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BLITZ

Development of a new intravital correlative microscopy procedure for dissecting the metastasis cascade in vivo at high resolution

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Tumor metastases are the main cause of human cancer deaths. Several imaging techniques allow to study the evolution of the primary tumor and the formation of metastasis in a distant organ. Nevertheless, none of them provide sufficient resolution for visualizing and understanding the implication of cancer cell machineries in the different steps of the metastasis formation.

We aim to image the ultrastructural alterations to the cancer cells and their microenvironment that enable their invasion, intravasation and extravasation. For this purpose, correlative light and electron microscopy (CLEM) which combines dynamic acquisitions *in vivo* with the nanoscale resolution provided by electron microscopy, is probably the most relevant and suited technique. However, there is currently no such procedure available for studying the metastasis cascade by CLEM in a relevant mouse model.

Herein, we describe a versatile correlative approach, combining three different microscopies, allowing to capture these rare events in differents biological models. Xenografted fluorescent cancer cells are imaged using multiphoton microscopy (2PEM). Near-infrared branding (NIRB) is used to mark the imaged area: using laser irradiation, a square is drawn right above the region of interest (ROI). The sample is then embedded in resin for electron microscopy and the resin block is imaged by X-ray computed tomography (micro-CT) allowing the vizualisation of the sample in the resin block. Upon imaging processing, the 2PEM and the micro-CT volumes are merged in order to calculate the position of ROI from the block face. Then, instead of approaching the ROI by serial sectionning, the block is precisely trimmed few micron above the ROI.

Using this pioneer technology, we show that we successfully retrieve the ROI from several sample modelizing the different steps of the metastasis formation, providing high resolution and detailed analysis of the cells ultrastructure[1].

In conclusion, we show here how we could track an interesting cellular behavior imaged *in vivo* using a correlative imaging approach. The CLEM protocol will be applicated to study three main step of the metastasis formation at the ultrastructual level. The implication of the metalloproteinase MT1-MMP in the basement membrane breakage, the involment of the invadopodia, an degradative structure allowing the cancer cells to invade the extracellular matrix and finally the study of the extravasation of the cancer cell.

[1] Fast and precise targeting of single tumor cells *in vivo* by multimodal correlative microscopy
M. A. Karreman, L. Mercier, N. L. Schieber, G. Solecki, G. Allio, F. Winkler, B. Ruthensteiner, J.G. Goetz, Y. SchwabJ Cell Sci 2015 : doi: 10.1242/jcs.181842

Elucidation of the molecular mechanisms underlying the role of the biological clock in the retinal development

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Circadian clocks are molecular pacemakers based on transcriptional feedback loops involving “clock” gene encoded transcription factors (PER1-2, CRY1-2, BMAL1 and CLOCK) and are able to entrain gene expression programs over the 24h cycle. The retina contains an endogenous circadian clock which regulates a plethora of physiological processes sustaining adaptation of visual function to the daily light/dark cycle, such as: melatonin and dopamine synthesis, sensitivity of ion channels and photoreceptor renewal. However, little is known, regarding the direct dependence of the photoreceptor generation and survival on the circadian clock. The *Period1* (*Per1*) and *Period2* (*Per2*) genes underlying the retinal clock have been involved in cell division as well as in the retinal ontogeny modus operandi: previous work from the lab demonstrated that *Per1/Per2* double mutant mice show a delay in general photoreceptor differentiation, along with reductions in cone opsin mRNA and protein levels (*Ait-Hmyed; Eur J Neurosci 2013*).

In light of these observations, the aim of this PhD will be to elucidate the downstream events that link the circadian clock with photoreceptor development based on a transcriptomics approach. Whole embryonic (E15, E18), post-natal (P0, P3) eyes and microdissected adult photoreceptors from wild-type (WT) and *Per1/Per2* double mutant mice will be examined for gene expression by next-generation sequencing methodology. Phenotypical differences will also be evaluated by histology and immunohistochemistry. Comparative analysis between the wild type and mutant mice has the possibility to yield canonical pathways including the molecular networks involved in the retinal/photoreceptor development as well as the detailed findings of the upregulated or downregulated genes.

In conclusion, the non-redundant *Per1* and *Per2* clock gene mutations could be a first link in understanding the role of the clock genes in photoreceptor differentiation/function and can lead to interesting perspectives regarding functional studies both in normal and pathological processes of retinal development.

