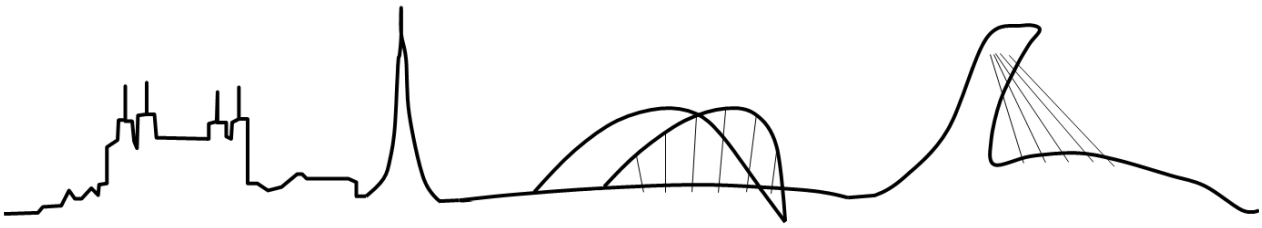

4th International conference on Stem Cells, Development and Cancer

Lyon, France May 16-17, 2022

Mérieux Auditorium, ENS Lyon, France

MEETING LEAFLET

AURA Stem
Auvergne Rhône Alpes
Stem Cell Network




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We dedicate the 4th International Conference on Stem Cells, Development and Cancer to our colleague and friend,
Dr. **Maxime Bouchard** (Department of Biochemistry, McGill University), who passed away peacefully April 21st, 2022.
Maxime was an exceptional scientist, mentor and a founding member of the Conference organizing committee.

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Program

Monday, May 16, 2022

TIME	EVENT
09:00 - 09:15	INTRODUCTION - Véronique Maguer-Satta and Claire Chazaud
09:15 - 12:15	DEVELOPMENT AND REGULATION OF STEM CELL FATE - Chairs: Claire Chazaud and Olivier Raineteau
09:15 - 09:45	Endocrine control of the mammary stem cell - Katrin Brisken, EPFL, Lausanne
09:45 - 10:15	Mechanisms Underlying Adult Neural Stem Cell Quiescence Exit - Darcie Moore, University of Wisconsin - Madison
10:15 - 10:30	Calcium signals triggered by the microenvironment regulate stem cell self-renewal: from adult neural stem cells to glioblastoma stem cells. - Valérie Coronas, Laboratoire 4CS, Poitiers - Selected talk
10:30 - 11:00	COFFEE BREAK
11:00 - 11:15	Neonatal brain injury unravels transcriptional and signaling changes underlying neural stem cell regenerative potential in the postnatal subventricular zone - Louis Foucault, Stem Cell and Brain Research Institute, Bron - Selected talk
11:15 - 11:45	Mechanical feedback in embryonic self-organization - Jérôme Gros, Institut Pasteur, Paris
11:45 - 12:15	New insight into the heterogeneity of HSCs and their clonal evolution during aging - Estelle Duprez, Centre de recherche en cancérologie, Marseille
12:15 - 14:00	LUNCH & POSTER VISIT
14:00 - 16:00	TUMORIGENESIS AND CANCER PROGRESSION - Chairs: Nathalie Mazure and Claude Caron de Fromentel
14:00 - 14:30	Drivers of Spontaneous Stem Cell Mutation - Allison Bardin, Curie Institute, Paris
14:30 - 15:00	Molecular mechanisms of brain tumor initiation and progression - Frédéric Charron, Institut de Recherches Cliniques de Montréal
15:00 - 15:15	ACLY defines a therapeutic vulnerability in PTEN-null T-ALL - Melania Tesio, Laboratory of Onco-Hematology, Paris - Selected talk
15:15 - 15:30	Control of disseminated breast cancer cell dormancy in the bone marrow by TGFB2 and BMP4 signaling - Emma Risson, Centre de Recherche en Cancerologie de Lyon - Selected talk
15:30 - 16:00	A Single Coactivator Simplifies Cancer to Binary Classes that Interchange to Evade Therapy - Rod Bremner, Lunenfeld-Tanenbaum Research Institute, Toronto
16:00 - 16:30	COFFEE BREAK
16:30 - 17:30	KEYNOTE: Deciphering Oncohistone Pathogenesis in Human Cancers - Naba Jabado, Research Institute of the McGill University Health Center, Montreal- Moderators: Colin Crist and Michel Cayouette
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Tuesday, May 17, 2022

TIME	EVENT
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09:00 - 09:30	The H3K9 methyltransferase SETDB1 safeguards muscle stem cell identity - Fabien Le Grand, Institut Neuromyogène, Lyon
09:30 - 10:00	Hematopoietic Lineage Determination by SWI/SNF Chromatin Remodeling Complexes - Julie Lessard, Institut de recherche en immunologie et en cancérologie, Université de Montréal
10:00 - 10:15	A tight coupling between ribosome biogenesis and chromatin remodeling rewires embryonic stem cell fate - Mathieu Gabut, Centre de Recherche en Cancerologie de Lyon - Selected talk
10:15 - 10:30	The comparative roadmaps of reprogramming and transformation unveiled that cellular plasticity is broadly controlled by Bcl11b and Atoh8 - Aurélia Huyghe, Cellular reprogramming, stem cells and oncogenesis Laboratory, Lyon -Selected Talk
10:30 - 11:00	COFFEE BREAK
11:00 - 11:30	Whole body regeneration requires a rewired embryonic gene regulatory network logic - Eric Rottinger, Institute for Research on Cancer and Aging, Nice
11:30 - 12:30	BIO-ENGINEERING - Moderators: Stéphanie Gobert-Gosse and Anne Mey
11:30 - 12:00	BioactiveCoatings: a new way to study cell signaling and differentiation - Catherine Picart, CEA/IRIG Grenoble
12:00 - 12:15	Growth factors alone can induce a non-canonical differentiation of fibroblasts into functional neural progenitors - David Knapp, Institut de Recherche en Immunologie et en Cancerologie, Montreal - Selected talk
12:15 - 12:30	The interplay between epithelial to mesenchymal transition and cell fate specification potentiates gastruloids self-organization - Alexandre Mayran, Institut Suisse de Recherches Expérimentales sur le Cancer (EPFL), Lausanne -Selected talk
12:30 - 14:00	LUNCH & POSTERS VISIT
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14:30 - 15:00	Cardiovascular disease modelling using human pluripotent stem cells in organs-on-chip - Christine Mummery, Leiden University Medical Center
15:00 - 17:30	ENVIRONMENT OF STEM CELLS - Chairs: Lydia Campos and Sylvain Lefort
15:00 - 15:30	Cytoplasmic material exchange between sensory neurons in vivo - Valerie Wallace, University Health Network, Toronto
15:30 - 15:45	Norrin/Frizzled4 signalling controls the microenvironment to suppress medulloblastoma - Nenad Pokrajac, Krembil Research Institute, Toronto - Selected talk
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16:00 - 16:30	COFFEE BREAK

TIME	EVENT
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17:00 - 17:30	Targeting muscle stem cells with bioactive lipids to enhance myogenesis in Duchenne Muscular Dystrophy - Nicolas Dumont, Centre de recherche du CHU Ste-Justine, Université de Montréal
17:30 - 17:45	POSTER AWARD & CLOSING REMARKS - Bénédicte Chazaud, Colin Crist and Michel Cayouette

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Poster #1

High-efficiency CRISPR-mediated HDR genome editing in CD34+ cells

Fanny-Meï Cloarec-Ung [1, @] , Jamie Beaulieu [1, @] , Arunan Suthananthan [1, @] , Bernhard Lehnertz [1, @] , Guy Sauvageau, Hilary Sheppard [2, @] , David Jhf Knapp [1, *, @]

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The combination of homology-directed repair (HDR) with CRISPR-Cas nucleases allows precise editing in the genome. While powerful, this technique suffers from low efficiencies, with 10-20% rates reported in human CD34+ cells which limits greatly its application in disease modelling or corrective therapeutic editing. To improve this efficiency, we performed a systematic comparison of ribonuclear protein (RNP) concentrations, HDR donor formulations, dosages, and small molecule additives in primary CD34+ cells. We found a strong effect of RNP concentration and a site-specific effect of Cas enzyme (Cas9 vs Cpf1) on cutting efficiency which was in turn a key determinant of HDR efficiency. Comparison between short oligonucleotide, long single-stranded DNA, and adeno-associated virus (AAV) HDR donors identified AAV as the most efficient and least toxic donor type. The addition and combination of different small molecules targeting the DNA repair system further increased efficiency to a mean of 78% at the SRSF2 locus and 56% for SF3B1 (≈ the RNP cutting efficiency). We observed homozygous and heterozygous clones for SRSF2 locus, and only heterozygous for SF3B1. With these optimizations, we are now approaching unit efficiency directly in CD34+ cord blood cells. This will open new avenues in both therapeutic strategies and disease modelling.

Topics : BIO-ENGINEERING

Keywords : CRISPR/cas ; homology ; directed repair ; Hematopoietic Stem Cells

Poster #2

Alzheimer's Disease: a new perspective for a brain-on-a-chip model

Adamantia Deligiannopoulou [1, 2, *, @] , Allessandro Leparulo [3, @] , Mattia Tambaro [4, @] , Claudia Cecchetto [3, @] , Marta Maschietto [3, @] , Stefano Vassanelli [3, 4, @]

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Alzheimer's Disease (AD) is one of the most devastating conditions of the elderly population worldwide. One of the major challenges is to build a detailed, multiscale, neuronal network model that reproduces key deficits of AD for drug screening. In this review we discuss the state-of-the-art of hiPSCs in the context of brain-on-a-chip approaches, in particular using Multi-Electrode-Arrays (MEAs) and nanotecnologies. A novel perspective is proposed by the Neureka project: using hiPSCs neurons exhibiting the pathology induced by AD patient brain extracts, the project aims to interface this culture with simulated neural circuits through nanowire structures (NW) that are distributed at multiple subcellular locations, similarly to real synapses, giving access to subcellular compartments of diseased neurons. Finally, we discuss implications of these advances in the stem cell technology for computational neuroscience and to to better reproduce 'in vitro' a wide spectrum of neurodegenerative diseases for drug development.

