

## Bankhead-Coley Cancer Research Program

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**Project Title:** AKT1 Function and Carcinogenesis

**Project Summary:** AKT1 is a cancer-causing gene, which plays a pivotal role in human oncogenesis. However, the molecular mechanism of AKT1 in human oncogenesis remains elusive. We have recently identified a specific AKT1-associated protein, TZP, which is an uncharacterized nuclear protein. We have shown that TZP is a transcription factor and induces p53 and p27 at mRNA levels. Further, TZP forms a complex with FOXO3a. Expression of TZP inhibits cell growth, DNA synthesis, and cell survival. AKT1 phosphorylates TZP in vitro and in vivo, suggesting that TZP is a substrate of AKT1. This prompted us to examine the effect of AKT1 on p53 transcriptional levels. Unexpectedly, we found that p53 mRNA level is negatively regulated by AKT1, i.e., AKT1-knockout cells express high levels of p53 mRNA, whereas reconstitution of AKT1 considerably reduces p53 expression. Further, TZP was epigenetically inactivated in a subset of leukemia cell lines examined. Moreover, we have demonstrated that AKT1 phosphorylates and inhibits pro-apoptotic MST1 kinase, suggesting that AKT1 cross-talks with MST1/WW45/Lats pathway. In addition, MST1 interacts with and phosphorylates TZP, leading to an increase of TZP function toward p53 and p27. Based on these findings, we hypothesize that AKT1/MST1/TZP cascade plays an important role in the control of cell survival, proliferation, and transformation induced by AKT1. The broad, long-term objective of this project is to elucidate the normal cellular function of the AKT1 protein and determine the importance of perturbations of the AKT1 pathway in human carcinogenesis. The specific aims are:

I. Define the role of the AKT1-associated protein TZP in AKT1 signaling. This will be achieved by examining the effects of AKT1 phosphorylation of TZP on (1) TZP induction and transactivation of p53 in wild-type and shRNA knockdown Mdm2 cells, (2) TZP tumor suppressor function in p53-null and wild-type p53 cells, as well as by determining (3) the effects of TZP on AKT1 oncogenic activity.

II. Examine the effects of AKT1 phosphorylation of MST1 on MST1/WW45/LATS function. We will examine the effects of AKT1 phosphorylation of MST1 on (1) AKT1 inhibition of MST1 cleavage and MST1-induced apoptosis, (2) MST1 kinase activity and nuclear translocation, (3) downstream target of MST1, (4) MST1/WW45/Lats complex formation and phosphorylation of Lats 1 and Lats 2, and (5) MST1 dimerization.

III. Define the MST1 regulation of TZP and TZP/FOXO3a complex as well as the effect of AKT1 on MST1/TZP/FOXO3a Cascade. This will be accomplished by defining (1) the phosphorylation site(s) of TZP induced by MST1, and examining (2) the effect of MST1 phosphorylation of TZP on p53 expression at mRNA level and p53 promoter activity, (3) the effect of MST1 phosphorylation of TZP on TZP/FOXO3a/MST1 complex formation, and (4) the role of AKT1 in MST1/TZP/FOXO3a signaling.