BMP9 induces a fibrotic phenotype through ALK1 and ALK5 receptors.

José M. Muñoz-Félix[1], Nuria Perretta-Tejedor[1], Cristina Cuesta[1], Isabel Fuentes-Calvo[1], Nélida Eleno[1], José M. López-Novoa[1], Carlos Martínez-Salgado[1,2]

www.ufrc.es

[1]Unidad de Fisiopatología Renal y Cardiovascular, Instituto "Reina Sofía" de Investigación Nefrológica, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Salamanca, Spain
[2]Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL), Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain

INTRODUCTION

Synthesis of extracellular matrix (ECM) proteins by miofibroblasts in the renal tubular interstitium is one of the most important processes that occur, in renal tubule-interstitial fibrosis. Several cytokines have been described for years as profibrotic, due to their properties in the promotion of ECM protein synthesis, such as transforming growth factor beta 1 (TGF-β1) and connective tissue growth factor (CTGF/CCN2).

Bone morphogenetic proteins are members of TGF-β superfamily and play important roles in development and differentiation. BMP9 was described as a potent ligand of the ALK1 receptor in endothelial cells. ALK1 regulates ECM protein synthesis in several cell types studied such as fibroblasts, hepatocytes or chondrocytes.

MATERIALS AND METHODS

Our goal is to analyze the BMP9-induced effects on ECM protein synthesis in mouse embryonic fibroblasts (BMP9) and the possible pathways involved. We have stimulated MEFs with 20 ng/ml BMP9 and we have inhibited ALK1 receptor with dorsomorphin-1 and ALK5 receptor with SB431542. Moreover, we have inhibited MAPK/Erk1/2 pathway with U0126

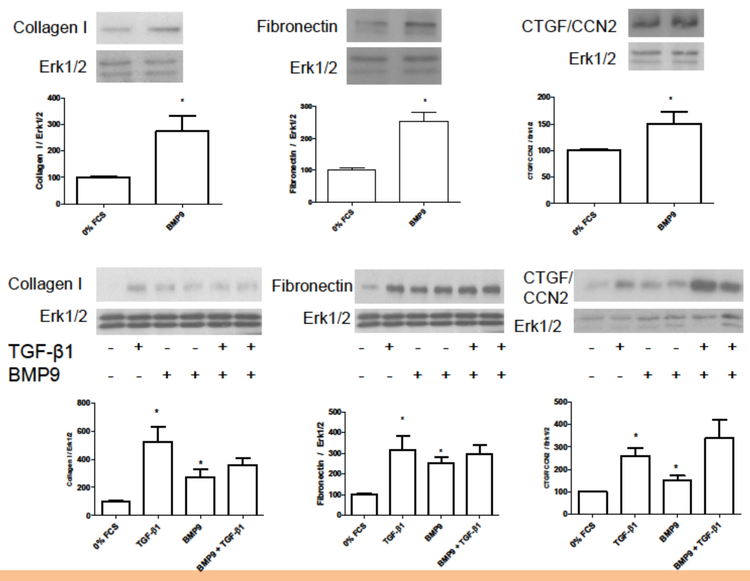
RESULTS

Our results indicate that BMP9 induces an increase in collagen I, fibronectin and CTGF/CCN2. Stimulation with BMP9 leads to a phosphorylation of Smad1/5/8 (through ALK1 receptor) and Smad2/3 (through ALK1 receptor) pathways. Inhibition of these receptors blocks BMP9-induced increase in ECM protein synthesis. Inhibition of MAPK/Erk1/2 pathway with U0126 blocks also BMP9-induced increase of ECM protein synthesis.

