CANCER CELL BIOLOGY PROGRAMME

ERWIN F. WAGNER Programme Director

The overall strategic goals of the Cancer Cell Biology Programme are to achieve a better understanding of the events leading to cancer development, progression and metastasis, and to discover molecular mechanisms that could provide a basis for novel therapies. The 4 Groups investigate how tumours grow as 'external organs' in close interaction with tumour - associated cells. The spectrum of investigations ranges from epithelial cancers such as liver, pancreas, skin and intestine, to bone and brain tumours. The research covers aspects of tumour cell biology, ranging from tumour stem cells, tumour cell interactions with host cells/environment such as tumour-associated macrophages and fibroblasts, to the role of inflammation, metabolism and metastasis. Powerful state-of-the-art mouse genetic models, human cellular systems, high-throughput genomic/proteomic and biochemical tools, as well as patient-derived materials, are employed. These aspects are successfully covered by the complementary research areas of 3 Senior and 1 Junior Groups.

The Senior Group, led by Francisco X. Real, studies epithelial tumours focusing mainly on pancreatic and bladder cancer. The Group employs an integrative approach to understand the molecular patho-physiology of these tumours and applies this knowledge in the clinical setting. Real's Group, with contributions from the Wagner lab, made an important discovery demonstrating an inflammatory transcriptional switch in pancreatic cancers involving the nuclear receptor NR5A2 and Jun/AP. Nabil Djouder's Group aims to dissect the contribution of various environmental stressors, including the nutrient and growth factor signalling pathways, to cancer development and associated diseases, in particular related to the gastro-intestinal tract. Massimo Squatrito's Group, which is partly supported by the Seve Ballesteros Foundation, studies how brain tumours, mainly glioblastomas, develop and how they respond to therapy. Finally, my own Group focuses on understanding the role of the transcription factor complex AP-1 (Fos/Jun) in physiological and pathological processes, with a strong focus on aspects of inflammation and cancer in liver, lung, skin and bone. We also investigate the role of AP-1 in inflammatory skin diseases, such as psoriasis, and aim to molecularly define the causes leading to lung fibrosis. We have continued to study how the whole organism responds to a locally growing tumour in the context of a complex immune-metabolic impairment in cancer-associated-cachexia.

"Our main goal is to keep CNIO globally competitive and to ensure that CNIO remains an international institution. Members of 13 different nationalities from 4 continents are represented in our Programme with the goal to perform top-level cancer cell biology as well as to train students and postdocs to become the next-generation of promising scientists."

GENES, DEVELOPMENT AND DISEASE GROUP

Erwin F. Wagner
Group Leader

Staff Scientists
Latifa Bakiri, Nuria Gago, María Jiménez, Liliana Mellor

Post-Doctoral Fellows
Kazuhiko Matsuoka, Álvaro Ucero

Graduate Student
Lucía T. Díez (until November)

Visiting Graduate Student
Pia Benedikt (*Karl-Franzens Universität GRAZ*, Austria, April-July)

Undergraduate Student
Jennifer Cascino (Fulbright Fellow, USA, until June)

Technicians
Vanessa Bermeo (until August), Ana Guío (TS)*

'Titulado Superior (Advanced Degree)

OVERVIEW

Our studies aim to analyse gene function in healthy and pathological conditions, e.g. in tumour development, using the mouse as a model organism, but also employing patient-derived samples. Specifically, the functions of the AP-1 (Fos/Jun) transcription factor complex regulating cell proliferation, differentiation and oncogenesis, as well as the cross-talk between organs, are being investigated. The goal is to define molecular pathways leading to disease/cancer development and to identify novel therapeutic targets (FIGURE). We focus on:

- → Elucidating a causal link between inflammation, cancer and AP-1 (Fos/Jun) expression, using cell type-specific, switchable genetically engineered mouse models (GEMMs).
- → Developing and characterising new GEMMs for cancer and human diseases, such as bone loss, arthritis, fibrosis and psoriasis, and applying these to preclinical studies.
- → Using multiple approaches to compare mouse models of disease to human disease and to identify therapeutically relevant targets.

