Exploring the role of centrosome inheritance in cell fate determination in human stem-like cells

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Introduction
Each cell cycle, the centrosome needs to duplicate. This duplication results in formation of two differently aged organelles that have different structure, composition and function (Fig.2). Interestingly, in several stem cells that divide asymmetrically, the age of the centrosome correlates with the fate of the daughter cell that inherits it.

Cancer stem cells (CSCs) can divide asymmetrically into two cells with different fates: one has a self-renewal potential (stemness), and the other one will differentiate into the bulk tumor mass, contributing to tumor maintenance and metastatic growth. However, whether such connection exists in human CSCs remains unclear. The goal of our project is to explore whether this connection exists in human CSCs and further explore how these cells utilize the centrosome to control cell-fate.

Methods
SNAP tag technology in SLCs

Results

Age of the centrosome correlates with cell stemness in SLCs.

Conclusion
We followed the inheritance of old and young centrosomes during division of human mammary stem-like cells (SLCs), using SNAP-tag derived technology, and found that the age of centrosome correlated with the stemness of the cell that inherits it.

Fig.1. Stem-like daughter cell preferentially inherits the old centrosome.

Fig.2. Centrosome asymmetry. The centrosome contains two centrioles - mother (orange) and daughter (brown). During the cell cycle, mother and daughter centrioles are separated and new centrioles (pink) are formed. This results in a lineage of four centrioles: grandmother, mother and two daughter centrioles.

Fig.3. Using the SNAP-tag to follow centrosomes in SLCs.

(A) Labeling of a SNAP-tag fusion with a fluorescent SNAP-tag substrate. BG, O6-benzylguanine (BG) (adapted from (2)).

(B) As a cell model, we used human SLCs recently identified in human mammary epithelial cultures (3). We prepared two stable cell lines, each of which stably expresses a centrosome marker fused with SNAP-tag. The first cell line contains a PCM marker: the PACT component, centrin (PACT) second has the centriolar component, centrin 2 (CETN2).

(C) SLCs form mammospheres in 3D cultures, which can be used as an in vitro assay of stemness.

Fig.4. The presence of old centrosomes correlates with stemness properties in SLCs.

(A) Schematic overview of sequential labeling with two SNAP-tag substrates: 647-SiR (old) and Oregon-Green (new) and visualization of unequal distribution of old and new centrosomal markers in mitotic PACT cells (fluorescence microscopy). Cells were synchronized with thymidine (G1/S arrest). (B) FACS sorting of two populations based on the high and low signal from old and new PACT or CETN2 (P1 and P2, respectively). (B-F) The cells that inherit old centrosomes are more stem-like by the criteria of mammosphere formation. (B) Schematic overview of sequential labeling with CellTrace Violet (blue) Cell Proliferation Kit and two SNAP substrates: TMR (old) and 647-SiR (new). (C) Proliferation analysis of cells after double thymidine treatment (G1/S arrest). (D) FACS sorting of two populations based on the high and low signal from old and new PACT or CETN2 (P1 and P2, respectively). (E-F) Quantification of mammosphere-forming capacity for each sorted cell population (PACT cells: 3 technical replicates, p value < 0.05 (E); CETN2 cells: 1 technical replicate (F)).

Future plan
We observed the correlation between the age of the centrosome and stemness in human SLCs. Next, we will combine SNAP-tag labelling with proteomics to identify stem cell-specific centrosome components, and explore their role in stemness maintenance. The final goal of these studies is to reveal novel mechanisms of centrosome-mediated control that influences the fate of dividing cancer SLCs, some of which might represent new targets for cancer therapies.