**Epigenetic regulation of the putative breast cancer metastasis suppressor gene SCN4B**

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**Background**

Breast cancer (BC) is still the leading cause of cancer related death in women worldwide. While SCN4B is considered to be a novel metastasis suppressor gene in breast cancer, very little is known about its epigenetic regulation – in particular its downregulation in cancer tissue. In this study, we explored the regulation of SCN4B by promoter methylation, the possibility of its re-expression and the clinical relevance of SCN4B in ductal adenocarcinomas of the breast.

**Materials & Methods**

For the investigation of epigenetic SCN4B regulation, we performed in silico methylation analysis of the promoter region (data from The Cancer Genome Atlas Program (TGA)) and evaluated the methylation and expression levels in vitro for several BC cell lines of various molecular subtypes by pyrosequencing (data not shown) and qPCR, respectively. Next, we treated several BC cell lines with methyltransferase inhibitor 5-aza-2'-deoxycytidine (AZA) and histone deacetylase inhibitor trichostatin A (TSA) to investigate a possible re-expression of SCN4B. To explore the clinical relevance of SCN4B in BC, we performed immunohistochemistry on a tissue micro array (TMA) containing tissues from 420 BC patients (Bavarian breast cancer cohort) and quantified the SCN4B expression by immunoreactive scoring (IRS) classification. For our analysis, we excluded all patients that received neoadjuvant chemotherapy. We analyzed the SCN4B expression for the remaining 202 patients with ductal adenocarcinomas of the breast with regard to molecular subtype, expression of the proliferation marker Ki67 and SCN4B dependent survival.

![Figure 1. In silico methylation of two SCN4B promoter CpG sites is associated with lower SCN4B expression (Data from TGA). (A) Methylation levels (B-solex) of 8 CpG sites within the SCN4B promoter (and first exon) for tumor and normal samples. Two neighboring CpG sequences (cg13566884 & cg7515458) are highly methylated in tumor but not in normal tissue. Red: high, white: low. (B) Linear correlation. (C) Moderate, negative correlation ( Spearman r = 0.32) between SCN4B promoter methylation (represented by cg13566884 & cg7515458) methylation and SCN4B mRNA expression in tumor.](Image 193x221 to 316x440)

![Figure 2. SCN4B is lost in many breast cancer cell lines and can be re-expressed through de-methylation and histone acetylation. (A) Relative SCN4B mRNA expression (B) ivermectin treatment (C) Epigenome editing of SCN4B is observed in various cancer cell lines, but especially in TNBC cell lines. Relative SCN4B expression (n = 3) for MDA-MB-231, T47D and SKBR-3 cells following treatment with 5 µM 5-aza-dC-deoxycytidine (AZA) for 72h, 50 µM trichostatin A (TSA) for 24h or a combination of both (72h AZA-TSA added for 1h after 5aza treatment) compared to DMSO controls. Elevated re-expression levels are found in T47D cells (204-fold) followed by MDA-MB-231 (174-fold) and SKBR-3 (60-fold). Assays will be reported in the future for statistical analysis.](Image 193x221 to 316x440)

![Figure 3. High SCN4B expression is correlated with lower Ki67 expression and a favorable distant disease (DSS) and local recurrence free survival (LRFS). (A) Representative breast tumor samples showing different SCN4B expression levels. (B) SCN4B expression levels in patients with various molecular BC subtypes (TMA, luminal A, luminal B, HER2+, triple negative) compared to other subtypes. (C) Correlation between higher SCN4B expression and lower Ki67 expression. (D) Kaplan-Meier survival curves showing improved DSS & AFSS for SCN4B positive patients (red line) compared to SCN4B negative patients. Blue line shows the decitabine- with intracellular Wilkinson method was used to compare survival curves.](Image 193x221 to 316x440)

**Results**

![Figure 3. SCN4B is downregulated in breast cancers by promoter methylation in silico & in vitro](Image 193x221 to 316x440)

**Conclusion**

- SCN4B is downregulated in breast cancers by promoter methylation in silico & in vitro
- SCN4B in vitro & in vivo downregulation is particularly strong in triple-negative breast cancers
- Loss of SCN4B in vivo leads to more aggressive tumors showing higher Ki67 expression
- Tumors retaining SCN4B expression have a favorable metastasis- and local recurrence prognosis

**Outlook**

- Further exploration of the epigenetic regulation of SCN4B including promoter methylation status in vivo and histone acetylation status in vitro
- Further exploration of the clinical relevance of SCN4B by expansion of the immunohistochemistry data set
- Exploration of downstream targets usually suppressed by SCN4B as new avenues for treatment of TNBC

**References**


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