Mdm2, A NEGATIVE REGULATOR OF p53, IS REQUIRED FOR RENAL ORGANOGENESIS.

Sylvia A. Hilliard, Susana Dipp, Xiao Yao, and Samir S. El-Dahr

Tulane University School of Medicine, Department of Pediatrics, New Orleans, LA

Mdm2 is a key negative regulator of p53 activity in the cell. Mdm2 complexes with p53 and negatively regulates p53-induced transcription of target genes, including the Mdm2 gene, p21, and pro-apoptosis genes. In the mouse, Mdm2 is required to restrict p53 activity and mdm2 null embryos die of p53-mediated apoptosis at the peri-implantation stage. However, the absolute requirement for Mdm2 in organogenesis is unknown. This study examined the role of Mdm2 in metanephric development. Mdm2 and p53 mRNAs are expressed at high levels during metanephric development in both ureteric bud (UB) and metanephric mesenchyme (MM) lineages. To explore Mdm2-p53 signaling in metanephrogenesis, Mdm2-conditional mice were bred with Hoxb7-Cre-transgenic mice that express Cre recombinase in UB lineage cells. Mdm2-conditional Hoxb7-Cre mice die soon after birth and display multiple kidney defects including severe hypoplasia, paucity of collecting ducts, almost complete lack of nephron precursors and glomeruli, and cyst formation. The majority of the cysts are Dolichos biflorus lectin-negative, suggesting a non-collecting duct origin. In vitro culture of E12.5 kidney explants resulted in arrested development of the UB and consequently fewer mesenchymal condensates in the null mutants relative to the heterozygous and wild type littermates. Although markedly reduced in number, the UB tips continued to express c-ret, Wnt11, and Emx2. However, there was a notable reduction in Wnt9b, Lhx-1 and Pax-2 expression levels in the conditional mutant kidneys. Curiously, the number of LTA-positive proximal tubules present in these null mutant kidneys appeared to be disproportional to the number of UB tips present. At E14.5-16.5, UB cells deleted for Mdm2 have elevated p53 activity, reduced proliferation rate (phospho-H3), and enhanced apoptosis (activated cleaved caspase 3). To determine if the mutant phenotype is mediated by p53, we first attempted an in vitro rescue using the p53 inhibitor, Pifithrin. At 20 micromolar concentrations, Pifithrin was able to partially rescue the UB branching defect of the Mdm2 null mutant kidneys. Remarkably, p53 deletion either from the entire kidney or specifically from the ureteric bud lineage completely restored kidney development in Mdm2-conditional Hoxb7 Cre mice. These results demonstrate that Mdm2 in the ureteric bud lineage is required for branching morphogenesis. Mdm2-mediated inhibition of p53 activity is a prerequisite for renal organogenesis.

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