NEDD9 is a New Biomarker of Drug Response in HER2+ Breast Cancer: From Bench to Bed.

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Abstract

NEDD9 is a scaffold protein frequently upregulated in HER2-positive breast cancer and associated with poor prognosis. In a clinical cohort (143), elevated NEDD9 expression correlated with advanced tumor stage (p = 0.0344) and lymph node involvement (p = 0.057), yet was also associated with improved pathological complete response (pCR) in a subgroup of NEDD9-high/ER-negative patients (p = 0.018) . To investigate the functional role of NEDD9 in chemotherapy sensitivity, HER2-positive/ER-positive BT474 cells were used to model high and low NEDD9 expression. Cells were treated with paclitaxel, cisplatin, or combination therapy. Interestingly, although BT474 cells are ER-positive, NEDD9-high cells displayed therapeutic sensitivity resembling ER-negative tumors, consistent with clinical data. This suggests that high NEDD9 expression may suppress ER activity, functionally mimicking an ER-negative phenotype. Similar to patient data, NEDD9-low cells exhibited increased chemoresistance, with approximately 20% higher cell viability and a 7–10-fold elevation in IC50 values across treatment conditions. This suggests that patients with low NEDD9 expression may not benefit from chemotherapy escalation and may require alternative therapeutic strategies. These findings identify NEDD9 as a predictive biomarker and support its evaluation in future clinical trials for HER2-positive breast cancer.

Introduction

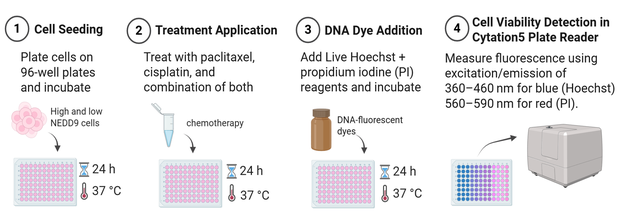
HER2-positive breast cancer comprises approximately 15% of all breast cancers and is typically treated with chemotherapy and HER2-targeted therapy. However, only a subset of patients derive significant benefit from chemotherapy, highlighting the need for biomarkers to guide treatment decisions. NEDD9 is a scaffold protein that coordinates integrin and receptor tyrosine kinase signaling, including HER2, and has emerged as a candidate predictive biomarker. Its association with treatment response may help identify patients suitable for chemotherapy de-escalation.

Hypothesis

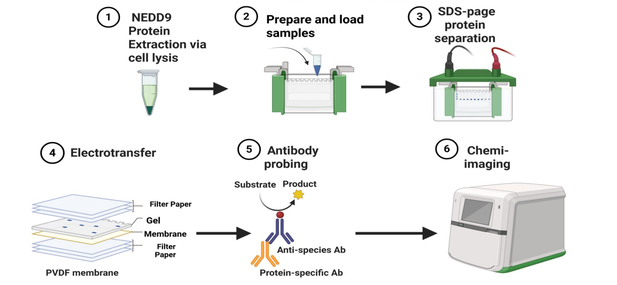
We hypothesize that NEDD9 modulates chemotherapy sensitivity in HER2-positive breast cancer. In both cell line and mouse models, NEDD9-low tumors are expected to exhibit greater resistance to chemotherapy, reflecting clinical observations and supporting the need for alternative treatment.

Methodology

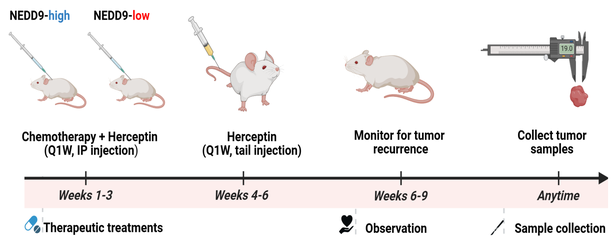
1 In Vitro Drug Treatment and Viability Analysis in NEDD9-High/Low BT474 Cells



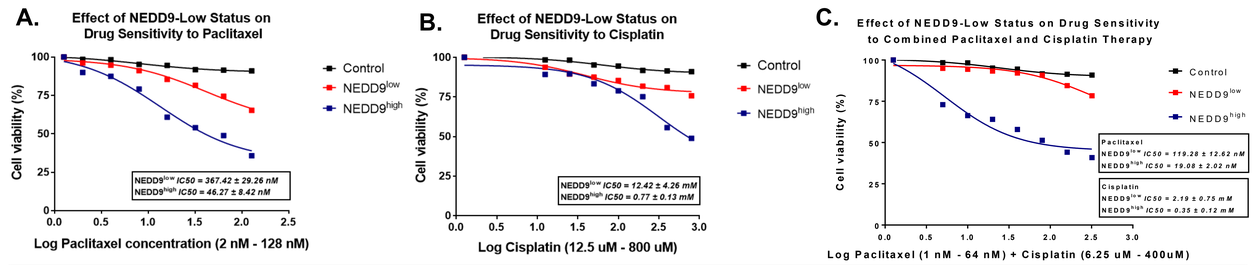
2 Western Blotting Workflow for NEDD9 Detection in BT474 Cells



3 In Vivo Treatment and Recurrence Monitoring in NEDD9-High/Low Tumor Models



Results



Graphs A-C. Graphs A–C. BT474 cells treated with Paclitaxel (2 nM – 128 nM), Cisplatin (12.4 µM – 800 µM), and combination therapy of Paclitaxel (1 nM – 64 nM) plus Cisplatin (6.25 µM – 400 µM) respectively. Cell viability was measured 24 hours post-treatment. Data were plotted using GraphPad Prism, and IC50 values were calculated with R program.