Topics	:	BIO-ENGINEERING
Keywords	:	Alzheimer's Disease ; hiPSC ; brain ; on ; a ; chip ; Multielectrodes array ; Nanowire electrodes array

Poster #3

Control of cell proliferation by apical trafficking in mouse intestinal organoids

Maela Duclos **1,** @

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Vesicular trafficking has a role in the control intestinal cells polarity, whose establishment and maintenance are essential to proper intestinal function. Interestingly, removal of the µ1B subunit of the clathrin adaptor AP-1 (*Ap1m2*), involved in polarised sorting, has been shown to lead not only to polarity defects but also to crypt hyperplasia in a mouse model, which enlightens a new role of vesicular trafficking in cell proliferation in the intestine. We confirmed both polarity defects and hyperproliferation using an inducible *Ap1m2* knockout strategy in mouse intestinal organoids. Further phenotypic analyses also unveiled a differentiation impairment of the secretory lineage and an expansion of the stem cell zone. Furthermore, we showed that *Ap1m2* depletion affects the localization of the apical polarity determinant Cdc42, whose depletion induces similar intestinal polarity, differentiation, and proliferation defects. We thus hypothesize that AP-1 regulates cell proliferation and differentiation by controlling the localisation of Cdc42. In conclusion, we showed that AP-1 plays a major role in the maintenance of the intestinal surface integrity by controlling both the polarity of the enterocytes and the proliferation/differentiation balance in the intestine. Future work will allow us to characterize the mechanisms by which AP-1 controls Cdc42 localization and the downstream pathways.

Topics	:	BIO-ENGINEERING
Keywords	:	Vesicular trafficking ; proliferation ; diffrentiation ; Cdc42

Poster #4

AQUIOS STEM System, a new flow cytometry automated method for the enumeration and analysis of CD34+ hematopoietic stem and progenitor cells

Lorena Barrientos [1,*,@] , David Faye [2,@] , Dan Flagler [3,@] , Eduardo Flores Fuentes [4,@] , Julia Bower [5,@] , Andreas Boehmler [3,@]

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AQUIOS STEM System is an automated solution for CD34+ cells enumeration by flow cytometry. Samples are just loaded on the system by the operator, and sample preparation and data analysis are performed automatically by the analyzer. The acquisition and analysis software follow the sequential gating strategy of the ISHAGE-Guidelines.
The AQUIOS STEM System for CD34+ enumeration by flow cytometry shows substantial equivalency for the simultaneous identification and enumeration of viable CD45+ cells and viable dual-positive CD45+/CD34+ cells count and percentage with the predicate stemCXP SYSTEM on FC500 analyzer.
The repeatability was done with clinical specimens. For CD34%, %CVs were 10.88%, 7.03% and 4.59% at the 25th, 50th and 75th percentiles. For CD34 count, %CVs were 7.55%, 6.67% and 4.89%. For CD45, %CVs were 2.28%, 2.30% and 2.35%.
The LoB, LoD and LLoQ established for AQUIOS STEM reagents are 0.49, 1.49 and 2.00 for CD34c and 31.33, 35.33 and 36.00 for CD45c respectively.
AQUIOS STEM system provides a comprehensive solution for automated CD34+ enumeration that minimizes the need for human intervention and that potentially enables laboratories to offer CD34+ enumeration outside regulatory lab office hours. Both aspects can increase the level of patient care and reduce time-to-result for this time-critical application.

Topics	:	BIO-ENGINEERING
Keywords	:	Flow Cytometry ; CD34+ cell enumaration ; AQUIOS STEM sytem ; automatization

Poster #5

Acetylation of PAX7 controls muscle stem cell self-renewal and differentiation potential

Marie-Claude Sincennes [1,@] , Caroline Brun [2,@] , Alexander Lin [3,@] , Tabitha Rosembert [3,@] , David Datzkiw [3,@] , John Saber [3,@] , Hong Ming [3,@] , Michael Rudnicki [3,@]

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Satellite cells are adult stem cells responsible for muscle regeneration upon injury. Satellite cell function has been suggested to be regulated by Acetyl-CoA and NAD+ availability, but the mechanisms underlying this type of regulation remain unclear. The transcription factor PAX7 is a critical regulator of satellite cell survival, self-renewal and proliferation. We identified two acetylation sites on PAX7 that positively regulate its transcriptional activity. Lack of PAX7 acetylation impairs its DNA binding ability, particularly to the homeobox motif. We identified two molecular regulators of PAX7 acetylation, the acetyltransferase MYST1 (regulated by Acetyl-CoA) and the deacetylase SIRT2 (regulated by NAD+). MYST1 and SIRT2 both control PAX7 acetylation levels, PAX7 target gene expression, and are important players in regulating the balance between satellite stem cell symmetric *versus* asymmetric division. Abolishing PAX7 acetylation using CRISPR/Cas9 mutant mice leads to an expansion of the satellite stem cell pool, reduced numbers of asymmetric stem cell divisions, and impaired muscle regeneration. Gene expression analysis confirmed that PAX7 acetylation affects target gene expression, specifically those harboring homeodomain binding motifs. Thus, our data demonstrate that acetylation levels regulate PAX7 transcriptional activity and function in satellite cells.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	muscle stem cells ; satellite cells ; myogenesis ; PAX7 ; transcription ; acetylation ; post ; translational modifications

Poster #6

Neonatal brain injury unravels transcriptional and signaling changes underlying neural stem cell regenerative potential in the postnatal subventricular zone

Louis Foucault [1, @] , Guillaume Marcy [2, 3, @] , Diane Angonin [3, @] , Célia Lentini [3, @] , Laurent Bezin [4, @] , Christophe Heinrich [3, @] , Vanessa Donega [3, 5, @] , Olivier Raineteau [3, *, @]

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Germinal activity persists throughout life within the subventricular zone (SVZ) of the forebrain, due to the presence of quiescent neural stem cells (NSCs) that are gradually reactivated throughout life. Accumulating evidence points at a role for NSCs during tissue repair following brain injuries and suggest their amenability to pharmacological manipulations. We used neonatal hypoxia as a rodent model of early brain injury, to investigate the contribution of SVZ NSCs to cellular regeneration within the neocortex. Our results reveal an increased proliferation and production of oligodendrocyte and glutamatergic neuron progenitors within the dorsal SVZ following neonatal hypoxia. Fate mapping demonstrates their contribution to de novo oligodendrogenesis and cortical neurogenesis, while transcriptional analysis reveals changes paralleling their reactivation. Finally, pharmacological activation of the Wnt/β-catenin pathway by intranasal administration of CHIR99021 following hypoxia promotes neuron and oligodendrocyte maturation. Labeling of NSCs in different states of activation demonstrates that pharmacological NSCs activation have no adverse effects on the reservoir of NSCs and on their longterm germinal activity. Altogether, our work reveals a regenerative potential for NSCs following early brain injury, identifying key transcriptional changes paralleling their activation and point at their amenability to pharmacological manipulation with no long-term detrimental effect on germinal activity.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	neural stem cells ; neonatal brain injury ; cellular regeneration ; glutamatergic neurogenesis ; transcriptionnal changes

Poster #7

The role of the Hippo/YAP pathway in spinal cord stem cell proliferation and fate control

Robert Chevreau [1,@] , Sandra Joppé [1,@] , Chantal Ripoll [1,@] , Valeria Hansberg [1,@] , Antoine Nouhaud [1,@] , Gaëtan Poulen [2,@] , Luc Bauchet [2,@] , Jean-Philippe Hugnot [1,*,@]

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Sensitive to traumatic injuries and neurodegenerative diseases for which no curative treatment exists, the adult mammalian spinal cord retains neural stem cells around the central canal. Regenerative species such as zebrafishes possess similar cells able to replace every cell after injury but in mammals, these cells proliferate, mainly differentiate into astrocytes at the expense of oligodendrocytes and neurons, failing to allow functional recovery despite *in vitro* multipotency. These stem cells represent an attractive therapeutic endogenous cell-source to alleviate spinal cord lesions, but require to understand their precise molecular mechanisms.
Previous RNA profiling on mice and human spinal cord revealed that the ependymal region specifically expresses Hippo/YAP pathway components, known to maintain stem cells in different organs. We hypothesized it could regulate mice and human spinal cord stem cells properties.
To investigate it, we cultured mice spinal cord stem cells and showed by genetic inactivation and pharmacological inhibition that the main Hippo pathway effector YAP1 is important for stem cell growth. We also showed by RNA-Seq and immunofluorescence that these stem cells spontaneously generate oligodendrocyte progenitors in culture, which seem to disengage from the Hippo and Notch signaling. Further analyses will reveal how Hippo/YAP pathway regulates spinal cord stem cell differentiation.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	spinal cord ; adult neural stem cells ; Hippo/YAP signaling ; differentiation ; proliferation

Poster #8

Increased myofiber contractile activity promotes muscle stem cell fusion, reduces inflammation and improves muscle function in a mouse model of cancer cachexia

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Cancer cachexia (CC) is characterized by systemic inflammation resulting in drastic body weight loss, skeletal muscle wasting (*i.e.*, reduced muscle size) and weakness (*i.e.*, reduced force production). CC reduces patient survival and no curative treatments exist. Emerging studies reveal that CC may result from dysfunction of muscle stem cells (MuSCs). Indeed, tumor-derived circulating factors block both MuSC differentiation and fusion leading to muscle atrophy. The regulation of myogenesis is also influenced by the dynamic interactions between MuSCs, myofibers and immune cells (i.e., macrophages).
We recently demonstrated that increased myofiber contractile activity by neuromuscular electrical stimulation (NMES) promotes MuSCs fusion in healthy muscles. We aimed to determine whether NMES improves MuSC fate and reduces muscle weakness, wasting and inflammation in the mouse model of CC bearing the C26 carcinoma.
We showed that the NMES training improves muscle force and mass in C26 mice together with changes in myofiber-type composition. These functional, structural and metabolic changes occur in association with MuSC fusion improvement and transition toward an anti-inflammatory status of macrophages in muscle, validated by both *in vivo* and *in vitro* studies.
These findings demonstrate that stimulated myofibers positively regulate MuSC fate and tissue inflammation to counteract the deleterious effects of CC.

