Rewiring Fibroblast Function in Breast Cancer

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Introduction

In the United States, breast cancer (BCa) is the second most common cause of cancer-related death for women and the most commonly diagnosed malignancy. Cancer-associated fibroblasts (CAFs) are a crucial component of the tumor microenvironment (TME) that influences breast cancer progression. By interacting with cancer cells and altering the extracellular matrix (ECM), CAFs actively promote tumor growth and metastasis. In contrast, normal fibroblasts restrict tumor progression. Despite the significant role of CAFs, little is known about the mechanisms underlying the regulation and conversion of normal fibroblasts into CAFs within the TME. Lysine Deficient Protein Kinase 1 (WNK1) is a serine/threonine kinase that is important for maintaining electrolyte balance and tissue homeostasis in the body. It is overexpressed in the BCa microenvironment, particularly in CAFs. This study investigated the role of WNK1 in regulating CAFs in BCa.

Methodology

Studies were conducted using BCa patient tissues, available public data, orthotopic mouse 4T1 tumor models, and *in vitro* assays to identify the effect of WNK1 on regulating fibroblast functions and BCa progression. WNK1 expression levels were determined in BCa tissues, orthotopic 4T1 BCa mouse embryonic fibroblasts (MEFs), and 4T1 BCa cells using Western blot analysis, RT-PCR, immunofluorescence, and confocal microscopy. Quantitative RT-PCR (qRT-PCR) and flow cytometry were used to identify the molecular changes associated with WNK1 manipulation in MEFs *in vitro*.

Results

WNK1 is highly expressed in human and mice breast cancer tissues and is associated with poor survival

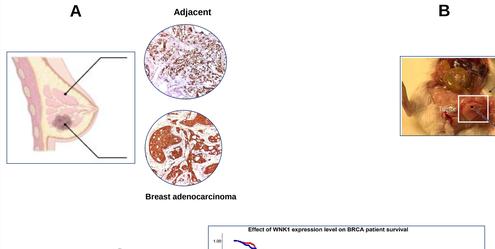


Figure 1. **(A)** WNK1 expression is significantly elevated in breast cancer (BCa) tissue compared to adjacent normal breast tissue. **(B)** WNK1 expression is significantly higher in 4T1 mice tumors compared to mammary fat tissue. Magnification 200X. **(C)** Effect of WNK1 expression level on BRCA patient survival (UALCAN).

Collagen and LOX are highly expressed in human breast cancer

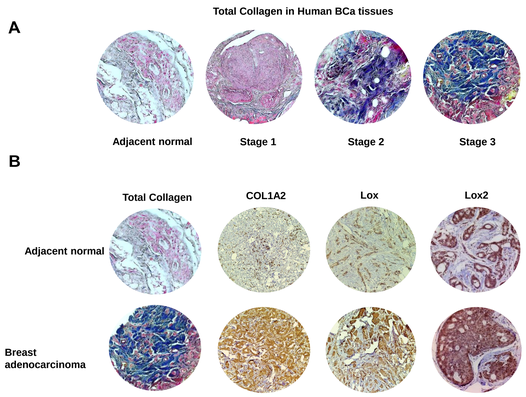


Figure 2. **(A)** Trichrome staining demonstrates that collagen deposition increases with BCa progression. Images captured at 200X magnification. **(B)** high expression of COL1A2, Lox and Lox2 in Breast adenocarcinoma.

Expression of WNK1 in 4T1 breast cancer cells and fibroblasts

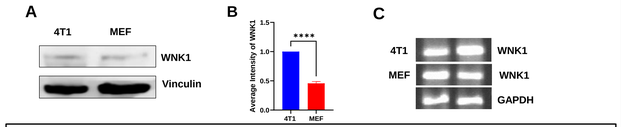
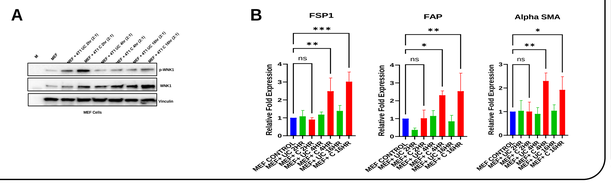


Figure 3. (A) Western blot analysis and **(B)** Densitometry, and **(C)** RT-PCR results show that WNK1 is expressed in both fibroblasts (mouse embryonic fibroblasts, MEFs) and BCa cells (4T1), the two predominant cell types in the tumor microenvironment (TME).