Western Blot Analysis of NEDD9 Protein in BT474 Cells with modified NEDD9 expression treated with standard of care Chemotherapy

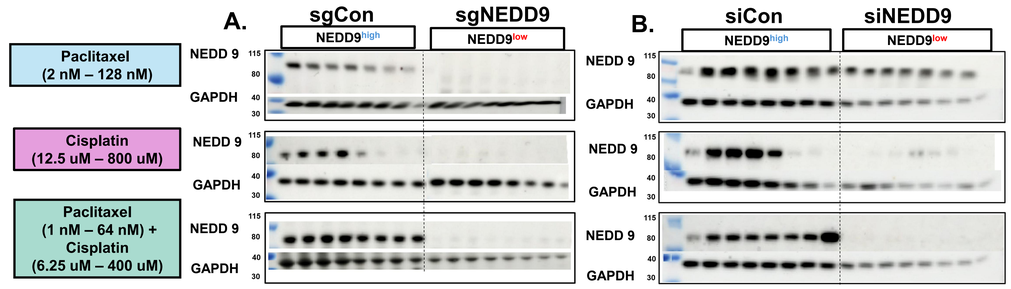
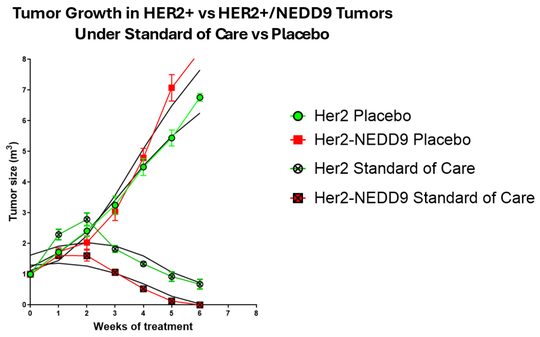


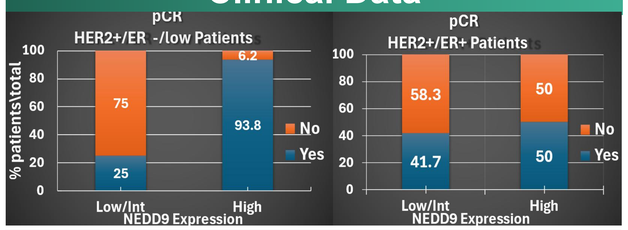
Figure A – B. Western blot analysis with NEDD9 antibodies confirms reduced NEDD9 expression in both NEDD9-low stable cell lines and siNEDD9- transfected BT474 cells. Mimicking chronic and acute changes in NEDD9 levels in patients following treatment with paclitaxel, cisplatin, or combination. GAPDH served as a loading control to ensure equal protein loading across all conditions.



Graph D. It illustrates tumor volume over a 6-week treatment period in late-stage HER2+ and HER2+/NEDD9 mouse models under standard of care (SOC) or placebo treatment. Mice with HER2+/NEDD9 tumors receiving standard of care (red line) showed the most significant tumor regression, with volumes approaching zero by week 6. In contrast, HER2+/NEDD9 placebo-treated mice (blue line) exhibited rapid tumor progression, reaching the highest final tumor size among groups. HER2 tumors treated with standard of care (purple line) displayed moderate regression, while HER2 placebo tumors (green line) grew steadily but less aggressively than NEDD9+ controls.

Interpretation: These results suggest that NEDD9 overexpression enhances response to standard chemotherapy. The differential responses support the role of NEDD9 as a potential predictive biomarker for chemotherapy efficacy in HER2+ tumors.

Clinical Data



Graph 1: HER2+/ER-/low patients with high NEDD9 expression had a significantly higher rate of pathological complete response (pCR) — 93.8% versus 25% in NEDD9-low patients — indicating strong treatment sensitivity.
Graph 2: In HER2+/ER+ patients, NEDD9 expression had no impact on pCR rates, suggesting NEDD9's predictive value is specific to ER-negative tumors.

Conclusion

- IHC analysis of archived HER2+ patient biopsies revealed distinct NEDD9-high and NEDD9-low groups.
- High NEDD9 expression was associated with improved pCR in HER2+/ER-negative patients.
- In BT474 cells, NEDD9-high cells showed greater chemotherapy sensitivity; NEDD9-low cells had elevated IC50s and ~20% higher viability.
- In mice, NEDD9-high tumors showed greater response to chemotherapy, with significantly reduced tumor growth compared to NEDD9-low controls.
- These results suggest patients with low NEDD9 expression may benefit from alternative treatment strategies

Future Directions

- Future studies will validate findings using NEDD9-low mouse models, complementing current work in NEDD9-overexpressing mice.
- Mice will be treated with standard-of-care versus reduced chemotherapy regimens.
- Tumor regression and recurrence will be measured to compare outcomes between NEDD9-low and NEDD9-high groups.
- In parallel, ER-negative SKBR3 cells will be compared to ER-positive BT474 cells to assess chemotherapy response across ER subtypes.
- These studies aim to clarify the role of NEDD9 expression in treatment sensitivity across different breast cancer models.

References

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