ImmunoTools *multiplex* Award 2014

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Identification of novel cytosolic and surface proteins in human platelets using multiplex array technology

Platelets play an instrumental role in hemostasis, immunity, and related pathological conditions such as myocardial infarction, cerebral vascular accident, transient ischemic attack, and various clotting disorders. Most of the platelet functions that are associated with hemostasis and immunity are mediated by either signaling molecules stored in granules, the content of which is released upon activation, and surface proteins, which play a major role in initiating signal transduction pathways as well as in mediating homotypic and heterotypic intercellular interactions (e.g., platelet-platelet binding and platelet-leukocyte or platelet-endothelial cell binding, respectively).

Accordingly, identification of the full spectrum of cytosolic and extracellular membrane proteins is critical for understanding the broad array of platelet functions under pathological conditions, especially in light of hemostasis and immune signaling.

Inasmuch as platelets are megakaryocyte-derived cell fragments that lack a nucleus and hence most of the transcriptional and translational machinery, genomic/transcriptomic techniques for the screening and identification of protein end-products is not a viable option in these cells. Instead, proteomic approaches are more suitable for such studies. Although many proteomic approaches have been employed to identify and characterize cytosolic and surface proteins in platelets, a complete data set regarding the platelet proteome is still missing (see table below). For these purposes, a technique employing a wide proteomic screening approach

would be most useful. The multiplex protein arrays are therefore a very interesting solution to study intracellular and surface proteins in human platelets.

Consequently, this project proposal aims to utilize the protein arrays to perform proteomic profiling of human platelets. We intend to include the data in a review we are finalizing for the journal *Blood*, entitled “Fluorescent labeling techniques for platelets.” In this review, we have compiled a data set of all known surface antigens on platelets as well as those that are expected to be present on the platelet membrane. With the multiplex protein arrays we want to focus specifically on the latter group so that we can include novel data in the review. Preferably, the proteomic profiling we want to perform includes: intracellular proteins involved in immune signaling (resting platelets), surface CD antigens on resting platelets, and surface CD antigens on activated platelets.

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includes free analysis of samples on several antibody arrays with large range of antibodies against human CDs, human cytokines, and others ...