Elucidating mechanisms of tumor permeability in Triple Negative Breast Cancer
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Elucidating mechanisms that contribute to breast cancer metastasis, and determining effective options to treat and eradicate cancer cells colonizing vital organs is important as >90% of TNBC deaths are directly attributable to growth of disseminated cells and the formation of metastases. The funding provided by the TNBCF provided resources that allowed for an extensive advancement in our understanding of processes that contribute to metastatic dissemination of TNBCs, as well as developing pioneering models to study the metastatic process, and which we are using to develop regimens to eradicate metastatic breast cancer. We are grateful for these resources, and have several reportable findings including:

Vascular properties of breast cancer cells contribute to metastasis.
In our 2014 *Clinical and Experimental Metastasis* publication(1), we reported the identification of a type of TNBC cell that exhibited endothelial morphologies and shared gene expression profiles with blood vascular endothelial cells. These “claudin-low” or “mesenchymal” cancer cells exist with varying proportions within most TNBCs and have migratory and cancer stem-cell characteristics that could contribute to cancer-vascular blood vessel mosaics, and thus promote metastasis. Therefore this project sought to identify genes within TNBC cells that we could target to inhibit endothelial-like tube morphologies of cancer cells. We focused on four genes that were potential strong candidates at mediating tubule morphologies and cancer/endothelial interactions, including CLP24, TMEM45A, SLPI, and SERPINE2. Reducing CLP24 and TMEM45a function through small interfering RNAs (siRNA) in the MDA-MB-231 breast cancer cell lines had little to no effect on *in vitro* tubule formation or *in vivo* vascular permeability as measured with acoustic angiography. Interestingly, inhibition or enforced expression of either SLPI or SERPINE2 was found to affect vascular tubule formation *in vitro* and promoted metastasis *in vivo*. Through collaborative studies with Simon Knott at Cold Spring Harbor, we identified that these two genes not only controlled endothelial-like tubules in TNBC tumors of the Claudin-low phenotype, but in addition they served as anticoagulants, ensuring perfusions of these extravascular networks which promoted lung metastasis(2). Through our bioinformatic analyses, we identified that these genes were not only important in these mouse models, but are in fact also validated proteins that likely promote metastasis in humans. This work was published in ***Nature,*** acknowledged TNBCF as a funding source, and has received considerable press from various news outlets around the world. We have included a copy of this exciting publication for your records.

Development of models to eradicate TNBC metastases.
The data from the study in the Nature paper showed the importance of TNBC vascular mimicry to intravasate, or get into, the vasculature. However, since TNBCs are often identified as metastases at

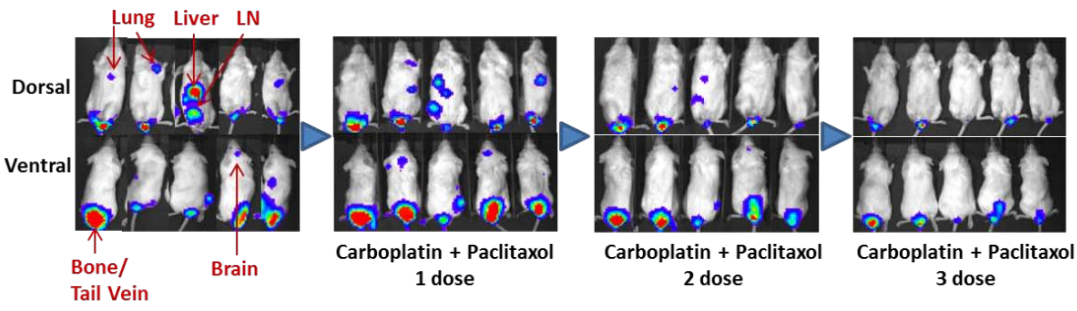

Figure 1: Curing TNBC Brain, Lung, Liver, and Lymph Node Metastases from the WHIM30 PDX BRCA mutant model. Bone metastases that formed at the cancer cell injection site were reduced but not eradicated.

the initial diagnosis, we are now expanding our models to utilize not only established breast cancer cell lines, but also patient-derived xenograft (PDX) models to find effective therapeutics for TNBC metastases. Our initial models have utilized two PDX lines, WHIM30 and WHIM2, which were not subjected to *in vitro* selection procedures found with established breast cancer cell lines. After labeling the cells with luciferase encoding lentivirus we were able to track metastatic outgrowths that arose after intravenous injection into the tail vein. Strikingly, in mice that had a high tumor burden of WHIM30, high dose treatment with carboplatin and paclitaxel was able to cure these mice of metastases in the brain, lung, liver, and lymph node. Interestingly, the cancer cells at the base of the tail, which we believe were incorporated into the bone did shrink, but were not eradicated. We are now expanding on these models and have received NIH RO1 funding for these studies; thus data collected as part of this grant were leveraged to obtain further NIH funding.

Other TNBCF supported studies that have been submitted for publication.

During the funding period for this project we have been approached by two independent laboratories to assist them with data analyses related to their research in TNBC; the Heide Ford Lab at the University of Colorado, and the Leslie Parise lab at the University of North Carolina. In both of these manuscripts, Drs. Harrell and Perou provided bioinformatic modeling support that was essential for completion of these studies. The Ford Lab studies identify mechanisms through which the SIX1 gene interacts with p53 to regulate TNBC development in mouse models(3). The Parise lab manuscript (now published and citing TNBCF support) identified a novel gene, CIB1, that when targeted successfully killed a large panel of different TNBC cell lines(4). Given the support provided to the Perou Lab, and the mission of the TNBCF, we felt it appropriate to acknowledge the TNBCF in these publications.

Career Advancement

During the course of these studies Dr. Harrell received and accepted a faculty position at Virginia Commonwealth University, which he started in July of 2015. He plans to continue his research into identifying other key genes that mediate endothelial characteristics and metastasis of TNBC. In closing, we thank the TNBCF for supporting our research, and we remain committed to finding a cure for TNBCs.

References

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