Assemblée générale de la SBCF

Date : 29 mai 2018 de 13h à 17h30

Lieu :
Institut Cochin - 22, Rue Méchain - 75014 Paris
Salle de séminaires Rosalind Franklin, 2ème étage

Programme :

13h-13h30	Accueil - Pause café
13h30 - 14h30	Assemblée générale SBCF
14h30-14h45	Présentation par Francesco Baschieri (IGR, Villejuif) : Bourse de voyage 2017
14h45-15h00	Présentation par Mathieu Cinato (I2MC, Toulouse) : Bourse de voyage 2017
15h00-15h15	Présentation par Anne Nehlig (IGR, Villejuif) : Bourse de voyage 2017
15h15-15h30	Présentation par Anca Radu (IAB, Grenoble) : Bourse de voyage 2017
15h30-15h45	Pause café
15h45-16h30	Présentation par Romain Levayer (Pasteur, Paris) : Prix Jeune Chercheur 2018
16h30-16h45	Présentation par Ralitza Staneva (Curie, Paris) : Bourse de voyage 2017
16h45-17h	Présentation par Virgnie Stevenin (Pasteur, Paris) : Bourse de voyage 2017
17h-17h15	Présentation par Diana Vargas-Hurtado (Curie, Paris) : Bourse de voyage 2017

Résumé des presentations :

Francesco Baschieri (IGR, Villejuif)

Flat Clathrin Lattices are new cellular mechanosensors

Cells constantly probe their surrounding environment to adapt their behavior based on physical and chemical properties of the extracellular matrix (ECM). For example, different degrees of ECM stiffness can drive cell differentiation, proliferation and migration. To read physical properties of the ECM, cells use mechanosensors such as integrins. Recently we showed that integrins located in clathrin coated structures can provide a link between the ECM and the cell. Clathrin-coated structures can be therefore adhesive structures. It has long been known that in addition to the small and dynamic clathrin coated pits, some cell lines form big immobile flat clathrin lattices (FCLs) at their plasma membrane, structures whose function is still unknown. By using micropatterns, we noticed that FCLs form only on the adhesive sides of the cells. In addition, cells growing on soft substrates (100 Pa – 5 KPa) were incapable of generating FCLs. We thus reasoned that FCLs could be ECM rigidity sensors. We showed that activated signaling receptors (EGFR, C-Met) accumulate in FCLs and signal from this location. In particular, signaling of EGFR from FCLs lead to an increased ERK phosphorylation ultimately resulting in increased cell proliferation. We concluded therefore that FCLs are mechanosensors of the cell. FCLs formation, however, does not depend on the actin cytoskeleton. Instead, the integrin dimer AlphaV-Beta5 is strongly enriched in FCLs and removal of this receptor results in loss of FCLs without affecting other mechanosensitive structures such as focal adhesions. We propose that strong engagement of the integrin with the ECM results in frustrated endocytosis, thus leading to FCLs formation. Theoretical models suggest that integrin binding forces are lower on soft substrates because local recoiling of the substrate upon stochastic unbinding of the integrins lowers chances of integrin rebinding. This can explain why FCLs only form on stiff environments. In support of this hypothesis, inhibition of the integrin-ECM binding by cleavage of the integrin results in the release of the endocytic machinery with subsequent dissociation of the FCL into clathrin coated pits that are internalized.
We propose that a stiff extracellular matrix can drive increased proliferation via FCLs formation and given that one characteristic common to most tumors is increased stiffness, the study of FCLs could open the road to new cancer therapies.

Mathieu Cinato (I2MC, Toulouse)

The lipid kinase PIKfyve in cardiac fibroblasts activation: a potential target to control cardiac fibrosis

