Crosstalk between NADPH oxidases and the Hippo pathway coactivator Yes-Associated Protein promotes the cardiac response to hypoxia


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Background: The Hippo pathway is an evolutionarily conserved regulator of organ size and tumorigenesis. YAP is the main effector downstream of the Hippo pathway. Hippo activation induces an inhibitory YAP serine phosphorylation and nuclear exit/proteolytic degradation, thereby negatively regulating YAP activity. YAP activates TEAD transcription factors, thereby controlling growth and death in many cell types. In the adult heart, YAP has been shown to contribute to the regenerative response in conditions characterized by hypoxia and increased load of reactive oxygen species (ROS). While the multicomponent family of NADPH oxidases with its essential subunit p22phox contributes to ROS generation in the compromised heart, it is not known whether there is a link between YAP and NADPH oxidases.

Aim: We investigated whether YAP is sensitive to hypoxia and interferes with NADPH oxidases in vitro in cardiomyoblasts and in vivo in murine hearts.

Methods: H9C2 cardiomyoblasts or isolated adult murine cardiomyocytes (CMCs) were exposed to 0.1% O2 for different time points. H9C2 cells were transduced with constitutively active YAP or transfected with siRNAs against Yap and p22phox. For in vivo experiments, mice deficient in p22phox and wildtype mice were exposed to 10% O2. ROS levels were measured by DHE fluorescence or electron paramagnetic resonance. Cell cycle analysis and cell counting were performed by FACS. Localization and activation status of YAP were assessed by immunofluorescence, western blot and nuclear fractionation. DNA binding was analyzed by electro mobility shift assay (EMSA) and chromatim immunoprecipitation (ChIP).

Results: Hypoxia increased YAP protein levels, nuclear translocation and activity while Yap phosphorylation was decreased in H9C2 cells and in CMCs. Treatment with antioxidants or depletion of p22phox prevented these responses. In line, hypoxic exposure for only one day was already sufficient to increase YAP expression in hearts from wildtype mice but not in hearts from p22phox-deficient mice. Furthermore, depletion of p22phox and of YAP reduced number, cell cycle progression and hypertrophy of H9C2 cells under hypoxia. Similarly, H9C2 cells stably expressing constitutively active YAP showed increased cell numbers, cell cycle progression and cell hypertrophy dependent on p22phox and ROS. Interestingly, YAP overexpressing H9C2 cells showed increased ROS generation, concomitant with elevated expression of p22phox. In line, p22phox and Yap levels were enhanced in mice lacking the YAP upstream kinase Mst1. Reporter gene assays, EMSA and ChIP analyses showed that TEAD and YAP bind to the p22phox promoter in H9C2 cells.

Conclusion: These findings show that hypoxia increases YAP expression and activity dependent on ROS derived from its target p22phox in the heart. This feed forward loop between YAP and NADPH oxidases might promote pulmonary hypertension and other cardiovascular diseases associated with low oxygen availability.