Topics : DEVELOPMENT AND REGULATION OF STEM CELL FATE

Keywords : cancer cachexia ; muscle wasting ; muscle stem cell fate ; inflammation

Poster #9

Characterization of muscle stem cell lineage establishment during postnatal growth

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During prenatal development, muscle architecture is established throughout the body in a spatiotemporal manner. However, the main muscle growth and the establishment of the adult muscle stem cell (MuSC) quiescent pool occurs in the postnatal life, during which, a significant fraction of the muscle stem/progenitors acquires features of the adult MuSCs. To address postnatal population dynamics as well as the potential heterogeneity of muscle stem progenitors across postnatal growth, we have performed sort lineage experiments coupled to single-nucleus RNAseq at different postnatal stages. For that, I used an inducible Pax7CreERT2 allele coupled to Rosa26LoxH2B::GFP mouse model in which, upon tamoxifen induction, Pax7+ MuSCs and their lineage are genetically marked with nuclear Histone2B::GFP. Initial analysis of postnatal days P3, P10 and P22 snRNAseq datasets revealed four major cell clusters: quiescent and self-renewing stem/progenitors, differentiating cells and newly fused myonuclei, all connected by a continuum of intermediate populations. We have identified stage-specific quiescent MuSC populations which seem to be distinct from the adult MuSCs. Moreover, a non-myogenic population that corresponds transcriptionally to Fibro-Adipogenic progenitors (FAPs) emerges from Pax7+ cells in all stages studied, and clusters separately within the FAP cluster from whole muscle experiments, an indication of potential functional heterogeneity.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	muscle stem cell ; cell lineage ; postnatal growth

Poster #10

Transcriptional programs controlling closure of pallial germinal activity

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The ventricular-subventricular zone (V-SVZ) is the largest neurogenic region of the postnatal forebrain, containing neural stem cells (NSCs) emerging from both the embryonic pallium and subpallium. Despite of this dual origin, glutamatergic neurogenesis declines rapidly after birth, while gabaergic neurogenesis persists throughout life. Here, we performed single-cell RNA-sequencing (scRNA-Seq) of the postnatal dorsal V-SVZ for unravelling the mechanisms leading to pallial lineage germinal activity silencing. We identify cell lineage-specific NSCs primed for the generation of neurons or glial cells, as well as a large population of so far uncharacterized quiescent NSCs (qNSC). Pallial qNSCs enter a state of deep quiescence, whilst in contrast, subpallial qNSCs remain transcriptionally primed for activation. In addition, neurogenesis by pallial NSCs is paralleled by acquisition of subpallial traits and impaired neuronal differentiation. Altogether, our results reveal that multiple mechanisms converge in silencing pallial germinal activity early after birth.

Topics : DEVELOPMENT AND REGULATION OF STEM CELL FATE

Keywords : subventricular zone ; single ; cell RNA ; sequencing ; neural stem cells ; quiescence

Poster #11

Bmpr1a integrates TGFβ/BMP-signaling to synchronize quiescence induction and blockade of neuronal differentiation to rapidly silence cortical neurogenesis after birth

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Cortical neurons are produced by dorsal (i.e. pallial) neural stem cells (NSCs) during embryonic development. While the period of cortical neurogenesis stops shortly before birth, postnatal dorsal NSCs continue producing glutamatergic progenitors that accumulate within the SVZ, i.e. the main germinal region of the postnatal brain, at postnatal stages. We performed scRNA-Seq analysis at different postnatal timepoints to investigate the transcriptional specificities of pallial NSCs and of their progeny following birth. Our results reveal enrichment of the transcription factor Hopx within pallial NSCs, together with sustained activity of the TGFβ/BMP-signaling pathway. In depth analysis of TGFβ/BMP related transcripts identified Bmpr1a as the most likely receptor involved in mediating its activity. Further, gain and loss of function manipulations of a constitutively active as well as an inactive form of Bmpr1a, result in 1) a rapid modulation of NSCs activity, 2) a dramatic effect on neuron production and maturation. Together, our results highlight a central role of TGFβ/BMP-signaling in synchronizing quiescence induction and blockade of neuronal differentiation/maturation, to rapidly silence pallial germinal activity after birth.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	corticogenesis ; transcriptomic ; postnatal forebrain ; sub ventricular zone ; electroporation

Poster #12

Comparative transcriptional analysis of subventricular & subgranular zones neural stem cells reveals differences in their activation status and signaling pathways integration

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There are two neurogenic niches within the postnatal forebrain, i.e. the subventricular zone (SVZ) and the sub granular zone (SGZ). Both regions contain neural stem cells (NSCs) which emerge from the embryonic pallium and express the transcription factor Hopx. However, while SGZ stem cells remain active throughout life, SVZ NSCs activity rapidly declines following birth. Here we have explored the transcriptional correlates of these strikingly different dynamics. To this end, we took advantage of proprietary as well as publicly available single cell RNA-sequencing datasets covering embryonic and postnatal development of the SVZ and SGZ. Integration and comparison of these datasets reveal that NSCs enter distinct types of quiescence within both structures. These different levels of quiescence correlate with differential activity of key signaling pathway (i.e. Wnt/β-catenin and TGFβ/BMP signaling), as well of their respective receptors. Genetic and pharmacological manipulations were used to further explore the instructive role of these signaling pathways over postnatal pallial NSCs activation. Altogether our work unravels mechanisms contributing to regional differences in the activity of pallial NSCs within the postnatal SVZ.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	Hopx ; TGFβ signaling ; single ; cell ; sequencing ; neurogenesis ; olfactory bulb

Poster #13

Impairment of myogenesis by interferon mediated epigenetic remodeling in inflammatory myopathies

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Inflammatory myopathies (IM) are a group of auto-immune diseases that are all characterised by severe muscle impairment. In spite of the use of immunosuppressive drugs, a significant proportion of patients show residual muscle weakness. IM patients are characterised by a strong IFN signature. IFN treatment of muscle stem cells (MuSC) *in vitro* has been shown to impairs differentiation, while inhibition of IFN-signaling prevented this dysregulation. A recent publication confirmed the link between myogenesis impairment in IM and IFN-signaling.
Interestingly, it has been shown that HIRA upon IFN stimulation is relocated to PML-nuclear bodies (PML-NB) and in parallel to IFN-stimulated genes (ISG) and promote their expression in fibroblasts. Deletion of HIRA during myogenesis *in vitro* and *in vivo* has also been shown to inhibit the expression of muscle lineage genes and impair muscle regeneration.
Our hypothesis is that abnormal IFN stimulation of MuSC in IM patients induces loss of HIRA in myogenic genes and its relocalization to ISG. This would leads to persisting impairment of muscle regeneration and sustained inflammation. Investigation of the link between IFN, myogenesis and HIRA-PML might uncover a novel epigenetic signalization that regulate muscle regeneration dysregulation in IM.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	inflammatory myopathies ; IFN signaling ; muscle stem cells ; myogenesis ; HIRA ; PML_NB

Poster #14

General transcription factor TAF4 antagonizes epigenetic silencing by Polycomb to maintain stem cell functions

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Introduction.
Constant renewal of the intestinal epithelium fueled by stem cells (SCs) is regulated by tissue-specific transcription factors interacting with the pre-initiation complex of transcription (PIC). Here, we have studied the role of the general transcription factor TAF4, a component of PIC, in the murine intestinal epithelium.
Results.
Taf4 gene inactivation in the developing intestine altered gut morphogenesis and compromised the emergence of adult-type SCs, while at the adult stage Taf4 loss perturbed SCs and the SC niche. In intestinal organoids, Taf4 inactivation prevented the formation of crypt-like buddings. Bulk RNAseq, ATACseq, single-cell RNAseq and immunocytology showed that without Taf4, SCs were lost, the SC niche was perturbed, and cell proliferation was abrogated. Regulon analysis revealed the involvement of the Polycomb Repressive Complex PRC2 in the phenotype induced by Taf4 deficiency. Indeed, treating Taf4-inactivated organoids with EPZ6438, an inhibitor of the EZH2 component of PRC2, restored SCs, the SC niche, cell proliferation and organoid buddings.
Conclusion.
This study reports the essential function played by a PIC component, TAF4, in the biology of the developing and adult intestine epithelium. It highlights a novel mechanism of action of TAF4 through antagonizing PRC2-mediated repression of the SC gene expression program.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	General transcription machinery ; Polycomb Repressive Complex ; Intestine ; Stem cells

Poster #15

Generation of embryo chimeras with pluripotent stem cells in non-human primates.