Fibrotic remodeling of cardiac tissue is a key determinant in the progression of heart failure, a leading cause of death worldwide. Cardiac fibrosis is characterized by the activation of cardiac fibroblasts and their differentiation to myofibroblasts. Activated fibroblasts express the contractile protein α-SMA and produce and secrete inflammatory cytokines (IL-6), pro-fibrotic factors (TGF-β), and extracellular matrix proteins (collagen I/III, fibronectin), leading to the formation of scar tissue which ultimately impedes cardiac normal functions.
The enzyme PIKfyve is a dual-substrate lipid kinase which produces the phosphatidylinositol-5 phosphate (PI5P) and PI(3,5)P2. It is localized on the endosomes and is known to regulate the endosomal maturation and recycling of cargoes to the plasma membrane. In this study, we investigate the involvement of PIKfyve in the control of cardiac fibroblasts activation. We demonstrate that pharmacological inhibition of PIKfyve reduces inflammatory and pro-fibrotic factors production by activated cardiac fibroblasts. We demonstrate here that PIKfyve controls cardiac fibroblasts differentiation by regulating the Smad-dependent transforming growth factor-β (TGF-β) signaling pathway.
Taken together, our results demonstrate that PIKfyve could be a potentiator of cardiac fibrosis, therefore opening the way to new therapeutic perspectives to control its development in cardiovascular diseases.

Anne Nehlig (IGR, Villejuif)

Microtubule-associated tumor suppressor ATIP3 controls Kif2A and aurora kinases to maintain mitotic spindle length

Maintaining the integrity of the mitotic spindle is essential to ensure proper chromosome segregation and normal cell division. Any defect in spindle length or symmetry leads to aneuploidy, which is a hallmark of cancer.
ATIP3 is a Microtubule-Associated-Protein encoded by candidate tumor suppressor MTUS1 gene whose expression is markedly down-regulated in aggressive breast tumors of the triple-negative subtype. ATIP3 presents potent anti-growth and anti-metastatic effects, making it an interesting therapeutic target for novel anti-cancer strategies (1-3).

Over the past few years our group has been focusing on the molecular aspects of ATIP3. We have shown that in interphase, ATIP3 localizes at the centrosome and along the microtubule (MT) lattice (1). ATIP3 is a potent MT stabilizer whose depletion increases MT dynamic instability at the plus ends through direct interaction with End-Binding protein EB1, a major marker/regulator of MT dynamics (4, 5).
During mitosis, ATIP3 co-localizes with the mitotic spindle and spindle poles. Time-lapse videomicroscopy analyses have shown that ATIP3 delays mitosis by increasing the time spent in metaphase. However, the role of ATIP3 in cell division remains unknown.

Our recent results indicate that ATIP3 depletion induces several mitotic abnormalities, including multipolar and asymmetric spindle as well as reduced spindle length. We provide evidence that ATIP3 effects on the mitotic spindle involve the depolymerizing kinesin Kif2A and the mitotic kinase Aurora A (AurKA). We show that ATIP3 maintains spindle length in metaphase by decreasing Kif2A recruitment at the poles. Of interest, ATIP3 interacts with Kif2A in an AurKA-dependent manner. Whether a novel ATIP3-Kif2A-AurKA axis is involved in spindle integrity is under investigation.
Together these results may shed light on novel mechanisms regulating mitotic spindle integrity and may deepen our understanding of how loss of ATIP3 may promote aneuploidy, with major consequences in breast cancer.

Anca Radu (IAB, Grenoble)

"LKB1 regulates cell fate by controlling pyruvate-alanine transamination and limiting p53 signaling"

The tumor suppressor LKB1 (also named STK11) codes for a serine/threonine kinase. Germline mutations of LKB1 are responsible for the Peutz-Jeghers syndrome, a dominantly inherited cancer disorder and somatic mutations of this gene have been associated with various cancers including lung and cervical tumors. LKB1 acts as a key regulator of energy metabolism through the activation of the AMP-activated protein kinase (AMPK), a sensor that adapts energy supply to the nutrient demands of cells facing situations of metabolic stress (Shackelford and Shaw, 2009). To achieve metabolic adaptations, AMPK phosphorylates numerous substrates which inhibit anabolic processes while activating catabolic reactions. In particular, AMPK inhibits the nutrient-sensor kinase mTOR. In addition to AMPK, LKB1 also phosphorylates 12 AMPK-related kinases that regulate cell polarization, axon branching of cortical neurons and hepatic neoglucogenesis.

