The role of p38γ and p38δ in modulating the TME and tumorigenesis

Alberto Bigogno[1], Jose Martìn-Gòmez[1], Ester Dìaz-Mora[1], Daniel Mora-Diego[1], Pilar Fajardo Flores[1], Juan Jose Sanz[1], Ana Cuenda[1]
[1]Department of Immunology and Oncology, Centro Nacional de Biotecnologia (CNB-CSIC), Madrid, Spain

Alternative p38 mitogen-activated protein kinases (p38MAPKs), p38γ and p38δ, are important regulators of inflammation and environmental stress, modulating cell responses to inflammatory cytokines and pathogens of a wide range of disease and cancer type. Our group has shown that p38γ and p38δ have a pro-tumorigenic role in the development of colitis-associated colon cancer (CAC), although their function seems to be dependent on the cellular context. Among the various cell types involved in the cancer development, intestinal fibroblasts (IFs) play a key role in the development of CAC by leading the response to colon mucosal damage and regulating the proliferation of epithelial cells.

p38γ and p38δ deletion in fibroblasts attenuates tumor burden and weight loss in the AOM-DSS mouse model

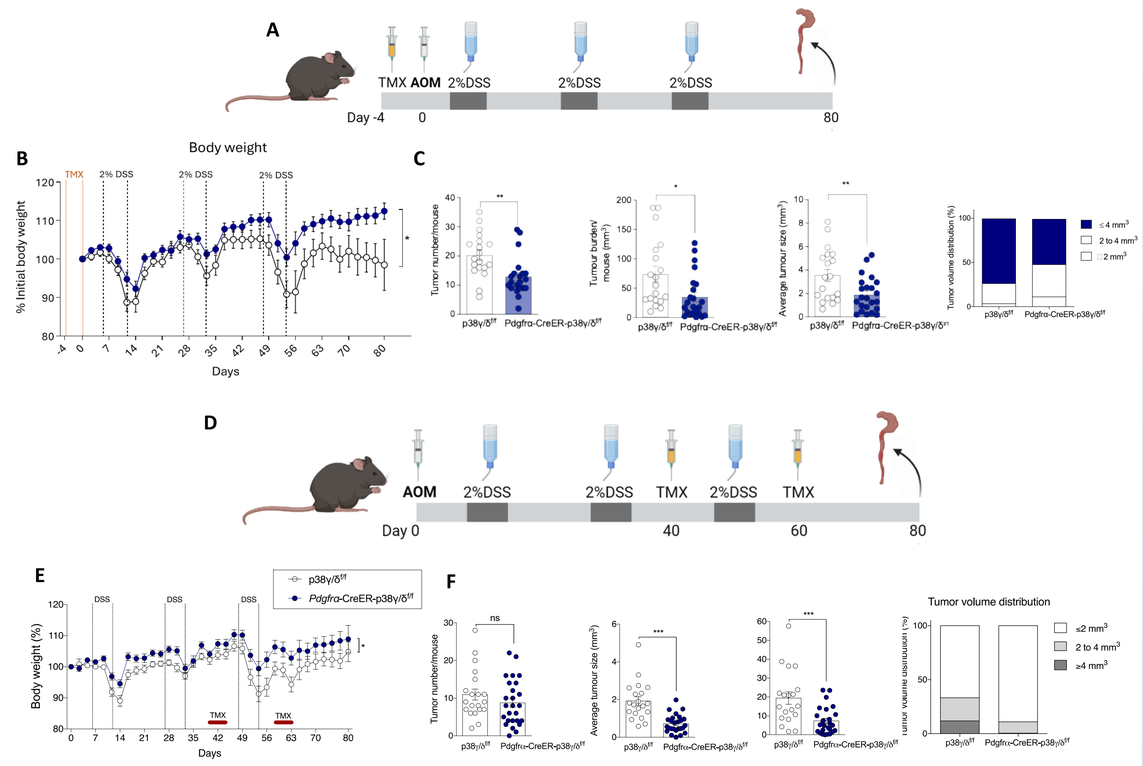