"Our goal was for the CNIO to remain an international and competitive institution. At present, 3 out of 4 Group Leaders in our department are foreigners, one of whom is partly funded by the Seve Ballesteros Foundation. Thirteen different nationalities from 4 continents are testimony to an international science culture, all focussing on unravelling the mysteries of inflammation, metabolism and cancer."

RESEARCH HIGHLIGHTS

We have developed a powerful technology for switchable, reversible and tissue-specific ectopic gene expression of specific AP-1 monomers/dimers in the liver, lung, skin and bone. We use mouse and human tissue samples for large-scale studies, such as deep sequencing (RNA-Seq, ChIP-Seq) and mass spectrometry analyses. We evaluate possible biomarkers and therapeutic approaches in small-scale preclinical studies based on these screens.

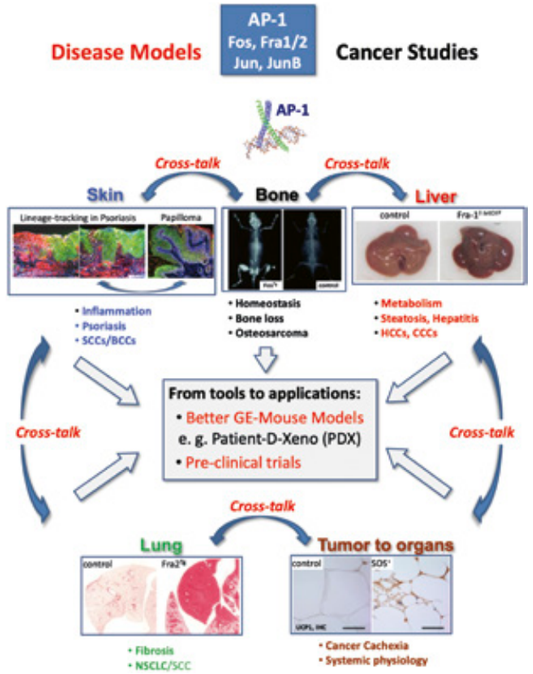

Figure Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse models to human disease and to identify novel targets. Furthermore, we are investigating the systemic response of the mouse organism to a growing tumour in cancer cachexia. Preclinical studies are performed using different genetically engineered mouse models (GEMMs) with compounds that target the identified molecules to determine the potential of translating our findings for the treatment of human disease.

Bone development, osteosarcomas and arthritis

We are studying the function of AP-1 proteins in bone development and disease using loss-(LOF) and gain-of-function mouse models. In mice, transgenic c-Fos expression leads to osteosarcomas (OSs). Using an inducible bone-specific Wntless LOF GEMM, we found that loss of Wnt signalling delays Fos-induced OS development. Our data also demonstrate that increased Wnt7b and Wnt9a and non-canonical Wnt signalling are causally involved in OS.

Rheumatoid, Psoriatic and Osteoarthritis (OA) are destructive joint pathologies linked to chronic inflammation. Using cell type-specific and inducible AP-1 LOF mouse models, combined with experimental arthritis models, we found that c-Fos is a key regulator of surgery- and age-induced OA.

Using mice with inducible epidermal deletion of JunB and cJun (DKO*) that develop skin inflammation and a psoriatic-arthritis-like (PsA) disease, we aim to elucidate potential therapeutic targets to alleviate skin and joint inflammation. We previously identified the S100A8/A9 complex as highly elevated in our GEMM as well as in human psoriatic skin samples. We have now generated new DKO*-GEMM with epidermal and global deletion of S100A9 to determine the specific role of keratinocyte-derived and neutrophil-derived S100A9 in skin or joint inflammation.

Liver disease—metabolism, fibrosis, inflammation and cancer

AP-1 proteins are important modulators of hepatic lipid metabolism as specific AP-1 dimers can either activate or repress PPARγ transcription. Therefore, fatty liver disease and obesity most likely depend on AP-1 dimer composition. In addition, ectopic expression of specific Fos or Fra-2, but not Fra-1-containing AP-1 dimers in hepatocytes, leads to liver dysplasia, inflammation, fibrosis and tumours with HCC signatures. Mechanistically, molecular analyses point to the involvement of pathways connected to human hepatocellular carcinoma (HCC), such as the Wnt/ß-catenin and Myc pathways and/or to altered cholesterol and bile acids metabolism. A robust connection between c-Fos expression and the activity of the LXR/RXR pathway, an important regulator of cholesterol homeostasis, was unravelled and most likely contributes to the oncogenic function of c-Fos in hepatocytes. We are currently testing whether any of the pathways we discovered can be exploited therapeutically to treat liver cancer in preclinical models.

