MOLECULAR ONCOLOGY PROGRAMME

MANUEL SERRANO Programme Director

It is my pleasure to introduce the highlights of the Molecular Oncology Programme in 2016. First of all, my enthusiastic and warm welcome to Alejo Efeyan, who joined the CNIO early this year to lead the Metabolism & Cell Signalling Junior Group. Alejo is a brilliant young scientist who trained as a postdoctoral fellow with David Sabatini, at the Massachusetts Institute of Technology, Cambridge. Cancer cells are metabolically hyperactive and an exciting discovery in recent years has been the realisation that cancer cells have mutations in the pathways that detect nutrient availability. Understanding how the nutrient sensing mechanisms contribute to cancer is the main goal of the Metabolism & Cell Signalling Junior Group. Alejo’s outstanding career and his original project have been awarded with a prestigious and generous grant from the European Research Council. His Group is now settled and fully operative. We are all very proud of having him here with us and we wish him all the best!

It is also very gratifying that the two other Junior Groups that joined the CNIO during 2015 have continued to successfully consolidate their teams and their projects throughout 2016. The Brain Metastasis Junior Group, led by Manuel Valiente, now has promising candidate small compounds that inhibit metastasis initiation in brain slices. Likewise, the Microenvironment & Metastasis Junior Group, led by Héctor Peinado is making impressive progress towards detecting how the vesicles shed by tumours (known as exosomes) are distributed throughout the organism, modifying it, and thereby making it more receptive for metastatic seeding.

In the following pages, you will read about several surprising discoveries that expand our understanding of cancer and that might pinpoint new therapeutic strategies in the near future. For example, a protein that regulates mRNA stability, CEBP4, and contributes to cancer progression (Melanoma Group, Nat. Commun. 2016); or telomere-derived transcripts that play a key role in chromosomal integrity through the stabilisation of telomeres (Telomeres & Telomerase Group, Nat. Commun. 2016).

It is also very gratifying that the two other Junior Groups that joined the CNIO during 2015 have continued to successfully consolidate their teams and their projects throughout 2016. The Brain Metastasis Junior Group, led by Manuel Valiente, now has promising candidate small compounds that inhibit metastasis initiation in brain slices. Likewise, the Microenvironment & Metastasis Junior Group, led by Héctor Peinado is making impressive progress towards detecting how the vesicles shed by tumours (known as exosomes) are distributed throughout the organism, modifying it, and thereby making it more receptive for metastatic seeding.

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Other discoveries concern basic mechanisms that are altered in cancer. For example, it has been found that the infliction of damage to tissues triggers cell plasticity in the surrounding cells and that cytokine IL-6 is a key factor in this process (Tumour Suppression Group, Science 2016). You will also read about a rather unique DNA polymerase named PrimPol (DNA Replication Group), about how cohesins regulate transcription, an unsuspected role for these proteins traditionally involved in sister chromatid cohesion (Chromosome Dynamics Group), and about a kinase named MASTL that is upregulated in cancer and its inhibition blocks the proliferation of some cancer cells (Cell Division & Cancer Group).

The identification of preclinical anti-cancer treatments is the ultimate goal of the Molecular Oncology Programme. In this regard, we are proud of two relevant contributions: a novel therapeutic approach for lung adenocarcinoma based on two inhibitory molecules that are effective even against aggressive cancers lacking p53 (Experimental Oncology Group, Nat. Med. 2016); and the identification of compounds that block DNA repair with therapeutic efficacy against acute myeloid leukaemia (Genomic Instability Group, Sci. Signal. 2016).

“Congratulations to Marcos Malumbres and Óscar Fernández-Capetillo for being elected as members of the European Molecular Biology Organization (EMBO).”
TUMOUR SUPPRESSION
GROUP

OVERVIEW

Tumour suppressors are genes that can prevent the development of cancer. All our cells have a functional set of these genes, but they can become defective over time. The affected cells thus become partially unprotected and, combined with additional mutations in other genes, can give rise to cancer. Understanding how tumour suppressor genes work may help us to design drugs that block cancer. Tumour suppressor genes are now known to control many aspects of cell biology and organismal physiology, such as cellular pluripotency, cell senescence, and metabolism. We aim to achieve an integrated understanding of cancer protection.

Our goals are to:

→ Understand the mechanisms of tumour suppression and identify new tumour suppressor regulators.
→ Study the interplay between tumour suppression and ageing.
→ Analyse the involvement of tumour suppressors in the regulation of metabolism and protection from metabolic damage.
→ Characterise cellular senescence as a tumour suppression mechanism.
→ Investigate cellular pluripotency and the involvement of tumour suppressors in the process of reprogramming to induced pluripotent stem (iPS) cells.
→ Explore the role of cell plasticity in cancer, tissue regeneration, and ageing.
→ Search for new frontiers in cell plasticity.

“We have found that damaged cells secrete factors that promote reparative activities in the surrounding cells, including loss of differentiation and plasticity. This could be beneficial to repair the damaged tissues, but it could also favour the expansion of dormant cancer cells.”

TUMOUR SUPPRESSION
GROUP

Manuel Serrano
Group Leader

Staff Scientists
Susana Llanos, Bárbara Martínez, Daniel Muñoz (until August), Cristina Pantoja

Post-Doctoral Fellows
Timothy Cash, Gian J. Lynch, Gianluca Varleti

Graduate Students
Noelia Akzour, Raquel Bernad, Salom Chao, Dalfo Chondromatou, Elena López-Guadamillas (until September), Lluc Monleón, Miguel Rovira

Technician
Maribel Muñoz (TS)*
*Titulado Superior (Advanced Degree)

Student in practice
Isabel Calvo
molecular mechanism by which p21 levels are linked to the activity of p21 protein as well as mTOR activation. We have unravelled the biopsies showed that about half of them possess high levels of the pathologies. Stratification of patients has been limited, until now, to a percentage of patients with this pathology has hardly improved over the last decade. 4E-BP1 binds to p21 and promotes p21 stabilization. Non-phosphorylated 4E-BP1/p21 complex ensuing stable p21 degradation of DDR1 and Notch signaling is a therapeutic target. 4E-BP1/p21 complex formation is required for the production of NADPH. We found that G6PD transgenic mice have overall higher levels of NADPH and, consequently, a better protection against oxidative damages. Importantly, these mice are not predisposed to cancer and, indeed, have a modest increase in longevity. These observations point to a novel strategy to delay ageing-related diseases, including cancer.

