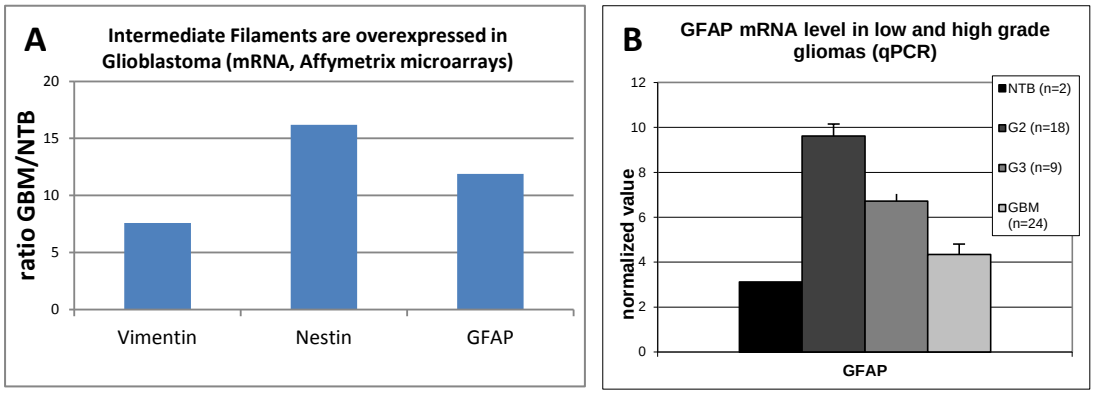
Descriptif du Projet de Recherche Doctoral

Role of intermediate filaments in the mechanics of astrocytes and gliomas

Background

The number of studies on the mechanics of tumour tissues has been increasing steadily for the past ten years, showing differences between normal and cancer cells. Most of these studies have been performed at the scale of the tumour or the tissue and shown that tumours exhibit increased tissue stiffness, which can in part be explained by an increase in the stiffness of the extracellular environment in tumours. Recent studies have focussed on the physics of the extracellular matrix [1] or on the mechanics of the plasma membrane [2]. In contrast, how the **cell interior** contributes to cell rigidity is poorly documented and the reported increase in stiffness at the tissue level may also be due to an increase in the intrinsic stiffness of the cytoplasm of tumour cells [3]. Surprisingly however, studies performed at the single cell level seem to indicate that cancer cells are more deformable [4] and their cytoplasm is less visco-elastic than normal cells [5]. The **cytoskeleton** is a group of biopolymers supporting most mechanical properties of the cells. The cytoskeleton includes actin microfilaments, microtubules and intermediate filaments (IFs). Until now, the actin and microtubule dynamics have been extensively studied but much less is known about IF dynamics.

This PhD project will be focused on the mechanics of **glioma cells**. Gliomas are the most common and lethal primary intracerebral tumors. They form the majority of primary brain tumours and originate from astrocytes or their precursors. Grades I and II generally evolve into grades III then IV (glioblastoma). The cytoskeleton, especially **intermediate filaments** which sustain cell rigidity, appears as a central player in the control of astrocyte polarity and migration, two essential cell functions that are deregulated in gliomas. A recent transcriptomic analysis has identified modifications in the expression of intermediate filament proteins (GFAP, nestin and vimentin) in gliomas (see Figure).



GBM = glioblastoma, NTB= non tumoral brain

Figure: The expression of intermediate filament (IF) proteins is increased in gliomas. (left) Transcriptomic analysis of intermediate filament proteins in gliomas showing increased expression levels of vimentin, nestin and GFAP in glioblastomas (grade IV). (right) qPCR analysis of samples from gliomas of different grades showing that the GFAP expression levels depend on the grade of the tumours. Collaboration with Sandrine Etienne-Manneville (Institut Pasteur, Paris).

Aims of the project

The goals of the project are 1) to compare the microrheological properties of normal astrocytes and of glioma cells and 2) to determine the contribution of IFs to these properties. Two techniques will be used. The first technique (developed in the group of the PhD supervisor) measures the intracellular visco-elastic properties, while the second technique (developed in the group of the co-

supervisor) integrates the mechanics at the scale of the whole cell. We hope to discriminate gliomas of different grades based on their mechanical properties, potentially leading to a diagnostic and prognostic tool for gliomas, and to decipher the role of IFs in the molecular mechanisms and in the signalling controlling intracellular mechanics.

