Fibroblastic Mutant *Trp53* in the Stroma Promotes Mammary Tumor Development via Enhanced Paracrine Factor Secretion

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Abstract

Tumor progression critically depends on stromal contributions, particularly from fibroblasts that regulate growth factors, angiogenesis, immunity, and ECM remodeling. While *TP53* is the most frequently mutated tumor suppressor and its cell-autonomous roles have been extensively studied, emerging evidence shows its stromal functions actively shape tumor-stroma crosstalk. However, the molecular mechanisms by which mutant stromal *TP53* influences tumor development remain undefined. This study examined whether fibroblast-specific expression of p53R172H influences HER2/neu-driven mammary tumorigenesis. We generated a female mouse cohort with genotypes *MMTV-neu*; *Fsp-Cre*; $Trp53^{wm\text{-}R172H/+}$ (NP) and *MMTV-neu*; *Fsp-Cre* or $Trp53^{wm\text{-}R172H/+}$ (N) as controls. In NP mice, Cre recombinase drove recombination of the conditional $Trp53^{wm\text{-}R172H/+}$ allele, converting wild-type p53 to mutant p53R172H specifically in fibroblasts. NP mice developed mammary tumors significantly earlier, with tumor-free survival of 520 days compared to 620 days in controls (p = 0.0007). Although tumor burden and metastatic frequency were unchanged, RNA sequencing revealed 855 upregulated and 1,927 downregulated genes unique to *NP* tumors. Gene Set Enrichment Analysis indicated activation of multiple pathways, notably PI3K/AKT/mTOR. In addition, transcriptomic profiling of NP versus N mammary glands showed elevated expression of secretory proteins SP1, SP2, and SP3, previously linked to PI3K/AKT-driven cell growth. In vitro assays using synthetic peptides of these proteins and HER2/neu+ mammary tumor cell lines confirmed that SP1, SP2, and SP3 promote tumor cell proliferation and migration. Analysis of TCGA and METABRIC datasets further demonstrated increased SP1 and SP2 expression in *TP53*-mutant human breast cancers, supporting their relevance in patients. Together, these findings reveal that fibroblastic mutant p53 accelerates breast tumorigenesis through paracrine signaling that activates PI3K/AKT/mTOR. These results highlight stromal mutant p53-mediated tumor-stroma crosstalk as a critical and potential therapeutic target for breast cancer biology.

Introduction

Breast cancer progression is strongly influenced by the tumor microenvironment, particularly cancer-associated fibroblasts (CAFs). *TP53*, the most frequently mutated tumor suppressor gene, has well-characterized cell-autonomous roles, but its stromal functions remain poorly understood. Using a conditional mutant *Trp53* allele, this work explores how mutant p53 in fibroblast accelerate HER2-positive mammary tumor development and reshape signaling pathways through paracrine mechanisms.

Methods and Materials

A conditional mouse model was employed to induce p53R172H mutation specifically in fibroblasts while maintaining wild-type p53 in other compartments. Tumor-free survival was monitored, and mammary tumors were analyzed by histology and immunofluorescence. RNA sequencing of tumors and mammary glands identified differentially expressed genes and enriched pathways. Secretome analysis was performed using the Vertebrate Secretome Database, and functional validation of candidate secretory proteins was conducted through proliferation and migration assays in HER2-positive tumor cell cultures. TCGA and METABRIC datasets were analyzed to correlate *TP53* mutation status with expression of secretory factors.

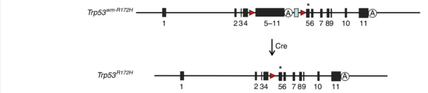


Figure 1. Schematic representation of the *Trp53*$^{wm\text{-}R172H}$ allele.

Results

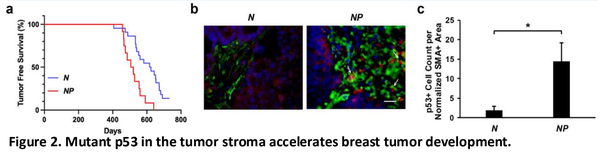


Figure 2. Mutant p53 in the tumor stroma accelerates breast tumor development.