**RESEARCH HIGHLIGHTS**

**Model of mTOR-mediated regulation of p21.** Non-phosphorylated 4E-BP1 binds to p21 and promotes p21 degradation, and this is associated to more aggressive cancers. 4E-BP1 degradation initiates more aggressive squamous cancers, whereas the 4E-BP1/p21 complex ensuing stable p21 accumulation, and this is associated to less aggressive cancers.

**New tumour markers for the prognosis of head and neck cancer**

Head and neck cancers include a heterogeneous group of tumours located in the oral cavity, pharynx and larynx. The survival rate of patients with this pathology has hardly improved over the last decade. Stratification of patients has been limited, until now, to a clinical classification and not a molecular one. Analysis of patients’ biopsies showed that about half of them possess high levels of the p21 protein as well as mTOR activation. We have unravelled the molecular mechanism by which p21 levels are linked to the activity of mTOR (FIGURE 1). When the mTOR protein is inactive, it dictates the degradation of p21, and, conversely, when mTOR is active, p21 becomes stable. The presence of both markers, active mTOR and high levels of p21, predict a less aggressive evolution of the disease. This may help in choosing from amongst different therapeutic options for these patients.

Antioxidant defences delay ageing and age-related diseases

Accumulation of cell damage plays an important role in ageing. There is no clear answer about which types of cellular damage are more relevant for ageing. Although the accumulation of oxidative damage with ageing is undisputed, the large majority of attempts to prove that oxidative damage is relevant for ageing have failed. All these attempts, however, have manipulated only one component of the complex network of antioxidant defences. In contrast to these previous attempts, we have approached this issue by increasing the levels of NADPH, a simple co-factor required for almost all antioxidant enzymes and whose levels are known to determine the global antioxidant capacity of cells. To achieve this, we generated transgenic mice with an increased expression throughout their bodies of glucose-6-phosphate dehydrogenase (G6PD), one of the most important enzymes for the production of NADPH. We found that G6PD transgenic mice have overall higher levels of NADPH and, consequently, a better protection against oxidative damages. Importantly, these mice are not predisposed to cancer and, indeed, have a modest increase in longevity. These observations point to a novel strategy to delay ageing-related diseases, including cancer.

**Senescence cells provide critical signals for cellular reprogramming**

The mechanisms involved in the reprogramming of differentiated cells inside a living organism remain to be elucidated. Senescence is a cellular response to damage characterised by an abundant production of cytokines and other secreted factors that, together with the recruitment of inflammatory cells, results in tissue remodelling. We have shown that in vivo expression of the reprogramming factors OCT4, SOX2, KLF4 and cMYC (OSKM) triggers two divergent cellular outcomes: most cells undergo senescence, while other cells undergo reprogramming, both occurring in close physical association. OSKM-induced senescence requires the tumour suppressor locus Inka/Arf, which, via the production of the cytokine IL6, creates an optimal tissue environment for in vivo reprogramming. We concluded that tissue injury or ageing, through cellular senescence and cytokine IL6, favours in vivo reprogramming through the accumulation of senescent cells.

**Figure 1** Model of mTOR-mediated regulation of p21. Non-phosphorylated 4E-BP1 binds to p21 and promotes p21 degradation, and this is associated to more aggressive cancers. 4E-BP1 degradation initiates more aggressive squamous cancers, whereas the 4E-BP1/p21 complex ensuing stable p21 accumulation, and this is associated to less aggressive cancers.

**Figure 2** Tissue damage and ageing favour in vivo reprogramming. Expression of OSKM in vivo induces the reprogramming of a small population of cells, as well as damage and senescence in many other cells. Senescent cells release factors that promote the reprogramming of neighbouring cells, and IL6 is a critical mediator. Tissue injury and ageing also favour in vivo reprogramming through the accumulation of senescent cells.
OVERVIEW

KRAS oncogenes have been implicated in about one fifth of all human cancers including lung and pancreatic adenocarcinomas, 2 of the tumour types with the worst prognosis. Unfortunately, identification of suitable therapies to treat these tumours remains elusive and patients are still treated with cytotoxic compounds approved over 2 decades ago. The recent discovery that these tumours display intra-tumour heterogeneity adds another layer of complexity that needs to be addressed. Hence, our laboratory has decided to search for novel therapeutic targets that may contribute to the early stages of lung tumour development, hoping that these targets will be present in all tumour cells – including cancer initiating cells and cancer stem cells – and not only in limited populations of evolving clones. In addition, we have continued our quest to validate known targets (mainly those of the MAPK and PI3K pathways) using genetically engineered mouse tumour models with the ultimate goal of establishing rational combination therapies that may provide significant therapeutic benefits in the clinic.

Significance

→ We have shown that human lung tumours respond efficiently to combinations of DDR1 and NOTCH inhibitors in PDX models.
→ We have provided a mechanistic explanation for the exclusive presence of K-RAS or EGFR mutations in human lung adenocarcinomas.
→ We have demonstrated that the different incidence of H-RAS and K-RAS oncogenes in human tumours is due to the signalling intensity of their respective oncoproteins.
Identification of novel therapeutic targets for the treatment of K-Ras driven lung adenocarcinoma

The recent discovery that lung tumours display significant levels of clonal heterogeneity (Govindan, Science, 2014) implies that effective therapies must target early oncogenic events/alterations present in all tumour cells and not only in clonal variants that appear during tumour development. To provide potential solutions to this key issue we decided to search for novel therapeutic targets present in the earliest stages of lung tumour development, expecting that such targets will be present in the entire tumour population including the putative cancer initiating/stem cells. Among the most highly expressed druggable genes we identified Ddr1, a locus that encodes a tyrosine protein kinase receptor. As reported early this year (Ambrogio et al., Nat Med, 2016), genetic and pharmacological inhibition of Ddr1 prevented progression of K-Ras driven p53 wild type, but not p53 mutant tumours. Yet concomitant inhibition of Ddr1 and Notch, a downstream mediator of Ddr1 activity, led to a significant anti-tumour effect even in aggressive K-RasG12V, p53 mutant adenocarcinomas. More importantly, this treatment induced regression of K-Ras, p53 mutant patient-derived lung ortho- xenografts (FDX) with a therapeutic efficacy superior to standard chemotherapy. Identification of additional targets present in these early K-Ras mutant driven lung cells should expand the therapeutic opportunities to treat K-Ras mutant tumours in the clinic, thus by-passing the challenges derived from the development of intra-tumour heterogeneity.