Fig1. (A) Model of AOM/DSS treatment for the induction of colitis-associated colon cancer (CAC) in Pdgfrα-CreER-p38γ/δ$^{f/f}$ v p38γ/δ$^{f/f}$ with TMX before AOM injection. (B) Body weight during the AOM/DSS treatment. (C) Tumor number, average tumor volume, total tumor volume, and tumor volume distribution. (D) Model of AOM/DSS treatment for the induction of colitis-associated colon cancer (CAC) in Pdgfrα-CreER-p38γ/δ$^{f/f}$ v p38γ/δ$^{f/f}$ with TMX injection at day 40 and day 60. (E) Body weight during the AOM/DSS treatment. (F) Tumor number, average tumor volume, total tumor volume, and tumor volume distribution.

Pdgfrα-CreER-p38γ/δ$^{f/f}$ mice have reduced proliferation and increased death in the colon epithelium upon treatment with AOM-DSS

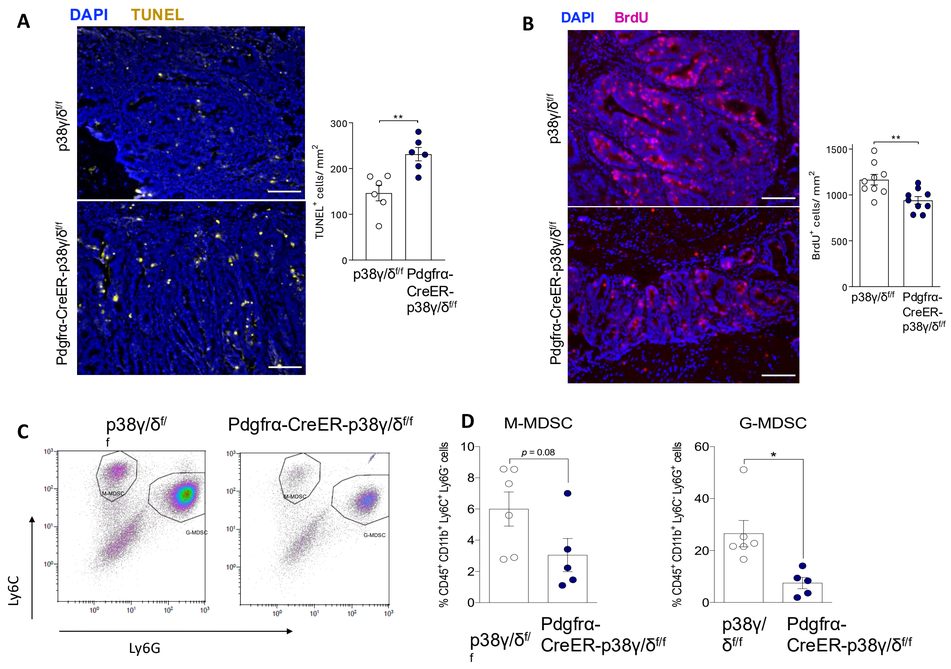

Fig2. (A and B) Colon sections from AOM/DSS- treated mice at day 80 of treatment stained with an anti-BrdU antibody (A) and TUNEL (B) and their respectively quantification. (C) Representative flow cytometry plots of Ly6C vs Ly6G of p38γ/δ$^{f/f}$ and Pdgfrα-CreER-p38γ/δ$^{f/f}$ highliting the populations of M-MDSC and G-MDSC. (D) Quantification of the two population M-MDSC and G-MDSC

p38γ and p38δ in fibroblasts regulate in epithelial proliferation pathways in colitis

