Utilizing Androgen Receptor Degraders for Breast Cancer Therapy

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Background

- Breast cancer is categorized by various molecular characteristics and is commonly divided into four different subtypes: luminal A, luminal B, HER2-positive, and triple negative.
- Luminal A is characterized by the presence of the estrogen receptor (ER) and lack of human epidermal growth factor receptor 2 (HER2).
- Luminal B is denoted by the presence of HER2 but can either have or lack ER and progesterone receptor (PR).
- HER2+ is a moderately aggressive subtype including the presence of HER2 and a lack of ER.
- Triple-negative breast cancer (TNBC) is a subset of breast cancer which lacks expression of ER, PR, and HER2.
- Due to the lack of clearly defined molecular targets and the robust invasive and proliferative capabilities of TNBC cells, treatment of patients with TNBC is incredibly difficult. Frequent recurrence, higher risk of metastasis, and lower survival rates are all characteristics of TNBC patients as compared to other breast cancer subtypes.
- Androgen receptor (AR) is a steroid hormone receptor that translocates to the nucleus after a ligand has bound.
- AR binds to the enhancer and promoter regions of the targeted genes which initiates transcription for cell proliferation.
- In TNBC tumors, AR signaling progresses tumor development and can potentially be considered an emerging target for clinical therapies.
- Proteolysis-targeting chimeras (PROTAC) are a class of emerging therapeutic inhibitors which utilize the ubiquitin E3 ligase to selectively degrade proteins.

Significance

- We evaluated an AR directed PROTAC as a potential therapeutic strategy to target TNBC.
- We aimed to decrease the survival rate of breast cancer cells via an AR degrader drug.
- This was evaluated using western blot to analyze protein expression of AR and MTT assay to determine the efficacy of the drug

Breast Cancer Subtypes

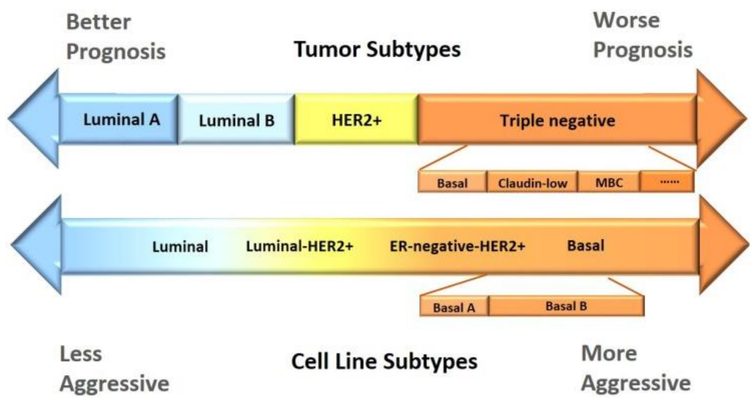

Figure 1: Comparison of the current subtyping schemes between breast cancer cell lines and tumors.

Dai X, Cheng H, Bai Z, Li J. Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping. J Cancer. 2017 Sep 12;8(16):3131-3141. doi: 10.7150/jca.18457. PMID: 29158785; PMCID: PMC5665029.

Androgen Receptor Signaling

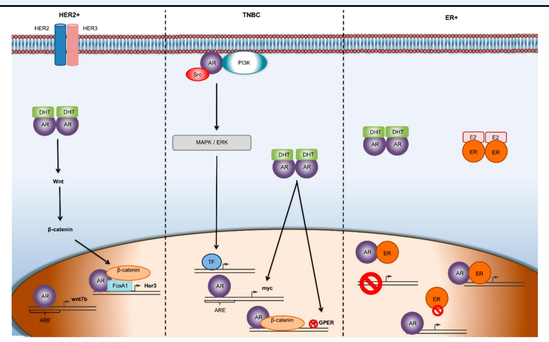

Figure 2: Mechanisms of AR mediated gene transcription in different subtypes of breast cancer.