Identification of genes causing congenital myopathies

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Congenital myopathies are genetic disorders characterized by distinctive morphological abnormalities in skeletal muscle fibers. They define a class of severe muscle diseases with a strong impact on patient survival and quality of life. The main subclasses include nemaline myopathy (with protein aggregates or rod), cores myopathy (central core or multi-minicore, well demarcated areas devoid of mitochondria) and centronuclear myopathy (centralization of nuclei). A large number of genes has already been described for causing congenital myopathies. However, about half of the patients do not have a genetic diagnosis supporting the implication of a large number of yet unidentified genes.

Massively parallel sequencing offers an unbiased and integrated approach to accelerate the identification and characterization of the genetic basis of congenital myopathies. Myocapture is a consortium of research teams, clinicians and sequencing platform working together to characterize the clinical, histological and genetic data of patients. The strategy was to sequence 1000 exomes of patients and their family, previously excluded for known myopathy-causing gene. For exome data analysis, efficient bio-informatic pipelines have been developed in house and shown to be very powerful to identify the mutations responsible for the disease.

Within this project, we studied a non-consanguineous Franco-Lebanese family with three affected children suffering from severe neonatal hypotonia, swallowing troubles and weak limb reflexes. Structural abnormalities on biopsy were not specific of any classical congenital myopathy. We sequenced the exome of the six family members, the two parents and their four children and filtered the variants according to a recessive mode of inheritance. All affected members carried two variants in SCN4A, a sodium channel highly expressed in muscle, compatible with a compound heterozygous segregation. A missense mutation in a well conserved amino-acid was transmitted by the father and a mutation affecting an essential splice donor site was transmitted by the mother. The mutations were well covered and confirmed by Sanger sequencing.

This example of integrated approach helped to expand the phenotype of diseases associated with mutations in SCN4A, previously described in other diseases such as congenital myotonia and potassium-related periodic paralysis. We identified SCN4A as a gene causing a new type of myopathy characterized by a clinical improvement over time and an overlap between classical congenital myopathy and dystrophy on muscle biopsy. Thus, this integrated clinic-molecular approach refines the classification of myopathies.

The identification, validation and characterization of novel implicated genes in congenital myopathies such as SCN4A will allow the development of novel diagnosis protocols to improve genetic counseling, including eventual prenatal or pre-implantation diagnosis. Moreover, the identification of novel genes is an important step for the discoveryof new therapeutic targets.

Oxytocin Neuron Cross-Talk Attenuates Nociception

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Abstract:

Oxytocin (OT) is a small neuropeptide synthetized within the paraventricular and supraoptic nuclei of the hypothalamus. OT is well known for its neurohormonal effect on the reproductive system, allowing uterine contraction during parturition and milk ejection after birth. In the central nervous system, oxytocin has also a wide range of effects, including a role in social and maternal interaction, anxiety and pain.

In the context of pain, OT neurons are activated in response to acute or inflammatory pain and have anti-nociceptive properties. Two modes of action of OT to control pain have been identified, the first one being the release of OT in the blood circulation by magnocellular neurons, where it decreases the activity of DRG nociceptive neurons; the second one being a direct projection of parvocellular OT neurons to sensory spinal cord neurons to decrease the intensity of the nociceptive information. The two component of OT analgesic action have always been studied independently, suggesting that they rely on separated neuronal populations. The aim of our study was to determine if these two complementary analgesic actions of OT could depend on a communication between the two OT nuclei.

We identified a subpopulation of approximatly 30 parvocellular neurons which contact both magnocellular cells within the SON and neurons of the deep layers of the dorsal spinal cord. Using in vivo extracellular recording of sensory neurons in the spinal cord and behavioral study, we demonstrated that these neurons are able to suppress nociception and promote analgesia in an animal model of inflammatory pain, by both central (release of OT within the spinal cord) and peripheral (release within the blood circulation) mechanisms.

Eliava*, Melchior*, Knobloch*, Wahis* et al., A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing, Neuron (2016) * Co-first author

Programmation of the α5β1 integrin expression in glioma stem cells confers aggressiveness to glioblastoma.

