CP5*: Analytical determination of biomarkers for diagnosis of breast cancer metastatic progression
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Associated TRD: TRD3.2

Significance: Dr. Raman's laboratory has made pioneering contributions towards determining the role of developmentally regulated genes, such as Twist, in cancer biogenesis and in developing imaging probes directed towards better understanding the metastasis process [1-8]. Monitoring and treating metastatic progression remains a formidable task due to many gaps in our knowledge including an inability to monitor specific differential adaptations in different tissue types. This is a consequence of the fact that visceral organs differ vastly from one another with unique attributes of metabolism, developmental programs, microenvironments, and function resulting in defined physiological identities. Consequently, metastatic cancer cells growing in an organ outside of the breast have likely diverged from the cancer cells found in the primary tumor and ought not to be considered as simple primary tumor implants. While an abundance of data has been produced using "-omics" approaches, well-characterized limitations have been noted [9] and our understanding of the fundamental dependencies and molecular mechanisms in organ-specific metastatic progression remains limited [10,11]. Accordingly, in order to develop new targeted treatment regimens aimed at controlling and ablating metastatic progression, there is an urgent need to evaluate the distinct phenotypic differences that exist between isogenic tumor cells growing at different metastatic sites.

Approach: To aid in evaluation of molecular distinctions between metastatic lesions and primary tumor, Dr. Raman's laboratory has established isogenic (analogous to patients) metastatic breast cancer (IMBC) cell lines derived from: brain, liver, lung, and spine from the primary tumor cell line (MDA-MD-231). These cell lines are of the aggressive triple-negative phenotype (TNBC), i.e., lack expression of estrogen and progesterone receptors as well as human epidermal growth factor receptor (HER2), and as such representative of metastatic disease that has limited treatment routes. Preliminary biological evaluations of these new metastatic cell lines indicate site-to-site phenotypic distinctions that reflect likely altered biophysical and molecular characteristics as well as drug resistance differences. As shown in **Fig. 1**, cells isolated from the brain were found to be significantly more resistant to paclitaxel as compared to the cells isolated from the lung, which reinforces the necessity for ascertaining therapeutic drugs targeted to organ-specific metastases. The LBRC and the Raman lab will collaborate to objectively define the biochemical and mechanical distinctions between these established IMBC cell lines. By adapting the plasmonic vertical nanopillar platform (TRD 3.2), we will build a catalogue of spectral and biophysical markers that constitute each metastatic site and validate/correlate them against immunohistochemistry as well as metabolomic and proteomic analyses. Working with the Raman lab, we will also determine the chemotherapeutic efficacy of four major classes of FDA-approved drugs - antimetabolites (gemcitabine), anti-mitotics (taxol), antibiotic/DNA replication disruptor (doxorubicin) and alkylating agents (cyclophosphamide) - by monitoring the identified mechanical and molecular markers and testing against standard MTS and clonogenic assays.

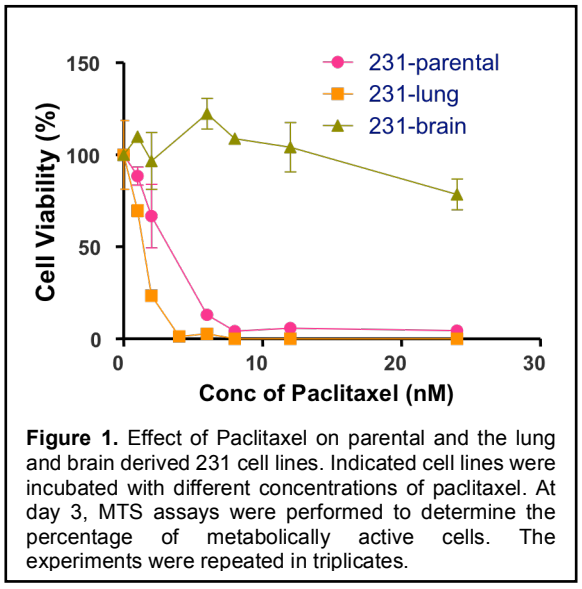

Figure 1. Effect of Paclitaxel on parental and the lung and brain derived 231 cell lines. Indicated cell lines were incubated with different concentrations of paclitaxel. At day 3, MTS assays were performed to determine the percentage of metabolically active cells. The experiments were repeated in triplicates.

Push-Pull Relationship: For non-destructive differential characterization of the IMBC cell lines, the LBRC **pushes** a novel plasmonic vertical nanopillar platform that can inform both on the adhesion and nanoscale deformability characteristics and on the molecular differences. Dr. Raman's lab seeks to further advance the use of Raman spectroscopic analyses to metastatic lesions in mouse models and flash frozen matched primary and metastatic human breast cancer samples **pulling** the LBRC to develop wide-field Raman microscopy approaches that can offer stain-free chemical images on the tissue specimen without necessitating long mapping times.

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