The Swiss Institute for Experimental Cancer Research (ISREC) has continued its contributions to the School of Life Sciences at EPFL, via cutting-edge research, mentoring young scientists, and classroom teaching. In addition, ISREC is playing a key role in the new Swiss Cancer Center Lausanne, a joint venture with the University of Lausanne and its Hospital and Medical Center (CHUV). This new cancer center, announced in January 2013, has a mission statement to become the first comprehensive cancer center in Switzerland, as defined by depth and breadth in basic and translational cancer research, in clinical research and clinical trials of new therapies, and excellent care of cancer patients. ISREC, with 15 faculty research groups focused on cancer research or fundamental cell and developmental biology, brings exceptional strength and talent to this new cancer center.

ISREC has been centrally involved in community-building initiatives for the SCCL, including a series of annual faculty-only and faculty plus staff retreats, held in the spring and fall, respectively. Both retreats - initiated in 2013 and continued annually henceforth under the excellent stewardship of Etienne Meylan (ISREC, SV, EPFL) and Olivier Michielin (CHUV, UNIL) – have proved successful at building bridges across the multiple sites in Lausanne that house cancer-related faculty.

The Lola and John Grace Distinguished Lectures in Cancer Research – sponsored by the Grace family – bring in eminent cancer scientists for a once-monthly lecture at EPFL that is televised to the CHUV and Biopole/Epalinges sites of the SCCL. In addition, ISREC sponsors a monthly faculty-only research presentation, and a weekly informal seminar series for ISREC students and postdocs.

Denis Duboule was recently elected to the prestigious College de France, established in Paris in 1530, a society consisting of 52 members whose endeavors span the pursuit and public dissemination of knowledge across the spectrum of academic disciplines. Michele de Palma and Oliver Hantschel were awarded highly competitive ERC Consolidator Grants. On the occasion of the EPFL graduation ceremony - the Magistrale 2016 - Etienne Meylan received the EPFL Life Sciences Teaching Prize, ‘For excellence of his teaching judged from the last 3 years’.

http://sv.epfl.ch/ISREC
Introduction

Research in Dr. Brisken’s laboratory focuses on the cellular and molecular underpinnings of estrogen and progesterone receptor signaling in the breast and the respective roles of these hormones and hormonally active compounds in carcinogenesis. The aim is to understand how recurrent exposures to endogenous and exogenous hormones contribute to breast carcinogenesis in order to better prevent and treat the disease. The laboratory has pioneered in vivo approaches to genetically dissect the role of the reproductive hormones in driving mouse mammary gland development and shown how they control intercellular communication. Dr. Brisken’s group has developed ex vivo and humanized mouse models using patient samples to study hormone action in human tissues in normal settings and during disease progression.

Keywords

Hormones, mammary gland development, breast carcinogenesis, paracrine signaling, estrogen, progesterone, RANKL, Wnt-4, stem cells, preclinical xenograft models

Results Obtained

Progesterone and Wnt4 control mammary stem cells via myoepithelial crosstalk

Ovarian hormones increase breast cancer risk by poorly understood mechanisms. We reveal that progesterone receptor (PR) signaling controls mammary epithelial stem cells through Wnt-4. Wnt-4 is secreted by PR positive luminal cells and activates canonical Wnt signaling in basal cells.

A preclinical model for ERα positive (ER+) breast cancer

Ninety percent of new drugs in oncology fail, partly because the preclinical models used to test them are not adequate. Breast cancer is the leading cause of cancer-related death among women worldwide and we lack in vivo models for the ER+ subtypes, which represent more than 75% of all cases. We show that ER+ tumor cells can be successfully established as xenografts when injected into the milk ducts of immunocompromised mice. Traditional grafting into subcutaneous fat induces TGFβ/SLUG signaling and basal differentiation and prevents in vivo growth. Intraductally, SLUG is suppressed and ER+ tumor cells grow like their clinical counterparts. Disease progression with invasion and metastasis can now be studied in a physiologic endocrine milieu.

The in vivo function of the secreted metalloproteinases ADAMTS18

We generated Adamts18-deficient mice and demonstrated a 100% penetrant eye defect resulting from leakage of lens material through the lens capsule. Adamts18 is also required for bronchiolar branching and vaginal opening. Thus, the orphan protease is essential in the development of distinct tissues and the new mouse strain is likely to be useful for investigating ADAMTS18 function in human disease, particularly in the contexts of infertility and carcinogenesis.
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Selected Publications

Introduction

Using genetic and biochemical approaches, we investigate how proprotein convertase family and their substrates govern stem and progenitor cell renewal and differentiation in the mammalian embryo, and how cancer cells redepoly them for tumor progression. Such road maps of progenitor cell differentiation are important both for regenerative medicine and to find ways to reduce tumor aggressiveness. We have shown that proprotein convertases control TGFβ-related activities and other master regulators of early lineage differentiation. However, what determines the substrate specificities and paracrine range of action of these proteases during development and in cancer is unclear. To address this, we generated convertase gene deletions and high resolution live imaging probes that reveal when and where these enzymes are active in normal and cancerous cells and tissues.

Several cancer hallmarks are shared by heritable polycystic kidney diseases. In such patients, renal epithelial tubule cells or their progenitors are reprogrammed to form fluid-filled cysts as they fail to protrude functional primary cilia into the tubule lumen to sense urine flow and possibly other stimuli. We study the role of the RNA-binding protein Bicc1 in signal transduction pathways such as Wnt, PKA and mTOR that control cell growth, repair and metabolism up- and/or downstream of primary cilia.