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Background: Glioblastomas (GBM) represent the most aggressive brain tumor with 15 months median survival after diagnosis because of a recurrence of the disease. Indeed, some cells are able to spread out of tumor beyond the limits of the resection. Understanding the mechanisms causing this process is a new challenge to prevent GBM cells from invading the host tissue and increasing life expectancy of patients. We previously discovered a correlation between integrin α5β1 expression and glioblastoma grade and patient survival. Recent studies showed that GBM contains self-renewing, tumorigenic cancer stem cells (GSCs) implicated in tumor initiation, recurrence and therapeutic resistance. Our goal is to determine if integrin α5β1 plays a role in GSCs behavior and agressiveness.

Methods: Two GSCs were used in this study, NCH644 et NCH421k. GSCs are cultured either as neurospheres in DMEN medium supplemented with growth factors (EGF and bFGF) or as adherent differentiated tumoral cells after addition of serum in the medium. Expression of proteins was analyzed by Western Blots with specific antibodies and mRNAs by RT-qPCR with specific primers. Several markers either associated with stemness (CD133, CD44, nestin, sox2…) or with differentiation in glial, neuronal or oligodendroglial lineages (GFAP, Tuj, BMP…) were investigated as well as the expression of integrins. Phenotypical characteristics are evaluated by in vitro proliferation and migration assays and by the capacity of GSC to initiate tumors in vivo when xenografted in nude mice. NCH421k cell line was also engineered to express α5 integrin by transduction of a lentiviral vector containing the human integrin gene.

Results: The two GSC lines did not express α5 integrin when cultured in neurosphere medium (undifferentiated form). However, when forced to differentiate, only NCH644 cells express the integrin and this expression confered proliferative and migratory advantages in vitro and an increase in tumor aggressiveness in vivo. Similar results (enhanced proliferation/migration and tumorigenesis) were obtained when α5 expression was forced in NCH421k cells suggesting that the integrin was indeed involved. Interestingly, expression of α5 integrin in NCH421k spheroids induced the loss of proneural stemness markers (nestin, sox2, oct4 and CD133) without increasing the differentiation markers (GFAP, tuj). However the mesenchymal stem cell markers CD44, serpine1, AXL, cMet were dramatically increased.

Conclusions: Although α5β1 integrin does not appear as a glioma stem cell marker, its expression is differentially observed when stem cells differentiate to form the bulk tumor in vitro and in vivo. Forced expression of α5 integrin in stem cells seemed to induce a switch from proneural to mesenchymal stem cells, these latter already proposed to be more aggressive in patients. Our results confirm that α5β1 integrin is an important player in glioblastoma aggressiveness and that it may represent a pertinent therapeutic target.

Structure of the 70S ribosome from *Staphylococcus aureus*

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During last few decades a lot of knowledge have been accumulated about structural aspects of protein synthesis in Gram-negative bacteria, such as *Escherichia coli* or *Thermus thermophilus*, but not Gram-positive bacteria. However, Gram-positive bacteria have evolved different strategies to regulate their gene expression, which are often directed by small deviations in the structure of their ribosomes. Moreover, the resistance to certain antibiotics is often driven by modifications of the ribosomal RNA.

Staphylococcus aureus is a Gram-positive pathogenic bacterium which causes numerous of healthcare associated infections in humans from minor skin infections to lethal diseases. In addition, *S. aureus* is extremely resistant to most of ribosome-targeting antibiotics widely used in medicine. Thus, high resolution structure of *S. aureus* ribosome will provide fundamental insights for design of a new drugs against this pathogen.

We established the protocol for purification of the ribosomes from *S. aureus* suitable for structural studies. Pure, homogeneous and stable ribosomes were obtained and were used for cryo-electron microscopy. Cryo-EM data were collected using the in-house spherical aberration (Cs) corrected Titan Krios S-FEG microscope (FEI, Eindhoven, Netherlands). The structure of full 70S ribosome from *S. aureus* was solved at 3.9 Å The structure revealed/confirmed several features of the ribosome unique for Gram-positive bacteria, particularly *S. aureus*. The model of vacant 70S ribosome from *S. aureus* will be used later for solving structures of this ribosome in complex with different ligands. Obtained results will provide us better understanding of the regulation of translation, synthesis of virulence factors and mechanisms of antibiotic resistance of this pathogen.