Anestis, A., Zoi, I., Papavassiliou, A. G., & Karamouzis, M. V. (2020). Androgen Receptor in Breast Cancer—Clinical and Preclinical Research Insights. *Molecules*, *25*(2), 358. https://doi.org/10.3390/molecules25020358

PROTAC General Mechanism

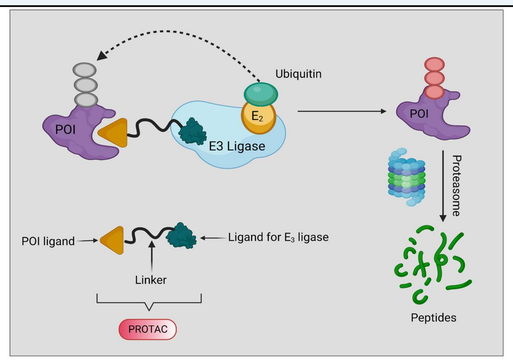

Figure 3: PROTAC mechanism

Sincere, N. I., Anand, K., Ashique, S., Yang, J., & You, C. (2023). PROTACs: Emerging Targeted Protein Degradation Approaches for Advanced Druggable Strategies. *Molecules*, *28*(10), 4014. https://doi.org/10.3390/molecules28104014

Results

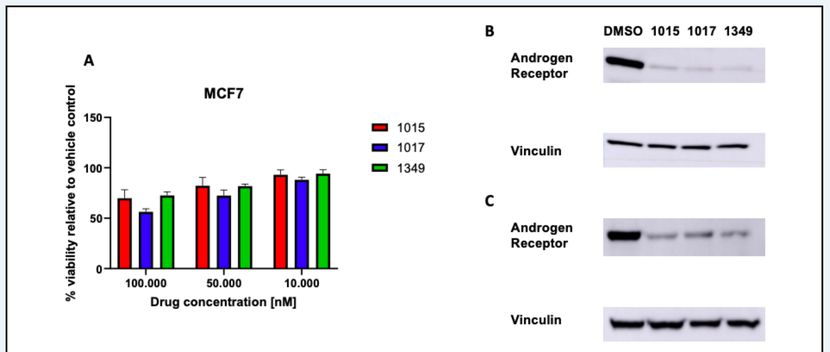

Figure 1: Androgen degraders inhibit breast cancer cell proliferation and androgen receptor protein expression. Breast cancer cells were plated onto 96-well plates for overnight and the cells were treated with different doses of 1015, 1017, 1349, or DMSO for 72 hours. The viability was determined by MTT assay. Data was plotted using GraphPad prism (**A**). BT474 cells were plated onto 6-well plates for overnight and the cells were treated with 100nM of androgen degraders for 24 hours, and the lysates of these cells were immunoblotted with androgen receptor. Vinculin was used as a loading control (**B**). MCF7 cells were plated onto 6-well plates for overnight and the cells were treated with 100nM of androgen degraders for 24 hours, and the lysates of these cells were immunoblotted with androgen receptor. Vinculin was used as a loading control (**C**).

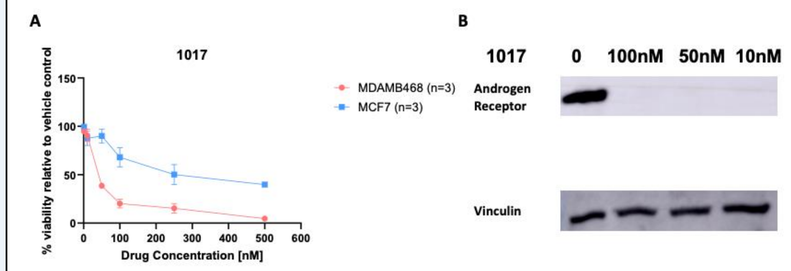

Figure 2: Androgen degrader 1017 inhibits breast cancer cell growth and androgen receptor protein expression in nanomolar dose range. MDA-MB-468 and MCF7 cells were plated onto 96-well plates for overnight and the cells were treated with different doses of 1017 or DMSO for 72 hours. The viability was determined by MTT assay. Data was plotted using GraphPad prism (**A**). MDA-MB-468 cells were plated onto 6-well plates for overnight and the cells were treated with varying doses of androgen degrader 1017 for 24 hours, and the lysates of these cells were immunoblotted with androgen receptor. Vinculin was used as a loading control (**B**).

Conclusion

- Our AR directed PROTAC has been shown to downregulate the proliferation of cancer cells while also mediating protein degradation of androgen receptors.
- This approach to therapeutics has the potential to serve as the basis for a development of treatment for breast cancer.

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