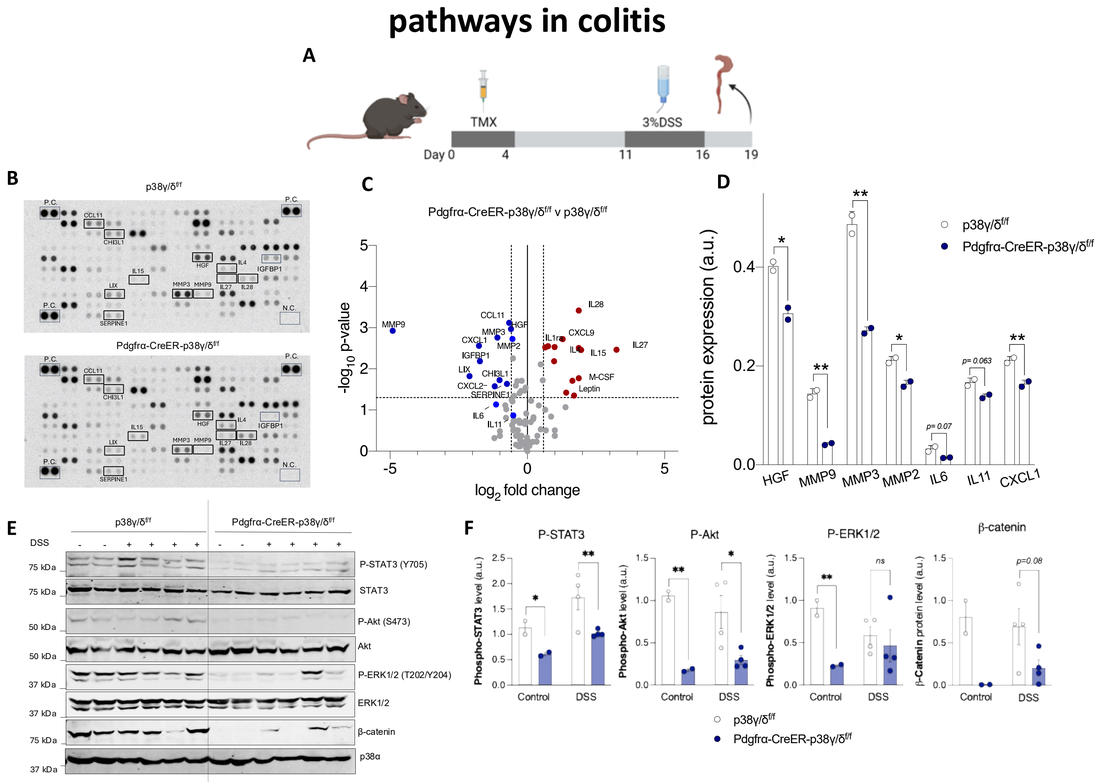

Fig3. (A) Model of DSS treatment to induce colitis in the colon of p38γ/δ$^{f/f}$ and Pdgfrα-CreER-p38γ/δ$^{f/f}$ (B) **Colon** protein lysates from p38γ/δ$^{f/f}$ and Pdgfrα-CreER-p38γ/δ$^{f/f}$ mice at day 80 of the AOM/DSS treatment immunoblotted using a commercial proteome profiler array kit. Proteins with a differential expression have been highlighted. (C) Protein levels quantified and represented in a Volcano plot showing the proteins with a differential expression in each genotype. (D) Protein expression quantified of some higlited proteins. (E) Tumor protein lysates from p38γ/δ$^{f/f}$ and Pdgfrα-CreER-p38γ/δ$^{f/f}$ mice at day 80 of the AOM/DSS treatment immunoblotted using the indicated antibodies. p38α was used as a loading control. (F) Relative expression of P-STAT3, P-Akt, P-ERK1/2, and β-catenin.

p38γ/p38δ modulate cancer cell proliferation and gene expression implicated in matrix remodeling and cytokine production