To determine how LKB1 coordinately regulates cell polarity and energy metabolism during cell fate decision, we spatio-temporally deleted the Lkb1 gene in embryonic multipotent neural crest cells (NCC). These cells originate from the neural tube and give rise to various tissues including the peripheral nerves and the enteric nervous system. We showed that LKB1 governs several aspects of cephalic NCC development that are crucial during vertebrate head formation (Creuset et al., 2016). We also reported that mutant mice exhibited hypopigmentation, hindlimb paralysis and intestinal pseudo-obstruction. We described that Lkb1 is essential for the differentiation and maintenance of two NCC-derivatives, Schwann cells and enteric cells. Using a model of neural crest stem cell line, we demonstrated that Lkb1 is key for glial differentiation. Mechanistically, Lkb1 loss led to increased alanine levels as also observed in vivo. Interestingly, inhibition of pyruvate-alanine transamination rescued glial differentiation of Lkb1-null NCC, in a dependent manner of mTOR. Furthermore, AICAR rescued glial differentiation of Lkb1-deficient NCC and treatment of Lkb1-deficient mice with AICAR corrected the Schwann cells and enteric phenotypes (Radu et al., submitted).

Interestingly, abnormal activation of the tumor suppressor p53 has been described in some NCC disorders and p53 inactivation in neurocristopathy mouse models rescues the pathological phenotype (Van Nostrand and Attardi, 2014). By using the NCC line differentiated in glial cells in vitro, we have shown that Lkb1 silencing triggers aberrant p53 hyperphosphorylation on Serine15. The transcription factor p53 is phosphorylated in response to cellular stress such as ROS and DNA damage and plays a central role in cell cycle arrest, autophagy and metabolism. We have found that p53 is hyperphosphorylated in Lkb1-silenced NCC as a response to oxidative DNA damage induced by increased ROS production. Exploring the contribution of p53 signaling in the NCC phenotype due to Lkb1 loss is currently under progress.

Altogether, these studies uncover an essential function for the LKB1 pathway in the formation of the neural crest lineage in part due to an original metabolic regulation of pyruvate-alanine cycling. These data also expand our understanding of the role of LKB1 and its crosstalk with p53 signaling in NCC development. Our data further suggest that LKB1 signaling contributes to the pathogenesis of neural crest diseases called neurocristopathies.

Romain Levayer Prix Jeune Chercheur SBCF 2018 (Pasteur, Paris)

From fitness comparison to spatial constrains: the many roads of cell competition

Developing tissues have an amazing plasticity which is mostly based on the capacity of every single cell to adapt their behavior to local and tissue scale information. This is well illustrated by cell competition, a process that drives the elimination of viable but suboptimal cells (so called “losers”) by fitter cells (so called “winners”) through apoptosis. In the past years, the number of genetic and tissular contexts leading to cell competition has been constantly increasing. Yet, the mechanism allowing recognition and elimination of suboptimal cells is still actively debated. By performing for the first time long term live imaging of Myc-dependent competition in Drosophila, we could show that the probability of elimination of loser cells correlates with the surface of contact shared with winner cells. This was suggesting that the shape of the interface between the two cell populations could modify the outcome of competition. Moreover, we characterized an active mechanism of cell mixing, which through cell-cell intercalation increases the surface between the two cell types and accelerates loser cell elimination (Levayer et. al, Nature, 2015). The combination of cell mixing and accelerated elimination of neighbouring healthy cells could promote tissue invasion by pretumoral cells.
We then focused on the regulation of spontaneous cell extrusion in the Drosophila pupal notum (a single layer epithelium). While we could confirm that tissue crowding was necessary and sufficient to drive cell elimination in the midline region, as previously suggested (Marinari et al., Nature, 2012), we found however that caspase activation was preceding and necessary for every cell extrusion event (Levayer et al., Current Biology 2016). This was suggesting that cells could have a differential sensitivity to crowding depending on their sensitivity to apoptosis. Indeed, inducing fast growth in clones resistant for apoptosis (activation of the oncogene RasV12) was sufficient to induce ectopic compaction of the neighbouring WT cells and their elimination. This mechanism could therefore lead to cell competition independently of the contact based mechanism described above. Altogether, we provided the first clear evidence of independent mechanisms of cell elimination that can both contribute to cell competition. We will then present our most recent evidences showing that downregulation of ERK through tissue compaction can trigger cell elimination in vicinity of active Ras clones. This is the first in vivo evidence of the mechanosensitivity of ERK, which could play a more general role in the fine tuning of cell elimination during tissue homeostasis and could promote tumor progression.