Keywords

Protease imaging, mRNA silencing, stem cells, cancer, polycystic kidney diseases

Results Obtained

The first lineage decision in mammalian embryos occurs at the morula stage when outer cells become polarized by asymmetric contacts and activate the transcription factor YAP to form trophoderm. By contrast, symmetric contacts of the adhesion molecule E-cadherin in inner cells inhibit this pathway and maintain the pluripotency of the cells that will form all body parts.

Our PC7 gene knockout and live imaging showed that Furin and PC7 jointly initiate morula compaction at least in part by stimulating E-cadherin cleavage and stability, thus identifying the most upstream regulators of ICM formation known to date. However, a related convertase (Pace4) was activated a few hours later specifically in outer cells and significantly compensated for combined loss of Furin and PC7. Thus, during inner cell mass formation, E-cadherin precursor processing involves not only one but as many as three functionally overlapping proprotein convertases that are dynamically regulated. Ongoing work addresses whether other substrates rely on fewer PC family members for cleavage due to differential trafficking, and whether their processing in specific subcompartments would be a viable cancer drug target.

Mutations in Bicc1 instigate cysts in kidney and pancreas. Our candidate search for the first direct targets identified AC6 and PKI6 mRNAs that affect cAMP signaling. However, why cAMP accumulates in polycystic kidney disease patients with ciliary defects is unclear. To elucidate how Bicc1 enables mRNA silencing, we modeled the structure of its SAM domain. In this model, the SAM organizes Bicc1 as a helical polymer with RNA binding sites arrayed at the surface. In line with this prediction, the bpk mutant Bicc1 allele and point mutations blocking SAM polymerization abolished the localization and silencing of associated reporter mRNA in cytoplasmic Bicc1 foci (Fig. 1). Whether Bicc1 polymerization is regulated by cilia or vice versa is under investigation.
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Selected Publications


In L11-stained cyst-lining cells of bpk mutant polycystic mouse kidneys (*), isoform A of Bicc1 is not polymerized by its SAM domain in cytoplasmic foci (A-E). Enrichment of Bicc1 (pink) and bound mRNA (yellow) in foci is also inhibited if SAM polymerization is blocked by point mutation (mutD, C-H).
Introduction

Our lab has contributed to elucidating the pro-angiogenic functions of monocytes/macrophages in mouse models of cancer, as well as the molecular and functional heterogeneity of macrophages in both experimental and human tumors. We have also characterized VEGF-independent modes of tumor angiogenesis, and illustrated the therapeutic opportunities afforded by inhibiting angiopoietin signaling in de novo models of metastatic cancer. Currently, we employ genetic cancer models and cell-engineering strategies, largely based on lentiviral gene transfer, to dissect the interactions among macrophages, blood vessels and T cells in tumors, primarily by focusing on angiogenic signaling, immune checkpoints, microRNA regulation, and secreted exosomes. By tackling these processes, we aim to reprogram the immunosuppressive tumor microenvironment to a form that facilitates the deployment of anti-tumor immunity and enhances the efficacy of anticancer therapies.

Current research topics include:
1. Engineered dendritic cell vaccines for cancer immunotherapy;
2. microRNA regulation of macrophage activation in tumors;
3. Anti-angiogenesis as a tumor-conditioning strategy for improving cancer immunotherapy;
4. Mechanisms of tumor resistance to antiangiogenic therapy;
5. Tumor-derived exosomes and cancer progression.

Keywords

Cancer, macrophage, angiogenesis, microRNA, exosome, immunotherapy, lentiviral vector.

Results Obtained

In one recent study, we identified a mechanism regulating the immunosuppressive functions of macrophages in tumors. Tumor-associated macrophages (TAMs) largely express an alternatively activated (or M2) phenotype, which entails immunosuppressive and tumor-promoting capabilities. Reprogramming TAMs toward a classically activated (M1) phenotype may thwart tumor-associated immunosuppression and unleash anti-tumor immunity. We found that conditional deletion of the microRNA (miRNA)-processing enzyme DICER in macrophages prompts M1-like TAM programming, which is characterized by hyperactive interferon (IFN-γ)/STAT1 signaling. This rewiring abated the immunosuppressive capacity of TAMs and fostered the recruitment of activated cytotoxic T lymphocytes (CTLs) to the tumors. CTL-derived IFN-γ exacerbated M1 polarization of Dicer1-deficient TAMs and inhibited tumor growth. Remarkably, we found that DICER deficiency in TAMs negated the anti-tumoral effects of macrophage depletion by anti-CSF1R antibodies, and enabled complete tumor eradication by PD-1 checkpoint blockade or CD40 agonistic antibodies. Finally, we showed that the genetic rescue of Let-7 miRNA activity in Dicer1-deficient TAMs was sufficient to partly restore their M2-like phenotype and decrease tumor-infiltrating CTLs. These findings indicate that DICER/Let-7 activity opposes IFN-γ-induced, immunostimulatory M1-like TAM activation, with potential therapeutic implications.
Selected Publications


The figure shows specific labeling of tumor-associated macrophages (TAMs; green) in mT/mG mice, which express GFP upon Cre-mediated recombination. The 3yz2-Cre transgene was used to induce macrophage-specific GFP activation. The 3yz2-Cre transgene was also used to conditionally delete Dicer in TAMs (see publication Baer et al., Nat Cell Biol., 2016).
Introduction

Our laboratory has started operating at EPFL during 2007. Its major aim is to study principles of mammalian embryological development by using the recent tools of functional genomics. A special focus is given to those similarities and differences that exist between the embryological development of vertebrates (to whom mammals belong) and those of other animals (invertebrates), from whom vertebrates derive.

