UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XLII CICLO

Research Topic ID: XLII – 1.8

Project Tutor: Davide Prosperi

Project Supervisor: Metello Innocenti

Project Title: A cytoskeleton-driven macropinocytic pathway as a vulnerability in cisplatin-resistant cervical cancer

Scientific background & Objectives

Cervical cancer remains a leading cause of cancer-related mortality worldwide, and cisplatin-based chemotherapy represents the standard-of-care for advanced disease. However, resistance to cisplatin severely limits therapeutic efficacy. Emerging evidence suggests that macropinocytosis, an actin-driven endocytic process, contributes to drug uptake and metabolic adaptation in cancer cells, yet its regulation and impact on chemoresistance remain poorly defined.

Recent findings from our laboratory identified a novel DIAPH1-CFL1 signalling axis that coordinates actin remodelling and macropinocytosis in cervical cancer cells. Loss of DIAPH1 impairs macropinocytic uptake and markedly sensitizes cells to cisplatin, suggesting a functional link between cytoskeletal dynamics and therapeutic response.

This project aims to (i) dissect the mechanisms whereby DIAPH1-dependent actin remodelling regulates macropinosome formation; (ii) determine how macropinocytosis influences cisplatin uptake and resistance; and (iii) validate the clinical relevance of this pathway in patient-derived samples.

By integrating mechanistic cell biology with translational analyses, this work will uncover cytoskeleton-dependent vulnerabilities in cervical cancer.

Project's Networks, Sustainability & Mobility

a) the coherence of the suggested project with competences/tools of the hosting lab
b) intradepartmental or external collaborations
c) at least one pertinent research article published by the proposer/s
d) 1 (or more) putative foreign institutions for achieving the required ordinary mobility (6 months)

a) The Innocenti laboratory has long-standing expertise in actin cytoskeleton regulation and cancer, formin (mDia proteins) and Arp2/3 complex biology, Rho GTPase signalling, and advanced imaging technologies.
The lab possesses CRISPR/Cas9 tools, proteomics platforms, 2D and advanced 3D culture systems, and *in vivo* models to dissect cytoskeleton-dependent endocytic processes and cancer drug responses.

b) Active collaborations ensuring strong mechanistic and translational integration include:

- Proteomics and quantitative mass spectrometry (**K. Gevaert**, VIB, Belgium) for interactomics and phosphoproteomics.
- Clinical collaboration with pathology units for tissue microarray analyses of cancer specimens (**K. Jalink**, NKI, Netherlands, **S. Werner**, ETHZ, Switzerland).

- Intradepartmental expertise in multiplexed imaging and spatial analysis to correlate actin remodelling, macropinocytosis markers, and clinical parameters (**F. Granucci**, BtBs, UNIMIB), and in the development of nanomedicines (**D. Prosperi**, BtBs, UNIMIB).

c) Recent PI's publications (14 as corresponding author) relevant to this PhD project**:**

Cangkrama, M. *et al.* A pro-tumorigenic mDia2-MIRO1 axis controls mitochondrial positioning and function in cancer-associated fibroblasts. *Cancer Res* (2022).

Argenzio, E. & Innocenti, M. The chloride intracellular channel protein CLIC4 inhibits filopodium formation induced by constitutively active mutants of mDia2. *FEBS Lett* (2020).

Argenzio, E. *et al.* Profilin binding couples chloride intracellular channel protein CLIC4 to RhoA-mDia2 signaling and filopodium formation. *J Biol Chem* **293**, 19161–19176 (2018).

Beli, P., Mascheroni, D., Xu, D. & Innocenti, M. WAVE and Arp2/3 jointly inhibit filopodium formation by entering into a complex with mDia2. *Nat Cell Biol* **10**, 849–57 (2008).

Isogai, T. *et al.* Quantitative Proteomics Illuminates a Functional Interaction between mDia2 and the Proteasome. *J Proteome Res* **15**, 4624–4637 (2016).

Isogai, T. *et al.* Proteomic analyses uncover a new function and mode of action for mouse homolog of Diaphanous 2 (mDia2). *Mol Cell Proteomics* **14**, 1064–78 (2015).

Isogai, T., van der Kammen, R. & Innocenti, M. SMIFH2 has effects on Formins and p53 that perturb the cell cytoskeleton. *Scientific reports* **5**, 9802 (2015).

Isogai, T. *et al.* Initiation of lamellipodia and ruffles involves cooperation between mDia1 and the Arp2/3 complex. *J Cell Sci* **128**, 3796–810 (2015).

Cangkrama, M. *et al.* A paracrine activin A-mDia2 axis promotes squamous carcinogenesis via fibroblast reprogramming. *EMBO Mol Med* **12**, e11466 (2020).

Kedziora, K. M. *et al.* Rapid Remodeling of Invadosomes by Gi-coupled Receptors: DISSECTING THE ROLE OF Rho GTPases. *J Biol Chem* **291**, 4323–4333 (2016).

Dual-Targeting Strategy to Repurpose Cetuximab with HFn Nanoconjugates for Immunotherapy of Triple-Negative Breast Cancer. Barbieri L *et al.* ACS Appl Mater Interfaces. 2025 Jun 11;17(23):33648-33663.
Del Nero, M. *et al.* Advanced Cell Culture Models Illuminate the Interplay between Mammary Tumor Cells and Activated Fibroblasts. *Cancers* **15**, 2498 (2023).

di Miceli, N. *et al.* TGF-β Signaling Loop in Pancreatic Ductal Adenocarcinoma Activates Fibroblasts and Increases Tumor Cell Aggressiveness. *Cancers (Basel)* **16**, 3705 (2024).

Rocca, G. *et al.* Multiplexed imaging to reveal tissue dendritic cell spatial localisation and function. *FEBS Letters* 1873-3468.14962 (2025).

Leyton-Puig, D. *et al.* Flat clathrin lattices are dynamic actin-controlled hubs for clathrin-mediated endocytosis and signalling of specific receptors. *Nature communications* **8**, 16068 (2017).

Leyton-Puig, D. *et al.* PFA fixation enables artifact-free super-resolution imaging of the actin cytoskeleton and associated proteins. *Biology open* **5**, 1001–9 (2016).

The Arp2/3 complex controls the development of homeostatic microglia.
Safaiyan S, *et al.* EMBO Rep. 2026 Feb 27.

mDia1 Assembles a Linear F-Actin Coat at Membrane Invaginations To Drive Listeria monocytogenes Cell-to-Cell Spreading. Dhanda AS, *et al.* mBio. 2021 Dec 21;12(6):e0293921.

d) The PhD candidate may be seconded to the labs of the foreign collaborators listed in *b)* for the mandatory stay abroad.