PUBLIC/LAY ABSTRACT

Extracellular vesicles regulate metastatic breast cancer dormancy in the liver

This proposal focuses on helping the many people who unknowingly harbor latent metastatic breast cancer by finding ways to prevent recurrence. One of the most common sites of breast metastases is the liver and its involvement correlates with the worst prognosis. Metastatic breast cancer remains largely incurable and the danger stems from a subset of tumor cells that lie in a dormant, non-proliferating state for years to decades before re-emerging as clinically detectable metastases, even after seeming curative removal of the initial tumor. Breast cancer cells can spread to distant sites even at the earliest stages of primary tumor development, well before detection, making strategies to prevent dissemination largely ineffective. Instead, the key to mitigating metastatic disease lies in identifying the mechanisms that drive tumor cells into a non-proliferating state and leveraging them to identify novel therapeutic approaches to further delay or prevent the onset of recurrence.

Scientific rationale: The mechanisms as to why some metastatic breast cancer cells initially enter a dormant state and what enables them to remain that way for a period of time remain unclear, particularly with respect to the liver. Cells and cues within the metastatic organ are known to be critical regulators of dormancy, with those belonging to immune and stromal components being the leading players in these interactions. Within the liver, the relevant cells are termed non-parenchymal cells (NPCs). The NPCs comprise about a third of the cellular composition of the liver and are both more sensitive to and the primary source of cues and signals. The role activated NPCs play in promoting the outgrowth of metastatic breast cancer cells is well characterized. However, our preliminary data indicated that inclusion of normal, quiescent NPCs (qNPCs) in a 3D liver tissue mimic reduced breast cancer proliferation and that their extracellular vesicles (EVs; nanometer-sized vesicles containing various signaling molecules) induced changes suggestive of reduced proliferation and invasiveness, which are key indicators of dormancy. Together these data lead us to ***hypothesize that EVs from qNPCs ($qNPC^{EV}$) suppress the proliferation of metastatic breast cancer cells upon colonizing the liver, allowing them to become dormant.*** Within the context of dormant liver metastasis, this hypothesis will be examined by discerning the individual contributions of the resident immune and stromal cells of the liver (NPCs) that are responsible for inducing the functional change in metastatic breast cancer cells via the secretion of EVs, and if this in turn promotes latent dormancy.

Innovation: Dormancy in breast cancer has been a challenging aspect of metastasis to study due to the paucity of relevant model systems. While animal models have provided valuable insights, they are not fully representative of the human situation due to issues of interspecies functions (*e.g.* immune and metabolic), and such studies also use immune compromised or artificially engineered mouse hosts. To address this, microphysiological systems (MPSs) – mini organ-on-a-chip models of human tissues and organs – have been developed which both complement and overcome some of the limitations of the aforementioned models. The Clark laboratory and collaborators have been involved in pioneering these MPSs and have established an all-human biologically complex Liver MPS which reproduces dormant breast cancer metastasis that is reflective of the human situation, and will be utilized for the proposed hypothesis.

Clinical applicability & impact: The major goal of this project is to turn metastatic breast cancer into a clinically irrelevant, manageable disease by maintaining metastatic breast tumor cells in a non-proliferating and dormant state. Successful completion of the proposed work will advance metastatic breast cancer research by elucidating the cells and cues that promote and maintain dormancy in metastatic breast cancer cells. This information will be leveraged to investigate targeted interventions to inhibit, delay or minimize recurrences. Transitioning towards the development of target therapies will initially involve interrogating the contents of EVs to ascertain the signaling molecule(s) that are key to regulating and maintaining quiescence. Once targets are identified, experiments will move forward to preclinical testing (testing that occurs prior to human clinical trials) of therapeutic drug candidates using the Liver MPS. The MPS will provide unmatched benefits and advantages, as the liver is both the main site of drug metabolism and dose-limiting toxicity for most common cancer therapies. Effectively, this means we will be able to more closely predict the efficacy and toxicity of drug candidates in humans. Notably, this attribute of the system for preclinical predictability is validated by its recent recognition by the Federal Drug Administration (FDA), speeding the translation to clinical trials in just a few years. The time to patient impact for these findings will depend on whether such agents are already in clinical use (*e.g.* repurposing of drugs is accelerated over new approvals).

In summary, the successful completion of the proposed project will identify mechanisms promoting dormancy whose activities can be leveraged to prevent or delay recurrence. The high and unchanging mortality rate for metastatic breast cancer make this an urgent area of research that is critical for the next generation of breast cancer patients to become breast cancer survivors.