To achieve this task, we use the developing mouse embryo in vivo as an experimental system, and try and apply the methodology developed following the sequencing of complex genomes. Our major aim is the understanding of the regulation of a critical family of transcription factors during the construction of the animal body plan, referred to as architect genes (the Hox gene family).

These genes have a special interest in the study of both our ontogenesis (our development as individuals) and our phylogeny (our origin as a group of individuals) and the detailed understanding of their regulations and functions will be an important step in our understanding of our own histories. More recently, in collaboration with the Martinez-Arias (Cambridge) and Lutolf (EPFL) laboratories, we have started a new research program using in vitro grown organoids as models of mouse embryos.

Keywords

Embryos, development, evolution, transcription, epigenetic regulation, Hox gene clusters, enhancers, chromatin.

Results Obtained

Over the past two years, progresses have been made in several lines of research. Importantly, by using biochemical, genetic and epigenetic approaches, we have finally obtained a fair understanding of the collinear mechanism at work during limb development, a project that started in 1989 with the discovery of this intriguing phenomenon. We deciphered the chromatin structure around the HoxA and HoxD gene clusters and reported that they both had evolved related topologically associating domains (TADs) regulatory organization, likely implying that such a structure was already present in an ancestor animal that only had one gene cluster.

During 2015, we have terminated a series of experiments whose aim was to determine which mechanism implements the transition between the regulation from one such TAD to the other, and we demonstrated that the Hox13 proteins themselves are involved in this important switch either by repressing the telomeric, or by activating the centromeric regulation. These opposite effects of the same proteins explain how these two regulations are exclusive from another and hence how the transition between the forearm and the hands is organized, with the mesopodial articulation (the wrist) in between. In the same context, we have shown how regulations in this system could be hijacked to serve another purpose in the course of evolution, as illustrated by the necessary function of these genes during the emergence of the mammary glands.

In parallel with these molecular studies, we have well progressed in our projects to try and better visualize the spatial organization of these genetic loci, as well as their long-range contacts with other genomic loci, either in expressing tissues, or in tissues where these genes are silent. In the former case, we set up a collaboration with the Sulyana Manley laboratory from the school of physics at EPFL, to try and picture the gene cluster by using STORM microscopy at very high resolution, in comparison with either SIM or more ‘classical’ FISH technology.
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Selected Publications

Introduction

We are interested in understanding fundamental cell division processes and focus on two in particular: centriole assembly and asymmetric division. To uncover the underlying mechanisms, we use a combination of genetic, functional genomic, biochemical, proteomic and cell biological approaches, primarily in C. elegans embryos and human cells.

Centriole formation: Centrioles are evolutionarily conserved organelles essential for the assembly of cilia, flagella, and centrosomes, and which are characterized by a 9-fold radial symmetry of microtubules. We and others identified five proteins required for centriole formation in C. elegans, which are likewise crucial in other organisms. In collaboration with the Steinmetz laboratory, we discovered that one of these protein families (SAS-6 proteins) forms 9-fold symmetric rings at the root of the 9-fold symmetry of centrioles.

Asymmetric cell division: Asymmetric division is crucial for generating diversity during development and stem cell lineages, and relies notably on proper positioning of the mitotic spindle. We and others showed that spindle positioning requires an evolutionary conserved ternary complex, which anchors the minus end directed motor protein complex dynein at the cell cortex. There, dynein is thought to generate pulling forces on astral microtubules that emanate from the spindle poles, thus positioning the mitotic spindle.

Keywords


Results Obtained

We pursued our multidisciplinary research program to gain insights notably into the mechanisms of centriole assembly, as well as of centrosome positioning, in particular during asymmetric division. Two studies illustrating our efforts are highlighted below.

Persistence of paternal centrioles
The two gametes contribute differently to the zygote at fertilization. Apart from the genetic material, the oocyte contributes the bulk of cytoplasmic constituents, whereas in most animal species the sperm contributes two centrioles. How long such paternally contributed centrioles persist in the developing embryo was not known in any system. We set out to track the fate of paternally contributed centriolar components in C. elegans embryos. Our analysis revealed that several evolutionarily conserved centriolar components exhibit exceptional persistence over many cell cycles in the embryo. These findings raise the intriguing possibility that centrioles could act as information carrier, and that paternal centrioles may contribute information to the zygote. See Balestra et al.; 2015.

Centrosome separation
The two centrosomes present at the onset of mitosis must separate accurately to ensure proper bipolar spindle assembly. The minus-end directed motor dynein plays a key role in centrosome separation, but the underlying mechanisms remained elusive. We addressed these questions in the one-cell C. elegans embryo using a combination of 3D time-lapse microscopy and computational modeling. Our analysis revealed that centrosome separation is powered by the joint action of dynein at the nuclear envelope and at the cell cortex. We demonstrated that dynein at the cell cortex acts as a crosslinker that transmits polarized actomyosin cortical flows initiated by the centrosomes earlier in the cell cycle. This novel mechanism elegantly couples the early events of cell polarization with centrosome separation, thus ensuring faithful cell division. See also De Simone et al.; 2016.
Computer simulation of centrosome separation in C. elegans embryo. Sperm (blue disk) and oocyte (dark green disk) pronuclei are shown. The two centrosomes (small green disks) nucleate microtubules (white lines). Dynein motors at the surface of pronuclei (blue points) or the cell cortex (red points) bind microtubules, exert force on them, and thus separate centrosomes.