Methodology

Two microrheological techniques will be applied to study the mechanics of astrocytes and gliomas. We have recently developed an active microrheology technique to measure the **intracellular visco-elastic properties** of individual cells. The technique is based on optical trapping of internalized microspheres coupled with single particle tracking by fast scanning confocal microscopy and has been applied to study the role of acto-myosin contractility in the rigidity of the Golgi apparatus [6]. The **visco-elastic relaxation** of the bead position following an applied step stress (creep experiment) gives the visco-elastic parameters after fitting of the data. We will first identify the visco-elastic theoretical model that best fits the data with a minimum number of elastic (hookean springs) and viscous (dashpots) using the classically used Maxwell (dashpot and spring in series) and Voigt (dashpot and spring in parallel) elements.

The second technique has been developed in the group of Atef Asnacios (Laboratoire MSC, Paris, co-supervisor of the PhD) and probes the global **mechanics of the whole cell** [7]. The cell is adhered between two microplates, one of which is oscillated at varying frequencies (0.1-10 Hz) to determine the **complex shear modulus** (storage modulus G' and loss modulus G'').

In collaboration with Sandrine Etienne-Manneville (Institut Pasteur, France), we will work with primary rat astrocytes, as the model for normal astrocytes, and with glioma cell lines of different grades (U373 grade III, U138 and U87 grade IV) or with primary tumour cells from patients (collaboration between the groups of Sandrine Etienne-Manneville and Marc Sanson, ICM Pitié-Salpétrière, Paris).

Proposed research

1- Mechanical properties of normal astrocytes and gliomas

The first part of the project will be aimed at quantifying the mechanical properties of astrocytes and glioma cells. The two complementary experimental approaches described above will allow us to identify the contribution of the cell interior to the whole cell mechanics. In the intracellular approach, the results will strongly depend on which location within the cell is probed. To reduce variability in the measurements, we will perform these experiments in cells plated on polarized (crossbow shape) **adhesive micropatterns**. In this system, intracellular compartments and the cytoskeleton organize in a standardized and reproducible polarized geometry [8]. We will first map the microrheological properties within micropatterned cells to evaluate their spatial variations. We will then compare the visco-elastic measurements obtained **at a given position** in normal astrocytes with those obtained in glioma cells of different grades.

Thanks to this spatial mapping method, we hope to obtain statistically very significant differences between normal and cancer cell types with a low number of cells (N=15-20). If successful, this method could lead to the development of a **new diagnostic and prognostic tool** for gliomas. We will first obtain a data bank of visco-elastic parameters in glioma cell lines of known grades. We will then use patient cells and compare the results obtained in these cells with the data bank to try and predict the tumour grade and outcome.

2- Role of intermediate filaments in the mechanics of astrocytes and gliomas

To quantitatively understand the role of IFs in astrocyte and glioma mechanics, we will **overexpress or downregulate** specific IF proteins or a combination of several IF proteins among GFAP, nestin and vimentin. It was shown recently in fibroblasts that vimentin depletion reduces the shear modulus of the cytoplasm but does not affect the mechanics of the cortex [9]. Since IF proteins appear to be overexpressed in gliomas (see Figure), depletion of IF proteins by siRNA in glioma cells should tune the visco-elastic properties towards the levels measured in normal astrocytes. Conversely, the visco-elastic properties should be similar in normal astrocytes overexpressing IF proteins and in glioma cells. A precise comparison between cells of different grades (cell lines or primary cells from patients) should further correlate the mechanical properties with the level of expression of IF proteins. Comparing the results at the intracellular level and at the whole cell level should also yield valuable information on the coupling between IFs and global cell mechanics.

We will also measure the mechanical properties of **individual IFs** (or more likely bundles of IFs) in normal astrocytes and in gliomas. Since the mechanical properties of IFs depend on their composition [2] and since IF composition is altered during glioma development, we should detect significant differences. Here, we will use our intracellular microrheology technique to push the bead against IFs at a constant speed (typically 2 µm/min) and visualize the corresponding **deformation of the IFs** by fast confocal microscopy. A force-displacement curve and a threshold force for bead ejection from the optical trap will be obtained. To visualize IFs, cells will be transfected with fluorescent constructs of IF proteins, such as vimentin-GFP. To dissect the role of each individual IF protein in the mechanics of IF bundles *in cellulo*, we will deplete a specific IF protein in cells expressing a fluorescent version of another IF protein (for instance, depletion of nestin and/or GFAP in vimentin-GFP expressing cells). In collaboration with Cécile Leduc in the group of Sandrine Etienne-Manneville (Institut Pasteur, Paris), we will compare the rigidity of IFs we measured *in cellulo* with the rigidity of IFs of defined composition polymerized *in vitro*. By testing several IF composition *in vitro*, we should identify which IF proteins contribute most to IF rigidity.

Keywords
Cytoskeleton, intermediate filaments, optical tweezers, microrheology, visco-elasticity, cancer, astrocytes, gliomas

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