Exploring the role of calpain proteases in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is characterized by a lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2) overexpression. The absence of these therapeutic targets limits treatment options and puts patients at risk of developing drug-resistant metastatic disease. My research explores the effects of genetic calpain disruption on TNBC tumorigenesis, metastasis, and drug sensitivity. Calpains are involved in both pro- and anti-apoptotic signaling, and these opposing roles may be exploited to improve treatment response while protecting healthy cells from off-target cytotoxic effects. Using shRNA knockdown, I have engineered a calpain-1/2 deficient TNBC cell line for use in a repurposing screen for synthetic lethal interactions between calpain deficiency and clinically approved drugs. Candidate drugs will be validated *in vitro* before moving on to mouse models of tumorigenesis and metastasis. Using CRISPR-Cas9 knockout of *capn1*, *capn2* and *capns1* in AC2M2 mouse mammary cancer cells, I demonstrated that genetic calpain disruption impedes migration and invasion *in vitro* and reduces the metastatic potential of TNBC cells in a mouse orthotopic engraftment model. We and others have shown that calpain contributes to activation of the pro-survival AKT kinase. We hypothesize that calpain may regulate AKT activation by cleaving three regulatory phosphatases, PTEN, PP2A and PHLPP. Furthermore, our lab recently demonstrated that conditional deletion of *capns1* in the mammary epithelium attenuates HER2-mediated tumorigenesis in a transgenic mouse model. Calpain-1/2 knockout in mammary tumor epithelial cells (MTECs) derived from these mice reduced their tumorigenic potential. To mimic the effects of systemic pharmacologic calpain inhibition, I am working to develop a doxycycline inducible *capns1* knockout MTEC system for use in orthotopic mouse engraftment models. Combined, these models will contribute to our understanding of calpain's role in drug sensitivity/resistance and may lead to novel combinatory therapies to improve outcomes for patients diagnosed with aggressive breast cancer subtypes.

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