Selected Publications

Introduction

The Hanahan group investigates tumor development and progression using genetically engineered mouse models of cancer that recapitulate important characteristics of human cancers, with strategic goals to elucidate pathogenic mechanisms underlying multi-step tumorigenesis and malignant progression, and to develop new therapeutic strategies based on knowledge of mechanism for translation toward clinical trials aiming to improve the treatment of human cancers. Currently the lab focuses on melanoma, glioblastoma, and pancreatic, breast, and cervical cancers. Topics include mechanistic studies on acquired capabilities – hallmarks of cancer – including the capabilities for ‘invasion and metastasis’ and ‘evading immune destruction’. A crosscutting theme is the role of the heterotypic tumor microenvironment and the accessory cells that collaborate with cancer cells to manifest malignant disease. In addition, the lab is studying mechanisms of adaptive resistance to therapies targeting these and other hallmark capabilities, which represent fascinating perturbations into corrupted regulatory systems, and offer potential avenues to circumvent such drug resistance with combinatorial therapies.

Keywords

Cancer mechanisms & therapeutic targeting, mouse models of cancer; tumor microenvironment.

Results Obtained

The lab has made exciting progress on multiple fronts during 2015/16. We have, for example:

• Reported that glioblastoma brain cancers are hypersensitive to drugs that elevate the cellular recycling system called autophagy to levels that cause cell death, impairing tumor progression, and that such autophagy-associated cell death can be instigated by repurposing two classes of clinically approved drugs, originally developed to be anti-depressants or anti-coagulants, which are now appreciated to hyper-stimulate autophagy (Shchors et al, Cancer Cell, 2015).

• Extended this concept, where in on-going studies we have found that these autophagy-inducing drugs can be combined with anti-angiogenic therapy, producing added benefit, exemplifying a conceptual strategy of co-targeting distinct hallmarks of cancer, aiming to limit adaptive resistance to cancer therapies (unpublished).

• Reported that pancreatic neuroendocrine tumors can be defined as two molecular subtypes, of which one is preferentially associated with metastasis (Sadanandam et al, 2015, and unpublished).

• Described a new form of adaptive resistance to anti-angiogenic therapy – metabolic symbiosis – whereby cancer cells, faced with vascular insufficiency, adopt compartmentalized metabolic states to share limited supplies of blood-borne glucose, with one instead utilizing as fuel lactate that is produced by the other’s metabolism of glucose (Allen et al Cell Reports, 2016).

• Investigated mechanisms of resistance to cancer immunotherapy, using a mouse model of cervical carcinoma induced by the HPV16 oncogenes, wherein multiple micro-environmental barriers to infiltration and killing by cytotoxic T cells are implicated. In the context of a Sinergia grant and two pharma collaborations, we are characterizing the immune barriers and testing mechanism-based therapies in logical combinations, seeking to break down the barriers and unleash efficacious immunotherapy (unpublished).
Metabolic symbiosis induced by anti-angiogenic therapy. Vascular regression elicits reorganization of tumors into a hypoxic compartment that is glycolytic and a normoxic compartment that imports and metabolizes lactate. Inhibition of mTOR disrupts this symbiosis (Allen et al., Cell Reports 2016).

Selected Publications

Introduction

Protein kinases are strongly involved in oncogenesis. The inhibition of aberrantly activated kinases is considered to be beneficial for cancer treatment. Since 2001, 30 inhibitors of a few oncogenic driver kinases in hematological and solid tumors have entered clinical practice. Despite remarkable clinical responses that could be achieved in selected diseases, most kinase inhibitors merely improve progression-free survival, but not overall survival, which is due to various mechanisms of evasive and adaptive resistance. Moreover, it is difficult to develop highly selective kinase inhibitors, as there are more than 500 kinases in humans with a conserved structure. Therefore, side effects caused by the inhibition of off-target kinases may limit its clinical utility.

The Hantschel lab studies oncogenic kinase signaling by using interdisciplinary approaches at the interface of biochemistry, proteomics, chemical biology and protein engineering with the aim to identify innovative ways for therapeutic intervention.

Main research avenues include:
- Structure-function analysis of protein kinases
- Targeting of intracellular protein-protein interactions and post-translational modifications with engineered high-affinity protein antagonists
- Analysis of oncogenic signaling networks using interaction- and phospho-proteomics.
- Mechanism-of-action and specificity studies of kinase inhibitors

Keywords

Leukemia, kinase inhibitors, protein engineering, phosphorylation, proteomics.

Results Obtained

Despite the success of ATP-competitive kinase inhibitors, the development of secondary drug resistance severely blunted initial clinical responses in most cases. In addition, only few human protein kinases have been targeted with the required degree of specificity by inhibitors targeting the ATP binding pocket of the protein kinase domain. An alternative strategy is the identification and targeting of sites other than the ATP binding pocket that are critical for kinase activity and that may down-modulate oncogenicity. This may provide an alternative handle to provide more specific kinase inhibitors, as the targeted site would be unique to only a few kinases and could decrease the overall incidence of drug resistance. We have demonstrated that the SH2 domain of the cytoplasmic tyrosine kinases oncoproteins ABL and FES acts as an allosteric activator, which is critical for high kinase activation and oncogenicity.

Based on the promising results we have embarked on a systematic survey of most classes of cytoplasmic tyrosine kinases for allosteric activation that may be mediated by their modular protein interaction domains, e.g. SH2 and SH3 domains (see Figure). Using both quantitative in vitro enzymological assays with purified kinases (from bacterial, insect cell and mammalian expression systems), structure-function analysis as well as models in cancer cells, we have mapped such novel allosteric interactions and currently study their molecular mechanism-of-action. This provides the rationale for the functional testing of these interactions in cancer models with the prospect of their targeting with engineered protein inhibitors (monobodies) and small molecule chemical inhibitors.

