PROJECT 2 (optional)

DoS: Ivan de Curtis

Title: Mechanisms of assembly of a molecular network promoting cell invasion.

Curriculum: Basic and Applied Immunology and Oncology

Link to OSR/UniSR personal page:
https://research.hsr.it/en/divisions/neuroscience/cell-adhesion.html

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

We have identified a molecular network including the proteins liprin-alpha1, ERC and LL5 that are essential for cell migration and invasion. These proteins interact with each other to form plasma membrane associated platforms (PMAPs), dynamic membrane-less structures that promote protrusion at the cell edge by recruiting scaffolds and regulatory enzymes (Astro and de Curtis, 2015; Ramella et al, 2022). Interestingly, PMAPs are associated to focal adhesions at the front of migrating tumor cells, affecting protrusion turnover (Astro et al, 2016; de Curtis, 2021). PMAPs also promote invadosome-mediated extracellular matrix degradation, which is required for tumor cell invasion (Sala et al, 2018). Aim of this PhD project is to explore the mechanisms regulating the assembly and function of PMAPs in cell migration/invasion. Data from the lab support the hypothesis that PMAPs are formed by liquid liquid phase separation (LLPS) of ERC1 to form biomolecular condensates at specific sites within migrating tumor cells (Sala et al, 2019). LLPS of specific macromolecules (proteins and RNAs) leads to the formation of several intracellular biomolecular condensates. Our recent work has identified ERC1 as the driver of intracellular condensates that may underlie the formation of PMAPs in migrating tumor cells (Sala et al, 2019). The PhD student will investigate the mechanisms that underlie the assembly of PMAPs, and the consequences of perturbing the dynamics and composition of PMAPs on tumor cell invasion. Combination of established functional assays with high resolution time-lapse imaging by confocal microscopy/TIRF/FRAP will be used to study the effects of ERC1 mutations on the biophysical properties of LLPS-induced condensates. ERC1 mutants will be tested for their ability to form condensates, and to affect the formation/dynamics of endogenous PMAPs in migrating tumor cells. Relevant mutants will be tested for effects on the interaction with PMAP partners by biochemical analysis, and on migration/extracellular matrix degradation by invasive tumor cells. Bioinformatic analysis with available programs will be used to identify residues of structurally disordered regions of ERC1 implicated in LLPS. This information will be used to analyze the effects of these mutations on LLPS and protein-protein interactions, and the functional consequences of their expression in migrating tumor cells. Interesting mutants may be tested in vivo using invasive tumor cell lines with silenced/mutated protein, to address the effects on the development of tumors and metastases. Results will highlight new mechanisms influencing the invasive behavior of malignant cells, and may potentially reveal new targets for therapy.

Skills to be acquired by the student (*Number of characters*, *including spaces*: max 600):

The student will acquire skills in molecular cell biology including in vitro and in vivo cell motility and invasion assays; confocal, FRAP, TIRF imaging on fixed and live cells; cell biochemistry and protein structure-function analysis.

References (max. 15)

Ramella M, Ribolla LM, de Curtis I. Liquid-Liquid Phase Separation at the Plasma Membrane-Cytosol Interface: Common Players in Adhesion, Motility, and Synaptic Function. J Mol Biol. (2022) 434:167228.

de Curtis I. Biomolecular Condensates at the Front: Cell Migration Meets Phase Separation. Trends Cell Biol. (2021) 3:145-148.

Pehkonen H, de Curtis I, Monni O. Liprins in oncogenic signaling and cancer cell adhesion. Oncogene. (2021) 40:6406-6416.

Sala K, Corbetta A, Minici C, Tonoli D, Murray DH, Cammarota E, Ribolla L, Ramella M, Fesce R, Mazza D, Degano M, de Curtis I. The ERC1 scaffold protein implicated in cell motility drives the assembly of a liquid phase. Sci Rep. (2019) 9:13530.

Sala K, Raimondi A, Tonoli D, Tacchetti C, de Curtis I. Identification of a membrane-less compartment regulating invadosome function and motility. Sci Rep. (2018) 8:1164.

Astro V, Tonoli D, Chiaretti S, Badanai S, Sala K, Zerial M, de Curtis I. Liprin-α1 and ERC1 control cell edge dynamics by promoting focal adhesion turnover. Sci Rep. (2016) 6:33653.

Astro V, de Curtis I. Plasma membrane-associated platforms: dynamic scaffolds that organize membrane-associated events. Science Signal. (2015) 8(367):re1.

Astro V, Asperti C, Cangi MG, Doglioni C, de Curtis I. Liprin-α1 regulates breast cancer cell invasion by affecting cell motility, invadopodia and extracellular matrix degradation. Oncogene (2011) 30:1841-9.

Di Cesare A, Paris S, Albertinazzi C, Dariozzi S, Andersen J, Mann M, Longhi R, de Curtis I. p95-APP1 links membrane transport to Rac-mediated reorganization of actin. Nature Cell Biol. (2000) 2:521-30.