Cancer-associated cachexia (CAC)

CAC is a complex wasting syndrome characterised by loss of muscle and fat along with 'browning', a switch from white to brown fat, as previously described. Our aim is to understand the systemic events taking place in CAC and to identify novel biomarkers and therapeutic targets. Systemic inflammation is a consistent event in CAC with innate immune cells, such as neutrophils, as a major cell type. Interestingly, Lipocalin-2, an adipokine important in innate immunity is highly upregulated in CAC and may be a potential new biomarker. We found that CAC is not prevented in a neutropenic situation suggesting that neutrophils may not be the key factor. Ongoing studies show that the Renin-Angiotensin-Aldosterone System (RAAS) is dysregulated in CAC in humans and mice, potentially leading to cardiac dysfunction. We are now dissecting, in mice and in human CAC samples, the involvement of the central and peripheral nervous system, the RAAS as well as the tissue-specific role of Ucp-1 (in collaboration with R. Señarís, Spain, M. Petruzzelli, UK, H. Watzke, M. Poglitsch, P. Benedikt and R. Zechner, Austria).

Fra-2 in lung fibrosis and cancer

Lung fibrotic diseases and non-small cell lung cancer (NSCLC) lack effective treatments and lead to high mortality. Using GEMMs we found that Fra-2, an AP-1 transcription factor, contributes to both diseases. Fra-2 expression is increased in lung fibrosis patient samples and correlates with poor survival in human NSCLC. In lung fibrosis, Fra-2 is associated with macrophage-specific expression of Type VI collagen in a type2 immune response and mediates disease progression, while in NSCLC, Fra-2 promotes growth in K-Ras-mutated tumours. We aim to find new therapeutic targets and potential disease biomarkers downstream of AP-1. The lung fibrosis studies are conducted in collaboration with Acceleron Pharma (USA), and the cancer studies with Mariano Barbacid's and Luis Paz-Ares' Groups at CNIO and Silvestre Vicent in Pamplona.

Skin inflammation, cancer and human disease

Characterisation of the systemic inflammatory disease in epidermal-deficient JunB GEMMs indicated a skin inflammation to bone cross-talk by Il-17A-mediated inhibition of Wnt signalling in osteoblasts. These mice also suffer from dysbiosis and chronic *S. aureus* colonisation, which is exacerbated in the absence of adaptive immunity. We have also generated several GEMMs to define the role of the antimicrobial proteins (AMPs), such as S100A8/A9 and Lipocalin-2, in inflammatory skin diseases with a focus on the systemic effects beyond the skin.

Using lineage tracing in the psoriasis-like mouse model, we found that mutant epidermal stem cells (ESCs) initiate epidermal hyperplasia and skin inflammation by priming neighbouring non-mutant epidermal cells to acquire a psoriasis-like phenotype. Mechanistically, TSLP neutralisation reduces non-mutant keratinocytes proliferation and VEGFα expression, an important pro-inflammatory mediator in psoriasis. These findings unravel specific roles of epidermal populations in psoriasis-like disease and provide novel mechanistic insights into epidermal cell interactions under inflammatory conditions.

It has been suggested that psoriatic patients have decreased skin cancer risk. Using our psoriasis-like mouse model and the well-established DMBA/TPA chemical carcinogenesis protocol, we observed that psoriasis-like mice with severe phenotype have a significant decrease in DMBA/TPA-induced skin papillomas compared to controls. Detailed characterisation suggests that in the context of chronic skin inflammation, elevated expression of senescence markers may modulate papilloma formation. ■

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EPITHELIAL CARCINOGENESIS GROUP

Francisco X. Real
Group Leader

Post-Doctoral Fellows
Enrique Carrillo (until March), Irene Felipe, Eleonora Lapi, Miriam Marqués, Sumit Paliwal

Graduate Students
Isidoro Cobo (until December), Ana M. Maldonado, Irene Millán, Catarina Pereira, Mónica Pérez

Technicians
Miguel Deblas, Natalia Del Pozo, Jaime Martínez

Titulado Superior (Advanced Degree)

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial carcinoma (UC), with a disease-oriented approach. We use patient samples, cultured cells, and genetically modified mice, giving a similar weight to the 3 model systems. Observations made at either of these levels are then extended through additional work. To translate the findings, we bring this knowledge to a 'population' level leveraging on information and samples from large patient cohorts.