Lack of selective advantage for lung cells expressing K-RAS and EGFR oncogenes

Activating mutations in KRAS and EGFR, the 2 most frequent oncogenic drivers in human lung adenocarcinoma, occur in a mutually exclusive manner suggesting functional redundancy and implying lack of positive selection. By means of a mouse model engineered to induce expression of mutant EGFRL858R in advanced tumours driven by a resident K-RasG12V oncogene, we show that, instead, their co-expression is detrimental for the progression of lung adenocarcinoma. In vivo expression of EGFRL858R in KrasG12V-driven tumours triggers an immediate response with hallmarks of replicative stress resulting in apoptosis. Yet, a fraction of tumour cells survive, but enter a transient cystostatic state incompatible with tumour development that is fully reversible upon discontinuation of EGFRL858R expression. Ultimately, continuous co-expression of both mutants results in the attenuation of the overall oncogenic signalling to levels compatible with cell proliferation and tumour growth. In sum, our results indicate that the mutual exclusivity of KRAS and EGFR activating mutations occurs as a combination of cellular toxicity and signal adjustment that results in the lack of selective advantage for those cells expressing both oncogenes.

Whereas the wild type H-Ras and K-Ras proteins are bioequivalent, their oncogenic isoforms H-RasV12 and K-RasV12 induce different tumour spectra

We have provided genetic evidence demonstrating that the H-Ras and K-Ras proteins are fully bioequivalent in mice. Previous studies have shown that replacement of the K-Ras alleles by H-Ras coding sequences resulted in viable mice (Potenza et al., EMBO Rep, 2005). Yet, these mice displayed cardiovascular defects. Now, we have shown that these defects were due to the presence of the H-Ras expressing alleles in these mice. Ablation of the 2 endogenous H-Ras alleles, hence generating mice that only express the H-Ras protein from the 2 targeted K-Ras alleles, is absolutely normal.

These results appear to be at variance with the well-established observation that H-RAS and K-RAS oncogenes are involved in different human tumour types. To determine whether the oncogenic versions of the H-Ras and K-Ras proteins are also bioequivalent, we knocked-in H-RasV12 oncogene sequences into the K-Ras locus. Germline expression of H-RasV12 or K-RasV12 from the K-Ras locus resulted in equal embryonic lethality. However, their expression in adult mice led to different tumour phenotypes. Whereas H-RasV12 elicited papillomas and haematopoietic tumours, K-RasV12 induced lung tumours and gastric lesions. The reason why H-RasV12 expression failed to cause lung tumours is due to the induction of a senescence-like state due to excessive MAP kinase signalling. Likewise, H-RasV12 but not K-RasV12 induced oncogene-induced senescence in mouse embryonic fibroblasts (MEFs). Label-free quantitative mass spectrometry revealed that minor differences in H-RasV12 expression levels led to drastically different biological outputs, suggesting that subtle differences in MAP kinase signalling influence the differential tumour spectra induced by RAS oncogenes.
We study the mechanisms by which tumour cells are immortal and normal cells are mortal. Immortality is one of the most universal characteristics of cancer cells. The enzyme telomerase is present in more than 95% of all types of human cancers and absent in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes, essential for chromosome protection and genomic stability. Progressive shortening of telomeres associated with organism ageing leads to ageing. When telomeres are altered adult stem cells have a maimed regenerative capacity.

Our research aims are:

- Generating mouse models to validate telomeres and telomerase as therapeutic targets for cancer and age-related diseases.
- Deciphering the interplay between telomeres and DNA repair pathways.
- Studying the role and regulation of non-coding telomeric RNAs or TERRA.
- Testing telomerase gene therapy in ‘telomere syndromes’ and age-related diseases.
- Elucidating the role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to iPS cells.

“We have demonstrated that TERRA long non-coding RNAs are essential for telomere protection.”
Fighting aplastic anaemia using a therapy designed to delay ageing

Aplastic anaemia is a rare, potentially fatal disease of the blood, by which the bone marrow is unable to generate blood cells at the appropriate pace. The disease can be hereditary or acquired and develops at any stage of life. A subgroup of the inherited form is caused by replicative impairment of haematopoietic stem and progenitor cells owing to very short telomeres due to mutations in telomerase and other telomere components. An abnormal telomere shortening is also described in cases of acquired aplastic anaemia. We have tested the efficacy of our telomerase gene therapy, originally designed to delay ageing, in two independent mouse models of aplastic anaemia due to short telomeres. We found that a high dose targets the bone marrow compartment, including haematopoietic stem cells. Telomerase treatment following telomere attrition in bone marrow cells rescues aplastic anaemia and mouse survival. Improved survival is associated with a significant increase in telomere length in peripheral blood and bone marrow cells, as well as improved blood counts. Our telomerase gene therapy represents a novel therapeutic strategy to treat aplastic anaemia provoked or associated with short telomeres.

Mice with hyper-long telomeres and unaltered genes

Telomere length is genetically determined, but in the past we were able to generate mouse embryonic stem (ES) cells with telomeres twice the size of normal ones. We have now described such ES cells with ‘hyper-long’ telomeres, traceable thanks to the co-expression of green fluorescent protein (GFP), to generate chimeric mice containing cells with both hyper-long and normal telomeres. We showed that chimeric mice contain GFP-positive cells – bearing hyper-long telomeres – in all mouse tissues (FIGURE 1), display normal tissue histology, as well as normal survival. Both hyper-long and normal telomeres are transcriptionally active, but the latter are significantly longer and fewer cells with short telomeres and less DNA damage with age, and express lower levels of p53. Cells with hyper-long telomeres function with normal cell cycle progression and generate chimaeric mice containing cells with both hyper-long and normal telomeres. Both hyper-long and normal telomeres tissues (FIGURE 1), display normal tissue histology, as well as normal survival.

Telomerase RNAs are essential to maintain telomeres

Despite their especially compact structure, which is difficult to access, telomerase transcribes information like the rest of the DNA generating long non-coding RNAs known as TERRA. Deciphering the role of TERRA was one of the unsolved issues of telomere biology in the past decade. This was, in part, due to a lack of genetic studies. We had already shown that mouse TERRA arise from two loci, the 20q locus is the main origin of human TERRA. Thus, both in mice and in humans, TERRA arise from one, or at most two loci (FIGURE 2). Ablation of 20q-TERRA in human cells results in a dramatic loss of telomere sequences and in the induction of a massive DNA damage response. These latter findings represent the first demonstration, in any organism, of the essential role of TERRA in the maintenance of telomeres.

RESEARCH HIGHLIGHTS

Figure 1. Representative image of an eye of chimeric mice. Cells bearing hyper-long telomeres are visualised in red. Telomeres appear in green.