Our work provides important insight into the regulation of a large class of therapeutically important protein kinases, may identify additional targetable sites and provide the framework for future cancer drug development efforts.
In cytoplasmic tyrosine kinases (CTKs), protein interaction domains (PID) are involved in autoinhibition. Upon oncogenic activation, PIDs contribute to allosteric activation of CTKs and increase substrate recruitment. Engineered proteins or drugs can be used to disrupt allosteric activation.

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Selected Publications

Introduction

Cancer stem cells (CSC) or tumor-initiating cells have been identified as subpopulation of tumor cells at the origin of cancer development and as a major driver for long-term tumor growth, tumor progression and metastasis. We are studying the biology of these cells with an emphasis on their interaction with other tumor cells and with the tumor stroma. In particular the role of cancer stem cells in the control of anti-tumor immune reactions has been a major topic of our research in recent years. Based on the principles we have learned from detailed analysis of pre-clinical tumor models, we are currently exploring several novel therapeutic options to interfere with these cells. We concentrate on breast and colon cancers for which we were able to show that eliminating cancer stem cells can cure disease even in advanced stages of cancer progression.

Keywords

Cancer stem cells, metastatic colonization, differentiation therapy, immunotherapy

Results Obtained

Most cancers, even in an advanced stage, resemble their tissue of origin indicating that tumor cells maintain parts of the normal differentiation program of their non-transformed ancestors. We now identified the homeobox transcription factor HoxA5 as an important inducer of intestinal epithelial differentiation. In colon cancer, HoxA5 is down-regulated during cancer progression, but when re-activated can induce loss of the cancer stem cell phenotype and can strikingly block-tumor growth and metastasis in vivo. HoxA5 is interconnected with the Wnt pathway in a negative feedback loop which ensures definitive bimodal fate decisions enforcing cells to halt cell cycling and exit the stem cell pool. Since HoxA5 expression can be triggered by retinoids, this may allow to treat colon cancer patients by Hox-mediated elimination of cancer stem cells.

Apart from generating all other tumor cells, we now find that cancer stem cells have an important function in controlling anti-tumor immune responses. For breast cancer, we have identified mechanisms which enable cancer stem cells to induce an immune suppressive microenvironment. This appears to be in particular important during metastatic seeding when a small number of cancer stem cells arrives in a new target organ and has to modify the stroma to become tumor-supportive. An important aspect of this ability of cancer stem cells is their immanent resistance to cell death which makes them withstand a number of hostile influences from the tumor stroma. We are working to decompose this immune suppressive activity in order to design targeted strategies for metastasis prevention which overcome these resistance mechanisms.
Cancer initiation in the intestinal epithelium of the pre-clinical cancer model APClox/lox, a frequently mutated tumor suppressor gene in human colorectal cancers, can be prevented by treatment with vitamin A (retinoic acid). This enforces differentiation of cancer stem cells preventing cancer growth.

Selected Publications

**Introduction**

Telomeres are nucleoprotein structures at the ends of eukaryotic chromosomes. They have crucial functions as tumor suppressors and they protect chromosome ends from degradation and rearrangements. Telomere length and chromatin defects cause telomeropathies, which are characterized by damage in highly proliferative tissues early in life. Telomeres also serve as cellular clocks. They shorten in normal human cells with every round of DNA replication due to the DNA end replication problem and the absence of telomerase. Short telomeres elicit a DNA damage response triggering a permanent cell cycle arrest termed cellular senescence. Thus, the replicative potential of primary human cells is limited. While cellular senescence may contribute to organismal aging, it is beneficial to restrain the growth of pre-cancerous lesions. During progression towards malignancy, senescence is overcome by mutations in cell cycle regulators such as p53 and pRB. Furthermore, cancer cells acquire mutations that reactivate the telomerase enzyme, which stabilizes telomere length.

Through telomerase activation, cancer cells acquire an immortal phenotype representing a cancer hallmark. Our laboratory combines telomeric chromatin analysis by mass spectrometry, biochemistry and molecular genetics to study the function, the dynamics and maintenance of telomere structures in normal development and disease.

**Keywords**

Telomeres, TERRA long noncoding RNA, chromatin, cellular senescence, telomeropathies.

**Results Obtained**

Oxidative damage of telomeres can promote cancer, cardiac failure, and muscular dystrophy. Specific mechanisms protecting telomeres from oxidative damage had not been described. In collaboration with Viesturs Simanis, we analyzed telomeric chromatin composition by QTIP (Nat Comm. 4, 2848 (2013); Methods 114, 28 (2017)) during the cell cycle and showed that the antioxidant enzyme peroxiredoxin 1 (PRDX1) is enriched at telomeres during S phase (Cell Reports 17, 3107 (2016)). Deletion of the PRDX1 gene leads to damage of telomeric DNA upon oxidative stress, revealing a protective function of PRDX1 against oxidative damage. We also found that oxidized nucleotide orDNA substrates cause premature chain termination when incorporated by telomerase. Thus, PRDX1 safeguards telomeres from oxygen radicals to counteract telomere damage and preserve telomeric DNA for elongation by telomerase.