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In contrast to rodent pluripotent stem cells (PSCs), which self-renew in the naïve state of pluripotency, conventional non-human primate PSCs (NHP-PSCs) self-renew in the primed state of pluripotency. As a result, they are unable to colonise pre-implantation embryo to form somatic chimeras. We developed a strategy to reprogram NHP-PSCs to naïve-like pluripotency using LIF, Activin, and chemical inhibitors of PKC and WNT signalling. The resulting cells, called 2CLA, acquired gene expression profile closer to the pluripotent cells of primate embryos. To study chimeric competency, NHP 2CLA cells expressing constitutive GFP were injected into morula-stage rabbit and cynomolgus monkey embryos, which were cultured to the late blastocyst stage. While conventional NHP-PSCs returned only 40% of positive embryos harbouring less than 10 GFP+ cells, 90% of rabbit blastocysts analysed displayed 10 to 50 GFP+cells in the epiblast and trophoblast after injection of naïve-like cells. Similar results were obtained after injection of NHP-PSCs into cynomolgus monkey embryos. Thus, after reprogramming to naïve-like pluripotency, NHP-PSCs acquire competence to colonise the epiblast and trophoblast. Chimeric embryos are currently undergoing single-cell RNA-seq analysis to characterize them. This work will lead to better understand the mechanisms involved in chimera generation for studying primate development.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	chimerism ; pluripotent stem cells ; non human primate

Poster #16

Control of cellular identity by Bcl11a and Bcl11b during pluripotent reprogramming

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A key challenge for developing embryos is to establish the identity of the cells by restricting progressively their plasticity. In contrast, such plasticity is regained during the conversion of somatic cells into induced pluripotent stem cells (iPSC) by the combined expression of *Oct4*, *Sox2*, *Klf4* and *c-Myc* (OSKM). For these reasons, a better understanding of cellular identity maintenance and cellular plasticity acquisition might have profound implications for regenerative medicine. In the light of our recent findings on the reprogramming roadmaps leading to cellular identity loss, this study aims to understand how the two transcription factors *Bcl11a* and *Bcl11b*, subunits of the SWI/SNF complex, could be involved in regulating cellular identity and plasticity *(i)* and to identify the molecular mechanisms implied *(ii)*. In OSKM induced iPSC we showed that loss of cellular identity is correlated with a switch in the expression of *Bcl11b* and *Bcl11a.* Moreover, using gain- and loss-of-function approaches, we revealed that the modulation of this balance controls somatic cell identity loss and the emergence of iPSC in various cell types. Therefore, transcriptomic and epigenomic analysis in these gain- and loss-of-function approaches unveil the molecular mechanisms constraining reprogramming and iPSC generation.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	Stem cells ; cell identity ; OSKM ; pluripotent reprogramming ; cellular plasticity ; SWI/SNF complex

Poster #17

Netrin-1 signalling activity is dynamic and cell cycle-regulated in mouse embryonic stem cells

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Mouse embryonic stem (mESC) cells are an incredibly useful model to study pluripotency and development *in vitro*.
We recently demonstrated that Netrine-1 (Ntn1) was a regulator of naive pluripotency (Huyghe et al., 2020). To dissect the dynamic of expression of Ntn1 and its receptors Neo1 and Unc5b, we established a tricolor fluorescent knock-in (KI) reporter mESC line for their endogenous expression using the CRISPR-Cas9 technique. We first demonstrated that subpopulations of cells expressing or not Ntn1, Neo1 and Unc5b coexist, and that they are interconvertible. We further characterized them at the functional, proteic and transcriptomic level. Bioinformatic analysis showed that netrin-1 signalling activity is tightly regulated by the cell cycle. Next, using Fucci ESC, we revealed that Ntn1 and Neo1 are predominantly expressed during the S phase of the cell cycle. We then showed that their expression is severely repressed in the G1 phase. Finally, we performed a shRNA screen that allowed us to conclude that they were differentially regulated by cell cycle actors. Altogether, these results highlight for the first time how a signalling pathway sustaining pluripotency is regulated during the cell cycle, potentially modulating the sensitivity to differentiation cues.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	Cell cycle ; Netrin ; 1 ; pluripotency ; stem cells ; CRISPR

Poster #18

A novel inhibitor of T-Cell Factor transcriptional activity in maintenance of the cerebellar rhombic lip germinative zone.

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Cerebellar Granule Neuron Progenitors (GNPs), the most numerous neuronal population of the brain, emerge from the upper rhombic lip (uRhL), a germinal area with stem cell niche properties. T-Cell Factors (TCF) are transcriptional effectors that act downstream of Wnt signalling. Whereas TCFs are transcriptional active within the uRhL, neither their function(s) nor their developmental regulators are known. In this study we investigated GNPs development in the amphibian *Xenopus,* and described similarities and differences with higher vertebrates. Using loss and gain of function approaches we showed that TCF as a transcriptional activator is necessary for both emergence and maintenance of the uRhL. We identified the transcription factor Barhl1, a target gene of *Atoh1* the master gene of GNP development, as the main inhibitor of TCF activity: Barhl1 physically interacts with TCF, and Barhl1 Knock-Down (KD) increases TCF activity within GNPs. Barhl1 KD increases the size of the uRhL, and delays GNPs differentiation, a phenotype compensated by a concomitant decrease in TCF activity. Our data demonstrate that through Wnt/TCF transcriptional inhibition Barhl1 drives GNPs into a committed, low proliferating, non-differentiated state. Our work unravels a new regulatory mode of Wnt/TCF signalling, active in a neural stem cells niche.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	Rhombic Lip ; Granule Neuron Progenitors ; Wnt/TCF ; Barhl1

Poster #19

Microtubules network dynamic in myonuclei positioning in muscle fibers

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Skeletal myofibers are syncytium with hundreds of myonuclei regularly positioned at their periphery. While the loss of peripheral myonuclei organization is the hallmark of numerous myopathies, how myonuclei are maintained at the periphery and how this contributes to muscle integrity and functionality remains elusive.
In our previous studies, we identified microtubule-related functions of MACF1, a member of the spectraplakin family, in the control of myonuclei dynamics in skeletal muscle fibers. We discovered that MACF1 is a key actor in preventing the myonuclei internalization process, mainly through its control on microtubules organization, myonuclei shape and mitochondrial biogenesis. Interestingly, following MACF1 knock-out, myonuclei internalization occurs in concomitant with a shift in the balance between microtubules dynamic and stable forms, reflected by alteration in levels of Tyrosinated versus De-Tyrosinated tubulins.
To address the contribution of microtubule dynamic along skeletal muscle development and its impact on skeletal muscle fate, we studied skeletal muscles of SVBP KO mice in which microtubules do not undergo De-Tyrosination and remain highly dynamic. We show that microtubules network architecture and myonuclei content/shape within myofibers are altered in SVBP KO mice. Additionally, resting state of satellite cells is also affected, highlighting a new controlling pathway of skeletal muscle homeostasis.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	satellite cells ; skeletal muscle ; microtubules

Poster #20

The metabolic sensor AMPKα2 is a satellite cell intrinsic regulator of myonuclear accretion

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Myogenic fate progression of muscle satellite cells (MuSCs) is accompanied by a change in metabolism, which is coordinated by intra- and extracellular metabolic cues that are integrated through the metabolic sensor AMPKα2.To study the role of AMPKα2 in the regulation of myogenesis *in vivo*, muscle injury was provoked by cardiotoxin injection in mouse *tibialis anterior* (TA). Mice lacking AMPKα2 had a lower relative TA mass and force, as well as a higher number of eMHC+ fibers *per* section 14 days post injury (d.p.i.). This myogenesis defect was recapitulated *in vitro*, as FACS-sorted AMPKα2-KO MuSCs had a lower fusion index. This defect did not arise from a change in differentiation. Moreover, live imaging of pre-differentiated cells revealed no difference in their motility or number of myoblast-myoblast fusion events. However, in a specific assay we observed less fusion between AMPKα2-KO myoblasts and WT myotubes than between WT myoblasts and WT myotubes. Moreover, fusion of MuSCs labelled with EdU during 5-14 d.p.i. was diminished (-27%) in AMPKα2-KO mice. Finally, we confirmed *in vitro* that this role of AMPKα2 is mediated by its kinase activity. Together, this shows a MuSC intrinsic role for AMPKα2 in muscle regeneration, through the regulation of myonuclear accretion.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Topics	:	ENVIRONMENT OF STEM CELLS
Keywords	:	Cell fusion ; cell ; cell communication ; metabolism ; MuSCs ; regeneration

Poster #21

A neural stem cell niche with an embryonic-like dorsal-ventral regionalization conserved in the aged human spinal cord

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Anamniotes and rodents maintain neural multipotent cells in the ependymal zone (EZ) around the central canal of the spinal cord. Our previous RNA profiling showed that immature developmental genes are still expressed even in the young human EZ. These ependymal cells maintain an embryonic-like spinal cord organization with the expression of typical spinal cord developmental/stem cell transcription factors such as Arx, Msx1, Pax6 or Sox2, 4,6,11 and cilia transcription factor FoxJ1. We and others found that these cells are multipotent and can generate neurons and glial cells *in vitro*. The maintenance of these cells in the adult or aged human spinal cord is still debated. We addressed this pending issue by collecting fresh spinal cords from 10 ageing humans (from 53 to 83 y.). Using immunolabelling techniques, we found a persistent lifelong expression of spinal cord developmental factors (Arx, Pax6, Msx1, Sox2 and FoxJ1) in the human EZ. A dorsal-ventral regionalization and a central lumen are also observed in most cases at all ages. The persistence of these embryonic-like cells in the aged human spinal cord potentially represents an interesting endogenous cellular source to alleviate various spinal cord lesions.