- **PUBLICATIONS**

- **PATENTS**

- **AWARDS AND RECOGNITIONS**
  - Miguel Catalan Cancer Achievement Award, Regional Government of Madrid. Scientific Committee Member, Innovative Medicines Initiative 2 (IMI2) Joint Undertaking, Brussels, Belgium.
  - Member of the External Scientific Board of Instituto de Investigación Sanitaria y Biomédica de Aragón (ISABIAL), Zaragoza, Spain.
  - Member, Alumni Advisory Board, Universidad Autónoma de Madrid.
  - Founding Editor, Cell Stem Cell.
  - Editorial Board Member, Nutrition & Healthy Aging.
CELL DIVISION AND CANCER GROUP

Marcos Malumbres
Group Leader

Staff Scientists
Mónica Alvarez, Guillermo de Cárcer, Ignacio Pérez de Castro (until February)

Post-Doctoral Fellows
Begoña Hurtado, Carolina Maestre, María Salazar

Graduate Students
Ana F. Batalha-Martins, María Maroto, Diego Martínez, Belén Sanz, María Sanz

Technicians
Aicha El Bakkali (since March), María Guirola (since March) (PEJ), Elisabet Zapatero (TS) (TS)**

*Plan de Empleo Joven (Youth Employment Plan)
**Titulado Superior (Advanced Degree)

OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated. During the past few years, we have used different mouse models to understand the relevance of cell cycle regulators, including cell cycle kinases and phosphatases, as well as proteins involved in ubiquitin–dependent degradation, in the control of cell division and tissue physiology. Our interests are: i) to understand the basic control mechanisms that regulate the cell division cycle; ii) to characterise the physiological and therapeutic consequences of cell cycle deregulation; iii) understanding the function of microRNAs in cell biology and tumour development; and iv) to understand how progenitor cells and cancer stem cells control their self-renewal and proliferative properties. As a final goal, we aim to generate information that may be useful for improving therapeutic strategies against cancer cell proliferation.

“In 2016, we investigated the relevance of several mitotic regulators during cancer progression and therapy, with special focus on kinases that are currently under preclinical and clinical evaluation.”
RESEARCH HIGHLIGHTS

Controlling the proper number of cell divisions

The mammalian cell cycle is regulated by at least 2 families of inhibitors, the INK4 and Cip/Kip proteins. While elimination of individual members of these families is a frequent finding in human cancer, the consequences of eliminating this inhibitory mechanism in mammalian cells have not yet been explored. Using a combination of mutant alleles in the mouse, we have now observed that a major physiological function of cell cycle inhibitors is to prevent replicative stress. In a mouse model insensitive to INK4 proteins and deficient in p21<sup>WAF1</sup> and p27<sup>KIP1</sup>, we observed that these inhibitors prevent the accumulation of DNA damage due to replicative stress in different tissues including the nervous system. Ablation of these inhibitors prevents mouse development. This effect is most likely due to hyperactivation of cyclin-dependent kinases as the replicative stress can be prevented by slightly inhibiting the enzymatic activity of these proteins (Quereda et al., 2016).

Cell cycle kinases as new targets for cancer therapy

Cell cycle progression is controlled by phosphorylation events and cell cycle kinases are currently the focus of multiple therapeutic strategies. Inhibitors of the Aurora and Polo-like kinases are evaluated in clinical trials with promising results, at least in haematopoietic malignancies. Over the last few years, we have generated mouse models with specific mutations in these kinases in order to understand their roles in different tissues and cell types. Our recent data have uncovered an unexpected function of Polo-like kinase 1 (Plk1) in the cardiovascular system, a role that we are studying in detail in order to understand possible toxicities derived from the use of Plk1 inhibitors in patients.

A relatively new serine/threonine kinase, known as MASTL (or Greatwall in flies and Xenopus), has been characterised as a critical node in cell division. We have previously shown that MASTL is essential for mouse embryonic development and cell cycle progression (Figure). This is due to mitotic collapse after nuclear envelope breakdown (NEB). MASTL is exported from the nucleus to the cytoplasm in a CRM1-dependent manner before NEB. Once the cytoplasm, Greatwall inhibits the PP2A-B55 complexes to maintain the mitotic state. These findings have therapeutic implications since MASTL acts by blocking the function of the PP2A phosphatase, a tumour suppressor frequently altered in human cancer. This implies that the inhibition of MASTL could, at the same time, slow down cell division and reactivate tumour suppressor PP2A, a protein capable of inhibiting many of the oncogenic molecular pathways involved in cancer development.

Over the past few months, we have tested this hypothesis by studying the relevance of MASTL in tumour cell proliferation and its possible use as a cancer target. In collaboration with Miguel Quintela’s Group at the CNIO and Carlos Caldas at Cancer Research UK, we analysed MASTL expression in breast cancer. Our data suggest that this protein is overexpressed in a significant number of breast tumours with poor outcome and correlates with poor prognosis. In collaboration with researchers at Pfizer, we used different RNAi and CRISPR techniques to analyse the effect of MASTL knockdown or knock out in breast cancer cells both in vitro and in vivo. These data indicate that some breast cancer cells require MASTL kinase activity for proliferation, suggesting that a subset of breast tumours may benefit from strategies aimed at inhibiting this kinase. We are currently studying the consequences of inhibiting MASTL in the activity of the PP2A phosphatase. Since MASTL specifically inhibits PP2A-B55 complexes, we are also characterising the relevance of the B55 family members present in the human genome.

Figure  Schematic representation of the control of the mammalian cell cycle by cyclin-dependent kinases (Cdkks) and MASTL. MASTL phosphorylates 2 small proteins, Ensa and Arpp19, which are both PP2A-B55 inhibitors. The proper balance between cell cycle kinases and phosphatases is crucial, not only for cell cycle progression but also for the structure and function of different tissues.

- PUBLICATIONS
OVERVIEW

Our laboratory has centred its research on trying to understand how cells respond to ‘replicative stress’ (RS), which is frequent in cancer and induced by several anticancer agents. In mammals, RS is suppressed by a signalling cascade initiated by ATR and CHK1 kinases. Throughout the years, our laboratory has developed a wide battery of cellular and animal tools for the study of RS. These tools include mice with enhanced or limited ATR-CHK1 function, cell lines in which the pathway can be activated at will, and chemical inhibitors of the ATR kinase. Our studies have revealed the impact of RS on cancer and ageing, have led to drugs that can be used to test our ideas on cancer therapy, and have also unveiled the mechanisms by which these drugs kill cancer cells. Altogether, our main aim is to understand how genome maintenance is safeguarded – particularly during replication – and to exploit this knowledge as a way to fight against cancer.

