2023-12-21

International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Active sorting of cancer cells and fibroblasts

General information

Call	2024
Reference	2024-13-SILBERZAN
Keyword(s)	Biological physics; Active matter; Cell sorting; Ewing sarcoma; Cancer Associated Fibroblasts

Director(s) and team

Thesis director(s)	Pascal Silberzan
Research team	Biology-inspired Physics at MesoScales
Research department	UMR 168 – Physical Chemistry Curie

Description of the PhD thesis project

Abstract

Ewing sarcoma represents the second most frequent primary bone tumor among teenagers and young adults. Initially mostly localized in the skeleton, its propensity to develop metastases makes it particularly aggressive.

Motivated by in vivo experiments monitoring the morphology of xenografts of Ewin tumors in mice, we propose here an interdisciplinary project that aims at investigating the processes involved in the segregation of cancer cells and fibroblasts in vitro. This segregation is related to the cell/cell adhesive properties, but also to the fibroblasts' nematic organization and to their active contractility, both properties affecting the tumors' structure. In particular, it is not clear whereas the fibroblasts impair the migration of the cancer cells out of the tumor or favor it at particular locations.

In close collaboration with O. Delattre's lab, we will use an Ewing cell line whose adhesion and invasiveness properties can be controlled. Experiments in well-controlled conditions will allow describing the coexistence between clusters of cancer cells and fibroblasts. The observations will be interpreted with physical theories encompassing adhesion and active nematic order. These well-controlled in vitro experiments may bring new insights in the knowledge of the mechanisms at stake in the formation of these tumors in vivo.

Background

Ewing's sarcoma is a highly aggressive cancer forming mostly in bone and affecting primarily teenagers and young adults[i]. These sarcoma corelate with the fusion protein EWS-FLI1[ii]. Karine Laud-Duval in the Delattre's lab (Institut Curie) with whom we collaborate in the present project has developed an Ewing's cell line model (called 1C) in which the expression of EWS-FLI1 can be silenced with Doxycycline: 1C-DOX(-) (high EWS-FLI1) is proliferative and 1C-DOX(+) (low EWS-FLI1) is invasive[iii].

Preliminary in vitro experiments with the minimal system of 1C tumor cells and NIH3T3 murine fibroblasts have demonstrated cell sorting where the shape and size of the clusters of 1C cells are controlled by the

proportions of both cell types - as it is the case in vivo. These experiments have also shown that NIH-3T3 cells form "nematic" phases where cells tend to align with each other. These active nematic properties, together with the different cell-cell interactions, result in clusters of tumor cells surrounded by 3T3 cells oriented along the boundary between the two tissues. Although cell sorting has been investigated before[iv], there is no theory based on active matter that describes such systems. The aim of the present project is to understand **the impact of physical parameters (activity, adhesion,...)** in the sorting of these cells. In the same line, other systems including breast cancer cells vs. cancer-associated fibroblasts[v] will also be investigated.

Objectives

The aim of this interdisciplinary project is to characterize the complex architecture between the tumoral tissue and the surrounding fibroblasts cells and to understand it in physical terms. We first ask by in vitro experiments whether **cell sorting in the 1C/3T3 system correspond to an active "phase separation"**. And if the observed patterns corelate to the in vivo observations on comparable tissues upon the activation/silencing of the EWS-FLI1 gene.

Then, on these well-controlled in vitro experiments, we will address questions that would be difficult to be dealt with in vivo. In particular, we will quantify the impact of the **nematic ordering** and the **activity** of the fibroblasts[vi] on the dynamics and morphology of the resulting composite tissue. We will also conclude if such composite active tissues are prone to stabilize the cancer cells in compact isolated clusters or if they allow them to switch to an invasive phenotype. These results will be interpreted in the theoretical framework of **active matter**, together with our collaborators in the lab.

Along the development of the project, we will turn toward other systems such as **breast cancer cells and cancer associated fibroblasts (CAFs)** (in collaboration with F Mechta-Grigoriou's lab). We also have the expertise in optogenetics[vii] to control the expression of some proteins of interest (Rho GTPAses, or oncogene) on demand, using light.

