

GENES, DEVELOPMENT AND DISEASE GROUP

Erwin F. Wagner
Group Leader

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

Post-Doctoral Fellows
Kazuhiko Matsuoka, Álvaro Ucero

Visiting Graduate Student
Jennifer Cascino (Fulbright Fellow,
since September)

Technicians
Vanessa Bermeo, Ana Guío (TS)*

Graduate Student
Lucía T. Díez

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 is that CNIO remains 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. Seventeen different nationalities from 5 continents are a testament to an international science culture, all focussing on unravelling the mysteries of inflammation, metabolism and cell differentiation in 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-function (LOF) and gain-of-function (GOF) mouse models. In mice, transgenic c-Fos expression leads to osteosarcomas (OSs). Using an inducible bone-specific Wntless (Wls) LOF GEMM, we found that loss of Wnt signalling delays Fos-induced OS development. Our data also demonstrate that canonical Wnt signalling is not a determining factor, while increased Wnt7b and Wnt9a suggest the involvement of non-canonical Wnt signalling.

Rheumatoid (RA), Psoriatic (PsA) and Osteoarthritis (OA) are destructive joint pathologies linked to chronic inflammatory diseases. We are studying the function of AP-1 factors in the development of arthritis using GEMMs and experimental arthritis models. Using cell type-specific and inducible AP-1 LOF mouse models, we found that c-Fos and JunB are key regulators of surgery- and age-induced OA with distinct functions. We are also investigating how a cross-talk from skin to bone contributes to the development and progression of different types of arthritis, as well as whether inflammation generated from arthritic joints can influence 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, ectopic expression of Fra-2, but not Fra-1-containing AP-1 dimers in hepatocytes, leads to liver dysplasia. 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 effect. Ectopic c-Fos or expression of

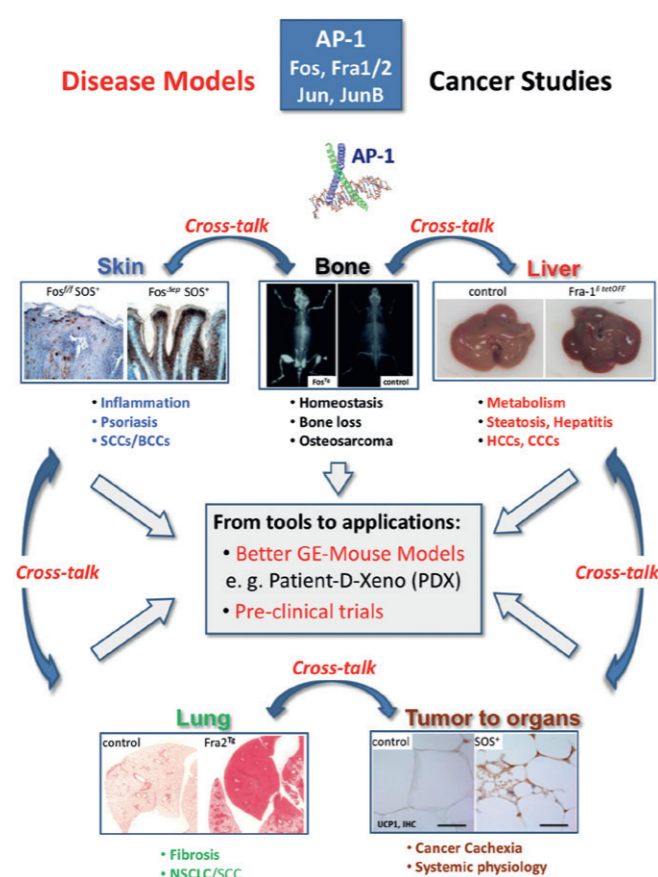


Figure Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse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

Fos-dimers lead to altered cholesterol and bile acids metabolism, inflammation, fibrosis, hepatocyte/bile duct proliferation and tumours with HCC signatures. A robust connection between c-Fos expression and the activity of the LXN/RXR pathway, an important regulator of cholesterol homeostasis, was unravelled and 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, contributes to the wasting process in CAC and also documented the involvement of β -adrenergic signalling and IL-6 in this process. Using GEMMs and syngeneic transplantation models, we are dissecting the switch from a local inflammation-associated tumour to the systemic effects of CAC, with the ultimate aim to identify biomarkers and therapeutic targets. Our recent studies demonstrate a deregulation of the immune system with dramatic changes in lymphoid and myeloid populations. Ongoing studies in mice and in human samples aim to dissect the involvement of the central and peripheral nervous system, the Renin-Angiotensin-Aldosterone system, as well as the tissue-specific role of Ucp-1 during CAC development (in collaboration with Drs. R. Senaris, Spain, M. Petruzzelli, UK, H. Watzke, M. Poglitsch, and R. Zechner, Austria).