"We have shown that, in the pancreas, the control of cell differentiation and the suppression of inflammation depend on similar transcriptional regulators indicating that both processes are tightly linked."

In PDAC, a main hypothesis is that cell differentiation is a potent tumour suppressor mechanism acting early in carcinogenesis. We use the excellent genetic mouse models available because these processes cannot be readily studied in humans. In mice, PDAC can originate in pancreatic progenitors and in adult acinar and ductal cells. Understanding the contribution of early molecular events is crucial to design better strategies for early tumour detection and prevention in subjects at risk.

In UC, we focus on identifying new genes, using them for improved tumour taxonomy, characterising the mechanisms of action, and applying this knowledge for improved prediction of outcome and therapy.

RESEARCH HIGHLIGHTS

Pancreas cancer molecular pathophysiology

The genetic/genomic changes associated with PDAC have been extensively described over the last few years by the genome consortia, but the contribution of precursor lesions and the molecular changes that precede tumour development are less well established. Our lab has pioneered the notion that incomplete acinar cell differentiation is associated with a scenario of pre-inflammation or inflammation and with predisposition to PDAC development using mutant *KRas*-driven genetic mouse models. These studies provide the basis for the pharmacological – or genetic - manipulation of acinar differentiation as a tumour preventative strategy.

NR5A2 is an orphan nuclear receptor for which putative endogenous ligands as well as pharmacological agonists have recently been identified. In mice, *Nr5a2* germline heterozygosity is associated with a pre-inflammatory state that sensitises the mice to the oncogenic effects of mutant *KRas*. Deletion of one *Nr5a2* allele is sufficient to cause a striking genomic redistribution of the protein in cooperation with AP-1 components. To further explore how this occurs, we have analysed the NR5A2 interactome using immunoprecipitation and mass-spectrometry. We find that reduction of NR5A2 protein levels by 50% (either genetically or during pancreatitis) is also associated with profound effects on the interactome, highlighting the relevance of subtle changes in protein dosage in cells; one of the proteins identified is the ubiquitous transcription factor NFIC (FIGURE 1A,B). At the transcriptomic level, *Nfic*$^{-/-}$ pancreata display a mild defect in acinar cell maturation as well as a significant down-regulation of the protein synthesis machinery. NFIC is a novel regulator of acinar differentiation playing an important role in the endoplasmic reticulum stress response. Similar to knockouts of other genes coding for proteins involved in acinar homeostasis, constitutive *Nfic*-null mice developed significantly more PanINs in a mutant KRAS context. The function of NFIC in acinar cells appears to be highly conserved between mice and humans (FIGURE 1C).

Urothelial carcinoma (UC) genetics, biology, and clinical translation

We are interested in refining our understanding of new genes involved in UC, using organoids to unravel their function, and to apply this knowledge in the clinical setting.

Through exome sequencing we identified mutations in *STAG2*, coding for a cohesin subunit, and in *RBM10*, coding for a splicing regulator, as new UC genes that are more broadly involved in human cancer. We have generated conditional mouse models for these two genes and are exploring their role in development and urothelial biology as well as their cooperation with other bladder cancer genes.

RBM10 somatic mutations occur in several epithelial tumour types, including UC. Germline *RBM10* mutations are associated with TARP syndrome. Our preliminary studies indicate that *Rbm10*-null mice recapitulate facets of this developmental condition. We have generated *Rbm10*-null normal urothelial organoids and are characterising their biological features. In addition, we collaborate with J. Paramio (*CIEMAT*, Madrid) to identify how tumour cells bypass growth requirements in organoid cultures. Also, through single-cell RNA-Seq, we are identifying urothelial cell populations that could shed light on the cell of origin of UC.