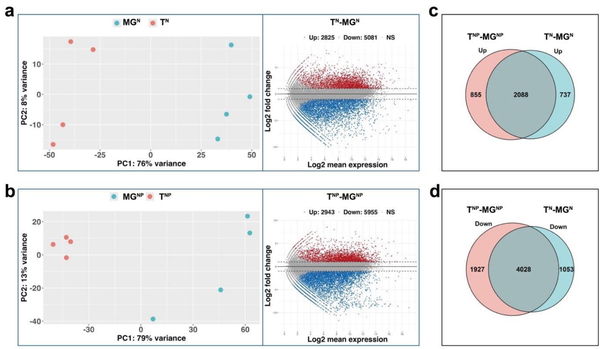


Figure 3. Stromal mutant p53 reprograms gene expression in mammary glands and tumors.

Results (contd.,)

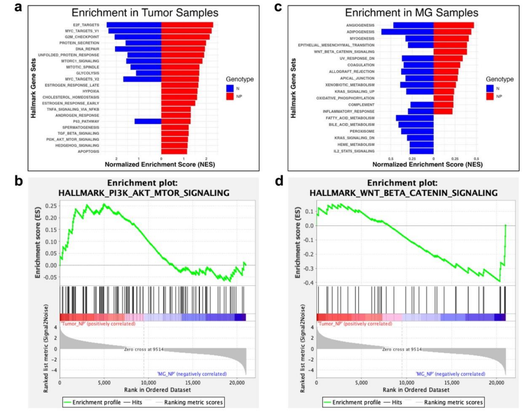


Figure 4. Stromal mutant p53 reprograms molecular signaling in tumors.

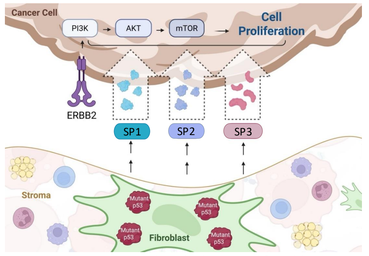


Figure 6. Proposed mechanism by which stromal fibroblastic mutant p53 promotes the progression of HER2+ breast cancer.

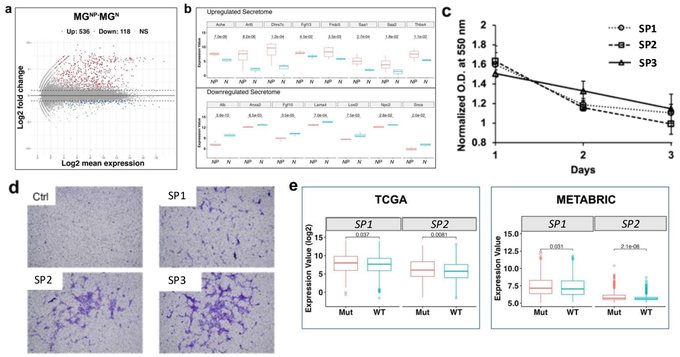


Figure 5. Fibroblastic mutant p53 upregulates SP1, SP2 and SP3 in the NP mammary gland, promoting neu tumor cell proliferation and migration.

Discussion

The specific stromal cell type producing SP1, SP2, and SP3 is unknown because bulk RNA-seq cannot determine cellular origin. These factors increased migration in vitro, but metastasis was unchanged in vivo, suggesting timing or microenvironment influences. Ki67 levels were similar at endpoint, likely reflecting proliferative plateauing rather than true biological equivalence. Mutant p53 effects may be oncogene-specific, and human dataset associations remain correlative rather than causative.

Conclusions

Fibroblastic mutant p53 accelerates HER2-positive mammary tumor development through non-cell-autonomous mechanisms by reprogramming fibroblasts to secrete factors that activate oncogenic signaling pathways, including PI3K/AKT, in adjacent epithelial cells. Upregulation of SP1, SP2, and SP3 highlights a paracrine mechanism that promotes tumor cell proliferation and migration. These findings reveal a novel aspect of tumor–stroma crosstalk and underscore stromal p53 status as a key determinant of the tumor microenvironment and a promising therapeutic target in HER2-positive breast cancer.

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