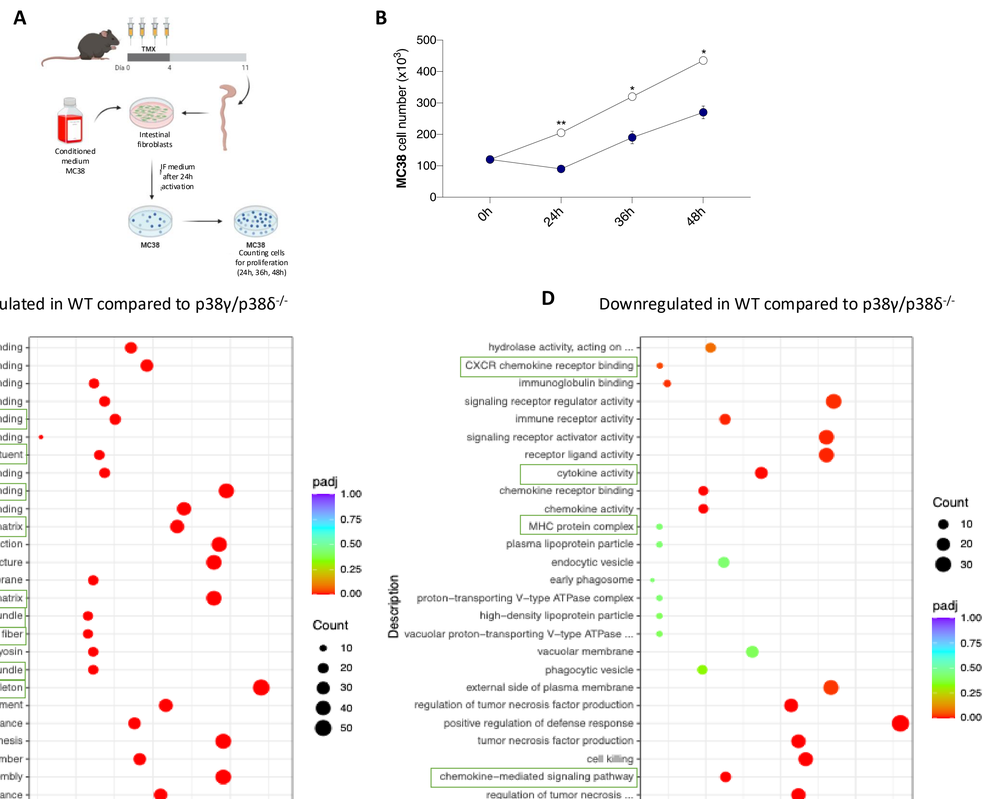

Fig4. (A) Experimetal design: intestinal fibroblast from p38γ/δ$^{f/f}$ and Pdgfrα-CreER-p38γ/δ$^{f/f}$ treated with MC38 supernatant, to later incubate the MC38 with the conditioned medium of the intestinal fibroblasts. (B) MC38 cell number at 0h, 24h, 36h, and 48h. (C and D) RNA-seq analysis of intestinal fibroblast islated from WT and p38γ/δ$^{-/-}$, representing the genes upregulated in WT mice compared to p38γ/δ$^{f/f}$ (C), and genes downregulate in WT compared to p38γ/δ$^{f/f}$ (D)

p38γ/p38δ regulates transcription factors that drive tumor proteases and cytokines expression

