

GENES, DEVELOPMENT AND DISEASE GROUP

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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 that lead 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, 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 is for CNIO to remain an international and competitive institution. At present, 4 out of 5 Group Leaders in our department are foreigners, one of whom is partly funded by the Seve Ballesteros Foundation. Fourteen different nationalities from 4 continents are a testament to our international science culture and we all focus 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 pre-clinical studies based on these screenings.

Bone development, osteosarcomas and arthritis

We are studying the function of AP-1 proteins and their targets in bone development and disease using loss-of-function (LOF) and gain-of-function (GOF) mouse models. In mice, c-Fos expression leads to osteosarcomas (OSs) and chondrogenic hyperplasias. We found that loss of Wnt signalling delays OS development in c-Fos GOF mice, pointing to a novel mechanism linking c-Fos/AP-1 and OS development.

Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA) and Osteoarthritis (OA) are destructive joint pathologies linked to chronic inflammatory diseases. We are studying the function of AP-1 factors and their target genes in the development of arthritis using GEMMs, experimental arthritis models and local gene manipulation approaches. Additionally, we are investigating how crosstalk from other organs, like skin and bone, may contribute to the development and progression of different types of arthritis, as well as whether inflammation generated from the joint, using arthritic models, can influence or induce disease development in adjacent and distant organs.

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 PPAR γ transcription. Therefore, fatty liver disease and obesity most likely depend on AP-1 dimer composition. In addition, while Fra proteins protect against steatosis, ectopic expression of Fra-2, but not Fra-1-containing AP-1 dimers in hepatocytes, leads to liver dysplasia in aged mice. Mechanistically, molecular analyses point to the involvement of pathways connected to human hepatocellular carcinoma (HCC), such as the Wnt/ β -catenin and Myc pathways.

Deletion of c-Fos in hepatocytes protects from chemically-induced liver carcinogenesis, whereas additional inactivation in immune cells abrogates this protective effect. Ectopic c-Fos or expression of Fos-dimers leads to altered cholesterol and bile

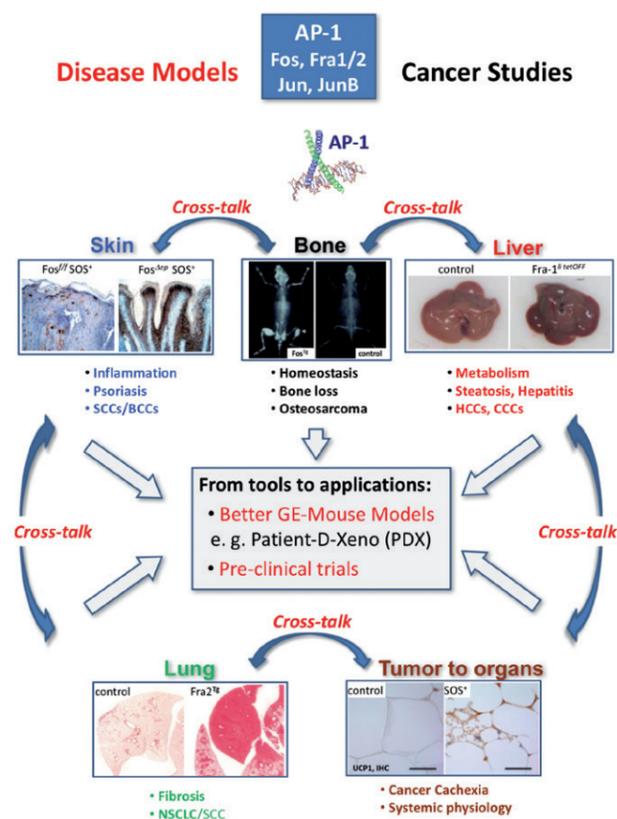


Figure Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse models of disease to human disease as well as 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 with compounds that target the identified molecules in order to determine the potential of translating our findings for the treatment of human disease.

acid metabolism, inflammation, fibrosis, hepatocyte/bile duct proliferation and tumours with human HCC gene signatures. A robust connection between c-Fos expression and the activity of the LXR/RXR pathway, an important regulator of cholesterol homeostasis, was observed and it most likely contributes to the oncogenic function of c-Fos in hepatocytes.

Cancer-associated cachexia (CAC)

We previously demonstrated that ‘browning’, a switch from white to brown fat, is a contributor to the wasting process in CAC, and we also documented the importance of IL-6 and β -adrenergic signalling. Using GEMMs, as well as syngeneic mouse models, we are investigating the role of inflammation in CAC and are also studying the systemic events in CAC, such as the role of the neuro-endocrine system, e.g. the renin-angiotensin-aldosterone system. In collaboration with the Medical University of Vienna and Attoquant Diagnostics (Vienna), we are analysing human serum samples from cancer patients to validate the findings from the GEMMs. Our goal is to understand the molecular switch from a local inflammation-associated tumour to the systemic effects of CAC, and to potentially identify novel biomarkers (in collaboration with Drs R. Senaris, Santiago de Compostella, Spain and M. Petruzzelli, Cambridge, UK).

Defining a function for AP-1 in lung disease

Lung fibrotic diseases and non-small cell lung cancer (NSCLC) share the same target organ as well as similar characteristics such as higher incidence in smokers, high morbidity and lack of effective treatments leading to high mortality. Our studies using GEMMs provide experimental tools for studying the important contribution of Fra proteins to these diseases. While Fra-2 is required for the innate immune response associated with disease progression in experimental lung fibrosis, Fra-2 promotes tumour growth in NSCLC. We are currently testing the therapeutic value of Fra-2 inhibition in our pre-clinical models for fibrosis and NSCLC, and are validating our findings using patients’ tissue samples. These studies are conducted

in collaboration with Daiichi Sankyo Company (Japan) and Mariano Barbacid’s Experimental Oncology Group at CNIO, respectively.

Skin cancer, inflammation and human disease

We have demonstrated that loss of epidermal Fra-2 protein results in skin barrier defects. Mechanistically, Fra-2 binds and transcriptionally regulates epidermal differentiation gene promoters. We are currently investigating the targets of Fra-2 in skin that play a role in barrier function and inflammation.

Characterisation of the epidermal inflammatory disease in mice lacking JunB suggests a skin to bone crosstalk. We have recently reported that IL-17A production in skin causes bone loss by inhibiting Wnt signalling in bone-forming osteoblasts. We have extended our studies and shown that psoriasis patients suffer from bone loss that correlates with IL-17A levels. We are currently evaluating the role of the microbiota in skin inflammation by antibiotic treatments, high-throughput microbiota sequencing and germ-free housing conditions.

High-throughput proteomics and transcriptomic analyses unravelled novel pathways and molecules for targeted therapies, such as S100A8/A9 and complement C3. We have now generated new GEMMs to define the role of these novel target molecules in inflammatory skin disease with a focus on the systemic effects beyond the skin in arthritis and bone loss.

Another angle of research in psoriasis involves the analysis of the role of epidermal stem cells in the disease initiation and progression using state-of-the-art lineage-tracking models. Recent data suggest that a subtype of epidermal stem cells is important for disease progression; we are currently characterising these cells and expanding these studies to patient samples. Finally, we are using GEMMs for Squamous Cell Carcinomas (SCCs), previously generated in the lab, for strategies to prevent skin cancers and to develop novel therapeutic approaches to treat peri-neural invasion with reduced incidence of metastasis. ■

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