The telomeric shelterin protein TRF2 suppresses the DNA damage response (DDR) and this function has been attributed to its abilities to trigger t-loop formation or to prevent massive decompaction and loss of density of telomeric chromatin. In collaboration with the group of Suliana Manley, we applied stochastic optical reconstruction microscopy (STORM) to measure the sizes and shapes of functional human telomeres of different lengths and dysfunctional telomeres that elicit a DDR (Genes & Dev, in press). Telomeres have an ovoid appearance with considerable plasticity in shape. Depletion of TRF2, TRF1 or both affected the sizes of only a small subset of telomeres. Co‐staining of telomeres with DDR markers further revealed that the majority of DDR-signaling telomeres retained a normal size. Thus DDR signaling at telomeres does not require decompaction. We propose that telomeres are monitored by the DDR-machinery in the absence of telomere expansion and that the DDR is triggered by changes at the molecular level in structure and protein composition.
Selected Publications


Quantitative telomeric chromatin isolation protocol (QTIP).

(a) Workflow of QTIP. (b) Effects of TRF2-depletion at telomeres. (c) Comparison of telomeric protein composition at long versus short telomeres. See Greimund, Aeby et al., Nature Communications 4: 2848 (2013) for details.

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Introduction

In our laboratory, we study signalling pathways that regulate crucial aspects of tumor metabolism or immunology. Our efforts are currently focused on non-small cell lung cancer (NSCLC), the principal type of lung cancer, which is the leading cause of cancer related deaths worldwide in women and men.

We use combinations of in vitro cellular systems, bioinformatics analyses and genetically-engineered mouse models of human cancer as well as human tissue specimens to accomplish our goal: to better understand how this malignancy develops and progresses to a fatal disease. An initial focus in our laboratory was to comprehend the molecular mechanisms explaining the regulation and function of a high affinity glucose transporter in NSCLC, GLUT3. Currently, we are expanding our research activities toward a more global understanding of pathways regulating the tumor environment. Hopefully, our projects will enable us to identify new vulnerabilities of this cancer, which could be amenable to future therapies to combat it.

We have also developed a new line of research on hepatoblastoma, a rare childhood liver cancer where we identified GLUT3 expression being strongly elevated.

Keywords

Lung cancer, mouse models, glucose metabolism, tumor immunology

Results Obtained

In 2015 and 2016 we have continued our work on glucose transporter GLUT3, and have begun to investigate GLUT1 in NSCLC. Based on our previous findings of GLUT3 being a target gene of the epithelial-mesenchymal transition (EMT) transcription factor ZEB1, we decided to explore how EMT perturbation would affect GLUT3 expression and, by extension, glucose metabolism in NSCLC.

To address these points, we used KrasLSL-G12D/WT; p53Flox/Flox mouse models of lung adenocarcinoma, where we increased or decreased the expression of an EMT transcription factor. Upon sacrifice, these lung tumors were isolated, and are currently under molecular and histopathological analysis. During our characterization of GLUT3 expression in tumor cells, we made the interesting observations that tumor cell lines from a rare pediatric liver cancer, hepatoblastoma, express GLUT3 to very high levels.

This prompted us to interrogate how GLUT3 is regulated in tumor cells from this malignancy, and if knowledge can be gained about the metabolism of these tumors. Experiments with cultured cells as well as actual human tumor tissue samples are ongoing.

We have also continued our investigations of signalling pathways that impact lung tumor development, and highlighted two proteins, RIP4 and RANKL, which are both known to be potent activators of NF-kappaB signalling, a pathway promoting lung cancer development. Through genetic or pharmacologic-based experiments, we modified their expression or activity directly in vivo, to reveal their function in the development of NSCLC. The consequences of RIP4 or RANKL blockade on tumor cells and the immune microenvironment are currently being investigated.

In parallel, we have elaborated a sophisticated methodology to extract and analyse in an unbiased manner the complex immune microenvironment of lung tumors from our mouse models. Hopefully, these analyses will provide new information about immune cell types causally linked to tumor progression.
General experimental setting: tumors are initiated by intratracheal virus-Cre, where a second gene or shRNA of interest can be added for study. Micro-CT reveals lung tumors a few months after initiation. At sacrifice, tumors are collected individually and prepared for multiple analyses.
Introduction

Research in Oricchio laboratory focuses on the genetics of lymphoma and its translation into new therapies. Lymphoma is a heterogeneous disease characterized by multiple genomic alterations. We combine genomic analyses of human tumors with functional in vivo studies using mosaic models of lymphomas to functionally annotate genes of interest. Moreover, we directly compare the impact of different genetic lesions on therapy response using highly controlled experimental systems that resemble the design of clinical trials in a physiological context. Our ultimate goal is to exploit our genetic and biological studies for the design of new therapeutic strategies.

Keywords

Cancer genetics, mouse models, therapy

Results Obtained

The research activity in the lab focuses on the lymphoma biology and recently we started a new project on Primitive Neuro–Ectodermal Tumors. We have 3 main projects ongoing in the lab.

Project 1. Identify novel therapeutic targets in Follicular and Diffuse Large B-cell lymphoma. We used an inducible CRISPR/Cas9 library targeting more than ~500 kinases to identify new essential targets in DLBCL lymphoma. We found that loss of specific kinases regulating B-cell receptor and mTOR signaling strongly impair B-cell proliferation. Moreover, we uncovered an unexpected synthetic lethal interaction between inhibition of B-cell receptor signaling and SRC-family kinases prompting the possibility to test new rational combination therapies.

Project 2. Identify chromosomal structural changes dictate by epigenetic and copy number alterations. Follicular lymphoma development is driven by multiple genomic alterations, including frequently mutated epigenetic modifiers (e.g. EZH2) and several copy number changes. The EZH2 gain of function mutation Y641X increases the H3K27me3 levels altering the heterochromatin organization and blocking the expression of several genes. Now, we are defining how epigenetic changes influence tri-dimensional organization of the genome. To analyze the chromosomal structural organization, we are using high-throughput Chromosome Conformation Capture method (Hi-C). We recently completed Hi-C analysis in lymphoma cell lines and in our preliminary data, we identified that the expression of several genes is concordantly regulated within specific chromosomal domains and is epigenetically controlled.

