



**Karolinska
Institutet**

Department of Microbiology, Tumor and Cell Biology

Inhibition of crucial oncogenes by pharmacologically activated p53

AKADEMISK AVHANDLING

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ABSTRACT

The tumor suppressor p53 is a transcriptional factor which is frequently inactivated in cancer, either by point mutations or by its negative regulators, such as Mdm2 and MdmX. Reactivation of p53 by small molecules is a promising strategy to treat cancer. The aim of this thesis is to elucidate the molecular mechanisms of the different biological responses induced by two p53-reactivating small molecules, RITA and nutlin.

We found that the induction of p53 pro-apoptotic target genes is not sufficient to induce a full-scale cell death; the inhibition of key survival genes is necessary to trigger robust apoptosis upon reactivation of p53. Our results reveal that two distinct transcriptional programs, activation of pro-apoptotic genes and repression of pro-survival genes are required to be orchestrated by p53 to produce a robust apoptotic outcome. In contrast to p53-mediated transactivation, transrepression by p53 is more strictly controlled by Mdm2 and requires a high ratio of p53/Mdm2 at the promoters of repressed genes.

Further investigation of the underlying mechanisms of the differential biological outcome upon p53 reactivation revealed that the inhibition of TrxR1 by RITA leads to the induction of ROS and activation of JNK. Activated JNK creates a positive feedback loop with p53 and converts p53 into an efficient transrepressor. We demonstrated that Wip1 is one of the crucial factors downstream of JNK, whose inhibition contributes to a robust and sustained transcriptional response by p53 and the subsequent cell death. Our data suggest that simultaneous activation of p53 and inhibition of TrxR1 lead to synthetic lethality in cancer cells. Our study points out that perturbing the redox system in tumors, which carry abnormally high level of ROS, might enable the pharmacologically reactivated p53 to selectively eliminate cancer cells.

Neuroblastoma is one of the most challenging childhood cancers. The ability of RITA to reactivate both wild type and mutant p53 prompted us to investigate the effect of RITA in a panel of seven neuroblastoma cell lines with different p53 status. We found that RITA induced apoptosis in all the neuroblastoma cell lines tested, irrespective of the status of p53. RITA-activated p53 induced a set of pro-apoptotic target genes. In addition, RITA-activated p53 repressed several key survival genes, including N-myc, Wip1, Aurora kinase, Mcl-1, Bcl-2, Mdm2 and MdmX. Moreover, RITA exhibited strong antitumor effect in xenograft models.

In summary, our data presented above demonstrate that concurrent activation of p53 in combination with inhibition of TrxR1 followed by the induction of ROS represent a promising strategy to treat cancer. Inhibition of pro-survival genes plays a fundamental role in a full-scale apoptosis induction in cancer cells upon pharmacological p53-reactivation.