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#  DEFENSE OF THE DOCTORAL

# DISSERTATION

**“Essential role of phosphatidylserine for the activation of Tyro3, Axl and MerTK (TAM) receptors by Growth Arrest Specific-6 (Gas6)”**

by

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**ABSTRACT**

The Tyro3, Axl, and Mertk (TAM) receptors are three homologous Type I Receptor Tyrosine Kinases that have important homeostatic functions in multicellular organisms by regulating the clearance of apoptotic cells (efferocytosis). Pathologically, TAM receptors are overexpressed in a wide array of human cancers, and often associated with aggressive tumor grade and poor overall survival. In addition to their expression on tumor cells, TAMs are also expressed on infiltrating myeloid-derived cells in the tumor microenvironment, where they appear to act akin to negative immune checkpoints that impair host anti-tumor immunity. The ligands for TAMs are two endogenous proteins, Growth Arrest-Specific 6 (Gas6) and Protein S (Pros1), that function as bridging molecules between externalized phosphatidylserine (PS) on apoptotic cells and the TAM ectodomains. Presently, the molecular mechanisms by which Gas6/Pros1 mediate TAM activation are not well understood. Using TAM/ IFNγR1 reporter cell lines to monitor functional TAM activity, we found that Gas6 activity was exquisitely dependent on Vitamin K-mediated γ-carboxylation and binding to PS, whereby replacing Vitamin K with anticoagulant warfarin, generating domain mutants, or substituting glutamic acid residues involved in PS binding, completely abrogated Gas6 activity as a TAM ligand. Consistent with this idea, externalized PS on apoptotic cells, on calcium-stressed cells, or on tumor-derived exosomes, opsonized Gas6 and all served as “cell-based ligands” to activate TAMs. Finally, using overexpression and CRISPR/Cas9 gene editing, we show that TMEM16F, a calcium-activated phospholipid scramblase, is required for 4T1 cancer cells to mediate calcium stress-induced PS exposure. When TMEM16F knockout (KO) 4T1 cells were orthotopically transplanted into mammary fat pads of Balb/c immune-competent mice, the KO cells developed smaller tumors in comparison with their wild-type counterparts. Taken together, our findings indicate that PS is critical for Gas6-mediated TAM activation. This further implies that TAMs are hyper-activated in tissues with high externalized PS found in tumor microenvironment. Our results provide new perspectives to better understand immune escaping mechanisms in tumor microenvironment and identify PS scramblases as potential targets for cancer therapeutics.