In collaboration with J. Valcárcel (*CRG*, Barcelona), we are analysing the mechanisms through which *RBM10* contributes to UC development using a combination of cellular, molecular and bioinformatics approaches. In addition, we are assessing whether RBM10-mutant tumours display specific therapeutic vulnerabilities using a variety of experimental strategies.

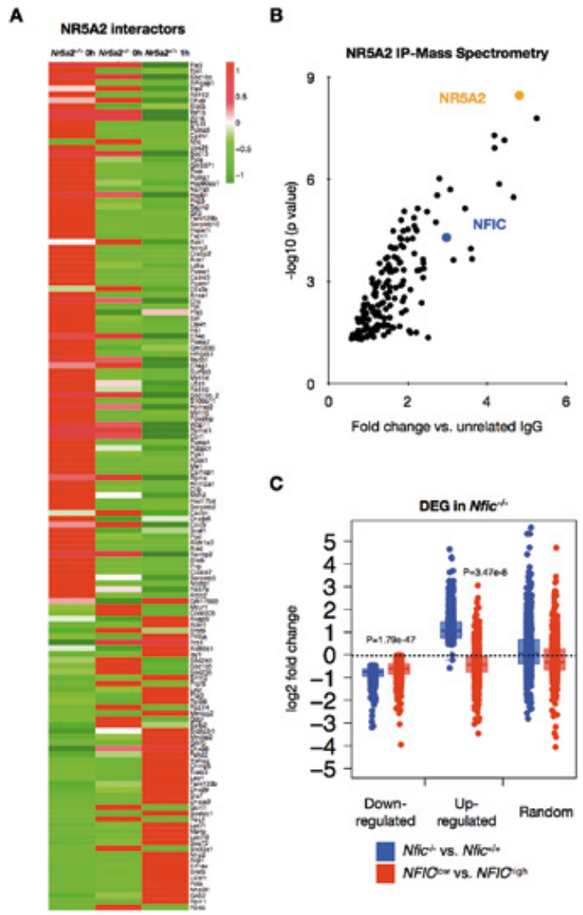

Figure 1 The NR5A2 interactome is dynamic and reveals novel players involved in acinar homeostasis. (**A**) NR5A2 interactors identified by immunoprecipitation with anti-NR5A2 antibodies and mass spectrometry using pancreatic tissue from wild type mice in basal conditions (*Nr5a2*$^{+/+}$ 0h), or 1 h after administration of one dose of caerulein (*Nr5a2*$^{+/+}$ 1h), and from *Nr5a2* heterozygous mice in basal conditions (*Nr5a2*$^{+/-}$ 0h). (**B**) NFIC is a novel NR5A2 interactor identified in all three analysed settings. (**C**) Genesets including differentially expressed genes (DEG) in the pancreas of young *Nfic*$^{-/-}$ vs. wild type mice: down-regulated genes are expressed at significantly lower levels in normal pancreas from subjects with low levels of *NFIC* (comparison of lower vs top decile of *NFIC* mRNA expression) (in collaboration with F. García and J. Muñoz, CNIO Proteomics Unit).

Our studies with patient samples have provided novel markers predictive of response to cisplatin-based chemotherapy and are guiding the design of novel clinical trials with targeted therapies and immune checkpoint inhibitors in collaboration with N. Malats, A. Font, D. Castellano, and an extended group of Spanish uro-oncologists. ■

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AWARDS AND RECOGNITION

- "Constantes y Vitales" Award for the best publication in Biomedicine 2018, *Atresmedia/La Sexta*, Spain.

GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

Nabil Djouder
Group Leader

Post-Doctoral Fellows
Hugo Bernard, Sebastián Thompson (until September)

Graduate Students
Almudena Chaves, Sergio De La Rosa, Amanda Garrido, Tatiana Grazioso, Irene Herrantz (since October), Ana Teijeiro

Technician
Eva Martínez (until February) (PEJ, CAM) *

Plan de Empleo Joven de la Comunidad de Madrid (Youth Employment Plan, Community of Madrid)

OVERVIEW

Our laboratory devotes effort to understand the molecular mechanisms linking environmental stresses to disease pathogenesis. Research in the last decade has focused mainly on understanding the functions and roles of newly discovered mutated genes in the development of cancer and associated disorders. However, the exposure to environmental factors, through the regulation and expression of virulent eukaryotic proteins, has often been an ignored permanent challenge for an organism.