Project 3. Define epigenetic and metabolic alterations in Primitive Neuro–Ectodermal Tumors (PNET). We developed a new in vivo model to study PNET pathogenesis. Our model is based on primary human neural precursor originated from induced pluripotent stem cells (human-iPS). We reported that PNET are dependent on MYC activity and genetic MYC inhibition alters the expression of metabolic genes such as PKM2 and LDHA and blocks cell proliferation. Now, we are exploring metabolic and epigenetic changes associated with PNET development. We are analyzing public available DNA methylation profile in PNET patients and we obtained in vivo preliminary data of the metabolic changes associated with these tumors.
Design and analysis of a genome editing screen using CRISPR to identify new therapeutic targets for lymphoma treatment.

Selected Publications

Introduction

Our group is interested in the molecular mechanisms controlling tissue self-renewal, differentiation and cancer. The basic principle of self-renewing tissues is that they continuously produce cells from a stem cell reservoir that gives rise to proliferating transient amplifying cells, which subsequently differentiate and migrate to the correct compartment. These processes have to be tightly regulated to ensure life-long homeostasis. Developmental signaling pathways such as Notch and Wnt signaling have been shown to play important roles in regulating self-renewing tissues. Moreover, these pathways are often deregulated during tumorigenesis due to mutations in key elements involved in these pathways. Using mouse genetics we study the role of evolutionarily conserved signaling pathways under physiological and pathological situations to gain a better understanding of their role in cancer. In addition, the lab optimizes and validates potential drug development candidates that target developmental signaling pathways to assess their mode of action and their efficacy in pre-clinical cancer models and in primary human tumor samples. The goal is to develop these drug development candidates further for clinical proof of concept in human studies. Another aspect of our current research is to study the influence of inflammation for tumor progression.

Keywords

Cancer, leukemia, stem cells, differentiation, immunity, notch, Wnt, preclinical drug development and trials.

Results Obtained

Dicer1 imparts essential survival cues in Notch driven T-ALL via miR-21 mediated tumor suppressor Pdcd4 repression:

The modulatory function of individual miRNAs in Notch driven T-ALLs has recently been established. Although pro-tumorigenic and tumor-suppressive miRNAs are implicated in disease onset in murine models of Notch-driven T cell leukemia, whether Dicer1-processed miRNAs are essential for Notch-driven T-ALL was unknown. We showed that Dicer1-processed miRs are essential at all stages of T-ALL development and maintenance. Lineage tracing experiments revealed that Dicer1 deficiency led to the induction of apoptosis in T-ALL cells whereas cell cycle progression remained unaltered. Through microarray-based miRNA profiling, we identified miR-21 as a previously unrecognized miRNA deregulated in both mouse and human T-ALL. We demonstrated that miR-21 regulates T-ALL cell survival via repression of the tumor suppressor Pdcd4.

Chronic inflammation imposes aberrant stem cell fate via mechanotransduction:

Chronic inflammation is associated with a variety of pathological conditions in epithelial tissues, including cancer, metaplasia and aberrant wound healing. We have delineated the effect of chronic inflammation on epithelial stem cells using the corneal epithelium as a model tissue. We demonstrated that chronic inflammation indirectly regulates stem cell fate choice by altering the mechanical properties of the surrounding microenvironment. Subsequently, aberrant mechanotransduction in corneal epithelial stem/progenitor cells induces epidermal differentiation via elevated β-catenin signaling. Corneal differentiation can be restored using small molecule inhibitors of mechanotransduction. Collectively, this study demonstrates that chronic inflammation and mechanotransduction are linked and act to elicit pathological responses in epithelial stem cells. This therefore establishes a new mechanism by which chronic inflammation can contribute to disease.
The schematic depicts the essential function of Dicer1-mediated miRNA biogenesis for induction and maintenance of Notch-driven T cell acute lymphoblastic leukemia (T-ALL) as well as novel signaling axis involving miR-21 and the tumor suppressor Pdcd4 that is essential for survival of T-ALL cells.

Selected Publications


Team Members

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Introduction

Cell division requires duplication of the genome followed by segregation of one copy to each daughter cell and cytokinesis. Errors in these events can result in cell death, or alter the cell’s behaviour, which can contribute to the development of diseases such as cancer. We use *S. pombe* model to study cytokinesis, the final event of the cell cycle. Our goal is to understand how cytokinesis is regulated and coordinated with other events in the cell cycle. In *S. pombe* a GTPase-regulated NDR-kinase signalling network known as the Septation Initiation Network (SIN) acts at multiple points during cytokinesis. Failure of SIN signalling results in the production of multinucleated cells that die, while inappropriate activation of the SIN promotes cytokinesis from any cell cycle stage. The SIN is considered to be the functional counterpart of the mammalian Hippo signalling pathway, which regulates growth and proliferation. The SIN also plays a role in meiosis, where is it essential for generating the spores/gametes following completion of the two meiotic divisions. Our primary tools are forward and reverse genetics, combined with cell biology and biochemical analysis. Our goal is to identify regulators and targets of the SIN in mitosis and meiosis.

