AP-3 dependent cargo in endothelial Weibel-Palade bodies.

Endothelial cells form the inner lining of the blood vessels and provide a dynamic barrier between blood (components) and the underlying tissue. Upon injury of the vessel wall, endothelial cells immediately respond by releasing their secretory organelles, the Weibel-Palade bodies (WPBs). By secreting hemostatic, inflammatory and angiogenic mediators that are stored in WPBs the vessel wall actively participates in minimizing blood loss and mounting inflammatory responses. However, the exact content of WPBs is not known. WPBs are formed at the trans-Golgi network (TGN) and is driven by the expression of its main cargo, Von Willebrand factor (VWF). Together with VWF a number of other secretory proteins such as chemokines, P-selectin and angiopoietin-2 are co-targeted into WPBs. After budding from the TGN, WPBs undergo a complex maturation process that includes trafficking of proteins such as CD63 from the endocytic compartment to WPBs in an AP-3 dependent manner. It is unclear which proteins other than CD63 are trafficked to WPBs in this step. In this project we will determine the content of WPBs using mass spectrometry, with a focus on determining the AP-3 dependent cargo.

The AP-3 complex is a heterotetrameric protein complex that interacts with specific cytoplasmic tail motives of proteins to facilitate their delivery to lysosome-related organelles (LRO), such as WPBs. There are mutations in the *AP3B1* gene, which encodes the AP3β3 subunit, which result in a polysystemic disorder called Hermansky-Pudlak syndrome type 2 (HPS-2). HPS-2 patients suffer from occulocutaneous albinism, bleeding tendency, pulmonary fibrosis and immunodeficiency, due to abnormal maturation of LROs in many different cell types. We have isolated blood outgrowth endothelial cells (BOECs) from an HPS-2 patient in which CD63 trafficking to WPBs is completely abrogated. We have also generated AP3B1 deficient BOECs using CRISPR/Cas9-gene editing, which display a similar phenotype.

To determine the AP-3 dependent cargo we will use a comparative approach, in which we will isolate WPBs from normal and HPS-2/AP3B1$^{-/-}$ BOECs and determine their content with mass spectrometry. WPBs will be isolated by subcellular fractionation using density gradient ultra-centrifugation. Alternatively, we will fluorescently label WPBs by lentiviral expression of GFP-tagged WPB cargo molecules and subsequently isolate fluorescent WPBs from endothelial homogenates by flow cytometry or by affinity isolation. We will localize newly identified candidates using immune fluorescent stainings and/or GFP-tagging followed by confocal microscopy.

Technics to be used

- Cell culture
- Virus production/transfection/transduction
- Confocal microscopy
- FACS
- Mass Spectrometry

Duration: 6-9 months
Education: university
Research group: Trafficking and secretion in the vasculature
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