1/ Purpose of the visit

PHYSICAL FORCES DRIVING FIBROBLAST-LED CANCER CELL MIGRATION

Cancer cell invasion and metastasis remain the leading cause of cancer-associated death. It is now clear that invasion is not simply a cancer cell autonomous process but the result of a complex interaction between tumor cells and their microenvironment. Among such non-cancerous cells, cancer associated fibroblasts (CAFs) are emerging as one of the most crucial cell types that drive tumor invasion. Recent evidence has established that CAFs are able to guide the collective migration of cancer cells. By co-culturing carcinoma cells and stromal fibroblasts in an organotypic system, Sahai and colleagues showed that cancer cells invaded the surrounding tissue by forming collective migratory chains and that the tip cell of every chain was always a fibroblast. While the ability of CAFs to rearrange the matrix and favor invasion is well established, how CAFs guide the forward motion of cancer cells remains unknown.

To decipher the biophysical features of the CAF-cancer cell interaction and their implication in cancer cell guidance, we propose to combine cancer cell invasion models with tools to measure inter- and intra-cellular forces. To do so, the labs of Dr Xavier Trepat, where the applicant is affiliated, and that of Dr Erik Sahai have established a collaboration.

Within the context of this collaboration, the purpose of this short visit is to share knowhow between both laboratories. The applicant will transfer to Sahai's lab the biophysical technology developed to measure cellular traction forces in Trepat's lab. Conversely, she will learn tools in cellular and molecular biology to better characterized the mechanical cross-talk involved between CAFs and cancer cells.

2/ Description of the work carried out during the visit

In the host laboratory, the applicant has implemented the main technical setups for leading Traction Force Microscopy (TFM) and micro-contact printing of proteins:

- TFM substrate fabrication (protein coated-polyacrylamide hydrogels of various stiffness containing fluorescent beads)
- Cell culture on TFM substrate of various stiffness (cancer cells, fibroblasts)
- Micro-patterning of proteins on glass coverslip and TFM substrate
- Cell culture on micro-patterned substrates

– TFM acquisitions (Live confocal microscopy imaging)

Furthermore, the applicant has improved her skills in:

– Spheroids fabrication
– 3D cell invasion assays in various ECM
– Live confocal microscopy imaging (3D spheroid invasion in ECM, 2D spheroid invasion on polyacrylamide gels).

Besides of technical skills, by interacting with all the members of Sahai's group the applicant had also the opportunity to deepen her knowledge regarding cancer cell invasion processes involving CAFs in various biological contexts.

3/ Description of the main results obtained

- **Co-working with Stefanie Derzsi, PhD.**

In order to go deeper into our understanding of the forces that drive the interaction between CAFs and cancer cells, two types of experiments were performed in parallel:

- 3D invasion of spheroids doublets: Visualizing the tension field generated by the CAFs and cancer cells through the matrix.
- 2D spreading of spheroids on TFM substrates: Visualizing the traction maps generated by collective cell migration during invasive process.

- **3D invasion:** Spheroids of CAFs (VCAFs-mcherry) and Cancer cells (A431-GPF) were fabricated by the standardized hanging-drop method. After one day of culture, one spheroid of each cell type was embedded in 3D ECM mixed with fluorescent beads. The distance between the two spheroids was adjusted manually to allow the visualisation of the spheroid doublet during the time lapse acquisition by confocal microscopy. A 24 hours time-lapse acquisition was performed by confocal imaging (1fr/30 min).

  Preliminary observations:

  Thanks to the fluorescent beads embedded in the ECM we managed to observe tensional field in the ECM induced by the two types of spheroids during the cellular invasion processes. We noticed that, even before cell invasion, spheroids of CAFs generate high displacement of beads through long-scale distance from the spheroid position. On the contrary, spheroid of cancer cell seems to generate less

tensional field in the surrounding matrix. The applicant foresees to repeat this type of experiment in her laboratory to further explore the effect of this ECM tensional modulation on the triggering of cancer cell invasion mediated by CAFs spheroid.

