Defining the Role for ZBP-89 in ATM-mediated DNA Damage Response to Irradiation in the Intestine

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ABSTRACT

ZBP-89 (ZNF148, Zfp148) is a zinc-finger transcription factor that inhibits cellular proliferation when overexpressed in cell lines. ZBP-89 forms a protein-protein interaction with p53 and Ataxia-telangiectasia mutated (ATM). However, it is unclear how the interaction modulates the function of these two proteins in vivo.

Double strand DNA breakage induced by □-irradiation induces ATM phosphoinositol kinase activity, which initiates a cascade of events culminating in cell cycle arrest, DNA repair or apoptosis. Elevated levels of ZBP-89 induce growth arrest and apoptosis in gastrointestinal cell lines. Therefore, we hypothesize that ZBP-89 facilitates cell growth arrest and activation of the DNA repair pathway after ATM activation.

To test this hypothesis, we irradiated 4 groups of mice—1) C57BL/6 mice without any genetic deletion; 2) mice with one or both copies of ATM deleted from the intestinal mucosa; 3) mice with the ATM deletion and one copy of Zfp148 deleted; 4) ATM deletion with both copies of Zfp148 deleted. After the intestines were fixed, paraffin-embedded and sectioned, I performed the de-paraffinization step for immunohistochemistry of ZBP-89, p53 and □H2AX. To stain for ZBP-89 protein, I performed an antigen retrieval step using sodium citrate, followed by blocking with 3% hydrogen peroxide and serum. I then added the primary antibody then the secondary antibodies. I used diaminobenzidine (DAB), a chromogenic detection method to visualize the antigen-antibody complex. I then counterstained with hematoxylin.

We predict that the mice with a deletion of ZBP-89 will exhibit more tissue damage as a result of the irradiation and DNA damage. Results are pending.

Keywords: ZBP-89, Cellular Proliferation, DNA Repair, Ataxia-telangiectasia Mutated

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