Topics	:	ENVIRONMENT OF STEM CELLS
Keywords	:	Cell determination and differentiation ; Human tissue ; Stem cells

Poster #22

Metabolic changes in obesity alter adipose stem cell metabolism and induce functional changes at transcriptional and non-transcriptional levels

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Obesity promotes the development of metabolic complications like type 2 diabetes, and a chronic inflammation mostly sustained by white adipose tissue. Preceding complications, glucose and lipid metabolisms are altered but inflammation is maintained at a low grade in the blood, defining a limit between healthy and morbid obesity. The mechanisms of transition are not understood and are independent of the body weight. We made the hypothesis that adipose stem cells (ASC) contribute to this limit. Indeed, ASC are adipocytes progenitors also supporting many regulating functions of their environment, among which the repression of activated immune cells. We performed a kinetic study of high fat diet induced obesity in mice and isolated ASC from functionally distinct white adipose depots to detect changes correlating with this transition. Interestingly, obese ASC acquired improved and depot specific immunosuppressive properties that were not influenced by the diet duration, differently from changes in cell metabolic pathways revealed NMR analysis. Proliferation and differentiation were not altered. Functional and metabolic switches were not supported by direct transcriptional activations and RNA seq analysis mostly showed transcriptional repression. Our results reveal obesity-induced features for ASC, setting a new basal state that precedes the shift towards altered functions in morbid obesity.

Topics	:	ENVIRONMENT OF STEM CELLS
Keywords	:	adipose (mesenchymal) stem cells – cell functions – metabolism – transcription – obesity

Poster #23

Deciphering CML LSCs TKI-backing dormancy within a standardized 3D BM model

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Despite therapeutic progress in Chronic Myeloid Leukemia (CML) thanks to Tyrosine Kinase Inhibitors (TKIs), it remains not yet curative since many CML patients still retain leukemic stem cells (LSCs) in their bone marrow (BM) after treatment arrest. Deciphering how the human complex BM dynamic ecosystem is involved in LSC dormancy will be crucial to get new insights on treatment escape. Thus, we developed a standardized 3D human BM-like model mimicking the medullar niche to decipher pathophysiological mechanisms that control LSC and take place during hematological pathologies. We generated a CD34+LSC model expressing FUCCI system, allowing a dynamic analysis of cell cycle phases in response to CML treatments. We used different TKIs such as Imatinib and Nilotinib but also Asciminib, which is the first allosteric inhibitor of BCR-ABL1. In addition, this model allows a dynamic tracking of quiescent CML cells localisation within our 3D model. We identified after LSC culture into the 3D system a higher BMP4 production, further increased by TKI treatment. Also, CML treatment induced immature cell dormancy when cultured on a stroma. Current analyses involve multiplex immunofluorescence of the retrieved BM-like niches using specific markers for each compartment to track quiescent LSCs localization within the system.

Topics : ENVIRONMENT OF STEM CELLS

Keywords : Stem Cell ; BCR ; ABL ; Persistence ; Dormancy ; 3D culture ; TKI ; Asciminib ; BMP4

Poster #24

Investigating the biological function of the stress response upon MuSCs quiescence exit

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Studying adult stem cells requires tissue dissociation and stem cells isolation, methods that we have recently shown to lead to major transcriptomic changes and introduce an artificial activation signature to the cells. In order to compare the transcriptional response in diverse cell types to niche disruption, we performed single-nucleus RNAseq on intact and dissociated skeletal muscle and liver. By doing this, we were able to identify a common stress core response induced in essentially all cell types during dissociation. This core response included immediate early genes, targets of the ERK1/2 MAPK pathway, and the polyamine synthesis pathway among many others. Moreover, we investigated the functional importance of this stress core in the early activation of quiescent stem cells. We used inhibitors against components of the polyamine synthesis and ERK1/2 signaling pathways to study their role in MuSCs early activation. We also combined ERK1/2 pathway inhibitors with mice overexpressing activated Notch, a pathway known to be required for MuSCs quiescence and were able to simulate quiescent-like MuSCs in culture conditions. Currently we are optimizing the culture conditions that will allow us to maintain but also induce quiescence, with the aim to enhance the regenerative potential of ex vivo amplified MuSCs.

Topics	:	EPIGENETICS AND GENE REGULATORY NETWORKS
Keywords	:	MuSCs ; quiescence ; MAPK ; Notch

Poster #25

Delineating the role of SETDB1 methyltransferase for regulating muscle stem cell function

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Epigenetic regulation allowing muscle stem cell (MuSC) progression into the myogenic lineage involves the H3K9 methyltransferase SETDB1 that controls chromatin compaction and gene transcriptional repression. Notably, we previously demonstrated that SETDB1 regulates differentiation in immortalized muscle cell line. As its role to control MuSC quiescence or proliferation remains unknown, we generated a conditional and inducible mouse model allowing the specific deletion of Setdb1 in MuSCs and undertook the analysis of SETDB1 for regulating MuSC function.
Remarkably, we showed that loss of SETDB1 dramatically impairs MuSC activation and proliferation and triggers cell death in both primary myoblasts and isolated myofibers. Gene expression analysis performed on sorted MuSCs revealed that the conditional knockout of Setdb1 increases the expression of genes associated to apoptosis and cell cycle defect as well as the re-expression of transposable elements that mainly belong to the endogenous retrovirus (ERV). As well, we are currently performing in vivo experiments using cardiotoxin-induced muscle injury to further evaluate the role of SETDB1 for controlling MuSC regenerative capacity. Our analysis performed at 7 days and 28 days after the injury confirmed the essential role of SETDB1 for muscle regeneration, thus highlighting the need to explore its role in skeletal muscle homeostasis.

Topics	:	EPIGENETICS AND GENE REGULATORY NETWORKS
Keywords	:	Histone methyl transferase ; SETDB1 ; MuSCs ; chromatin regulation

Poster #26

Setting up of colorectal organoids culture for preclinical/clinical investigations in cancer

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Colorectal cancer (CRC) is the 3rd world most diagnosed and the 2nd world most deadly cancer. Its development is based on the transformation of crypt epithelial stem cells and progenitors into cancer stem cells. The organoid technology allows studies on isolated primitive cells which proliferate and differentiate reconstituting crypt architecture *in vitro*. However, the recent first prospective experimental treatment of CRC patients based on organoid drug responses was disappointing and concluded on the required further optimization of culture conditions from biopsies and metastases for the clinical applicability of organoids.
We have set up a human CRC organoid culture aiming to be applied to heterogeneous CRC phenotypes and to be compared with organoids from normal tissues. The different types of structures found in organoid culture were characterized morphologically and for their growth and maintenance capacities. A fluidigm analysis of stem cell markers, proliferation and differentiation genes was realized. Compared to normal organoids, CRC organoids displayed disturbed epithelial polarization, a mixed epithelia-mesenchymal differentiation and higher capacities of maintenance along passages of the culture. Further experiments with our organoid culture conditions would allow to verify its interest for preclinical/clinical studies on therapeutic resistant and metastatic primitive cells.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Colorectal cancer ; stem cells ; organoid

Poster #27

Manipulating neural stem cells to create a transgenic independent model for glioblastoma

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Neural stem cells (NSCs) residing in the subventricular zone (SVZ) of the forebrain are considered to be the cells of origin of glioblastoma (GBM), the most frequent and aggressive of primary brain cancers. Indeed, somatic mutations in tumor suppressor genes in postnatal neural stem cells of the SVZ act as drivers for glioblastoma development. In order to better understand the etiology of the disease and the molecular processes involved in the onset of the GBM, it is important to develop mouse models that enables mechanistic studies. However, currently GBM induction implicates complex genetic backgrounds and the use of conditional mutations based on the CRE-Lox system, limiting genetic studies of new candidate genes or regulators potentially involved in gliomagenesis. To circumvent this limitation, we developed an in vivo glioblastoma model that is based on postnatal brain electroporation of NSCs in combination with the PiggyBac transposase system and the use of CRISPR technology. This easily applicable system does not implicate transgenic mouse lines nor CRE-LoxP system, freeing such approaches for functional studies.
We show that this model enables the induction and analysis of GBM development in a temporally and spatially highly regulated fashion and may be implemented in any mutated genetic background.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	glioblastoma model ; CRISPR/Cas9 ; PiggyBac system

Poster #28

Norrin/Frizzled4 signaling controls the microenvironment to suppress medulloblastoma

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Crosstalk between pre-tumour and stromal cells in the tumour microenvironment plays a pivotal role in tumour initiation and progression. In the cerebellum, canonical Wnt signalling mediated by Norrin/Frizzled4 (Fzd4) activation in meningeal endothelial cells inhibits preneoplasia and tumour progression in mouse Sonic hedgehog medulloblastoma (Shh-MB). We investigated the link between stromal signalling and tumour initiation in granule cell progenitors (GCPs), the cell of origin of Shh-MB. We found that Norrin/Fzd4 signalling maintains the activation of perivascular macrophages (pvMΦs) in the meninges during the critical window for tumour initiation. Depletion of pvMΦ during this critical window phenocopies the effects of Norrin deficiency on tumour initiation, indicating that pvMΦs suppress tumourigenesis. Finally, we show that both Norrin/Fzd4 and pvMΦs inhibit chemotaxis and proliferation of GCPs. Taken together our results identify an unanticipated cross talk between endothelial cells and pvMΦs in the control of preneoplasia in the cerebellum.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Medulloblastoma ; Microenvironment ; Perivascular Macrophages ; Endothelial cells ; Neurodevelopment