Ralitza Staneva (Curie, Paris)

Tissue explant imaging reveals spatially coordinated migration patterns in the tumor core

In early stages of metastasis, cancer cells exit the primary tumor and enter the vasculature. Although most studies have focused on the tumor "invasive front", cancer cells from the tumor core can also potentially metastasize. To address cell motility in the tumor core, we developed a pipeline to image tumor explants from spontaneously-forming tumors in real time using long-term two-photon microscopy. We find that cancer cells in the tumor core are remarkably dynamic and exhibit correlated migration patterns, giving rise to local "currents" and large-scale tissue dynamics. Collagen structures in the tumor core appear to influence the direction of these local currents. Although cells exhibit stop-and-start migration with intermittent pauses, pausing does not appear to be required during division. Our explant system provides new insight into the dynamics of cancer cells in the tumor core, opening new avenues of research in understanding the migratory properties of cancer cells and later metastasis.

Virgnie Stevenin (Pasteur, Paris)

Identifying parameters of host cell vulnerability during *Salmonella* infection by quantitative image analysis and modeling

Salmonella Typhimurium is a Gram-negative bacterium that invades non-phagocytic intestinal epithelial cells inducing membrane ruffles at the entry site. Thanks to its flagella, *Salmonella* swim on the surface of the epithelium and target specific site of infection. Strikingly, some cells are more likely to be infected than others. However, the parameters that determine this heterogenic host cell targeting remained mainly unknown. To address this issue, we quantitatively characterized the host cell "vulnerability" towards *Salmonella* infection based on imaged parameters. We performed successive infections of the same host cell population followed by automated high-throughput microscopy and observed that infected cells have a higher probability of being re-infected. Establishing a predictive model, we identified two combined origins of host cell vulnerability: the pathogen-induced cellular vulnerability emerging from *Salmonella* uptake and persisting at a later stage of the infection, and the host cell-inherent vulnerability. We linked the host cell inherent vulnerability with its morphological attributes such as the local cell crowding, and with host cell cholesterol content. This showed that the probability of *Salmonella* infection success can be forecast from morphological or molecular host cell parameters. Our approach can be extended to the study of the cooperation and targeting strategies of various kind of intracellular pathogens.

Diana Vargas-Hurtado (Curie, Paris)

Spindle morphology tailoring through time: Interplay between spindle architecture and morphogenesis of the mammalian brain

The mitotic spindle, a microtubule-based structure, provides the forces to segregate the chromosomes during mitosis. Centrosomes, the main microtubule-organizing centres (MTOCs) of animal cells, organize the spindle poles contributing to spindle orientation and bipolarity. Interplay between centrosomes, microtubules (MTs) and associated proteins form a dynamic steady-state spindle that is tuned to the cellular environment to ensure proper chromosome segregation.

From all the organs in the human body the brain is particularly vulnerable to chromosome segregation defects. Aneuploidy generated by centrosome dysfunction impairs embryonic neural progenitor survival, which culminates in severe brain size reduction at birth, a pathological condition known as microcephaly. The origin of this susceptibility is not known.

To unravel the mechanisms of error-prone mitosis in the neuroepithelium, we characterized mitotic spindle assembly during mouse neurogenesis in the WT brains. Strikingly, we found that the morphology of the spindle changes during neurogenesis.

While at early stages spindles of NSC contain longer astral MTs that contact the cell cortex, spindles at later stages have longer and thicker k-fibers appearing more robust. A comparative immunofluorescence analysis also showed that the distribution and levels of key spindle associated proteins were also varying between developmental stages. So far, we have identified Tpx2, a microtubule nucleating and bundling factor, as one key determinant of spindle morphology.

Our results indicate unexpected modifications in the pathways used by NSCs to build a bipolar spindle during neurogenesis, which confer a different chromosome segregation capacity. Indeed, by challenging spindle formation, we observed an improvement of mitotic accuracy during the course of neurogenesis. We thus propose that during mammalian neurogenesis not all the progenitors are equally competent to segregate chromosomes correctly.