

GENES, DEVELOPMENT AND DISEASE GROUP

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**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.

Bone development, osteosarcomas and arthritis

We are studying the function of AP-1 proteins in bone development and disease using loss-of (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 PPAR γ 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

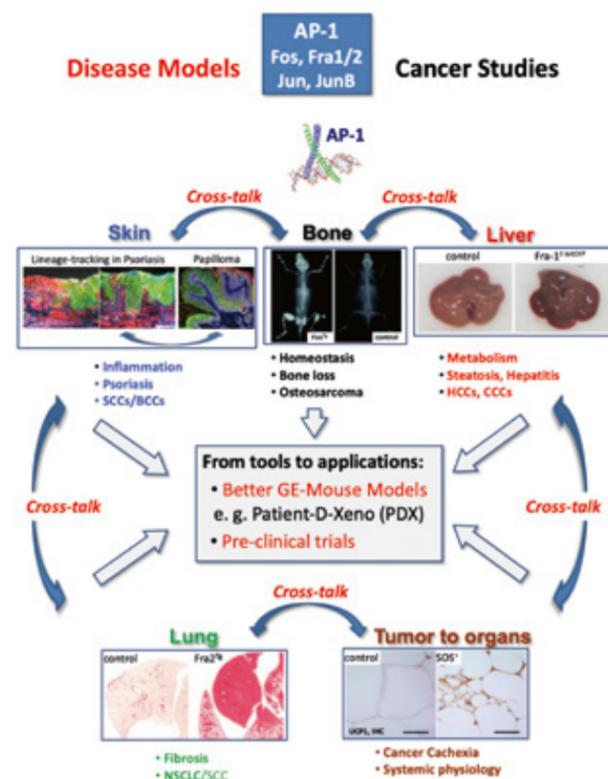


Figure Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse 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.

hepatocellular carcinoma (HCC), such as the Wnt/ β -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. Señaris, 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 VEGF α 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. ■

PUBLICATIONS

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