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

Xinpei Chen[1,3], Philipp Mutzel[1], Kilian Hierdeis[1], Johannes Trefz[2], Martin Schönfelder[2], Henning Wackerhage[2], Agnes Görlach[1,3,4], Andreas Petry[1]

1 German Heart Center Munich, Experimental and Molecular Pediatric Cardiology, Munich, Germany 2 Technical University of Munich, Department of Sport and Health Sciences, Munich, Germany 3 DZHK (German Centre for Cardiovascular Research), partner site Munich, Munich, Germany 4 Technical University of Munich, School of Medicine, Munich, Germany

Background: The Hippo pathway is an evolutionary conserved regulator of organ size and tumorigenesis that negatively regulates cell growth and survival. YAP is the main effector downstream of the Hippo pathway. Hippo activation results in inhibitory YAP phosphorylation and nuclear exit/proteolytic degradation, thereby negatively regulating YAP activity. YAP itself regulates the transcriptional activity of TEAD, thereby controlling growth and death in many cell types. In the cardiovascular system and the 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 small subunit p22phox contributes to ROS generation in the compromised heart and in vascular diseases, it is not known whether there is a link between YAP and NADPH oxidases under these conditions.

Aim: We investigated whether YAP is sensitive to severe hypoxia and interferes with NADPH oxidases in cardiomyoblasts and in murine models of hypoxia.

Methods:

Rat cardiomyoblasts H9C2 or freshly isolated adult murine cardiomyocytes were exposed to 0.1% O_2 for 4 hours. H9C2 cells were transduced with constitutively active YAP or transfected with siRNAs against Yap and p22phox. ROS generation was measured by DHE fluorescence or electron paramagnetic resonance. Cell cycle analysis and cell counting were performed by FACS. Localisation and activation status of YAP were assessed by immunofluorescence, western blot and nuclear fractionation. DNA binding was analysed by electro mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP). For in vivo experiments, mice with nonfunctionial NADPH oxidases were kept in an isobaric hypoxic chamber.

Results:

In H9C2 cells Yap nuclear translocation and activity were enhanced and YAP phosphorylation decreased at 0.1% oxygen in a p22phox- and ROS-dependent manner. YAP phosphorylation was also decreased in isolated adult cardiomyocytes after hypoxia. ROS induced YAP nuclear translocation and YAP activity in H9C2 cells. In a murine model of hypoxia increased YAP nuclear localization and induction of YAP target genes were shown dependent on p22phox.

H9C2 cells stably expressing constitutively active YAP showed increased ROS generation, increased cell numbers, cellular hypertrophy as well as increased cell cycle progression in a redox sensitive manner. p22phox mRNA and protein levels were enhanced as well as p22phox promoter activity. Binding to a specific TEAD binding site in the p22phox promoter could be proofed by EMSA and ChIP analyses.

Conclusion: These data identify YAP as a transcriptional coactivator of p22phox under hypoxic conditions in cardiomyoblasts whereas p22phox promotes YAP expression and activation under hypoxia. This feedback loop might be important in promoting pulmonary hypertension and other cardiovascular diseases linked to low oxygen availability.