“During 2016, we have investigated which tumour types could best benefit from a treatment with ATR inhibitors, the potential mechanisms of resistance to these drugs, as well as new pathways that suppress RS.”
Two new players that suppress replication stress in mammalian cells

Besides ATR, several other factors participate in limiting the impact of RS in mammalian cells. Recent works have identified that POLD3, a subunit of the DNA polymerase Polδ, participates in the repair of the breaks generated by RS, and also suggest that limiting its activity could be specifically deleterious for cancer cells. By developing a novel conditional knockout mouse strain we found that POLD3 deletion is lethal during embryonic development and also when depleted in adult mice. These severe defects were explained by a complete destabilisation of the POLG complex in the absence of POLD3, which abrogates DNA replication, raising serious doubts regarding the potential impact of RS in mammalian cells. Recent works have identified besides ATR, several other factors participating in limiting the impact of RS in mammalian cells.

Efficacy of ATR inhibition in two preclinical models of cancer

Reproductive stress (RS) is a widespread phenomenon in cancer cells that, when persistent, leads to DNA double-strand breaks and genomic instability. Besides from the basal level of RS that occurs in every cell division, the presence of oncogenes, or many of the agents used in chemotherapeutic agents, are potent inducers of RS. In mammals, RS is sensed and suppressed through a signalling cascade that is initiated with the activation of the ATR kinase. In mammals, RS is sensed and suppressed through a signalling cascade that is initiated with the activation of the ATR kinase. We previously hypothesised that due to the high levels of RS in cancer cells, they could be particularly dependent on a proficient RS response. In this regard, and in collaboration with the Experimental Therapeutics Programme, we have developed chemical inhibitors of ATR that presented some anti-tumour properties in vitro. During 2016, our work in this area focused on the identification of tumours that are particularly sensitive to ATR inhibition, as well as on the discovery of mechanisms of resistance to these chemicals. For the first area of focus, we have shown efficacy of ATR inhibitors, as monotherapy, in 2 mouse models of Ewing Sarcoma and Acute Myeloid Leukaemia (FIGURE 1). Regarding the second area of focus, we discovered – via genomewide CRISPR-Cas9 screening – that the levels of CDC23A, a key phosphate controlling mutotic entry, are a key determinant of the sensitivity to ATR inhibitors in mouse and human cells.

**PUBLICATIONS**

Our research focuses on a protein complex named cohesin that is essential for chromosome organisation. Cohesin mediates sister chromatid cohesion and, thereby, ensures faithful DNA repair by homologous recombination and proper chromosome segregation during cell division. It also plays a major role in the spatial organisation of the genome by promoting long-range DNA looping, which in turn contributes to transcriptional regulation, organisation of DNA replication factories and locus rearrangement by recombination. Mutations in cohesin have recently been found in several tumour types, most prominently in bladder cancer and acute myeloid leukaemia. Mutations in cohesin and its regulatory factors are also at the origin of a group of human syndromes collectively known as cohesinopathies.

Our goal is to understand how cohesin works, how it is regulated and how its dysfunction contributes to cancer and other human diseases. In particular, we are intrigued by the existence of different versions of the cohesin complex in somatic cells. We use mouse models carrying knockout alleles of genes encoding cohesin subunits to investigate their functional specificity, both at the cellular level and in the context of an organism. We also take advantage of the Xenopus egg cell-free system to explore additional aspects of cohesin regulation.

“We aim to define the specific contributions of cohesin-SA1 and cohesin-SA2 to genome organisation. Our work may uncover vulnerabilities in cancer cells carrying mutations in the gene encoding SA2, which is one of the twelve genes most mutated in cancer.”
Cohesin-S2A regulates transcription independently of CTCF

Cohesin consists of four core subunits, SMC1, SMC3, RAD21 and SA. There are two versions of the SA subunit in vertebrate somatic cells, SA1 and SA2. We have previously reported that cohesin-SA1 is required for telomere cohesion, while cohesin-SA2 plays a major role in centromeric cohesion. In terms of transcriptional regulation, much less is known about the potential differences between these two complexes. A study in HeLa cells reported a similar genomic distribution for cohesin-SA1 and cohesin-SA2 that largely overlapped with the distribution of the architectural protein CTCF. Our initial comparison of cohesin distribution and transcriptomes of wild type and SA1-null mouse cells suggested that cohesin-SA1 could be more important for the regulation of transcription than cohesin-SA2. First, we detected an almost double number of cohesin-binding sites genome wide in SA1 null cells, and than cohesin-SA2. First, we detected an almost double number of cohesin-binding sites genome wide in SA1 null cells, and second, we found that in a number of genes whose expression was altered in SA1 null cells, cohesin-SA2 could not replace cohesin-SA1 efficiently since cohesin-S1 occupancy was clearly reduced in these cells (Remeseiro, et al., 2012). When we analysed chromatin states during S phase by promoting cohesin acetylation and Sororin knock out (KO) alleles for these two genes. We showed that cells lacking Pds5A, Pds5B, or both, have distinct alterations in their transcriptomes when compared to wild type cells. Genome wide distribution of cohesin is not obviously altered in the absence of either Pds5 protein, but does change in the absence of both. Under this condition, the dynamic association of cohesin to chromatin, measured in Fluorescence Recovery After Photobleaching (FRAP) experiments, is significantly decreased. Much milder effects are observed in cells lacking only Pds5A or Pds5B. Aberrant accumulation of cohesin in axial structures, known as vermicelli, previously described in Wapl depleted cells, can be observed only in the absence of the two Pds5 proteins (FIGURE 2). Thus, Pds5 proteins are required for proper cohesin dynamics and cohesin distribution, although no clear specificities can be found for Pds5A and Pds5B, at least globally. The gene expression differences described above might therefore be caused by preferential interactions with chromatin regulators at specific loci. It is also possible that Pds5 proteins have functions independent of cohesin. We are currently exploring both these possibilities.

