ImmunoTools *special* Award 2024

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Secretory vesicles involved in vascular function: identification of protein cargo and study of secretion mechanisms.

Endothelial cells have a critical role in vascular homeostasis taking part in regulating processes like inflammation, angiogenesis, and hemostasis. Weibel-Palade Bodies (WPBs) are unique secretory organelles in these cells, storing critical proteins such as von Willebrand Factor (vWF) and P-selectin, which are released during vascular activation. Understanding the mechanisms of WPB exocytosis is crucial for deciphering the cellular responses that underlie endothelial dysfunction in vascular diseases.

To achieve our objectives, the selected ImmunoTools reagents will play a significant role in several key experiments:

1. Identify new WPB cargo molecules

• Anti-human CD62P (P-selectin): P-selectin is a well-established transmembrane marker of WPBs. By co-staining for P-selectin alongside newly identified potential cargo molecules, we can confirm their colocalization within WPBs. This approach provides a robust method to verify whether these newly identified molecules are stored in WPBs before secretion. Furthermore, using live-cell imaging techniques, tracking P-selectin dynamics during exocytosis will reveal the spatiotemporal behaviour of these vesicles and their associated cargo, contributing to our understanding of WPB biology.

2. Elucidating VEGFR2 Endocytosis Pathways

• Anti-human VEGFR2/CD309: VEGFR2 is a critical receptor for VEGF-mediated signalling. This antibody will be used to track VEGFR2 localization during endocytosis. The VEGFR2 antibody will be also essential for immunofluorescence

and western blot assays to confirm the presence of the protein.

3. Quantifying WPB Secretion via vWF Release

• PER-conjugated secondary antibodies: Quantitative analysis of vWF secretion is a primary readout for WPB exocytosis. PER-conjugated antibodies will be critical in ELISA assays to measure extracellular vWF levels under various experimental conditions. These secondary antibodies will also amplify detection in western blot assays, ensuring the robust validation of protein expression and secretion.

4. Functional Studies on Endothelial Cell Behavior

Many WPB cargos influence endothelial cell proliferation, migration, and angiogenesis. For this reason, we need the antibodies:

• CD31 and CD54, as they will be used to identify activated endothelial cells during different *in-vitro* assays.

Summary

The ImmunoTools reagents are crucial for our project, providing robust tools for studying endothelial cell biology and WPB dynamics. Anti-CD62P antibodies enable precise tracking of WPB exocytosis, while anti-VEGFR2 abs will allow understanding of VEGFR2 trafficking. The antibodies anti-CD31 and anti-CD54 will validate endothelial activation. The VEGFR2 protein, purified for high specificity, plays a key role in understanding the VEGF-mediated signaling pathways. By using the purified VEGFR2 protein, we can investigate its interactions with other cellular proteins. PER-conjugated secondary antibodies ensure sensitive and reproducible detection of vWF secretion, a key functional readout of WPB activity. Together, these reagents will support a comprehensive investigation of the mechanisms regulating WPB secretion and their broader implications for vascular health and disease. By employing these reagents in conjunction with advanced imaging and molecular techniques, our study will advance our understanding of endothelial cell function, potentially uncovering novel therapeutic targets for vascular pathologies.

ImmunoTools *special* AWARD for **Panagiotis Lentzaris** includes 9 reagents:

PER - conjugated polyclonal antibodies to mouse immunoglobulins (IgG), PER-conjugated polyclonal antibodies to rabbit immunoglobulins (IgG + IgM); PER-conjugated polyclonal antibodies to mouse immunoglobulins (IgG + IgM), PER-conjugated polyclonal antibodies to rabbit immunoglobulins (IgG)

monoclonal antibodies against human CD31 plus CD31 (TR17), CD62P (HI62P), CD54 (MEM-111)

recombinant human cytokines: rh Vascular Endothelial Growth Factor Receptor-2 (rh VEGFR2 / CD309)

DETAILS more AWARDS