IJAE
Vol. 118, n. 2 (Supplement): 199, 2013
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Relaxin antagonizes the effects of TGF-β/Smad3 axis on cardiac fibroblast-myofibroblast transition through the upregulation of Notch-1 signalling

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The hormone relaxin (RLX) is produced by the heart and has beneficial actions on the cardiovascular system. We previously demonstrated that RLX stimulates mouse neonatal cardiomyocyte growth, suggesting its involvement in endogenous mechanisms of myocardial histogenesis and regeneration. In the present study, we extended the experimentation by evaluating the effects of RLX on primary cultures of neonatal cardiac stromal cells. RLX inhibited TGF-β1-induced fibroblast-myofibroblast transition, as judged by its ability to down-regulate α-smooth muscle actin and type I collagen expression. We also found that the hormone up-regulated metalloprotease (MMP)-2 and MMP-9 expression and downregulated the tissue inhibitor of metalloproteinases (TIMP)-2 in TGF-β1-stimulated cells. Interestingly, the effects of RLX on cardiac fibroblasts involved the activation of Notch-1 pathway. Indeed,Notch-1 expression was significantly decreased in TGF-β1-stimulated fibroblasts and this reduction was prevented by the addition of RLX to TGF-β1-stimulated cells. Moreover, pharmacological inhibition of endogenous Notch-1 signaling by DAPT, a γ-secretase specific inhibitor, as well as the silencing of Notch-1 ligand, Jagged-1, potentiated TGF-β1-induced myofibroblast differentiation and abrogated the inhibitory effects of RLX. Interestingly, RLX and Notch-1 exerted their inhibitory effects by interfering with TGF-β1 signaling, since the addition of RLX to TGF-β1-stimulated cells caused a significant decrease in Smad3 phosphorylation, a typical downstream event of TGF-β1 receptor activation, while the treatment with a prevented this effect. These data suggest that Notch signaling can down-regulate TGF-β1/Smad3-induced fibroblast-myofibroblast transition and that RLX could exert its well known anti-fibrotic action through the up-regulation of this pathway. In conclusion, the results of the present study beside supporting the role of RLX in the field of cardiac fibrosis, provide novel experimental evidence on the molecular mechanisms underlying its effects.

Key words

Cardiac regeneration, fibroblast-myofibroblast differentiation, matrix metalloproteases, myocardial fibrosis, Notch-1; relaxin (RLX), Smad3, TGF-β1.