Keywords

Cytokinesis, cell division, meiosis, mitosis, signal transduction, yeast

Results Obtained

Association of SIN proteins with the spindle pole bodies (SPBs) during mitosis is important for SIN regulation. We used semi-automated image analysis (with the Unser lab, EPFL) to study SIN proteins localisation in wild-type and mutant cells. This analysis uncovered new facets of SIN regulation. First, the association of Cdc7p with the SPBs in early mitosis is asymmetric, favouring the new SPB. This requires Plo1p activity, and is unaffected by mutations that influence Cdc7p asymmetry in anaphase. Second, Cdc7p asymmetry in anaphase B is promoted by the 14-3-3 protein Rad24p, but delayed by the DYRK-family kinase Pom1p and the spindle assembly checkpoint. Finally, some SIN proteins show dynamic localisation patterns in early mitosis, which then become fixed in anaphase B. We are now investigating the molecular basis underlying the transition between the two states of the SIN.

In a companion study in collaboration with the Xenarios lab (SIB-UNIL), we adopted a Boolean modelling approach to describe the qualitative behaviour of the SIN and predict the behaviour of compound mutants that had not yet been constructed. Our extended Boolean model of the SIN comprised most SIN components and regulators as individual, experimentally malleable nodes. We used CDK activity levels as control nodes for the simulation of SIN related events in different stages of the cell cycle. The model was optimized using single knock-out experiments of known phenotypic effect as a training set, and was able to correctly predict a double knock-out test set. Moreover, the model made in silico predictions that have been validated in vivo, providing new insights into the regulation and hierarchical organization of the SIN.

We also collaborated with the Lingner lab (EPFL SV) to study the protein composition of telomeres at different cell cycle stages. This study revealed that peroxiredoxin1 associates with telomeres maximally during S-phase and helps protect telomeres from oxidative damage.
The image of the SIN scaffold Cdc11p-GFP illustrates progress from interphase (single SPB) to mitosis as a kymograph. The vertical axis is time, the horizontal represents SPB position. The inter-SPB distance decreases as the nuclei move to the middle of the daughter cells after spindle disassembly. In wild-type cells, CAR contraction begins at maximal SPB separation. The three phases of mitosis are shown to the right of the image. The “early” and “late” states of the SIN are shown to the right of the image. The “early” state requires Plo1p activity, and is characterised by faint SIN protein signals and unstable association of Cdc7p and Sid1p with the SPBs. The “late” state does not require Plo1p, but depends upon Spg1p and Etd1p. It is characterised by asymmetric localisation of some SIN proteins. The gradient between them indicates the fact that the precise timing of the transition varies from cell to cell.

Selected Publications

Introduction

New technologies allow for comprehensive characterization of the molecular changes that cause a healthy cell to become cancerous. These technologies produce vast amounts of data. We develop computational methods that will help to extract insights and knowledge from such data.

Our main focus is on gene regulation. Transcription factors are key elements of regulatory circuits that control gene expression. We are interested in the molecular processes that guide transcription factors to their target sites, in a developmental stage- and tissue-specific manner, and we are studying these processes by using computational approaches in conjunction with high-throughput functional genomics data such as CAGE and ChIP-Seq data.

We are further interested in the use of molecular profiling data for medical diagnosis. To this end we develop and test machine learning methods in the framework of open prediction challenges organized by the DREAM and sbv IMPROVER consortia.

Besides research, our group develops and maintains bioinformatics databases and web servers. Our best known resource is the Eukaryotic Promoter Database EPD, created in 1986 and regularly updated since then. The ChIP-seq server features web-based programs to access and analyze a large collection of public functional genomics data sets. The Signal Search Analysis (SSA) and PWMTools server offer DNA motif discovery and search tools. These three resources are tightly interlinked and together form a comprehensive web-based platform for gene regulatory regions analysis.

Keywords
Computational genomics, epigenetics, molecular diagnostics and machine learning

Results Obtained

Research

Building cell differentiation trees from ChIP-seq data. Following up on previous joint work with Bernard Moret’s group we successfully applied a new tree building algorithm to histone modification data from ENCODE. The novelty of this algorithms is that it can assign samples to internal nodes of a tree corresponding to the common progenitors of more differentiated cell types.

Building transcription factor specificity models from high-throughput experimental data: We successfully adapted a computational pipeline originally developed for protein binding microarray (PBM) to be used with high-throughput data generated with SMiLE-seq (Selective Microfluidics-based Ligand Enrichment followed by sequencing) technology recently developed in Bart Deplancke’s lab.

Promoter analysis: Making use of large volumes of recently published transcription start and nucleosome mapping data, we carried out a comparative study on the role of positioned nucleosomes in promoter regions in five model organisms. Our results show that the so-called +1 nucleosome plays an active role in transcription start site selection in those promoters that lack a core promoter element such as a TATA-box, initiator or DPE.

Annotation of SNPs with regard to TF binding potential. We developed a computational pipeline to identify all common variants that change the predicted affinity of a transcription factor binding site in the human genome. The resulting catalogue will be used to interpret histone modification profiles from a GWAS study carried by the SysGenetiX consortium.

Bioinformatics resources

The Eukaryotic Promoter database EPDnew was extended to three new model organisms: Arabidopsis thaliana, Saccharomyces cerevisiae and Schizosaccharomyces pombe. Several new feature has been added to the ChIP-seq server, including an application that generates genomic feature correlation plots in form of heatmaps and allows export of the corresponding numerical data in a format appropriate for follow-up analyses by statistics software packages such as R.
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Selected Publications


Input form and results page of the PWMScan server. Upper right: Input form. Center left: sequence logo of the position weight matrix entered. Bottom: results page with action buttons for saving the match list, for extracting surrounding DNA sequences or sending the results to another web application.