CONCLUSIONS

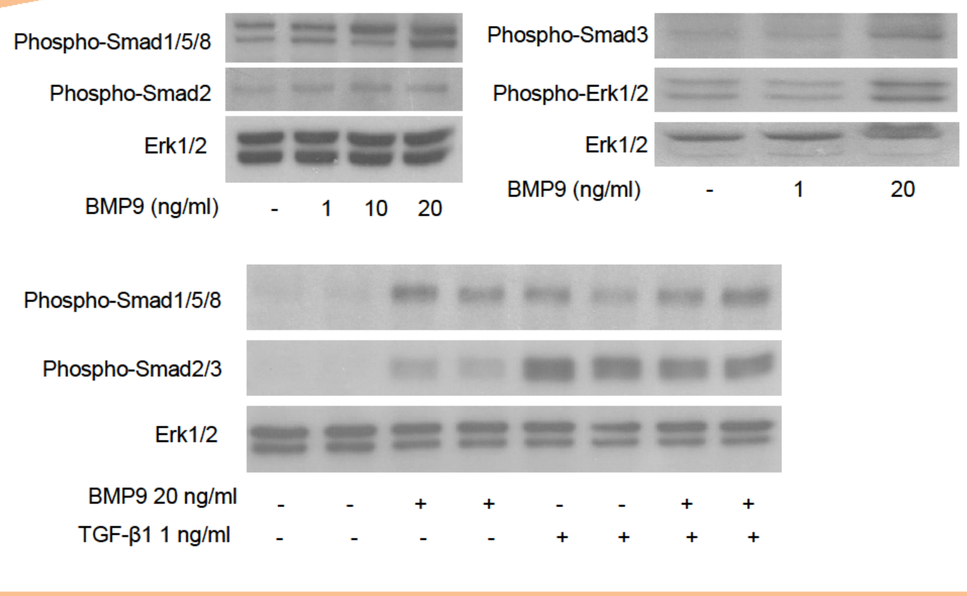
This work identifies BMP9 as a novel profibrotic factor in vitro.

BMP9 effects in ECM protein synthesis



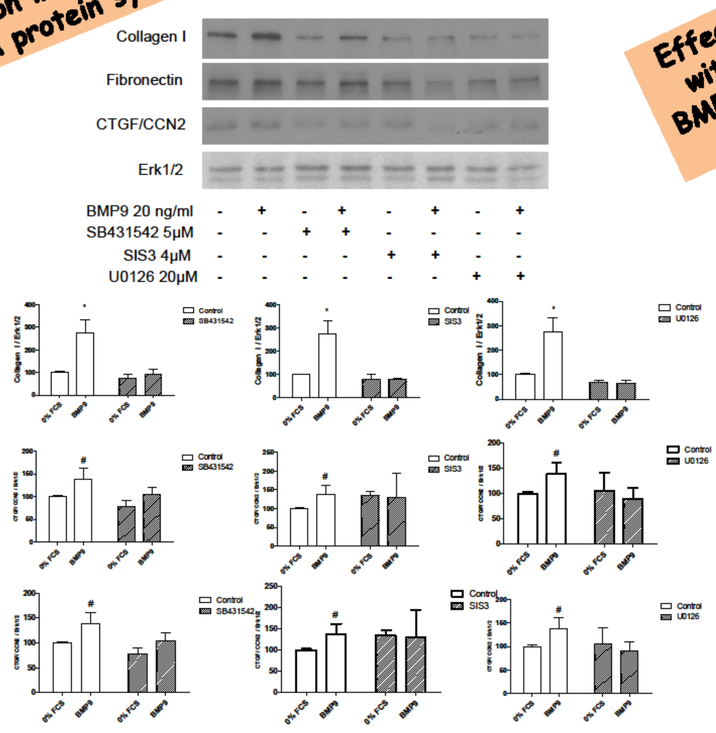
Expression of collagen I, fibronectin and CTGF/CCN2 after stimulation with 20 ng/ml BMP9 (upper panel) and 1 ng/ml TGF-β1 (lower panel). Histograms represent the mean ± SEM of the optical density of the bands of five experiments expressed as percentage over basal values. *P < 0.01 vs. MEFs in basal conditions.