Poster #29

In vitro characterization of cellular heterogeneity in Diffuse Low Grade Gliomas

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Diffuse IDH-mutant Low-Grade Gliomas (LGG, Grade-II) are primary rare brain cancers. They grow at a slow rate but often degenerate into High-Grade Gliomas (Grade-III, Glioblastomas) after few years. Low-Grade Gliomas show an intratumoral heterogeneity, which defeats current therapies. Despite surgery, the overall survival of patients remains poor. Furthermore, there are few cellular tools to study them. Development of these tools is essential to find markers which will allow isolation and targeting of each subpopulation present within tumor. We hypothesize that one of these subpopulations is made up of cells with stem-like properties allowing the maintenance of cellular heterogeneity and resistance to treatments. To investigate this, we have standardized methods to derive cells lines from patient tumoral resections. We were able by this method to generate a biobank of cell lines from patients. We focused on one cell line named LGG-275. We performed a single-cell RNA analysis to characterize cellular heterogeneity of LGG-275. We identified at least three cell subpopulations expressing different markers confirmed by immunostainings. From this data, our first objective is to isolate each subpopulation. Then, we will study their ability to restore the initial heterogeneous population. Finally, we will identify molecular pathways controlling the fate of these cells.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Diffuse Low Grade Gliomas ; Biobank ; Intratumoral Heterogeneity ; Stem like properties ; Single cell RNA sequencing

Poster #30

Pericyte stem cell polarize pro-tumoral macrophages in pancreatic cancer

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Pancreatic cancer is associated with an abundant stromal reaction, which accounts for up to 80% of the tumor mass. Within stromal reaction, immune cells communicate with several actors, from cancer associated fibroblast to soluble factors. Among immune cells, macrophages represent one of the major cell populations in tumor microenvironment. We recently discovered a pro-tumoral pericyte population sharing stem properties (CD45-EPCAM-CD31-CD106+CD24+CD44+) in early lesion of pancreatic cancer. We sought to determine the role of pericyte stem cells (PeSC) in impacting macrophages phenotype and function. We report here that both *in vitro* and *in vivo* PeSC induce macrophages polarization towards an immunosuppressive phenotype by expressing both CD206 and CD169 markers as well as downregulating antigen presentation capacity. We show that this macrophages population is upregulated in pre-cancerous conditions as well as in human pancreatic cancer. We also address that the crosstalk between macrophages and PeSC led to a significant increase of both immunosuppressive extracellular protein βig-h3 and CXCL12 chemokine production, associated with inhibited CD8+ proliferation. Finally, we showed that both PeSC and macrophages can inhibit CD8+ T cell in a PD1 dependant and independent manner respectively. This work may lead to the identification of a new macrophage population supporting pancreatic cancer development.

Topics : TUMORIGENESIS AND CANCER PROGRESSION

Keywords : Pericyte stem cell ; Pancreatic cancer ; Macrophages

Poster #31

The Endothelin signaling pathway in Diffuse Low-Grade and High-Grade Gliomas

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Diffuse IDH-mutant Low-Grade Gliomas (LGG) are slowly-growing tumors which often progress into secondary glioblastomas (GBM). These glial tumors are characterized by specific mutations such as a recurrent missense mutation in IDH1/2 gene coding for the isocitrate deshydrogenase, recurrent mutations in ATRX gene and a 1p/19q codeletion. Gliomas present an intratumoral heterogeneity that defeats currently used therapies. To bypass these hurdles, understanding the molecular mechanisms and pathways underlying the generation of this cellular diversity is crucial chiefly throughout cancer progression.
Among them, the **Endothelin signaling system** encompassing cytokines (ET1-3) secreted by vessels and two receptors (EDNRA and EDNRB) is highly expressed in the CNS. In the normal brain, this signaling is known to regulate stem cell properties such as maintenance and proliferation. We used cultures of low and high-grade gliomas and glioma sections to explore the expression and role of EDNRA and EDNRB. We found that the EDNRB is the main receptor expressed in glioma cells. By treating high- and low-grade glioma cells with ET-1 and ET-3, we found that these cytokines reduce proliferation. Our current work aims at deciphering the downstream signaling cascade and target genes mediating endothelin effects on glioma cells, paving the way for new treatments.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Brain tumors ; Diffuse Low ; Grade Gliomas ; Glioblastomas ; Endothelin ; EDNRB

Poster #32

Cooperation of bone morphogenetic protein and estrogens signalling pathways in the dynamics of transformation of mammary stem cells at the origin of breast cancer

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The mammary gland is regulated by different signalling pathways like bone morphogenetic proteins (BMP) and estrogens. Estrogens can be of endogenous origin like oestradiol or of exogenous origin like bisphenols. Deregulations of BMP and estrogens pathways can lead to breast cancer, particularly by deregulating breast stem cells function. We aim to understand the cooperation between BMP and estrogens signalling pathways in transformation of a human not transformed breast stem cell model (MCF10A). In combination with BMP2, we have demonstrated a specific effect of bisphenol S (BPS), compared to BPA or estradiol, on the immature phenotype assessed by mammospheres assays. Interestingly, this effect seems to be dependent on GPER, a non-canonical estrogens receptor. Besides, BPS treatment increase the number of soft-agar clones in a transformed cell line derived from MCF10A after long-term BMP2 exposure. Exploring the molecular basis of these functional effects, we found a physical interaction between the BMPR1B receptor and GPER, as well as ERα36, another non-canonical estrogens receptor. These results call into question the way to assess environmental pollutants like bisphenols based on their binding with the canonical estrogens receptor ERα66 and highlight interactions between BMP and estrogens pathways and potential mechanisms of breast cancer initiation.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	stem cells ; BMP ; estrogens ; bisphenols ; transformation ; breast cancer

Poster #33

Impairment In Mechanotransduction Pathways, A Key For AML Chemoresistance.

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In Acute Myeloid Leukemia (AML), the clonal expansion of the leukemic stem cells (LSC) in the bone marrow (BM) promote the modification of the physical properties of the BM niche, and reduce the overall space allocated to each cell populations. Thus, LSC intrinsic stiffness can be impacted by the niche via reciprocal mechanical responses. Together, these biomechanical changes are potential factors that will impair treatment responses.
Combining publicly available transcriptomics data analysis and using AML cell lines models, we found a correlation between treatment resistance and impairment of BMP and YAP/TAZ mechanotransduction pathways. To reproduce closely the BM existing conditions and characterize the intrinsic and extrinsic properties of the cells, we used physical and biological approaches such as tuned extracellular matrices with regulated stiffness, an original confinement system to reproduce AML hyperproliferation in the BM and a microfluidic system to measure intrinsic cell stiffness. With these tools, we evaluated differences between sensitive or resistant AML cell lines, and treatment responses of the resistant cells to the chemotherapy in combination with inhibitors of BMP and YAP/TAZ pathways.
Thus, we found evidences that forces and mechanotransduction participate to chemoresistance in AML and could be of interest as therapeutic targeting.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Acute Myeloid Leukemia ; Bone Morphogenetic Proteins ; Mechanotransduction ; Treatment resistance.

Poster #34

Deciphering the crosstalk between intercellular communication via tunneling nanotubes, the BMP pathway and mammary cell transformation.

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Breast cancer is the second leading cause of death by cancer in women. Understanding the underlying mechanisms of mammary cell transformation may enable the identification of new therapeutical targets. In constant interaction with their niche, mammary cells sense physico-chemical cues through transmembrane extracellular matrix proteins such as Syndecan-1. They also establish contact with neighboring and distant cells through the recently discovered actin-based tunneling nanotubes (TNTs). Syndecan-1 expression modulates bone morphogenetic proteins (BMP) signaling, both of which being altered during breast cancer progression. Using novel mammary cell lines of various degrees of transformation, we observed: (i) a progressive increase of TNTs number and length during transformation, allowing long-range intercellular communication between transformed cells; (ii) the formation of TNTs linking transformed to non-transformed cells; (iii) the presence of BMP receptors and Syndecan-1 within or/and along TNTs membrane. Overall, these results suggest a potential crosstalk between TNTs, the extracellular matrix and the BMP pathway, impacting on transformation initiation and spreading. Our dynamic and mechanistic ongoing investigations will help decipher this newly identified crosstalk.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Tunneling nanotubes ; BMP signalling ; Syndecan ; 1 ; breast tumor initiation

Poster #35

Regulation of BRCA1 and BRCA2 gene expression by BMP signaling in mammary epithelial stem cells and functional consequences

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Introduction: Breast cancer is the leading cause of cancer death in women worldwide. It is a heterogeneous disease with several molecular subtypes. The myoepithelial subtype shows the poorest prognosis with a higher probability of recurrence at the localised stage. These recurrences are due to the persistence of breast cancer stem cells after initial treatment. These immature cells are regulated by different signalling pathways, including the Bone Morphogenetic Proteins (BMP) pathway.

Materials and methods: We use in vitro the human mammary epithelial cell line MCF10A to model the mechanisms of stem cell tumour initiation in the mammary gland.

Results: We show that exposure to BMP4 ligand, produced by the mammary gland microenvironment, results in transcriptional repression of BRCA1 and BRCA2 genes in MCF10A cells. This results in a situation of simultaneous haploinsufficiency for these two genes. Functional consequences include preferential differentiation according to myoepithelial phenotype and increased sensitivity to PARP inhibitors, suggesting underlying phenomena of homologous recombination deficiency.