Pds5 proteins regulate cohesin dynamics

Two factors are associated with chromatin-bound cohesin, Pds5 and Wapl. Wapl promotes cohesin unloading and in its absence there is an excess of cohesin on chromatin, and chromosome organisation is altered both in interphase and mitosis. The role of Pds5 is less clear. Moreover, there are two versions of Pds5 present in vertebrate cells: Pds5A and Pds5B. In order to explore their specific functions we previously generated murine SA1 and SA2. There are two versions of the SA subunit in vertebrate somatic cells, SA1 and SA2. We have previously reported that cohesin-SA1 is required for telomere cohesion, while cohesin-SA2 plays a major role in centromeric cohesion. In terms of transcriptional regulation, much less is known about the potential differences between these two complexes. A study in HeLa cells reported a similar genomic distribution for cohesin-SA1 and cohesin-SA2 that largely overlapped with the distribution of the architectural protein CTCF. Our initial comparison of cohesin distribution and transcriptomes of wild type and SA1-null mouse cells suggested that cohesin-SA1 could be more important for the regulation of transcription than cohesin-SA2. First, we detected an almost double number of cohesin-binding sites genome wide in SA1 null cells, and than cohesin-SA2. First, we detected an almost double number of cohesin-binding sites genome wide in SA1 null cells, and second, we found that in a number of genes whose expression was altered in SA1 null cells, cohesin-SA2 could not replace cohesin-SA1 efficiently since cohesin-S1 occupancy was clearly reduced in these cells (Remeseiro, et al., 2012). When we analysed chromatin states during S phase by promoting cohesin acetylation and Sororin knock out (KO) alleles for these two genes. We showed that cells lacking Pds5A, Pds5B, or both, have distinct alterations in their transcriptomes when compared to wild type cells. Genome wide distribution of cohesin is not obviously altered in the absence of either Pds5 protein, but does change in the absence of both. Under this condition, the dynamic association of cohesin to chromatin, measured in Fluorescence Recovery After Photobleaching (FRAP) experiments, is significantly decreased. Much milder effects are observed in cells lacking only Pds5A or Pds5B. Aberrant accumulation of cohesin in axial structures, known as vermicelli, previously described in Wapl depleted cells, can be observed only in the absence of the two Pds5 proteins (FIGURE 2). Thus, Pds5 proteins are required
OVERVIEW

Our laboratory studies the molecular mechanisms that underlie genomic duplication in mammalian cells. The ‘replisome’ complex in charge of DNA replication encounters natural obstacles (e.g. unusual DNA structures, collisions with transcription proteins), as well as exogenous challenges such as ionising radiation, UV light and chemicals that modify the DNA structure and block DNA polymerases. The situations in which replication forks are forced to slow down, stall or collapse are generically referred to as replicative stress (RS). Our Group investigates the ‘DNA damage tolerance’ pathways that facilitate DNA replication in the presence of RS or damaged DNA. In recent years, we have identified 2 mechanisms that counteract RS: (1) the conditional activation of dormant replication origins; (2) the participation of PrimPol, a primase-polymerase enzyme, in the restart of stalled forks. We continue to characterise the different cellular responses to RS (FIGURE 1).

“We have developed genetic tools to investigate the physiological impact of defective DNA replication, including mouse strains that suffer from a high incidence of haematological cancers due to their inefficient response to DNA damage during replication.”
RESEARCH HIGHLIGHTS

Cellular functions of PrimPol protein

We have continued to characterise PrimPol, a DNA primase-polymerase that participates in DNA damage tolerance during chromosomal replication. With this aim in mind, we have generated human and mouse cells in which PrimPol expression is either downregulated or completely ablated. PrimPol-deficient cells display a marked sensitivity to UV irradiation, including the accumulation of unrepairable Thy dimers (CPDs and 6-4pp) in the DNA. The skin of PrimPol KO mice also presents an inefficient healing response to UV irradiation and a higher frequency of benign papillomas. The importance of PrimPol as a tumour suppressor gene is currently being investigated.

Effects of DNA re-replication in vivo

CDC6 and CDT1 proteins are responsible for the loading of MCM2-7, the DNA helicase, at replication origins. After CDC6 and CDT1 execute their ‘origin licensing’ functions in the G1 phase, their activities are inhibited until mitosis is complete in order to prevent origin reactivation and DNA over-replication within the same cell cycle. However, these strict control mechanisms may be partially overridden in some cancer types, notably non-small cell lung carcinomas, by the overexpression of Cdt1 or Cdc6 genes.

We have recapitulated the deregulated expression of Cdt1 and Cdt2 using mouse strains that allow the inducible expression of both proteins, alone or in combination. While individual deregulation of CDC6 or CDT1 has only mild effects, their combination is lethal for developing embryos and also for adult individuals. Using single-molecule analyses of DNA replication, high-throughput confocal microscopy and histopathology, we have identified origin re-firing events that are sufficient to cause DNA over-replication and DNA damage in different tissues. These mouse models will allow a complete study of the physiological impact of DNA re-replication in vivo.

Evidence for replicative stress in early embryonic cell cycles

Replicative stress is normally studied in the context of cells undergoing external challenges. However, it also occurs in the unperturbed S phase when the replication machinery reaches special DNA structures (e.g. G-quadruplexes) that are difficult to replicate, or when it collides with a transcriptional fork. In 2016, we participated in a collaborative study, led by Dr M. Lopes (University of Zurich, Switzerland), that identified unexpected levels of physiological RS. Mouse embryonic stem cells and early embryos by the blastocyst stage display a constitutive accumulation of RPA-covered ssDNA, fork slowing and fork remodelling events, all hallmarks of RS. These characteristics are related to the short duration of the G1 phase in embryonic stem cells and are lost upon the onset of cell differentiation. This result underscores the importance of the G1 phase to fully repair DNA that had been damaged in the previous cell cycle, before entering a new round of replication (Ahuja et al., 2016).

Single-molecule analyses of DNA replication

As replicative stress impinges on many cellular processes, the possibility of analysing DNA replication at the single-molecule level continues to attract the interest of many research groups at the CNIO and other institutions. In 2016, we collaborated in two projects led by Oscar Fernández-Capetillo (CNIO Genomic Instability Group) to demonstrate that USP7 ubiquitin protease targets SUMO and is essential for DNA replication (Lecona et al., 2016), and that PolD3 is haploinsufficient for DNA replication in mice (Murga et al., 2016). In the latter project, the analyses of replication in stretched DNA fibres revealed a striking accumulation of asymmetric forks in the absence of POLD3, a regulatory subunit of DNA polymerase ε (Figure 1). Finally, a collaboration with B. Freire (Hospital Universitario de Canarias) revealed a novel function for USP7 ubiquitin protease in the control of DNA replication (Hernández-Pérez et al., 2016).

