Basonuclin-1 in Inflammatory Breast Cancer: Tumor Suppressor or Oncogene?

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Background

Inflammatory Breast Cancer (IBC) is a rare and aggressive form of breast cancer with the highest mortality rate of all breast cancer types [1]. Unlike other breast cancers, the inflammatory nature of IBC can disrupt the early diagnosis process by producing symptoms such as redness, enlargement of the breast, pain, and itchiness, which are attributes often mistaken for mastitis, infection of the breast [2]. The need to characterize the role of genes expressed in IBC is crucial to identify novel therapeutics.

Preliminary studies involving RNAseq data sets uncovered differentially expressed transcription factors between IBC and non-IBC breast cancer cell lines (Fig. 1). An IBC cell line, SUM149, displayed the highest expression of basonuclin-1 (BNC1). SUM149, is a triple-negative inflammatory breast cancer cell line, lacking expression of HER2, ERα (estrogen receptor), and PR (progesterone receptor) [3]. Despite BNC1 having been previously associated with various other cancers, extensive research has not gone into defining its role in triple-negative IBC. This study aims to investigate expression of BNC1 in the triple negative cell model SUM149.

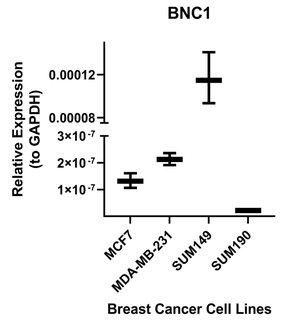
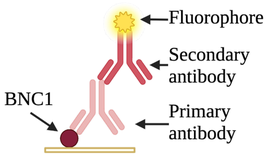


Figure 1. qPCR comparing gene expression of basonuclin-1 in breast cancer subtypes. This graph represents relative expression of basonuclin-1 to GAPDH, determined by a qPCR reaction with primers that could amplify both isoforms of BNC1. This experiment was employed to validate the differential expression of basonuclin-1 in different cell lines. The results demonstrate that there is an overexpression of basonuclin-1 in SUM149.

Hypothesis

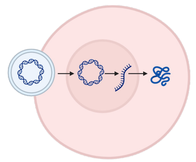
Basonuclin-1 acts as a oncogene, promoting cell proliferation and migration within triple-negative inflammatory breast cancer.

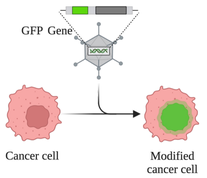
Methods



SUM149 cells were seeded onto a 4 well plate for indirect immunofluorescence. Wells were treated with a primary antiBNC1 antibody and a secondary antibody tagged with an ALEXA488 fluorophore.

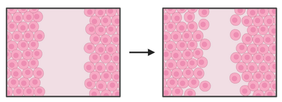
SUM149 cells were cultured and transfected with an siRNA targeting the basonuclin-1 mRNA and a scramble siRNA as a negative control.

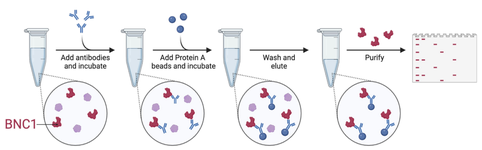




SUM149PT and non-tumorigenic breast cell line MCF-10A cells were transfected with a pLOC plasmid containing a coding sequence for BNC1 and a GFP reporter to induce BNC1 overexpression

Scratch assays were imaged at 0 hours, 19 hours, and 24 hours. Percentages of growth were calculated by determining the difference between the area at 0 hours and 24 hours.





Immunoprecipitation was performed on SUM149 cell lysates. Western Blotting techniques were used to determine the expression of BNC1 in all samples.

Results

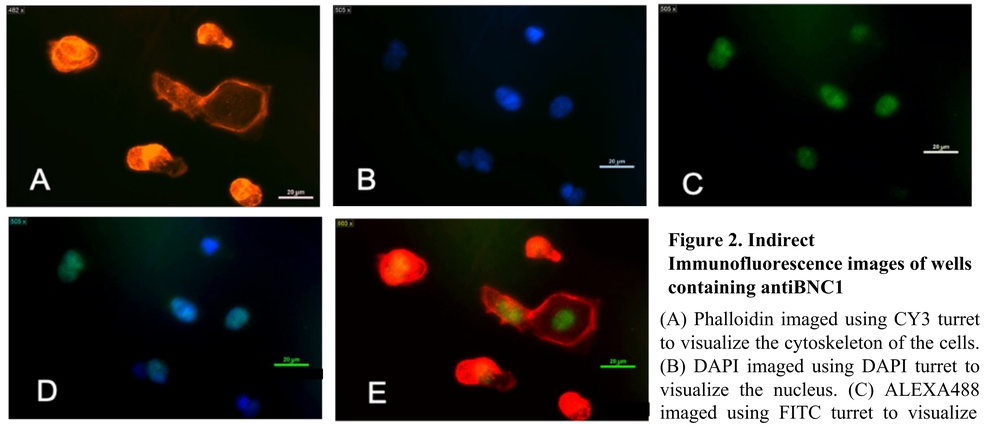


Figure 2. Indirect Immunofluorescence images of wells containing antiBNC1

(A) Phalloidin imaged using CY3 turret to visualize the cytoskeleton of the cells. (B) DAPI imaged using DAPI turret to visualize the nucleus. (C) ALEXA488 imaged using FITC turret to visualize the nucleus. (C) ALEXA488 imaged using FITC turret to visualize BNC1. (D) Images B and C merged to compare the location of BNC1 and the nucleus. The nucleus is blue and the BNC1 is green. (E) Images A and C merged to visualize where BNC1 is within the cell. Phalloidin stained cytoskeleton is in red and BNC1 protein is green.

