

ABSTRACT

THE ROLE OF DBF4-DEPENDENT KINASE
IN MAINTAINING GENOME STABILITY

By

Nanda Kumar Sasi

DBF4-dependent kinase (DDK) is a two subunit kinase composed of the CDC7 kinase and its regulatory subunit, DBF4. It is essential for initiating DNA replication at individual origins and also has less understood roles in DNA repair, mitosis, and meiosis. Both DDK subunits are highly expressed in many diverse tumor cell lines and primary tumors, which is correlated with poor prognosis. Inhibiting DDK causes apoptosis of tumor cells, but not normal cells, through a largely unknown mechanism. The aim of this dissertation is to improve our understanding of the role of DDK in maintaining genome stability, in tumorigenesis, and to identify ways to better utilize DDK as a target for tumor therapy.

First, we studied the role of DDK in initiating and maintaining the replication checkpoint pathway. This pathway ensures complete and accurate replication of DNA before chromosomes segregate during mitosis. We found a novel role for DDK in the nucleolytic processing of stalled replication forks, structures generated upon inhibition of DNA replication. DDK-mediated fork processing is essential for generating single stranded DNA at stalled forks, which in turn is required for activating a replication-checkpoint pathway. Our results suggest that high levels of DDK expression might enable tumor cells to tolerate replication stress, a by-product of increased rate of proliferation. Indeed, gene expression signature of tumors with high levels of DDK correlated with increased resistance to genotoxic chemotherapies. Surprisingly, the level of DDK expression is also strongly correlated with genome-wide gene mutation frequencies suggesting that increased DDK levels promote elevated mutation frequency. This is consistent with the role of DDK in promoting an error-prone trans-lesion DNA repair pathway, a possible mechanism for the increased rate of mutagenesis. Finally, using an RNA interference screen we identified 23 kinases and phosphatases that promote apoptosis of both breast and cervical carcinoma cell lines when DDK is inhibited. These hits include checkpoint genes, G2/M cell cycle regulators and known tumor suppressors. Initial characterization of the LATS2 tumor suppressor suggests that it promotes apoptosis independently of the upstream MST1/2 kinases in the Hippo signaling pathway. A clear understanding of this pathway would enable better use of DDK inhibitors for tumor therapy and also suggest possible mechanisms by which tumors might become resistant to DDK-targeting drugs.

These results have increased our understanding of DDK's role in cellular response to replication perturbation, an important function beyond its essential role in DNA replication. Our studies highlight the importance of DDK in tumor cells and explain the survival advantage gained by its increased expression. Finally, this work lays out strategies for targeting DDK to limit tumor growth and overcome resistance to existing genotoxic chemotherapies.