ABROGATING TRIPLE NEGATIVE BREAST CANCER METASTASIS THROUGH INHIBITION OF CALPAIN-1/2

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Clinical literature shows that high expression of calpain-1/2 correlates with poor survival in breast cancer. Proteolytic cleavage of calpain-1/2 substrates in cancer is known to affect cell survival signaling, migration, invasion, and sensitivity to chemotherapeutics. Therefore, we hypothesized that genetic abrogation of calpain-1 and/or calpain-2 activity will make MDA-MB-231 human triple-negative breast cancer (TNBC) cells less motile, more sensitive to chemotherapeutic challenges, and will reduce their tumorigenic and metastatic potential.

We developed a panel of *CAPN1*, *CAPN2*, and *CAPNS1* genetic knock-out and lentiviral add-back MDA-MB-231 cell lines. We showed that we can successfully abrogate/restore calpain expression and activity. We demonstrate a phenotype of diminished cell migration *in vitro*, induced by calpain-1 deficiency through CAPN1 or CAPNS1 knock-out and rescued by the respective add-back. We also demonstrate reduced metastatic potential of these calpain-deficient TNBC cells in a mouse orthotopic engraftment model, while growth of a primary tumor is unaltered.

To develop a pharmacological approach to induce the above-mentioned phenotypes, we developed a split NanoLuciferse reconstitution biosensor to measure the PEF-PEF interactions in calpain-1/2 and to screen for single amino acid substitutions that can disrupt the PEF-PEF dimerization that would be indicative of potential small-molecule binding sites. With the biosensor, we have demonstrated differences in PEF-PEF interactions of CAPN1/2 with CAPNS1, and we have employed these biosensors in an *in vitro* and an *in silico* high-throughput screening campaign with the eventual goal of developing allosteric isoform-specific inhibitors of calpain-1 and calpain-2 for *in vivo* applications.

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