

ity and lipid accumulation was affected by forced expression of miR-1, indicating miR-1 did not influence the differentiation into these cell types. Thus, a muscle-specific miRNA, miR-1, plays important roles in controlling myogenic differentiation and maturation in lineage-committed cells, rather than functioning in fate determination.

PE-440

TAZ and YAP Function as Critical Co-activators for TBX5 and Notch Signaling Represses TBX5 Activity

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The T-box transcription factor TBX5 plays essential roles in cardiac and limb development. Various mutations in the TBX5 gene have been identified in patients with Holt-Oram syndrome (HOS), which is characterized by congenital defects in the heart and upper extremities. In this study, we identified a WW-domain containing transcriptional regulator TAZ as a potent TBX5 co-activator. TAZ directly associates with TBX5 and markedly stimulates transcription driven by various TBX5-dependent promoters. Although TAZ does not have intrinsic histone acetyltransferase (HAT) activity, TAZ interacts with the HAT proteins, such as p300 and PCAF, to stimulate TBX5 activity. YAP, a TAZ-related protein with conserved functional domains, also stimulates transcription of TBX5-dependent promoters, possibly by forming a heterodimer with TAZ. In contrast, HRT and HES family proteins, which are down-stream targets of Notch signaling pathway, inhibit TBX5 activity through TAZ or YAP. These findings reveal key roles for TAZ and YAP in the control of TBX5-dependent transcription and suggest the involvement of these co-activators in cardiac and limb development.

PE-441

Essential Role of p300 in Post-natal Cardiac Mitochondrial Gene Expression and Maintenance of Normal Cardiac Function

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To evaluate the physiological role of p300 in the adult heart, we analyzed the mice carrying cardiac specific overexpression of dominant-negative mutant of p300 (C/H3 domain deletion mutant of p300; DN-p300). DN-p300 significantly inhibited the p300-induced activation of GATA- and MEF2- dependent promoter, confirming that C/H3 domain deletion operates as a dominant negative mutant, as previously described. DN-p300 transgenic mice (TG) showed the reduced survival rate compared to non-transgenic mice (NTG), and mostly died by 20 weeks of age. At 12 weeks of age, TG showed significant increases in the heart weight-to-body weight ratio and the lung weight-to-body weight ratio ($P < 0.01$). Echocardiography revealed the depressed ejection fraction (NTG [$n=8$], $65.5 \pm 1.5\%$; TG [$n=11$], $33.1 \pm 2.6\%$; $P < 0.01$) and the increased LV end-systolic diameter (NTG [$n=8$], 4.3 ± 0.12 mm; TG [$n=11$], 5.26 ± 0.11 mm; $P < 0.01$) in TG. In electron microscopic analysis, we found the markedly increased number and reduced size of mitochondria in TG myocytes. The expression of mitochondrial fatty acid β -oxidation genes was significantly decreased in TG. Tetramethylrhodamine-ethyl esters (TMRE) staining demonstrated the diminished mitochondrial membranous potential (MMP) in TG. The mRNA expression of PGC-1 α , a pivotal master regulator of mitochondrial genes expression, was significantly decreased in TG, and DN-p300 repressed the activity of MEF2-dependent PGC-1 α promoter. All these data indicate a critical role of p300 in the regulation of mitochondrial gene expression and the maintenance of normal cardiac function in post-natal heart.

PE-442

Endogenous TGF- β Negatively Regulates Cardiac Differentiation through Smad2 Activation in Embryonic Stem Cells

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We have demonstrated that in ES cell differentiation, Smad2 shows unique bimodal activation, and Smad2 activation in the early phase is indispensable for endodermal and mesodermal induction, while in the late phase, negatively regulating cardiomyogenesis. In this study, we analyzed the mechanisms by which cardiomyogenesis was suppressed by Smad2 activation in the late phase. Nodal/Cripto were expressed in the early stage and then downregulated, whereas TGF- β and Activin were expressed only in the late phase, suggesting TGF- β and Activin were responsible for the late Smad2 activation. Treatment with a neutralizing antibody against TGF- β in the late phase significantly enhanced cardiomyogenesis, while the effect of anti-Activin antibody was modest. Inhibition of TGF- β function by adenovirus-mediated expression of soluble TGF- β type II receptor decreased Smad2 activation in the late phase, and augmented cardiomyogenesis, indicating TGF- β accounts for Smad2-mediated inhibition of cardiomyogenesis. Using ES cells stably transfected with α -MHC promoter-driven EGFP and cell sorting, EGFP (+) myocytes and EGFP (-) non-myocytes were individually analyzed. Inhibition of TGF- β -mediated Smad2 activation resulted in a greater replicative potential in differentiated cardiomyocytes, which was coupled with increased expression of N-Myc, c-Myc, cdc25 and cyclinA2 in cardiomyocytes. Differentiation of non-myocytes into cardiomyocytes was also augmented by Smad2 inhibition. Thus, endogenous TGF- β negatively regulates cardiomyogenesis through Smad2 activation by modulating proliferation and differentiation of cardiomyocytes.

PE-443

Role of Glycogen Synthase Kinase-3 β in Cardiomyocyte Protection by Erythropoietin

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Background: The molecular mechanism by which erythropoietin (EPO) protects hearts from ischemia/reperfusion injury is unclear. Based on reported interaction between glycogen synthase kinase-3 β (GSK-3 β) and mitochondrial permeability transition pores, we examined the hypothesis that suppression of GSK-3 β activity and/or translocation of this kinase contribute to EPO-induced cardiomyocyte protection. Method: EGFP-tagged wild-type (WT), constitutive active (S9A) or dominant negative (K85R) GSK-3 β was transfected into H9c2 cardiomyoblasts. Apoptosis was induced by H_2O_2 (100 μ M, 2hr) with or without EPO pretreatment (10 U/ml), evaluated by Hoechst-33342 nuclear staining, and expressed as the ratio of condensed nuclei to total nuclei. Translocation of GSK-3 β was analyzed from EGFP signals and images of mitochondria by mitotracker staining and those of nuclei by Hoechst33342 staining. Result: Both the EPO receptor and common β receptor, subunits of a tissue-protective heteroreceptor, were detected in H9c2 cells by immunoblotting. H_2O_2 -induced apoptosis was increased in S9A-transfected cells compared with that in WT-transfected cells ($30.6 \pm 2.1\%$ vs. $44.4 \pm 5.7\%$, $p < 0.05$) and tended to decrease in K85R-transfected cells ($26.1 \pm 4.3\%$). EPO suppressed apoptosis in WT-transfected cells ($30.6 \pm 2.1\%$ vs. $21.9 \pm 2.5\%$, $p < 0.05$) but not in S9A-transfected cells ($44.4 \pm 5.7\%$ vs. $41.9 \pm 5.1\%$). Moreover, EPO failed to further protect K85R-transfected cells ($26.1 \pm 4.3\%$ vs. $26.4 \pm 3.0\%$). GSK-3 β was co-localized with 45.4% of mitochondria, which was not changed by EPO. Conclusion: EPO protects cardiomyocytes against apoptosis by inactivation of GSK-3 β without its additional recruitment into mitochondria.