Based on the integration of experimental mouse models, combined with the use of state-of-the art technologies and human data, we aim to provide a comprehensive study for a rational approach towards the development of novel mechanism-based therapies to prevent and treat diseases.

"We aim to understand mechanisms of disease by generating new mouse models that recapitulate pathological features of human syndromes in order to guide early prevention and treatment."

RESEARCH HIGHLIGHTS

Poor diets (under-nutrition, micronutrient deficiencies, over-nutrition, high-fat and low-fibre diets, etc.), alcohol consumption, ionising radiation, bacteria and virus infections, etc., are risk and pathogenic factors for disease development. How these environmental factors can alter the host's eukaryotic epithelial cells to cause various pathologies, potentially progressing to cancer, remains largely unknown. Finding new genes affected by environmental stressors, and understanding their functions and role in disease development, may pave the way for future therapies. In our lab, we therefore focus on the identification and understanding of mechanisms of likely causal links between environmental stresses and pathologies in order to develop new preventive and therapeutic options.

Unconventional prefoldin RPB5 interactor (URI)

The responses of eukaryotic cells to a variety of environmental stresses involve changes in the expression profile of molecular chaperones. These chaperones are essential to engage protective mechanisms to ensure cellular and protein homeostasis caused by injurious environmental stimuli. In our lab, we focus on studying the roles and functions of the unconventional prefoldin RPB5 interactor (URI), a member of the prefoldin chaperone family, whose expression is modulated by various pathogenic environmental factors. Principally, lessons from genetically engineered URI gain- and loss-of-function mouse models taught us that high URI expression may lead to uncontrolled protein substrate regulation, and decreased URI may induce over-functioning of protein clients – both conditions may lead to various pathologies.

Microspherule protein 1 (MCRS1)

We also recently discovered MCRS1 (Microspherule protein 1) with scaffolding activities regulating mTORC1 activity in response to amino acids.

Mechanisms of gastrointestinal tract disease

Our interest is therefore driven by the discovery of URI and MCRS1 proteins, both regulated by environmental stressors, which may compromise their functions and activate pleiotropic circuits supporting non-oncogene addiction functions and dependence, provoking severe outcomes. Using URI and MCRS1 mouse models generated in our lab, combined with cutting-edge technologies, we are studying mechanisms of disease predominantly associated to the gastrointestinal tract, often related to pathogenic environmental factors (ionising radiation, bacteria, viruses and poor diet), with the objective of developing new strategies for treatment. Our research is mainly focussed on the study of intestinal, gastric, pancreatic and liver disorders (FIGURE).

In this regard, we made the following discoveries:

- → Inflammatory cues up-regulate hepatic URI, which inhibits *de novo* NAD^+ synthesis causing DNA damage and thereby initiating hepatocellular carcinoma (HCC). Replenishing the pools of NAD^+ by using nicotinamide riboside prevents HCC. Our data suggest that metabolic alterations initiate tumorigenesis prior to genomic instability.
- → Nutrient overload increases hepatic URI, which results in NAD^+ deficit-induced DNA damage that activates metabolic inflammation-associated IL-17A to cause non-alcoholic steatohepatitis (NASH) and HCC. Boosting NAD^+ by using nicotinamide riboside or blocking IL-17A axis prevents NASH and HCC.
- → Hepatocellular carcinoma originates from transformed hepatocytes, whereas hepatic progenitor cells give rise to benign lesions including regenerative nodules and adenomas.
- → Cells exposed to prolonged inadequate glucose concentrations elicit first a protective and adaptive response to optimise glucose utilisation and suppress death, in order to give to the cells an opportunity to recover from metabolic stress. OGT regulation by URI is a sophisticated mechanism conferring c-MYC-dependent survival functions in response to glucose fluctuations.
- → MCRS1 has oncogenic and tumour suppressive activities by regulating mTORC1. Inhibition of mTORC1 via MCRS1 deletion in the intestine protects from APC loss-dependent tumorigenesis, whereas it promotes colitis-induced colorectal cancer (CRC). Our work reveals mTORC1 oncogenic and tumour-suppressive roles in intestinal epithelium and avenues to optimised and personalised therapeutic regimens for CRC.