Experimental approaches

Experiments are based on cocultures of cancer cells and fibroblasts. Cell mixtures will be first plated at various total cell densities and various ratios of each cell type on fibronectin-coated soft substrates. They will then be imaged with automated microscopes for up to several days (the different cells are fluorescently labelled with different colors to follow them dynamically). Velocity, density, proliferation fields will be quantified. Phase separation will then be investigated from a quantitative analysis of the videos. Other important parameters will be estimated from experiments in which the substrate is textured at different length-scales to independently impose boundary conditions and the orientation of the nematic director. Microfabrication will be performed in the on-site laboratory clean room and the impact of these multiscale cues will be included in the theory. Performing these experiments on a soft substrate is not only more physiological but it also allows measuring the traction forces exerted by the cells. These data will be compared to the predictions of the continuum hydrodynamic model developed by our collaborators[viii]. Finally, these experiments will be extended to other systems of cancer cells and associated fibroblast-like cells. In this last case, the stiffness of the substrate is a particularly important parameter as some of these CAFs are functional only on soft substrates.

International, interdisciplinary & intersectoral aspects of the project

International collaborations

We already work with several theory groups abroad (Netherland, Belgium, Israel…). Very recently, this problem of active demixing in active matter has been theoretically addressed in Oxford University (Yeomans' group). We have already discussed these questions with some of these groups and it is obvious that **there is a common interest in collaborating on these questions**. We would be thrilled to engage in a more formal collaboration on this project. A student dedicated to these experiments would be the necessary bridge to make this happen.

Interdisciplinary approaches

Physics/ biology: By essence, our project is an interdisciplinary biological physics project. The tools and techniques that we use are diverse, ranging from **biology** (cell culture, transfection, immunolabelling etc.) to **physics** (quantification of field of interest: force, orientation…, theoretical modelling) and **engineering** (image processing and microfabrication). Collaborations with the biology labs of O. Delattre and F. Mechta-Grigoriou are at the core of this project.

Theory /experiments: Although we perform experiments, we always work in close association with theory groups. The student involved in the project will interact with theoreticians for the interpretation of the results.

Recent publications

1. Sarkar T., Yashunsky V., Brézin L., Blanch Mercader C., Aryaksama T., Lacroix M., Risler T., Joanny J.-F., **Silberzan P**.: *Crisscross multilayering of cell sheets*, PNAS Nexus, **2**, (2023), pgad034

2. Yashunsky V., Pearce D.J.G., Blanch-Mercader C., Ascione F., **Silberzan P**.[+], Giomi L.[+]: *Chiral Edge Currents in Nematic Cell Monolayers* Phys. Rev. X **12**, (2022), 041017. ([+] equal contributions)

3. Moitrier S., Pricoupenko N., Kerjouan A., Oddou C., Destaing O., Battistella A, **Silberzan P**., Bonnet I.: *Local light-activation of the Src oncoprotein in an epithelial monolayer promotes collective extrusion*, Commun. Phys. **2**, (2019), 98

4. Blanch-Mercader C., Yashunsky V., Garcia S., Duclos G., Giomi L., **Silberzan P**.: *Turbulent dynamics of epithelial cell cultures*, Phys. Rev. Lett. **120**, (2018), 208001

5. Duclos G., Blanch-Mercader C., Yashunsky V., Salbreux G., Joanny J.-F., Prost J., **Silberzan P**.: *Spontaneous shear flow in confined cellular nematics*, Nat. Phys. **14**, (2018), 728 (**)

Expected profile of the candidate

We look for an experimentalist with a taste for interdisciplinarity (physics, biology). The candidate must possess a Master degree (or equivalent) in biological physics, quantitative biology, or related field, and have some experience in data analysis (preferably in image processing) and programming. Prior experience in experimental work is required; background in cell biology is a plus but is not mandatory. Knowledge of French language is not required but Advanced English level is expected.

[i] KA Ross et al. ISRN Oncol (2013):759725
[ii] Delattre et al Nature (1992) 359 :162
[iii] Tirode et al Cancer Cell (2007) 11 :421
[iv] Steinberg et al J Cell Sci (1975) 18 :385
[v] Pelon et al. Nat Commun (2020) 11:404
[vi] Duclos et al. Nat Phys (2018) 36 :728
[vii] Moitrier et al. Commun. Phys. (2019) 2:98
[viii] Marchetti et al Rev. Mod. Phys. (2013) 85:1143