4T1 conditioned medium increases the expression of WNK1 and matrix crosslinking genes in fibroblasts



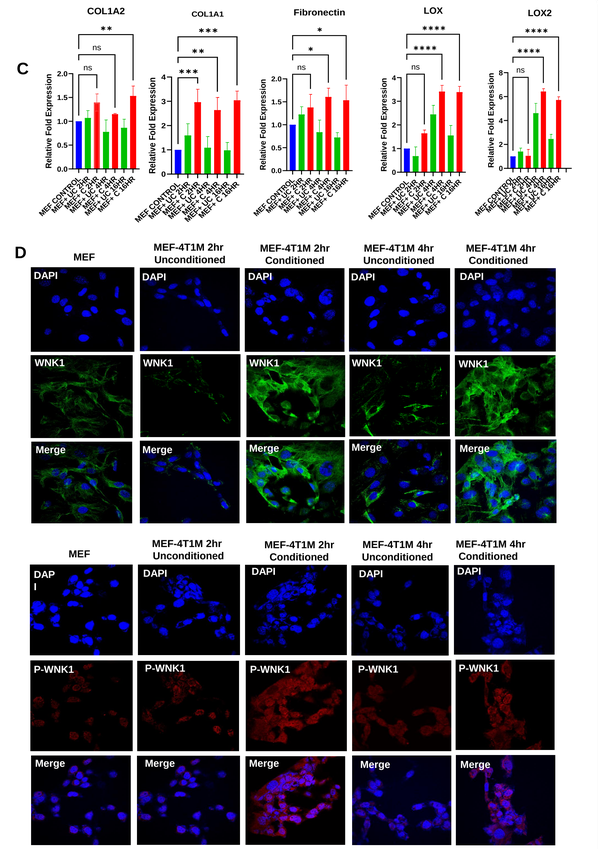


Figure 4. (A) Western blot analysis demonstrates the increased expression of WNK1 and p-WNK1 in MEF cells after exposure to 4T1-conditioned media. **(B and C)** qRT-PCR data show a significant increase in the expression of cancer-associated fibroblast (CAF) markers and extracellular matrix (ECM) proteins in MEFs following treatment with 4T1-conditioned media. **(D)** Confocal images further confirm the upregulation of WNK1 and p-WNK1 in MEFs treated with 4T1-conditioned media.

WNK1-overexpressing fibroblasts exhibit high CAF and matrix cross-linking marker expressions

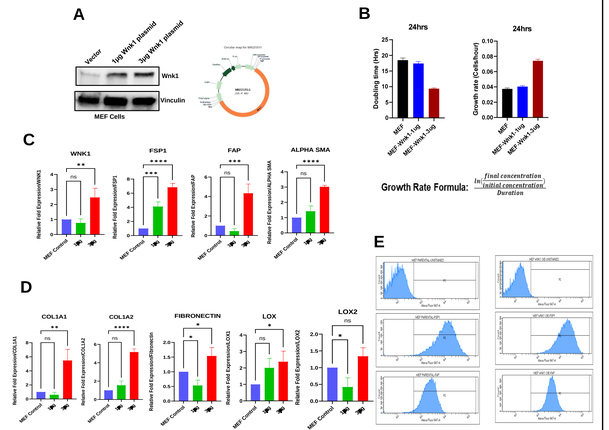


Figure 5. (A) Western blot analysis demonstrating the expression of WNK1 MEF (Mouse Embryonic Fibroblast) with empty vector cells compared to those overexpressing WNK1. **(B)** Graph illustrating the reduced doubling time and increased growth rate of WNK1-overexpressing MEFs compared to normal MEFs. **(C and D)** Quantitative real-time PCR results indicate significantly elevated expression levels of WNK1, cancer-associated fibroblast (CAF) markers (FSP1, FAP, and α-SMA), as well as matrix cross-linking genes (COL1A1, COL1A2, LOX1, LOX2, and Fibronectin) in WNK1-overexpressing MEFs. **(E)** The Flow cytometry graph shows the molecular changes associated with WNK1 manipulation in MEFs *in vitro*.

Conclusion

- Elevated WNK1 expression is associated with worse disease outcomes in breast cancer, suggesting its potential involvement in breast cancer progression and treatment outcome.
- WNK1 is expressed not only in tumor cells but is also significantly upregulated in cells of the tumor microenvironment (TME), particularly in cancer-associated fibroblasts.
- WNK1 contributes to the progression of breast cancer by regulating the crosstalk between cancer-associated fibroblast (CAF) and cancer cells.
- Data suggest that WNK1 may play a key role in fibroblast activation, therefore promoting tumor progression and metastasis via matrix deposition and TME remodeling in BCa.
- Our findings indicate that WNK1 contributes to BCa progression by regulating CAF conversion and ECM protein production. Our results suggest that WNK1 may be a potential therapeutic target in Bca.

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