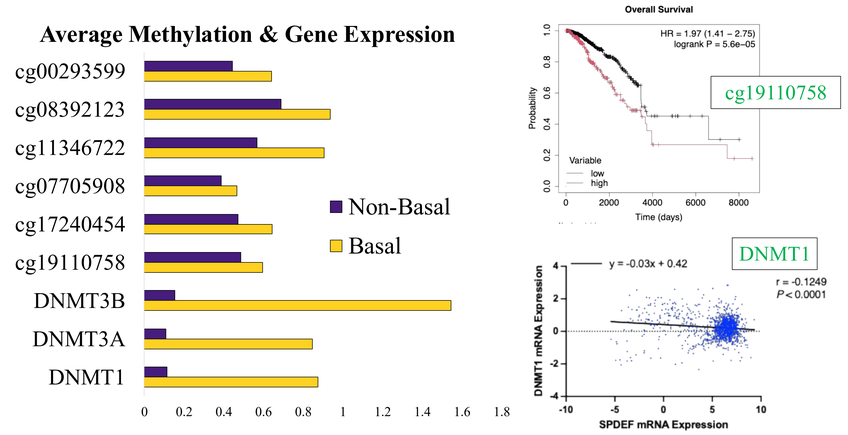
Figure 1: Genes selected for co-expression analysis

Results

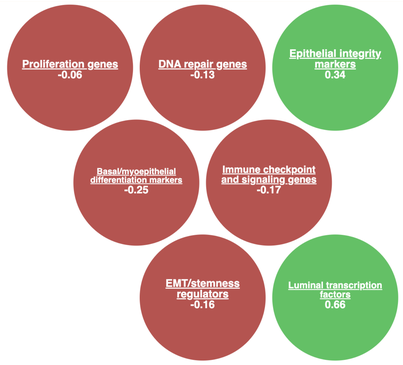
Low SPDEF expression is associated with **poor** prognosis, **Black or African American** race, **younger age** at diagnosis, & **Basal** subtype



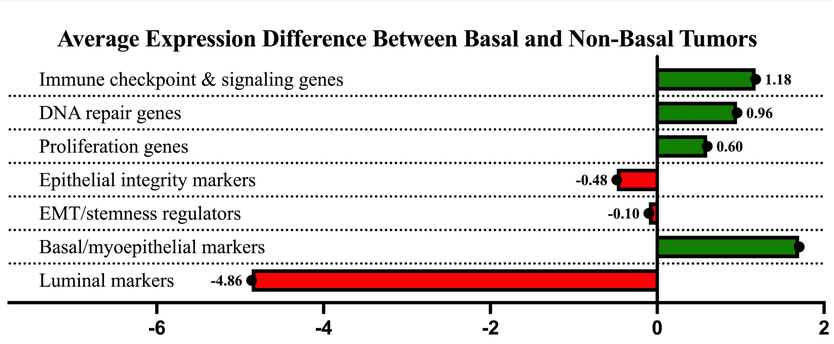
6 CpG islands & **3 DNMT genes** are associated with reduced SPDEF expression, poor BRCA survival, and increased activity in Basal tumors



Correlation between SPDEF and average gene family expression



Tumor subtype differences in gene family expression



Conclusions

- **This study provides compelling evidence that SPDEF functions as a tumor suppresser in breast cancer, with low expression strongly associated with adverse clinical and molecular outcomes.**
- Low SPDEF expression is linked with the Basal tumor subtype, Black or African American race, and younger age at initial pathologic diagnosis.
- Promoter methylation emerges as a possible driver of SPDEF silencing. Methylation of 6 CpG loci and expression of 3 DNMTs significantly correlated with SPDEF downregulation, decreased BRCA survival, and increased activity in Basal tumors.
- Low SPDEF expression co-occurs with high expression of basal/myoepithelial, proliferation, DNA repair, and immune-related genes and low expression of luminal differentiation genes. These trends support the link between SPDEF loss and epithelial-mesenchymal transition and increased stemness. A summary of our proposed tumorigenic mechanism can be found in **Figure 2**.
- **Ultimately, an enriched understanding of SPDEF's tumorigenesis role and its regulation can improve risk stratification and guide targets for novel therapy, particularly for tumor subtypes that are less sensitive to current treatments.**

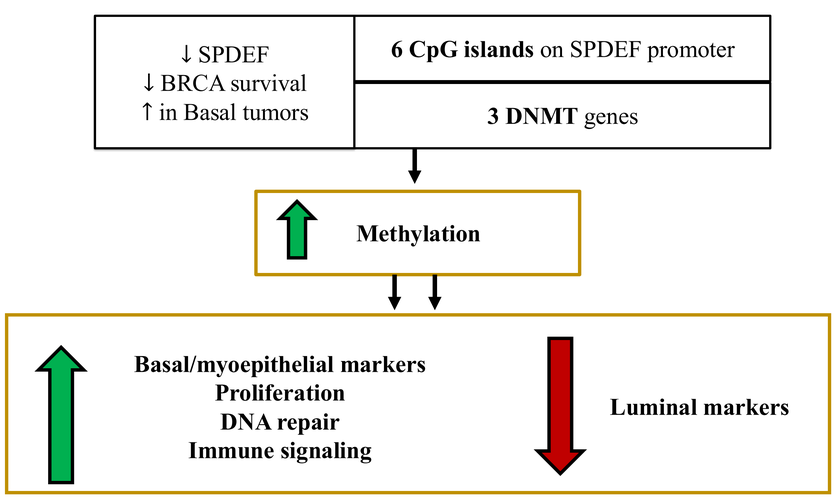


Figure 2: Proposed tumorigenic mechanism of SPDEF