Master 2 Internship

Title: **Breast cancer cell migration behavior under controlled substrate stiffness**

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Project:

Most animal cells release glycoproteins into the extracellular space, creating a structured meshwork called the extracellular matrix (ECM) with different biophysical features including topography, stiffness and porosity. Although these tissues govern the organization of the cells in the body, most of culture assays are still performed on 2D use substrates made of rigid materials such as glass or polystyrene. But, polystyrene and glass substrates exhibit stiffness values, which are orders of magnitude higher than that for ECM proteins causing the cells to show a non-natural behavior. Indeed, accumulative evidence emphasize the importance of matrix stiffness of naturally derived as well as engineered hydrogel substrates used for *in vitro* cell culture, in the maintenance of stem cell properties or the cell migration, spreading or adhesion of cancer cells (Engler, Sen et al., 2006, Zaman, Trapani et al., 2006).

Accordingly, the project proposes to perform comparative mobility characterization of breast cancer cell lines on 2D substrates with calibrated stiffness within physiological range corresponding to soft normal breast tissues and to stiffer breast tumor environment. Our preliminary data indicate that the expression of membrane receptor CD95 promotes cell migration/invasiveness possibly responsible for metastasis and poor clinical outcome. Mobility properties will thus be compared between a CD95 positive and CD95 deficient breast cancer cell line.

First, the stiffness of hydrogels formulated using a culture medium as solvent, will be characterized using atomic force microscopy (AFM) indentation. Second, 2D cell cultures will be performed on selected substrates. Migration of individual cells and cell population dynamics in 2D will be assessed based on long-term video imaging obtained with a lens-free optical phase microscope (cytonote 6W @IPRASENSE) fitted inside an incubator. These experiments constitute an initial step to understand how the presence of CD95 is involved for metastatic dissemination in breast cancer.

Scientific context:
This internship at LOMA is proposed in the setting of an interdisciplinary collaboration also involving the biology groups of Pierre Vacher (Institut Bergonié, Bordeaux) and Patrick Legembre (Inserm. Limoges), and the physics group of Benjamin Audit (Laboratoire de Physique, ENS de Lyon). The trainee will benefit from the complementary expertise provided by these groups in cancer biology and in data processing. The present project integrates within the general questioning addressed by the collaboration on the "Role of CD95/Fas in cancer stem cells regarding the extracellular matrix stiffness". The biophysical aspects of this project outlined below constitute a possible backbone for a follow-up PhD project.

Breast cancer (BC) is the most common cause of cancer in women (DeSantis, Fedewa et al., 2016). BC is a heterogeneous disease whose molecular classification has been significantly improved to distinguish luminal A and B expressing hormonal receptors including estrogen (ER) and/or progesterone receptors (PR), basal/triple negative breast cancer (TNBC), and human epidermal growth factor receptor 2 (HER2)-like tumors. Basal/TNBC patients present the poorest clinical outcome with no targeted therapies available as compared to other molecular subtypes. TNBC presents an intratumoral heterogeneity whose origin is mainly explained by a hierarchical organization of tumor tissues where several subpopulations of self-renewing breast cancer stem cells (bCSCs) sustain the long-term oligoclonal maintenance of the neoplasm (Kreso & Dick, 2014). Accordingly, CSCs are suspected to be the seed for the distant metastasis responsible for poor clinical outcome (Oskarsson, Batlle et al., 2014).

CD95 (Fas/APO-1/TNFRSF6) belongs to the tumor necrosis factor (TNF) receptor family (Peter, Hadji et al., 2015). Recent data highlighted that this receptor does not only induce apoptotic signaling but also non-apoptotic signals promoting oncogenesis (Barnhart, Legembre et al., 2004, Kleber, Sancho-Martinez et al., 2008). The cognate ligand of CD95, namely CD95L (CD178), is a transmembrane protein mainly found expressed by activated T lymphocytes and natural killer (NK) cells to eliminate infected and transformed cells through cell-to-cell contact (Suda, Takahashi et al., 1993). CD95L extracellular domain can also be cleaved by metalloproteases (Fouque, Debure et al., 2014) to release a soluble CD95L (s-CD95L). Unlike membrane-bound CD95L (m-CD95L), s-CD95L fails to trigger cell death but instead contributes to aggravating metastatic dissemination of cancer cells (Barnhart et al., 2004, Hoogwater, Nijkamp et al., 2010, Kleber et al., 2008, Malleter, Tauzin et al., 2013) by inducing non-apoptotic signaling pathways including MAPK (Chen, Park et al., 2010), NF-κB (O'Reilly, Rojo et al., 2006) and PI3K (Tauzin, Chaigne-Delalande et al., 2011). Although CD95L-expressing immune cells edit tumor cells by sparing cancer cells expressing low CD95 level at their plasma membrane (Strasser, Jost et al., 2009), CD95 is still expressed in TNBCs (Blok, van den Bulk et al., 2017) and the function of this receptor in these cancers remained unknown. Our preliminary data indicate that CD95 expression in TNBC cells sustains dedifferentiation of stem cells and promotes cell migration/invasiveness.

Cell mechanical properties (stiffness, viscoelasticity) are determinant components of their ability to spread, stretch, exert forces on substrates, shape change and migrate. This mechanics has been characterized by different methods during the past two decades (Van Vliet *et al.* 2003), among them, atomic force microscopy (AFM) was shown to conciliate both a very high spatial resolution (a few nm) and a very high sensitivity (a few pN resolution). Its application to cancer cells has been paramount for our understanding on how cell mechanics can be impacted by an alteration of different signaling pathways in strong relation with their cytoskeleton filament network compliance to stress (Suresh 2007, Laperrousaz *et al.* 2016). Recent approaches have stimulated the revision of more traditional model of cells mechanics as pure elastic bodies as complex visco-elastic systems with fractional temporal and spectral response (Gerasimova-Checkina *et al.* 2018) and to demonstrate that these power-law viscoelastic relaxation of cells encodes cell motility trends (De Sousa *et al.* 2020). Because the stiffness of breast tissues varies (*i.e.*, normal breast tissue exerts a mechanical pressure on cells

has been estimated by standard elastic models in the range of 1.2 kPa while breast tumor environments exhibit mechanical features comprised between 2.4 to 4.8 kPa (Keely & Nain, 2015) and CD95 contributes to stemness at least *in vivo* and in plastic 2D cell culture (Chen et al., 2010, Qadir, Ceppi et al., 2017), we hypothesize that the presence of CD95 in TNBC cells could promote their adaptation to pathological mechanical pressures favoring individual migration and/or inter-individual co-migration in elevated ECM stiffness and thereby favoring metastatic dissemination. Accordingly, this project proposes to combine biophysical tools and algorithms to investigate the effect of CD95 in TNBCs regarding maintenance of CSCs and migration/invasion in different normal/tumor environments in order to elaborate models of cell migration and population behavior to understand how the presence of CD95 is mandatory for TNBC cells to promote metastatic dissemination. The experimental tools are respectively a nano-probe indentation of the cells with an atomic force microscope (AFM) and a lens-free phase microscope.

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