PUBLICATIONS


Figure 1. Pathways that facilitate fork progression through damaged DNA. The yellow star represents a polymerase-blocking lesion. The main proteins involved in each pathway are mentioned: (A) Repriming downstream of the lesion, (B) Fork reversal and restart. (C) Translesion synthesis DNA polymerases. (D) Lesion skipping by template-switch. Adapted from Muñoz and Méndez (2016).

Figure 2. Detection of fork asymmetry in stretched DNA fibres. Top: representative images of replicating DNA molecules labelled with CBU (red) and IdU (green). Each image shows 2 forks moving away from a central origin. Bottom: quantification of fork asymmetry in DNA fibres prepared from B-cells from PolD3-competent (left) or PolD3-deficient (right) mice. Adapted from Murga et al (2016).
Melanomas are inherently aggressive cancers for which basic and translational research have significantly improved patient prognosis. Nevertheless, clinical responses are still incomplete. The long-term goals of our Group are to identify new progression biomarkers and therapeutic agents. Focusing on stress response programmes involving apoptosis, autophagy and endosome mobilisation, we have discovered lineage-specific oncogenes that define the melanoma “fingerprint”. Transcriptomic and proteomic analyses of the melanoma secretome have enabled us to define how tumour cells remodel the (lymph)angiogenic vasculature and avoid immune recognition. Moreover, we have generated a unique set of animal models for non-invasive imaging of melanoma progression in vivo. These systems have led to the validation of nanoparticle-based treatments that are currently being tested in clinical trials. Our ultimate objective is to improve the management of patients with otherwise refractory metastatic melanomas.

“We have identified oncogenic cascades that are uniquely deregulated in melanoma and as such, may represent novel targets for therapeutic intervention.”
CPEB4 and CUGBP1 in the regulation of mRNA stability, with unexpected targets involving master specifiers of the melanocyte lineage (Figure 2). We have also assisted F. Gehweiler’s laboratory (the Centre for Genomic Regulation, Barcelona) with the identification of pro-metastatic roles of the translation controller UNR in melanoma (Wirth et al., Cancer Cell 2016). Similarly, histopathological studies with our long-term collaborators, P. Ortiz-Romero and J.L. Rodríguez-Peralto (Hospital 12 de Octubre, Madrid), have validated the chromatin remodeler and RNA binding factor DEK as a risk factor for melanoma metastasis (Rivero-Falkenbach, Pigment Cell Melanoma Res, 2016).

We have also made great progress regarding one of the most pressing needs in the field of melanoma, namely, the mechanisms underlying immune suppression (reviewed in Cerezo-Wallis and Soengas, Curr Pharm Design. 2016). This was achieved by combining the analysis of human melanoma biopsies with a new class of ‘lymphoporter’ mouse models that we generated in collaboration with Sagarri Ortiz’s Transgenic Mice Unit at the CNIO. Moreover, we have expanded the use of dsRNA nanoparticles as immunomodulatory agents. This information will be used to support clinical trials that are currently being performed by Biostecnics Therapeutics, a biotechnology company cofounded by M. Soengas.  

**RESEARCH HIGHLIGHTS**

- **Aim 1:** Diagnosis

- **Aim 2:** Tumor Progression

- **Aim 3:** Imaging and Treatment

- **Figure 1** Main objectives of the CNIO Melanoma Group: objectives and model systems.

- **Figure 2** New melanoma drivers: the RNA binding protein CPEB4. (A) Upregulation of CPEB4 in malignant melanomas visualized by comparative histological analyses in benign nevi and malignant tumour specimens. CPEB4 is stained in pink. (B) Schematic representation of newly identified roles of CPEB4 in the control of lineage-specific tumour drivers (MITF and RASQ2). These functions link, for the first time, RNA binding proteins to mechanisms underlying tumour type identity.

**Lineage-specific oncogenic dependencies in melanoma**

One of the long-term objectives of the Melanoma Group is the discovery of new melanoma drivers. We previously identified a cluster of endosomatical-associates genes that distinguish melanoma from over 30 additional malignancies (Alonso-Curbelo et al., Cancer Cell 2014 and Oncotarget 2015). In collaboration with the group of P. Agostinis (University of Leuven, Belgium), we further explored therapeutically-relevant regulatory mechanisms and functions of the endosomysial architecture in different cell types (Mars et al., FEBS J, 2016). More recently, we have also discovered unique features of autophagy and its lysosomal-associated process in melanoma. Employing human melanoma biopsies, combined with newly-generated mouse models, we identified selective heterogeneous losses of ATG5 as a new risk factor for melanoma progression and as a main mediator of the resistance to targeted therapy (García-Fernández et al., Autophagy, 2016).

**RNA binding proteins and RNA-based anticancer agents in the control of melanoma cell proliferation and metastasis**

Malignomas are long-known for being associated with a plethora of changes in mRNA gene expression profiles. Still, the specific contribution of RNA binding proteins (RBPs), particularly, spliceosomes modulators, remains virtually unexplored in this disease. We have identified tumour-selective roles of RBPs in melanomas defined by selective allelic loss of ATG5 (Autophagy, 2012, 176–179). Pérez-Guajardo et al., Cancer Cell 2016). We previously showed that the protein CPEB4 and CUGBP1 in the regulation of mRNA stability, with unexpected targets involving master specifiers of the melanocyte lineage (Figure 2). We have also assisted F. Gehweiler’s laboratory (the Centre for Genomic Regulation, Barcelona) with the identification of pro-metastatic roles of the translation controller UNR in melanoma (Wirth et al., Cancer Cell 2016). Similarly, histopathological studies with our long-term collaborators, P. Ortiz-Romero and J.L. Rodríguez-Peralto (Hospital 12 de Octubre, Madrid), have validated the chromatin remodeler and RNA binding factor DEK as a risk factor for melanoma metastasis (Rivero-Falkenbach, Pigment Cell Melanoma Res, 2016).

- **PUBLICATIONS**


**AWARDS AND RECOGNITION**

Outstanding Research Award, the Society for Melanoma Research.

The elected Board Member of AIOM’s (Asso- ciazione Italiana di Oncologia Sperimentale) 2020.

**ANNUAL REPORT 2016**

**Aim 2:** Tumor Progression

- **Figure 1** Main objectives of the CNIO Melanoma Group: identifying new progression biomarkers and validating more efficient anticancer agents. Indicated are the main experimental systems and representative publications.

**ANNUAL REPORT 2016**

**Aim 2:** Tumor Progression

- **Figure 1** Main objectives of the CNIO Melanoma Group: aiming at identifying new progression biomarkers and validating more efficient anticancer agents, indicated are the main experimental systems and representative publications.