Defining a function for AP-1 in lung disease

Lung fibrotic diseases and non-small cell lung cancer (NSCLC) share some characteristics such as higher incidence in smokers, high morbidity and lack of effective treatments leading to high mortality. Using GEMMs we found that Fra/AP-1 proteins contribute to both diseases. While Fra-2 is associated with a fibrosis-specific innate immune response leading to disease progression, Fra-1 and Fra-2 promote tumour growth of K-Ras-induced NSCLC. Furthermore, Fra-2 expression is increased in lung fibrosis patient samples and correlates with poor survival in human NSCLC. Since AP-1 inhibition delayed lung fibrosis progression in preclinical models, our research focusses on deciphering the molecular mechanism and finding biomarkers and therapeutic targets downstream of AP-1. The lung fibrosis studies are conducted in collaboration with Daichi Sankyo Company (Japan) and Genentech (USA), and the cancer studies with Mariano Barbacid’s Experimental Oncology Group and Luis Paz-Ares’ Lung Cancer Clinical Research Unit at the CNIO.

Skin cancer, inflammation and human disease

Characterisation of the epidermal inflammatory disease in mice lacking JunB suggests a skin to bone cross-talk. We reported that IL-17A production in skin causes bone loss by inhibiting

Wnt signalling in bone-forming osteoblasts, and showed that psoriasis patients suffer from bone loss that correlates with IL-17A levels. Epidermal-deficient JunB GEMMs also suffer from dysbiosis and chronic *S. aureus* colonisation, which is exacerbated in the absence of adaptive immunity. We are currently evaluating the role of the microbiota in skin inflammation using antibiotic treatments, high-throughput microbiota sequencing and are investigating the functional contribution of autophagy to controlling skin infections.

Comparative analyses in GEMMs and psoriatic patient samples unravelled novel molecules for targeted therapies, such as the antimicrobial proteins (AMPs) S100A8/A9, Lipocalin-2 and complement C3. We have generated several GEMMs to define the role of AMPs in inflammatory skin disease with a focus on the systemic effects beyond the skin in arthritis and bone loss. Although global deletion of S100A9 in the psoriasis-like mouse model alleviated skin inflammation and psoriatic arthritis, the cellular source of S100A9, with a crucial role in disease development and progression, is still unclear. Thus, we generated a new psoriasis-like GEMM with epidermal deletion of S100A9. Preliminary data show that keratinocyte-expressed S100A9 did not affect skin or joint inflammation, but reduced psoriatic-associated local bone loss. We are evaluating the role of keratinocyte-expressing S100A9 in these events using GEMMs and *in vitro* cultures.

Despite an important contribution in skin homeostasis and cancer, the role of epidermal stem cells (ESCs) in chronic inflammatory skin diseases is unclear. Using a lineage-tracking system in psoriasis-like GEMMs, we observed a differential behaviour of distinct subpopulations of epidermal cells, including keratinocytes and ESCs. RNA-sequencing revealed important changes in metabolism and extracellular matrix proteins in psoriatic-like ESCs. *In vitro* assays and human patient samples are being utilised to further dissect the contribution of these subpopulations to psoriasis.

Finally, using GEMMs for Squamous Cell Carcinomas (SCCs), we aim to identify therapeutic strategies for skin cancer prevention and to treat peri-neural invasion and metastasis. ■

PUBLICATIONS

Bakiri L, Hamacher R, Graña O, Guío-Carrión A, Campos-Olivas R, Martínez L, Dienes HP, Thomsen MK, Hasenfuss SC, Wagner EF (2017). Liver carcinogenesis by FOS-dependent inflammation and

cholesterol dysregulation. *J Exp Med* 214, 1387-1409.
Fan F, Bashari MH, Morelli E, Tonon G, Malvestiti S, Vallet S, Jaharian M, Seckinger A, Hose D, Bakiri L, Sun C, Hu Y, Ball CR, Glimm H, Sattler M, Goldschmidt H, Wagner EF, Tassone P, Jaeger D, Podar

K (2017). The AP-1 transcription factor JunB is essential for multiple myeloma cell proliferation and drug resistance in the bone marrow microenvironment. *Leukemia* 31, 1570-1581.
Tsuji K, Li JT, Tsukui T, Ren X, Wagner EF, Sheppard D (2017). Fra-2 negatively

regulates postnatal alveolar septation by modulating myofibroblast function. *Am J Physiol Lung Cell Mol Physiol* 313, L878-L888.
Uluçkan Ö, Wagner EF (2017). Chronic systemic inflammation originating from epithelial tissues. *FEBS J* 284, 505-516.