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DISSERTATION

"Targeting Phosphatidylserine (PS) and PS Receptors in Oncology: Therapeutic mAbs and Mechanistic Implications"

by

David Calianese

Molecular Biology, Genetics, & Cancer Track

B.S., 2014, Providence College

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Professor
Department of Microbiology, Biochemistry, and Molecular Genetics

Friday, July 31st, 2020
1:00 P.M.

MSB-B619

ABSTRACT

The asymmetric distribution of lipids across the inner and outer leaflets of the plasma membrane, characterized by the distribution of anionic phospholipids such as phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) on the inner leaflet and phosphatidylcholine (PC), sphingomyelin on the outer leaflet, are important for membrane homeostasis. This arrangement also ensures proper functioning of proteins embedded in the membrane or bound to the extracellular or intracellular leaflets. Membrane asymmetry is irreversibly breached during caspase-dependent apoptosis, whereby externalized PS on dying cells interacts with a cohort of PS receptors, the best characterized being the TAM (Tyro3, Axl, and Mertk) and TIM (T cell/transmembrane, Immunoglobulin, and Mucin) trans-membrane receptors, that mediate engulfment of the cell corpse (a process called *efferocytosis* in recent years). PS is also reversibly externalized on activated and stressed viable cells through calcium and ATP-mediated processes. Immunologically, externalized PS, and subsequent interactions with PS receptors, initiates signaling cascades leading to the production of immunosuppressive cytokines and induction of inflammation resolution signals that maintain tissue tolerance and mitigate autoimmunity. Under physiological conditions, these signals preserve tissue homeostasis; however, in the context of pathological conditions such as cancer and infectious disease, suppressive PS signaling is hijacked and promotes disease progression. Due to a high level of metabolic stress and cell death within tumor microenvironments (TMEs), PS is constitutively externalized on tumor cells as well as the associated vasculature, and thus is an appealing target for therapeutics. Phosphatidylserine (PS) targeting monoclonal antibodies (mAbs), including 11.31 that directly targets PS, and Bavituximab (Bavi), that targets PS via β2-glycoprotein 1 (β2GPI) have been in pre-clinical and clinical development for over 10 years for the treatment of infectious diseases and cancer. The first part of my thesis investigated the distinction between PS-targeting mAbs 11.31 and Bavi, revealing a novel mechanism by which 11.31 modulates T-cell receptor mediated (TCR)-mediated T-cell activation in human healthy donor peripheral blood mononuclear cells (PBMCs). Confocal and AMNIS® Imagestream® imaging approaches combined with competition assays using PS-binding protein Annexin V show that while 11.31 and Bavi both preferentially recognize activated T-cells, they bind to different locations on the plasma membrane and appear to be functionally distinct. The second part of my thesis investigated the diversity of PS-targeting mAbs found clinically in HIV-1 infected patient plasma. Screening of 105 patient plasma samples revealed a broad array of PS-, as well as β2GPI-, reactive antibodies ranging in isotypes as well as IgG subtypes, indicating that a diverse repertoire of PS-targeting antibodies occur naturally. These may harbor previously uncharacterized immunomodulatory functions and may have therapeutic potential. The final part of my thesis aimed to characterize the signaling mechanisms of a cohort of PS-receptors, T-cell immunoglobulin and mucin-domain containing receptor (TIM), as well as develop a first-in-class family of PS-targeting constructs in order to further elucidate PS-signaling as well as design a novel approach to targeting within TMEs.