BMP9 effects in signaling pathways induction



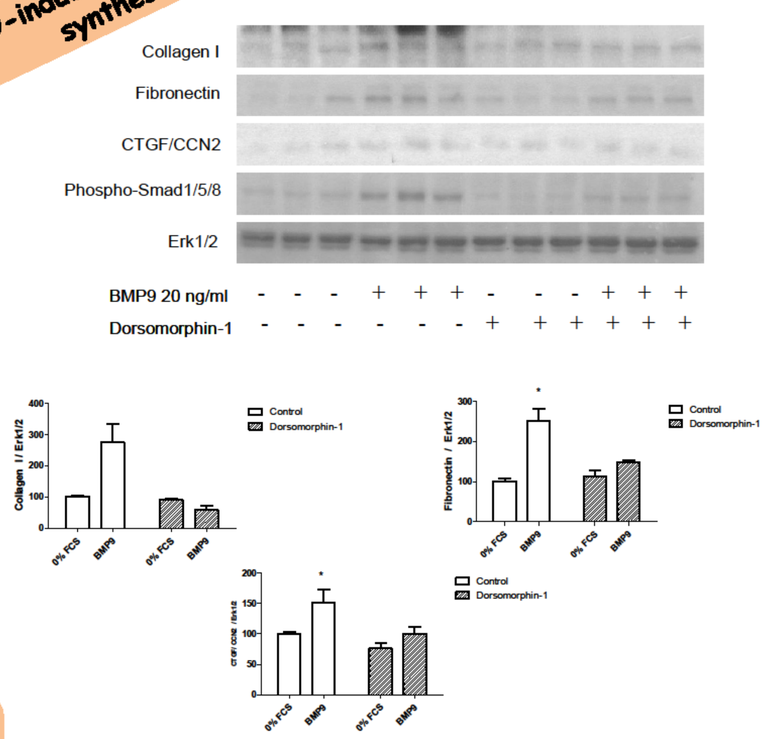
Expression of phospho-Smad1/5/8, phospho-Smad2, phospho-Smad3 and phospho-Erk1/2 after BMP9 stimulation at different concentrations (upper panel), and after stimulation with 20 ng/ml BMP9 and 1 ng/ml TGF-β1 (lower panel).

Effect of ALK5 and Erk1/2 inhibition in BMP9-induced ECM protein synthesis



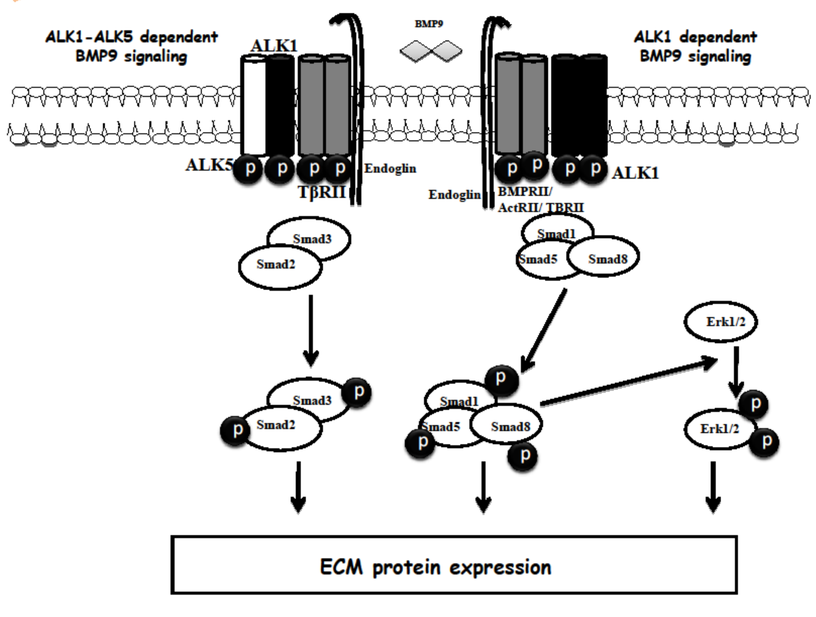
Expression of collagen I, fibronectin and CTGF/CCN2 after stimulation with 20 ng/ml BMP9 in cells pre-inhibited with the ALK5 inhibitor SB431542 (5μM),the Smad3 inhibitor SIS3 (4μM) and the MEK/Erk1/2 inhibitor U0126 (20μM).

Effect of ALK1 inhibition with dorsomorphin-1 in BMP9-induced ECM protein synthesis



Expression of collagen I, fibronectin, CTGF/CCN2 and phospho-Smad1/5/8 after stimulation with 20 ng/ml BMP9 in cells pre-inhibited with the ALK1/2/3/6 inhibitor dorsomorphin-1 (1μM).

Summary of the data



BMP9 binds ALK1 and activates the Smad1/5/8 and Smad2/3 pathways. ALK5 is also necessary for BMP9 to promote Smads activation. Moreover, BMP9 also activates the MAPK/Erk1/2 pathway, perhaps due to an indirect effect of Smad1/5/8 phosphorylation.
All these pathways contribute to the regulation of ECM protein expression in fibroblasts.

30--FP
52 ERA
J2) Chronic Kidney Disease. Pathophysiology, progression & risk factors.
Jose M Lopez-Novoa
DOI: 10.3252/pso.eu.52era.2015
ePosters supported by F. Hoffmann- La Roche Ltd.
52nd ERA-EDTA CONGRESS London2015 UNITED KINGDOM MAY 28 - 31
PosterSessionOnline.com