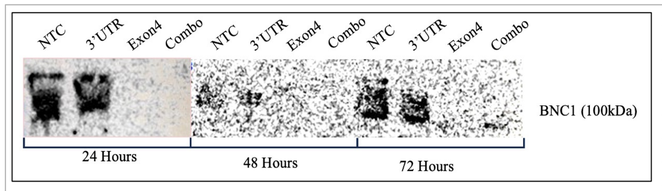


Figure 3. Identification of BNC1 expression to understand the efficiency of BNC1 knockdown with various siRNAs. Immunoblotting image of expression levels of BNC1 in SUM149PT following treatment of 1uM siRNAs of non-targeting negative control, 3'UTR, Exon4, and a combination of 3'UTR and Exon4.

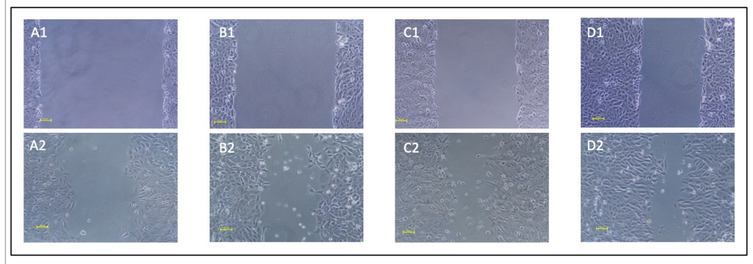


Figure 4. Motility assay of knockdown images taken at 0 hours and 24 hours. SUM149PT cells were treated with 1uM siRNAs of non-targeting negative control, 3'UTR, Exon4, and a combination of 3'UTR and Exon4. (A1) Treatment with non-targeting negative control siRNA 0-hours after scratch. (A2) Treatment non-targeting negative control siRNA 24-hours after scratch. (B1) Treatment with 3'UTR siRNA 0-hours after scratch. (B2) Treatment with 3'UTR siRNA 24-hours after scratch. (C1) Treatment with Exon4 siRNA 0-hours after scratch. (C2) Treatment with Exon4 siRNA 24-hours after scratch. (D1) Treatment with 3'UTR siRNA and Exon4 siRNA 0-hours after scratch. (D2) Treatment with 3'UTR siRNA and Exon4 siRNA 24-hours after scratch.

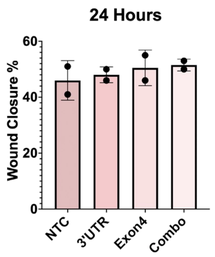


Figure 5. Comparison of cells treated with siRNAs in BNC1 knockdown in SUM149PT to determine any phenotypic differences. Total wound closure percent between 0-hours and 24-hours graphed.

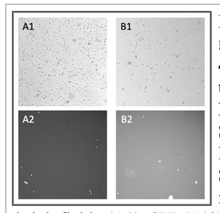


Figure 6. GFP reporter imaged 48 hours after transfection in MCF-10A and SUM149PT cell lines. (A1) SUM149PT cells imaged with brightfield. (A2) SUM149PT cells imaged with FITC, showing GFP localization. (B1) MCF-10A cells imaged with brightfield. (B2) MCF-10A cells imaged with FITC, showing GFP localization.

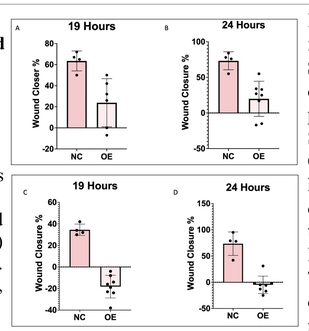


Figure 7. Statistical representation of the motility assay of MCF-10A cells and SUM149 cells, both with BNC1 overexpression from transfection of pLOC plasmid. (A) Control and overexpressed SUM149 cells 19 hours after the scratch. (B) Control and overexpressed SUM149 cells 24 hours after the scratch. (C) Control and overexpressed MCF-10A cells 19 hours after the scratch. (D) Control and overexpressed MCF-10A cells 24 hours after the scratch. The results on these graphs suggest that the overexpression of BNC1 greatly inhibits motility in both kinds of cell lines.

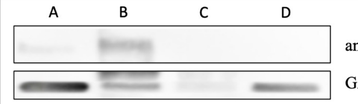


Figure 8. Immunoblotting image of expression levels of BNC1 in SUM149PT following immunoprecipitation. GAPDH expression used as loading control. (A) Whole cell lysate containing 5% of Immunoprecipitation sample protein concentration. (B) Immunoprecipitation sample treated with protein A magnetic beads and antiBNC1 antibody. (C) Negative control sample with lysates treated with IgA magnetic control beads. (D) Negative control sample with lysates treated with protein A magnetic beads.

Summary

➔ Indirect immunofluorescence confirmed the localization of BNC1 within the nucleus of SUM149 cells.

➔ Overexpression studies in the SUM149 and non-tumorigenic MCF-10A cell lines revealed that elevated levels of BNC1 lead to a marked decrease in cell motility. This significant insight suggests a suppressive effect of BNC1 on cancer cell migration

➔ Preliminary immunoprecipitation experiments designed to isolate BNC1 and its interacting proteins yielded successful pull-down results, implying successful isolation and potential identification of interacting partners.

Future Directions

Future directions in this research project include but are not limited to:

➔ Perform a BNC1 gene knockout using CRISPR-Cas9 and conduct motility assays

➔ Compare basonuclin-1 expression in multiple breast cancer cell lines using Western Blot techniques

➔ Study the viability of multiple breast cancer cell lines when to BNC1 overexpression or knockout

➔ Explore co-immunoprecipitation with SP1 to identify potential physical interactants of BNC1 and use mass spectrometry for further analysis.

Acknowledgements

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