The Isolation and Detection of Cell-Derived Microparticles in Murine and Human Plasma

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Microparticles (MPs) are 100nm to 1.5μm membrane blebs released from cells via cell activation-stimulated budding and/or during apoptosis. Cell-derived MPs circulate in the bloodstream retaining features of their parent cells, such as surface antigens, which can serve as “fingerprints” of cellular origin. In this way, MPs from particular organs or tumors can serve as biomarkers of an individual's physiological condition or disease state. Our research centered on the task of developing a reproducible method for the isolation and detection of cell-derived MPs from both murine and human plasma. We utilized the expression of cell surface antigens as well as Annexin-V recognition of exposed phosphatidylserine (PS) to detect and characterize MPs isolated from conditioned media via differential centrifugation. We confirmed the sizing of Annexin-V and antigen-positive MPs with flow cytometry, and further visualized and sized the MPs using transmission electron microscopy. Using platelet-poor plasma obtained from mice injected with human Mia-PaCa-2 pancreatic cancer cells, we were able to successfully detect and differentiate the cell line-derived MPs and the platelet-derived MPs from the mouse itself. Spike-in experiments indicated detection limits of approximately 20 MPs/ul plasma. Overall, we successfully demonstrated the ability to reproducibly isolate MPs from conditioned media, as well as murine and human plasma, and the ability to define the cellular origin of MPs through the presence of cell surface antigens. Our future steps will include the application of our isolation and detection methods to quantify and correlate the concentration of tumor-derived MPs with tumor size in murine tumor progression models.

Research advisor Katherine Landschulz writes, “Abe was able to successfully develop techniques for using flow cytometry to detect cell-derived microparticles in both human and non-diseased and diseased (tumor-laden) murine plasma. His future steps will include the quantification of these microparticles in order to correlate them with pancreatic tumor size in mice.”