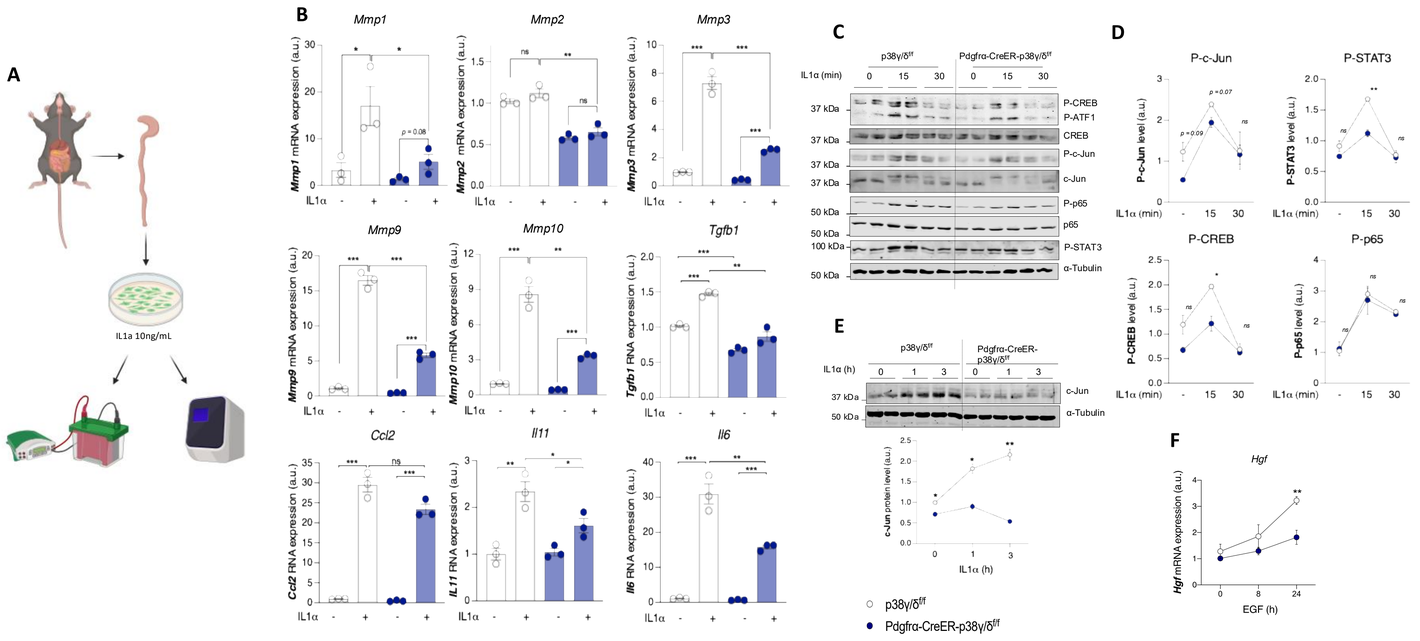

Fig5. (A) Experimetal design: intestinal fibroblast from p38γ/δ$^{f/f}$ and Pdgfrα-CreER-p38γ/δ$^{f/f}$ treated with 10ng/mL of IL1α, to later analyze it with qPCR and western blot. (B) Relative mRNA expression of Mmp1, Mmp2, Mmp3, Mmp9, Mmp10, Tgfb1, Ccl2, Il11, and Il6.(C) Intestinal fibroblast protein lysate immunoblotted using the indicated antibodies. α-Tubulin was used as a loading control. (D) Relative expression of P-c-Jun, P-STAT3, P-CREB, and P-p65. (E) Intestinal fibroblast protein lysate immunoblotted using c-Jun antibody. α-Tubulin was used as a loading control, and relative quantification. (F) mRNA expression of Hgf from p38γ/δ$^{f/f}$ and Pdgfrα-CreER-p38γ/δ$^{f/f}$ intestinal fibroblasts treated with EGF for 8h and 24h

Conclusion

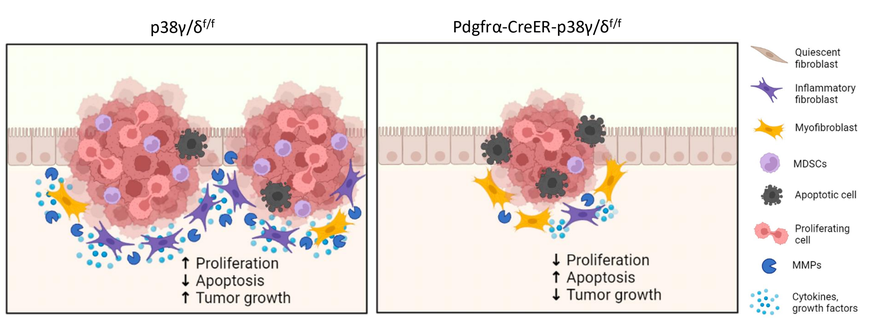

Fig5. Proposed model of the role of p38γ/δ in fibroblasts.

Altogether, our results indicate that the alternative p38MAPKs regulate the differentiation and the paracrine function of IFs, increasing the tumorigenic role of these cells in the pathogenesis of CAC. These observations support the potential use of p38γ and p38δ as therapeutical targets in this disease.

Funded by the European Union
OncoProTools
Centro Nacional de Biotecnología
Ministerio de Ciencia, Innovación y Universidades
CSIC
(PID2022-139944OB-I00). Ministerio de Ciencia e Innovación
Worldwide Cancer Research Fund-AECC Ref nº: 25-0404
European Comision (HORIZON-MSCA-DN-2021-101073231)
worldwide cancer research
asociación española contra el cáncer