**PDE6D Dependent Trafficking of K-Ras to Stemness Promoting Centriolar Organelles**

Rohan Chippalkatti, Bianca Parisi, Farah Yacoub Kouzi, Nesrine Ben Fredj, Daniel Abankwa

Cancer Cell Biology and Drug Discovery Group, Department of Life Sciences and Medicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg

The cancer driving properties of *KRAS*, *NRAS* and *HRAS* appear to correlate with their ability to drive stemness in normal and cancer cells. However, exactly how RAS proteins promote stemness is not understood.

The trafficking chaperone PDE6D transports prenylated proteins to the primary cilium (PC), which is found on stem and epithelial cells, and harbours several stemness signalling pathways. Upon re-entry of the cell cycle, the PC is internalized and its basal body becomes the mother centrosome. Asymmetric inheritance of the centrosomes is typically associated with asymmetric divisions, where one cell retains stemness. Given that PDE6D plays a critical role in regulating the sub-cellular distribution of KRAS, we hypothesized that it also facilitates localization of KRAS to the PC, which would enable asymmetric apportioning of KRAS via the mother centrosome.

We found that KRAS accumulates more at the PC than NRAS and HRAS, and in a PDE6D-dependent manner in muscle C2C12 cells. Modulation of KRAS on the phosphorylation-site Ser181 either pharmacologically or by introducing mutations decreased its interaction with PDE6D as measured by BRET in HEK cells and reduced KRAS accumulation at the PC in C2C12 cells. Ciliated, stem-like C2C12 myoblasts differentiate into non-ciliated myocytes/ -tubes within a few days upon switching to low serum, which transiently downregulates MAPK-signalling. In line with this, we find that overexpression of dominant-negative KRAS-S17N increases differentiation even under high serum conditions. Intriguingly, the opposite is true for oncogenic KRAS-G12V, which increases the stem-like fraction even under low serum conditions. Additional data suggest that reducing the accumulation of active KRAS on centriolar organelles drives differentiation. Our data indicate that the distribution of KRAS during the cell-cycle to centriolar organelles, such as the PC and centrosomes, may be critical for its normal and aberrant cancer-associated activities.