We intend to make significant progress over the next few years in order to elucidate mechanisms of disease associated to the digestive system. This will be made possible thanks to the specific environment at the CNIO providing state-of-the-art facilities and access to key technological platforms with advanced technologies, as well as the availability of various genetically engineered mouse models generated in our lab, patient-derived xenograft models, organoids, cell biological and biochemical techniques, and the large number of omics and human data. ■

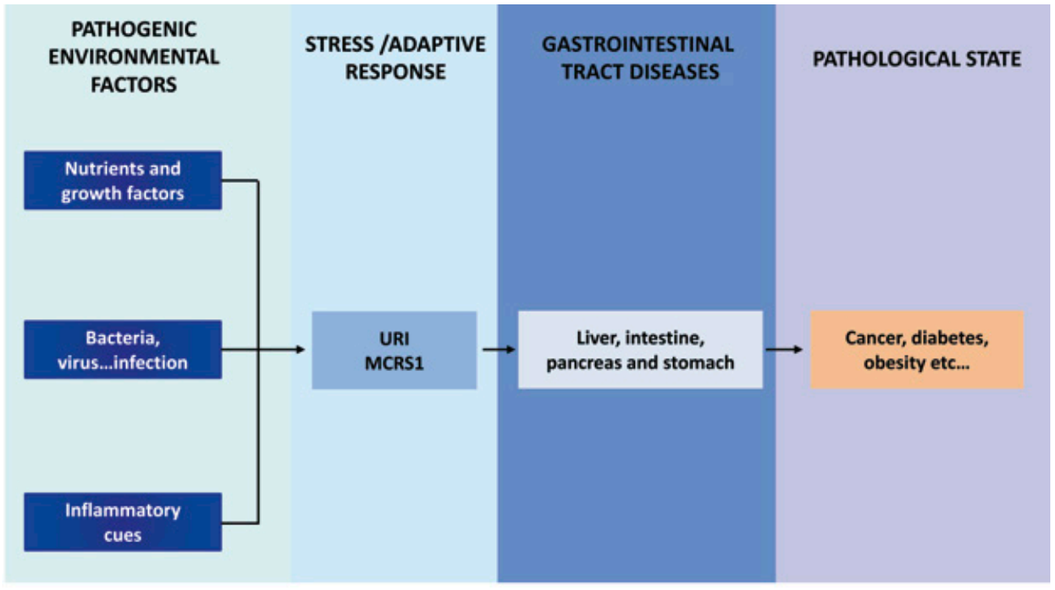

Figure Representation of our research directive. Our lab is mainly interested in understanding how pathogenic environmental factors lead to disease associated to the gastrointestinal tract. Molecular chaperones are essential to engage protective mechanisms to ensure cellular and protein homeostasis caused by injurious environmental stimuli. Environmental stress modulates thus URI and MCRS1 expressions to maintain cellular homeostasis or cause disease development.

PUBLICATIONS

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Book Chapters

- Djouder N (2018). Prefoldins: the new chaperones. Djouder, N (ed.); Advances in Experimental Medicine and Biology, vol. 1106. Springer Nature. ISBN 978-3-030-00736-2; ISBN 978-3-030-00737-9 (eBook).
- Chaves-Pérez A, Thompson S, Djouder N (2018). Roles and Functions of the Unconventional Prefoldin URI, vol. 1106:95-108. In: Advances in Experimental Medicine and Biology. Springer Nature. ISBN 978-3-030-00736-2; ISBN 978-3-030-00737-9 (eBook).

AWARDS AND RECOGNITION

- Member of the European Society for Clinical Nutrition and Metabolism (ESPEN).

SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito
Junior Group Leader

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OVERVIEW

Glioblastoma (GBM) is the most common and lethal primary central nervous system tumour in adults. Despite the recent advances in treatment modalities, GBM patients generally respond poorly to all therapeutic approaches and prognosis remains dismal. Radiation and chemo-resistance are characteristic of various cancer types, however it is not clear if this therapy resistance is a consequence of tumour progression or if it is intrinsically associated with the genetic events that lead to tumour formation in the first place. Gaining insights into the pathways that determine this poor treatment response will be instrumental for the development of new therapeutic modalities.

