Post-translational Regulation of p53 During Nephrogenesis.


Departments of Pediatrics** and Human genetics*, Tulane University Health Sciences Center, New Orleans, LA.

p53, a tumor suppressor/transcription factor, protects the genome by promoting cell cycle arrest and differentiation or apoptosis. In response to stress, p53 is stabilized and activated via phosphorylation/acetylation which prevent its association with the ubiquitin ligase mdm2 and enhance its DNA-binding affinity. We tested the hypothesis that the p53 protein is developmentally regulated via post-translational modifications. Western blots and EMSA of mouse kidney nuclear fractions showed that the ratios of phos-p53^Ser-6/9/15/20/37/392 as well as acetylated p53^K-372/383 to total p53 were maintained at high levels during nephrogenesis only to reach undetectable levels by adulthood. To determine the functional relevance of these modifications, we assessed the effects of ser-to-ala (p53-S/A 15, 20, 33, 37, 392) and lys-to-arg (p53-6K/R 320, 370, 372, 373, 381, 382) substitutions on the stability and transcriptional activity of p53. Expression of p53-S/A mutants on a p53-null background revealed that phosphorylation of Serines is interdependent and necessary for full stability of p53 but is not essential for its nuclear localization. In comparison, p53-6K/R mutants are retained in the nucleus and thus are more stable. Transient transfection assays revealed that p53-S/A 15,20,33,37 mutants had lower transcriptional activity (p<0.05), whereas p53-S/A392 retained wild-type activity. p53 acetylation deficient mutants were found to differentially regulate terminal renal markers. To determine whether altered p53 stability in vivo affects the embryonic kidney, E12.5 kidneys were treated with the mdm2 inhibitor, Nutlin, for 24-48 hrs. Nutlin stunted metanephric growth and induced premature expression of AQP2, eNaC, and CAII. Similarly, collecting duct-specific deletion of mdm2 induced renal hypoplasia and enhanced AQP2 expression. We conclude that: (1) early kidney development is characterized by a marked and sustained activation of p53 secondary to extensive post-translational modifications; (2) p53 acetylation is indispensable for p53 transcriptional activity, whereas p53 phosphorylation regulates p53 stability; (3) fine-tuning of p53 stability is essential for renal development.

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