CHARACTERIZATION OF THE MDM2 BINDING REGIONS OF RIBOSOMAL PROTEIN L5

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Dedication

This dissertation is dedicated to Ashlynne Nicole. You will always remain in my thoughts and within my heart.

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Abstract

Kevin D. Plummer

CHARACTERIZATION OF THE MDM2 BINDING REGIONS OF RIBOSOMAL PROTEIN L5

The MDM2-p53 feedback loop is a well-characterized pathway. p53 is a transcription factor and regulates the transcriptional expression of genes that encode proteins responsible for cellular senescence, cell cycle arrest, apoptosis, and DNA repair. Various cellular stresses can result in p53 activation, including hypoxia, DNA damage by agents such as UV or IR, oncogenic signaling, nucleotide depletion and nucleolar stress from perturbation of ribosomal biogenesis. Under normal conditions, MDM2's role in the pathway is to inhibit p53 function by directly binding to this protein and facilitating its ubiquitylation and 26S proteasome-mediated degradation. Under stressful cellular conditions, certain proteins interact with and rescue MDM2's inhibition of p53. For example, upon exposure to small amounts of Actinomycin D, rRNA transcript synthesis is stalled resulting in the release of various ribosomal proteins including RPL5, RPL11 and RPL23; each of which has been shown to bind MDM2 within its central acidic domain and inhibit its ability to destabilize p53. Although the RPL5 binding region of MDM2 have been mapped in prior investigations, the MDM2-binding region(s) of RPL5 have yet to be characterized.

By employing RPL5 deletion mutagenesis and *in vitro* GST-fusion protein-protein association assays with purified proteins, this dissertation attempts to elucidate those regions of RPL5 that may interact with MDM2. Normalizing RPL5-WT to 1.00, our study reveals that the basic N and C-terminals of RPL5 appear to bind with MDM2 while RPL5's central region displays negligible binding to the central acidic domain of MDM2. Also, the possible meanings of these RPL5 MDM2 binding domains are discussed along with their utilization in potential future applications.

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