Conclusion: We suggest a role for BMP4 in the early stages of carcinogenesis of myoepithelial breast tumours.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	breast cancer stems cells ; tumour initiation ; BMP pathway ; BRCA1/2

Poster #36

RMS organoids are new innovative tools for efficient translation of basic research into novel treatments regimens targeting apoptosis

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Rhabdomyosarcoma (RMS) are the most frequent soft-tissue sarcoma in children and adolescents. Despite the use of a multimodal treatment and the implementation of several clinical trials associating different chemotherapeutic drugs combinations, they remain a therapeutic challenge and survival rate does not exceed 20% in case of metastases or relapse. Although having embryonic myogenic attributes as a common denominator, RMS are a heterogeneous group of malignancies, sustained by a wide range of oncogenic programs, which explain the difficulties to challenge them as a sole entity in clinics. To help unravelling the biology of these cancers, we definitely need new relevant cancer models, which reproduce tumors' complexity. The recent advances in organoid technology have offered the possibility for the development of novel and robust human cancer models that accurately recapitulate inter- and intra- tumoral heterogeneity. By combining bionformatics' analyses and design of new and original RMS organoid models, we challenge the view of apoptosis as a lever to trigger tumor cell death, showing that targeting intra-tumor heterogeneity could help identifying new therapeutic combinations by exploiting therapeutic vulnerabilities.

Topics : TUMORIGENESIS AND CANCER PROGRESSION

Keywords : Rhabdomyosarcoma ; organoids ; apoptosis

Poster #37

Propagation of Human Colon, Mammary, and Lung Cancer Organoids in Growth Medium Utilizing Tissue-specific Reagent Kits and Ready-to-use Wnt-3a and R-Spondin1 Conditioned Media

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With the increased availability of cryopreserved human cancer organoids generated by academic laboratories, large-scale biobanking initiatives, and commercial sources, there is an unmet need for simplified, standardized, and cost-effective methods for preparation of the complex growth media required by these models. It contains a variety of recombinant proteins, small molecules, and other growth factors that are costly to purchase in small-scale, time consuming to reconstitute and aliquot, and demonstrate varying stability and shelf life once prepared. Organoid culture media often utilizes undefined conditioned media (CM) from one or more engineered cell lines that must be cultured separately, requiring additional time / resources. To address these challenges we are developing reagent kits containing essential growth medium components in an individually lyophilized format for long-term storage and easy single-use preparation of organoid growth media for varied tissue types including human colon, mammary, and lung. We compared growth medium formulated with our kit components, with small-scale “homebrew” preparations and found equivalent or better culture performance (as calculated by doubling times) and similar morphology. The results show that our tissue-specific kits and ready-to-use CM can provide a simplified, cost-effective method to support long-term propagation of human cancer organoids from multiple tissues and cancer types.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	human cancer organoids ; organoid conditioned media

Poster #38

Identification of a CD106+ pericyte stem cell leading to Ly6G+ cell accumulation responsible for resistance to immunotherapy in pancreatic cancer

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We report the identification of a cell population that shares pericyte, stromal and stemness features, does not harbour the *Kras*G12D mutation and drives tumoral growth *in vitro* and *in vivo*. We termed these cells pericyte stem cells (PeSCs) and defined them as CD45-EPCAM-CD29+CD106+CD24+CD44+ cells. We performed studies with *p48*-Cre;*KrasG12D*(KC), *pdx1*-Cre;*KrasG12D*;*Ink4a*/*Arf*fl/fl (KIC) and *pdx1*-Cre;*KrasG12D*;*p53R172H* (KPC) and tumor tissues from PDAC and chronic pancreatitis patients. We also performed single cell RNAseq analysis and revealed a unique signature of PeSC. Under steady-state conditions, PeSCs were barely detectable in the pancreas but present in the neoplastic microenvironment in both humans and mice. The co-injection of PeSCs and tumor epithelial cells into tumor-bearing mice led to increased tumor growth associated with the differentiation of Ly6G+ myeloid-derived suppressor cells and a decreased amount of F4/80+ macrophages and CD11c+ dendritic cells. This population had the potential to induce resistance to anti-PD-1 immunotherapy when coinjected with epithelial tumor cells. Our data reveals the existence of a cell population that instruct immunosuppressive myeloid cell responses to bypass PD-1 targeting and thus suggest potential new approaches for overcoming resistance to immunotherapy in clinical settings.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Pancreatic adenocarcinoma ; Pericyte stem cell ; Tumor microenvironment ; Immune resistance

SELECTED SHORT TALKS

Calcium signals triggered by the microenvironment regulate stem cell self-renewal: from adult neural stem cells to glioblastoma stem cells.

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Neural stem cells (NSC) ensure lifelong physiological renewal of neuronal and glial cells in the brain. Yet, there is growing evidence that mutations in NSC lead to the emergence of glioblastoma, one of the most deadly tumors in adults. Glioblastomas are believed to harbor a subset of cells called glioblastoma stem cells (GSC) that escape irradiation and chemotherapy and have the capacity to regenerate the tumor. Sharing functional properties, both NSC and GSC display prominence of calcium signaling pathways in their transcriptomes. Calcium signals are encoded by calcium channels among which store-operated calcium channels (SOC) transduce extracellular signals.
We investigated the roles of SOC in adult murine NSC and in human GSC. We found that NSC and GSC express SOC proteins (TRPC1 and Orai1) that support calcium entries into the NSC and GSC in response to extracellular signals. Pharmacological blockade of SOC drastically reduced NSC and GSC proliferation and colony-forming ability, suggesting that SOC regulate stem cell self-renewal. Furthermore, the SOC inhibitors shifted the division type of NSC from proliferative symmetric to asymmetric.
Our data support that SOC are major regulators brain stem cells, in both healthy brain and brain tumors, involved in the stem cell response to microenvironment signals.

Topics : DEVELOPMENT AND REGULATION OF STEM CELL FATE

Keywords : neural stem cell ; glioblastoma ; microenvironment ; calcium channel

Growth factors alone can induce a non-canonical differentiation of fibroblasts into functional neural progenitors

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Background: Evidence of direct lineage reprogramming based on transcription factor (TF) over-expression has suggested that cells possess substantial plasticity in their identity. The contribution of growth factors (GFs) to lineage plasticity remains largely unexplored, despite their role in normal differentiation. We investigated whether neural GFs could have a supportive or instructive role in the direct differentiation to neural progenitor cells (NPCs).
Results: The addition of neural GFs greatly enhanced the output of mature neurons (NESTIN-TUJ1+MAP2+) and NPC (NESTIN+TUJ1+/-MAP2-) from HEK293T induced to neural fate by Cas9a-mediated activation of neural TFs. Dermal and lung fibroblasts under these conditions showed >50% differentiation to NPC. Surprisingly, for fibroblasts, NPC differentiation could occur without neural TF activation by Cas9a but was dose dependent on neural GFs. Critically, fibroblast-derived NPC were able to generate even clonal neurospheres with equivalent frequency and size to iPSC-derived NPC. Spheres were composed of phenotypically and morphologically mature neurons. Fibroblast-derived NPC thus possess the functional capabilities of a true NPC.
Conclusions: Neural GFs have a potent instructive effect on fibroblasts, capable of eliciting a "reprogramming" level differentiation. These results highlight the importance of extrinsic factors on the determination of cell identity and suggest that lineage plasticity may even occur naturally.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	reprogramming ; differentiation ; growth factors ; neural progenitor ; fibroblast ; Cas9 activation

Neonatal brain injury unravels transcriptional and signaling changes underlying neural stem cell regenerative potential in the postnatal subventricular zone

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Germinal activity persists throughout life within the subventricular zone (SVZ) of the forebrain, due to the presence of quiescent neural stem cells (NSCs) that are gradually reactivated throughout life. Accumulating evidence points at a role for NSCs during tissue repair following brain injuries and suggest their amenability to pharmacological manipulations. We used neonatal hypoxia as a rodent model of early brain injury, to investigate the contribution of SVZ NSCs to cellular regeneration within the neocortex. Our results reveal an increased proliferation and production of oligodendrocyte and glutamatergic neuron progenitors within the dorsal SVZ following neonatal hypoxia. Fate mapping demonstrates their contribution to de novo oligodendrogenesis and cortical neurogenesis, while transcriptional analysis reveals changes paralleling their reactivation. Finally, pharmacological activation of the Wnt/β-catenin pathway by intranasal administration of CHIR99021 following hypoxia promotes neuron and oligodendrocyte maturation. Labeling of NSCs in different states of activation demonstrates that pharmacological NSCs activation have no adverse effects on the reservoir of NSCs and on their longterm germinal activity. Altogether, our work reveals a regenerative potential for NSCs following early brain injury, identifying key transcriptional changes paralleling their activation and point at their amenability to pharmacological manipulation with no long-term detrimental effect on germinal activity.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	neural stem cells ; neonatal brain injury ; cellular regeneration ; glutamatergic neurogenesis ; transcriptionnal changes

The interplay between epithelial to mesenchymal transition and cell fate specification potentiates gastruloids self-organization

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Mouse embryonic stem cells (ESC) can be stimulated to undergo gastrulation-like events forming gastruloids, 3D structures mimicking post-implantation embryos. Gastruloids undergo cell fate diversification, break symmetry, and self-organize along a single antero-posterior axis without any polarizing cues.
Here, we sought to investigate the mechanism underlying gastruloid self-organization and its interplay with cell fate specification.
First, we found that gastruloid self-organization is associated to epithelial to mesenchymal transition (EMT). Using a combination of single cell transcriptomic, and imaging of gastruloids with high temporal resolution, we found that the timing of EMT completion, but not its initiation is differentially regulated between different cell fates.
Then, we generated a serie of ESC lines to genetically dissect the EMT process and its function during gastruloid's development. Interestingly, not all EMT steps are required for self-organization. However, E-Cadherin repression is crucial for proper gastruloid organization, and in its absence gastruloids frequently elongate along multiple axes.
Finally, interfering with EMT also has a strong impact on gastruloid cell differentiation. Indeed, both the cell fate proportions and the differentiation pace of the two major gastruloid lineages is severely affected.
In summary, we propose that an interplay between cell fate acquisition and EMT is critical for gastruloid self-organization.