**ANNUAL REPORT 2016**
Microenvironment & Metastasis Junior Group

Héctor Peinado
Junior Group Leader
Staff Scientist
Susana García

Post-Doctoral Fellows
Marta Herguera, Claudia Savini (since November)

Graduate Students
Ana J. Alonso, Teresa González (since June); Lucia Robado

Overview

Our laboratory is focused on understanding metastatic progression. During this process, tumour cells communicate actively with the tumour microenvironment. Among all factors involved in metastasis, our laboratory is specifically interested in defining the role of secreted exosomes during pre-metastatic niche formation. Exosomes are actively involved in cell-to-cell communication during both physiological and pathological processes. Our data support that tumour-secreted exosomes are involved in: 1) pre-metastatic niche formation and metastatic progenitors depending on the integrin expression profile on their surface; and 2) stromal cell reprogramming by horizontal invasion of: 1) pre-metastatic niche formation and metastatic processes. Our data support that tumour-secreted exosomes are involved in the crosstalk between the adipose tissue, platelets and tumour cells during the metastatic process (FIGURE, A). We are dissecting the systemic effects of tumour-derived exosomes in adipose tissue as well as the involvement of platelets, determining their role in metastasis. Ultimately, we aim to determine specific signatures in circulating exosomes and platelets of cancer patients in order to define new prognostic and therapeutic markers that can be applied in the clinical setting.

Role of tumour-derived exosomes in lymph node metastasis

Melanoma-secreted exosomes have been shown to home to specific niches in lymph nodes. We are studying how tumour-secreted exosomes promote cellular and molecular alterations in the lymph node microenvironment, fostering metastasis (FIGURE, A). The goal of the current project is to determine the mechanisms through which tumour-secreted exosomes promote lymph node and distal metastasis. Our studies in melanoma patients will be the first ones evaluating the use of circulating vesicles in lymphatic fluid as biomarkers to predict relapse and metastatic potential.

Linking obesity with metastatic risk

Obesity has been associated with the increased risk of developing metastasis in certain cancers. Although the implication of obesity in cancer is clear, there is, to date, a lack of studies analysing the impact of obesity on metastasis. We are investigating the mechanisms involved in the crosstalk between the adipose tissue, platelet and tumour cells during the metastatic process (FIGURE, B). We are dissecting the systemic effects of tumour-derived exosomes in adipose tissue as well as the involvement of platelets, determining their role in metastasis. Ultimately, we aim to determine specific signatures in circulating exosomes and platelets of cancer patients in order to define new prognostic and therapeutic markers that can be applied in the clinical setting.

Novel pathways involved in neurofibromatosis progression

Although neurofibromatosis is a genetic disorder, in this project we aim to develop a very innovative concept, which focuses on unveiling unknown pathways involved in exosome secretion during neurofibromatosis progression. We are investigating the molecular signature of exosomes secreted from highly metastatic neurofibromatosis models. Our data support that tumour-secreted exosomes carry a specific signature that can be detected in the circulation. This approach will result in the development of new diagnostic tests and therapies to block neurofibromatosis progression.

Publications


Awards and Recognition

1. FERO Grant for Translational Research in Oncology (II BECA FERO 2016), FERO Foundation for Oncology Research, Spain.
2. Fundación Pfizer Research award 2016, Spain.
The Brain Metastasis Group is seeking to identify novel ways to target both cancer cells and the associated microenvironment in order to reduce metastatic burden in the brain.”

**RESEARCH HIGHLIGHTS**

- **Using a novel medium-throughput drug discovery platform, the laboratory identified two compounds with the potential to target established brain metastasis from experimental lung and breast cancer models.**

- **We identified two novel mediators of brain metastasis that are enabling us to explore the influence of epigenetics on brain colonisation as well as the ability of cancer cells to interact with neurotransmitters.**

- **We are evaluating the therapeutic potential of targeting specific components of the microenvironment that are only present surrounding metastatic lesions in the brain.** Our research suggests that the viability of brain metastasis is highly dependent on altered components of the microenvironment, thus highlighting potential vulnerabilities.

**OVERVIEW**

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is poor given that available therapies (i.e. surgery and radiation) have limited benefits for patients and the disease inevitably progresses. The rise in the number of patients with brain metastasis is partially due to the increasing number of systemic therapies that work extracranially but not in the brain. In this scenario, cancer cells present at this highly demanding secondary site have additional time to evolve and develop into clinically detectable lesions. In the laboratory, we study why and how cells from different cancer types (breast cancer, lung cancer and melanoma) are able to access the brain, survive and colonise this vital organ. We dissect the biology of these processes in vivo using experimental models in order to challenge the current status of this unmet clinical need.

**PUBLICATIONS AT OTHER INSTITUTIONS**


**AWARDS AND RECOGNITION**

- IV “Profesor Durántez” - Fundación LAIR Award 2016, Spain.
Mammals, including humans, have evolved in an environment where the ability to efficiently use limiting nutrient sources has been a key survival adaptation that has shaped all our responses to nutrients. Unprecedented nutrient overabundance is in conflict with our cellular and organismal responses, which are best tuned to operate under scarcity. These aberrant responses not only lie at the core of the pathogenesis of the metabolic alterations observed in diabetes, but are also key in cancer and the process of ageing. We use genetically engineered strains of mice as a physiological framework to understand the molecular bridges from elevated nutrient levels to human disease. In particular, we have genetically modified the RagA and RagC GTPases, key players in the sensing of nutrients that activate a master regulator of metabolism, a kinase called mTOR.

Mice with gain-of-function mutations in RagA − therefore unable to sense a drop in nutrient levels − have an increased glycaemia in spite of a normal food intake and decreased adiposity. Furthermore, these mice show intolerance to glucose, which means that when glucose is administered it remains in the circulation, and peripheral organs (such as liver and skeletal muscle) are unable to uptake it. These perturbations are tightly associated with the development of type 2 diabetes. Indeed, when we examined the ability of peripheral tissues to respond to insulin we observed an impaired response to insulin, also known as insulin resistance, which leads to increased levels of glucose in circulation. We are currently characterising other metabolic imbalances observed in these mice and are performing a genetic dissection of these alterations by deregulating nutrient sensing in an organ-specific manner.

“By means of novel strains of mice with deregulated nutrient sensing we identified metabolic alterations that drive uncontrolled proliferation of B lymphocytes and nutrient imbalances associated with diabetes.”

By Alejo Efeyan, Junior Group Leader

2016 Publications