- **2D invasion, TFM experiment**: Spheroids of CAFs (VCAFs-mcherry), Cancer cells (A431-GPF) and mixed CAFs/A431 (1:1 ratio) were fabricated by the standardized hanging drop method. After one day of culture, one spheroid of each cell type was seeded 3hours on TFM substrate previously fabricated (fibronectin coated-PA gels containing fluorescent beads, Young's modulus: 3kPa or 9kPa). A 24 hours time-lapse acquisition was performed by confocal imaging (1fr/30 min).

  Preliminary observations:

  A first set of TFM images was acquired on these different samples; we observed marked differences in cell invasion strategy depending on both the type of spheroid observed (CAFs, A431 or mixed cells) and the substrate stiffness.

  The applicant foresees to repeat this type of experiment in her laboratory to further analyse the traction forces generated by these different types of spheroids on different substrate stiffness.

- **Co-working with Nil Ege, PhD. Comparative study of traction force generated by breast Normal Fibroblasts (NF) versus breast Cancer Associated Fibroblast (CAF) on different substrate stiffness.**

As a preliminary experiment, polyacrylamide gels (PA gels) of two different stiffness (Young's modulus: 3kPa and 6kPa) containing fluorescent beads were fabricated and coated with fibronectin. Breast NF or CAF were then seeded overnight on each PA coated gels. Traction Force Microscopy experiment were then performed by confocal microscopy acquisition on living cells. The first set of data was obtained and is now in computational processing.

- **Co-working with Danielle Park, PhD. Traction force measurement during the streaming migration of Kepi cells.**

As a complementary approach within this project, TFM substrates of various stiffness were fabricated in the host laboratory (PA gels of 3kPa, 6kPa and 9kPa). Then Micro-contact printing of protein were performed on these substrates (by the use of polydimethylsiloxane (PDMS) stamps previously fabricated in the applicant's laboratory) in order to generate lines of proteins (fibronectin, lines width 5 microns) that will be used as a 2D scaffold for cell migration. Kepi cell were then

seeded overnight on this substrate and TFM confocal acquisition were performed. The first set of data was obtained and is now in computational processing.

- **Co-working with Dr. Sophie Acton. Effect of Podoplanine (Pdpn) expression on traction force generated by Lymph nodes fibroblastic reticular cells (FRCs).**

As a preliminary experiment, polyacrylamide gels (PA gels) (Young's modulus: 6kPa) containing fluorescent beads were fabricated and coated with Fibronectin. ^{WT} FRCs or $^{Pdpn-/-}$ FRCs were then seeded overnight on each PA coated gels. Traction Force Microscopy experiments were then performed by confocal microscopy acquisition on living cells. A first set of data was obtained and is now in computational processing.

4/ Future collaboration with host institution

-Current collaboration in Sahai's Group

- Stefanie Derszi (PhD student)

"Deciphering the mechanical processes that drive Cancer cell invasion mediated by Cancer-Associated-Fibroblasts."

-Future collaboration in Sahai's Group

- Danielle Park (PhD student)

1. *"Analysing the traction field of coordinate cancer cells (Kepi cells) within streaming migration vs. other cancer cell types (MDA-MB 231, TS1, TS2 cells)."*

2. *"Deciphering the persistence and the strength of the streaming migration of Kepi cells."*

- Nil Ege (PhD student)

1. *"Deciphering whether the recruitment of YAP in the nucleus modulate the traction forces generated by fibroblasts on an elastic substrate."*

2. *"Comparative study of TFM experiments: Investigating YAP localisation and traction forces in fibroblasts adherent on different substrate stiffness."*

-Future collaboration in other group from Cancer research UK

- Dr. Sophie Acton (Caetano Reis e Sousa's Research group)

"Does Podoplanin expression impact on the traction force generated by the fibroblastic reticular cells?"

5/Projected publication/articles resulting or to result from the grant

In the next following months the applicant will commit herself to lead the proposed collaborations as well as the aforementioned experiments implemented for her own project. Hence, as a future step, the results that will emerge from this research will be then published as a collaborative work and the applicant ensures that she will acknowledge the Quantissue Grant for having supported the project.