Topics	:	DEVELOPMENT AND REGULATION OF STEM CELL FATE
Keywords	:	Gastruloids ; pseudo ; embryos ; self ; organization ; EMT ; cell fate specification

Regulation of vascular cells in Duchenne Muscular Dystrophy

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In skeletal muscle, endothelial cells (ECs) form the niche, and interact with muscle stem cells to sustain myogenesis. In Duchenne Muscular Myopathy (DMD), skeletal muscle undergoes permanent and inefficient cycles of regeneration. We are interested in the functions of ECs, which remains poorly described in DMD.
Combining *in-vitro* and *in-vivo* technics, we observed higher proliferating and colonizing capacities in mdx-ECs, which resulted in an increase of the capillary density and the vessel volume in human and mdx, mouse DMD muscle. However, mdx capillaries were less covered by pericytes, mainly in fibrotic areas. Since FibroAdipoPrecursors (FAPs) are the main contributors to fibrosis in DMD, we investigated FAP:EC interactions. Matrigel plug assay showed that mdx-FAPs stimulated angiogenesis better than WT-FAPs, which was emphasized in mdx recipients. Analysis of gene expression in FACS-sorted dystrophic-ECs showed a dysregulation of genes involved in EC cytoskeleton organization and in basal lamina production. This change in EC function is manifested *in-vitro* by an alteration of cell shape, cell junction formation and by a reduction of *in-vitro* basal lamina production.
Our results show alterations of FAP:EC interplay in DMD muscle, that may lead to a dysregulated vascularization. We aim to dissect those interactions at the molecular level.

Topics	:	ENVIRONMENT OF STEM CELLS
Keywords	:	endothelial cells ; vessels ; Duchenne Muscular Dytrophy ; fibrosis

A tight coupling between ribosome biogenesis and chromatin remodeling rewires embryonic stem cell fate

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Embryonic stem cell (ESC) fate decisions are regulated by a complex circuitry that requires tightly coordinated gene expression regulations at multiple levels from chromatin organization to mRNA processing. Recently, ribosome biogenesis and translation have emerged as key pathways that efficiently control stem cell homeostasis. However, the molecular mechanisms underlying the regulation of these pathways remain largely unknown. We analyzed the expression, in mouse ESCs, of over 300 genes involved in ribosome biogenesis and we identified RSL24D1 as the most differentially expressed between self-renewing and differentiated ESCs. RSL24D1 is highly expressed in multiple mouse pluripotent stem cell models, an expression profile also conserved in human ESCs. RSL24D1 is associated with nuclear pre-ribosomes and is required for the maturation of 60S subunits in mouse ESCs. Interestingly, RSL24D1 depletion significantly modifies ESC's proteome as it impairs global translation, particularly of key components of the polycomb repressive complex 2 (PRC2). Consistently, the decrease of RSL24D1 expression significantly alters mouse ESC self-renewal and proliferation while having a moderate impact on lineage commitment and cell differentiation. Taken together, these results demonstrate that RSL24D1-dependant ribosome biogenesis is both required to sustain the expression of pluripotent transcriptional programs and silence PRC2-dependant developmental programs, which concertedly dictate ESC homeostasis.

Topics	:	EPIGENETICS AND GENE REGULATORY NETWORKS
Keywords	:	embryonic stem cell ; ribosome biogenesis ; PRC2 regulation ; pluripotency maintenance

ACLY defines a therapeutic vulnerability in PTEN-null T-ALL

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Loss of the tumor suppressor gene PTEN commonly occurs in T-cell acute lymphoblastic leukemia (T-ALL), where it defines patients at high risk relapse. Combining metabolomic analysis, transcriptomic profiling and distinct murine models, we characterized the genetic-oncogenetic-metabolic circuits which operate in this paradigmatic T-ALL subgroup.
Using a PTEN-deficient mouse model, we first demonstrated that ACLY, the major cellular source of cytosolic acetyl-coA, is strictly required for T-ALL initiation. Whereas PTEN mutant mice all died within 17 weeks, the concomitant ACLY deletion prevented disease development in 70% of the animals, which did not develop the disease as ACLY deletion abrogated the emergency of TCRalpha-cmyc rearrangement induced by PTEN loss.
We next translated our findings to the human pathology, performing a transcriptomic analysis (174 patients) and a metabolomic analysis (8 cases) of primary T-ALL cells. These analysis showed that *PTEN* genomic alterations associate to ACLY activation which is needed to fuel fatty acids and cholesterol synthesis. ACLY pharmacological inhibition reduced the survival of primary human T-ALL cells and its genetic silencing reduced the engraftment of primary T-ALL cells. Taken together, these results demonstrate that ACLY defines a novel metabolic vulnerability, which can be pharmacologically targeted in a subset of high-risk T-ALL cells.

Topics : TUMORIGENESIS AND CANCER PROGRESSION

Keywords : T ; ALL ; ACLY ; omics

Control of disseminated breast cancer cell dormancy in the bone marrow by TGFB2 and BMP4 signaling

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Breast cancer-related deaths are due to metastasis that can arise years after treatment due to the dormancy of disseminated tumor cells (DTCs) in secondary organs. The bone marrow (BM) is of major interest as Disseminated Tumor Cells (DTCs) detected in this site are associated to poor prognosis. TGFB2 was found to induce breast cancer cell (BCC) dormancy in the BM, but other pro-dormancy factors are present and several signals may be integrated. Among them, BMP4 has been implicated in regulating stem cell quiescence in several organs and cancers. Nevertheless, the precise role of BMP4 and its interplay with TGFB2, in BCC dormancy in the BM has not been assessed. Interestingly, when combined in 3D assays and with the FUCCI (Fluorescence Ubiquitination Cell Cycle Indicator) system, TGFB2 and BMP4 display a stronger anti-proliferative effect compared to individual ligands, in a synergistic fashion. Single-cell RNAseq analysis revealed the heterogeneity of the G0 compartment with a unique deep dormant cluster appearing in the TGFB2+BMP4 condition. Use of a 3D model of the BM niche to validate this findings in situ is ongoing. By providing a better understanding of in cancer dormancy, our results can open opportunities for patients by preventing cancer relapse.

Topics : TUMORIGENESIS AND CANCER PROGRESSION

Keywords : Breast Cancer Dormancy TGFB BMP Bone Marrow

Norrin/Frizzled4 signalling controls the microenvironment to suppress medulloblastoma

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Crosstalk between pre-tumour and stromal cells in the tumour microenvironment plays a pivotal role in tumour initiation and progression. In the cerebellum, canonical Wnt signalling mediated by Norrin/Frizzled4 (Fzd4) activation in meningeal endothelial cells inhibits preneoplasia and tumour progression in mouse Sonic hedgehog medulloblastoma (Shh-MB). We investigated the link between stromal signalling and tumour initiation in granule cell progenitors (GCPs), the cell of origin of Shh-MB. We found that Norrin/Fzd4 signalling maintains the activation of perivascular macrophages (pvMΦs) in the meninges during the critical window for tumour initiation. Depletion of pvMΦ during this critical window phenocopies the effects of Norrin deficiency on tumour initiation, indicating that pvMΦs suppress tumourigenesis. Finally, we show that both Norrin/Fzd4 and pvMΦs inhibit chemotaxis and proliferation of GCPs. Taken together our results identify an unanticipated cross talk between endothelial cells and pvMΦs in the control of preneoplasia in the cerebellum.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Medulloblastoma ; Microenvironment ; Perivascular Macrophages ; Endothelial cells ; Neurodevelopment

The comparative roadmaps of reprogramming and transformation unveiled that cellular plasticity is broadly controlled by Bcl11b and Atoh8

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Coordinated changes of cellular plasticity and cellular identity are critical for pluripotent reprogramming and oncogenic transformation. However, the sequences of cellular/molecular events that orchestrate these intermingled modifications have never been comparatively dissected. Here, we deconvoluted the cellular trajectories of reprogramming (via Oct4/Sox2/Klf4/c-Myc) and transformation (via Ras/c-Myc) at the single-cell resolution and revealed how the two processes intersect prior to bifurcate. This approach also led to identify the transcription factor (TF) Bcl11b as a broad-range regulator of cell fate changes, as well as a pertinent marker to capture early cellular intermediates that emerge simultaneously during reprogramming and transformation. Multi-omics characterization of these intermediates led to unveil a c-Myc/Atoh8/Sfrp1 regulatory axis that constrains rodent and human reprogramming but also cancer cell plasticity and neuron transdifferentiation. Mechanistically, we found that the TF Atoh8 restrains cellular plasticity, independently of cellular identity, by binding a specific enhancer network. This study provides insights into the partitioned control of cellular plasticity and identity for both regenerative and cancer biology.

Topics	:	TUMORIGENESIS AND CANCER PROGRESSION
Keywords	:	Pluripotent reprogramming ; oncogenic transformation ; transdifferentiation ; cellular intermediates ; cellular plasticity ; cellular identity ; bHLH transcription factors ; iPS cells ; single ; cell