In our laboratory, we use a variety of approaches – both genetic and small molecule drug screenings – coupled with *in vivo* GBM mouse models in order to identify genes involved in therapy resistance of gliomas. We reason that these studies will help to define new therapeutic targets for the treatment of brain tumours.

"The current most effective treatment for GBM patients is a combination of radiotherapy and alkylating agents. Increasing the sensitivity of the tumour cells to these therapies will possibly extend the survival of the patients."

Post-Doctoral Fellow
Miguel Jiménez (since June)

Graduate Students
Carolina Almeida, Alvaro Curiel (until July), Veronica Matía

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'Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

Novel therapeutic approaches for therapy-resistant malignant brain tumours

The standard therapies for GBM patients, IR and Temozolomide (TMZ), generate double-strand DNA breaks (DSBs), which are the most deleterious form of DNA damage. The DSBs are then responsible for the initiation of the DNA Damage Response (DDR) and consequently the activation of DNA repair pathways and cell-cycle checkpoints. We have previously presented evidence that alterations in key DNA repair and checkpoint proteins can modulate the GBM treatment response.

The DDR signalling is a very intricate pathway and many of its elements can be altered in a given tumour patient, offering both challenges and opportunities from a treatment perspective. Loss of components of a specific DNA repair pathway might be balanced by the increased activity of other components or pathways. Upregulated DNA repair pathways could lead to resistance to radiotherapy and DNA-damaging chemotherapy, therefore inhibitors of these pathways could potentially increase the sensitivity of the cells to these therapies. By contrast, pathways that are lost represent weaknesses in the DNA repair ability of the tumour cell and they could be exploited by choosing a suitable chemotherapy to induce unrepairable (more toxic) DNA damage. It is estimated that the efficacy of radiotherapy and chemotherapy would be improved if tumour cells could be rendered more sensitive without altering the sensitivity of normal tissues.

Through different functional genetic studies, we have observed that defects in components of the Mismatch Repair (MMR) system are significantly associated with resistance to TMZ. Moreover, we have discovered that chromosomal rearrangements of the O-6-methylguanine-DNA methyltransferase (MGMT) lead to overexpression of MGMT and contribute to TMZ resistance, both in high-grade and low-grade gliomas. Most importantly, we have identified another alkylating agent that is able to overcome these resistance mechanisms and that has a synergistic effect when used in combination with TMZ (FIGURE). ■

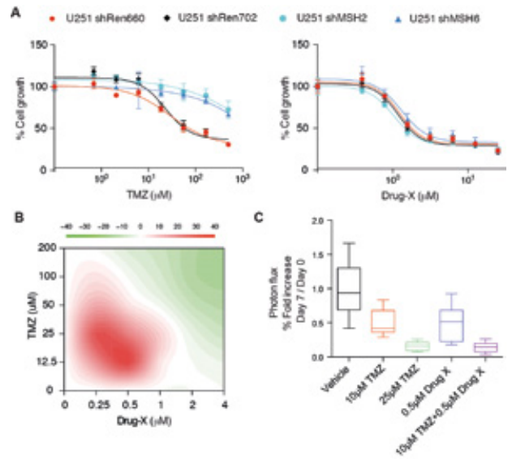

Figure Overcoming TMZ resistance. (**A**) Silencing of MMR component (MSH2 and MSH6) leads to resistance to TMZ (*left*) but not to the novel alkylating drug (*right*). (**B-C**) TMZ and Drug-X have synergistic effects both *in vitro* (**B**) and on brain-tumour slices cultured *ex-vivo* (**C**).

PUBLICATIONS

- Zhao N, Sebastiano V, Moshkina N, Mena N, Hultquist J, Jimenez-Morales D, Ma Y, Rialdi A, Albrecht R, Fenouil R, Sánchez-Aparicio MT, Ayllon J, Ravisankar S, Haddad B, Ho JSY, Low D, Jin J, Yurchenko V, Prinjha RK, Tarakhovsky A, Squatrito M, Pinto D, Allette K, Byun M, Smith ML, Sebra R, Guccione E, Tumpey T, Krogan N, Greenbaum B, van Bakel H, García-Sastre A, Marazzi I (2018). Influenza virus infection causes global RNAPII termination defects. *Nat Struct Mol Biol* 25, 885-893.
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