Title: Actin isoform specificity in tumor cell migration and invasion.

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Main aim: To study the localization of actin isoforms in metastatic tumor cells at high spatial resolution using state-of-the-art microscopy techniques.

Background:

Tumor cell migration is controlled by microfilaments composed of the small globular protein actin. To orchestrate cell migration, actin filaments organize in various structures such as lamellipodia, focal adhesions and invadosomes. While it is acknowledged that this organizational diversity of actin filaments is controlled through tightly regulated interactions with hundreds of different proteins, it is still poorly understood how actin filaments are able to differentially recruit these proteins in space and time.

Non-muscle mammalian cells express two actin isoforms, β and γ cytosolic actin. Although β- and γ-actin only differ four residues, they differentially integrate into actin structures and each isoform is non-redundant for various biological processes. As such, the relative expression levels or aberrant expression of particular actin variants is an important feature of pathological conditions such as wound healing, cardiovascular disease and tumor development. Specifically for tumor development, deregulated expression of actin variants can lead to tumor malignancy, metastatic development and therefore a bad prognosis in many of the main cancer types such as liver, colorectal and lung cancer. Interestingly, how β- and γ-actin together regulate these processes is largely unknown.

For this internship project, we hypothesize that the specific and differential integration of monomeric β- and γ-actin in filaments drives the organizational diversity of actin filaments and thereby the migratory and invasive properties of tumor cells.

Research plan:

Tagging actin variants is challenging, but recent preliminary data in our lab suggests that a specific site in actin is permissive for manipulation (Fig. 1). In this internship, advanced molecular cloning techniques (Gibson assembly) will be used to generate additional tagged variants of β and γ actin variants with different epitope tags. The tagged variants will be transfected in metastatic breast cancer cells and their localization into various actin-based structure will be studied using super-resolution fluorescence microscopy. In addition to these overexpression systems, metastatic tumor cell lines with genetic insertions of small tags will be generated using CRISPR/Cas9 and analyzed using fluorescence microscopy.

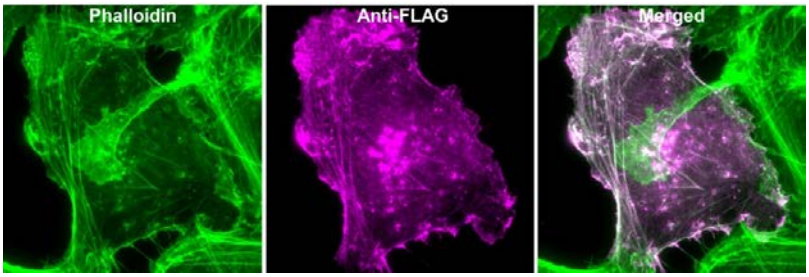

Fig. 1. FLAG-tagged β-actin integrates into actin filaments. A FLAG epitope tag is inserted into β-actin. This gene product is overexpressed in metastatic tumor cells and fluorescently labelled and imaged by widefield microscopy (magenta). Note the clear colocalization with actin filaments (Phalloidin staining, green).

Techniques:

- State-of-the-art fluorescence microscopy (Airyscan and STORM super-resolution)
- CRISPR/Cas9
- Advanced molecular cloning
- Cell culture
- SDS-Page and western blot

References:

1. Perrin et al. *Cytoskeleton (Hoboken).* 2010
2. Chen et al. *J Struct Biol.* 2012
3. Vedula et al. *Elife.* 2017
4. Patrinostro et al. *PNAS.* 2018