FALL 2018

Biochemistry and Molecular Biology Brown Bag Series

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"TIP60 Regulation of ΔNp63α is Associated with G2/M Progression"

Tuesday, November 13, 2018

11:00 AM

129 Medical Sciences Building

Lab: Madhavi Kadakia

Boonshoft School of Medicine
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TIP60 regulation of ΔNp63α is associated with G2/M progression

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The transcription factor ΔNp63α, a p53 family member, is found primarily in the basal stratum of the skin where it functions to promote epithelial morphogenesis, cell proliferation, and cell survival. ΔNp63α is up-regulated in non-melanoma skin cancers such as squamous cell carcinoma (SCC), implicating it as a proto-oncogene. Using cell culture and *in vitro* techniques, we have identified the acetyltransferase TIP60 as an upstream regulator of ΔNp63α. We observed that overexpression of ΔNp63α with increasing concentrations of TIP60 resulted in a dose-dependent increase in ΔNp63α protein levels. Also, co-expression of ΔNp63α with a TIP60 catalytic deficient mutant diminished this increase, thereby suggesting that TIP60 acetyltransferase activity contributed to increased ΔNp63α protein levels. Further, knocking down TIP60 reduced endogenous both ΔNp63α protein and transcript levels. Inhibition of *de novo* protein synthesis led to increased ΔNp63α protein half-life in the presence of TIP60. Finally, TIP60 decreased the ubiquitination and proteasomal degradation of ΔNp63α. Cumulatively, these results indicate that ΔNp63α expression is regulated by TIP60 at both the transcriptional and post-translational levels. We further demonstrated that TIP60 and ΔNp63α can interact with each other in cells as well using recombinant protein, and co-localize within the nucleus of an SCC cell line. We confirmed acetylation of ΔNp63α by TIP60 using an *in vitro* acetylation assay with recombinant protein and identified acetylated lysines via mass spectrometry. Moreover, site-directed mutagenesis demonstrates that these lysines are critical to the ability of TIP60 to regulate ΔNp63α protein levels. We also observed that knockdown of both TIP60 and ΔNp63α led to a reduction in SCC proliferation. Using flow cytometry analysis, we found that both TIP60 and ΔNp63α expression promoted G2/M progression. Our results therefore indicate that TIP60 mediates SCC growth in part due to regulation of ΔNp63α. The identification of TIP60 as a novel upstream regulator of ΔNp63α provides researchers with a novel therapeutic target to combat this disease.