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p53 reactivation by the small molecule RITA: molecular mechanisms

AKADEMISK AVHANDLING

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ABSTRACT

Inactivation of the tumor suppressor p53 is essential for the development and maintenance of cancer cells. Therefore, reactivation of p53 appears to be a promising strategy for anti-cancer therapy. We have previously identified the small molecule RITA that prevents interaction between p53 and its negative regulator Mdm2 by direct binding to p53 (Issaeva et al., 2004). RITA reactivates the transcriptional transactivation function of wild type p53 and induces p53-dependent apoptosis *in vitro* and *in vivo*.

In this thesis we addressed the molecular mechanisms of RITA action. In particular, we investigated which signaling networks are important for RITA-mediated cancer cell killing, characterized p53/RITA interaction and studied the effect of RITA on mutant p53.

We previously demonstrated that transactivation of pro-apoptotic genes is required for cell death induced by RITA-reactivated p53 (Enge et al., 2009). We found that the activation of pro-apoptotic targets is not sufficient for a full-scale induction of cell death by p53. Here, we showed that a dramatic and rapid downregulation of a number of critical oncogenes and oncogenic pathways by RITA-reactivated p53 is required for the induction of apoptosis. Importantly, our results indicate that induction of pro-apoptotic genes and inhibition of anti-apoptotic/survival genes represent two branches of p53 response, which are differentially regulated. Our results suggest that p53-mediated transrepression is more tightly controlled than transactivation and correlates with increased p53 and reduced Mdm2 abundance on chromatin.

To address the molecular mechanism through which RITA interferes with Mdm2, we mapped RITA-binding site in p53 using a series of deletion and point mutants. We found that RITA binds outside of the Mdm2 binding site and identified S33 and S37 as key p53 residues targeted by RITA. This implies that p53/Mdm2 interaction is prevented by RITA via allosteric mechanism. We propose that RITA/p53 binding induces a conformational trap locking Mdm2-contacting residues in an orientation unfavorable for the p53/Mdm2 binding. Moreover, we found that the conformational change induced by RITA prevents the binding of another p53 inhibitor, MdmX.

Half of human tumors carry point mutations in the p53 gene that abolish p53 binding to DNA. This correlates with poor prognosis and often confers increased resistance to conventional chemo- and radiotherapy. The ability of RITA to induce a conformational change in p53 prompted us to test whether RITA can also restore mutant p53 activity. Here, we found that RITA suppressed the growth and induced apoptosis in a p53-dependent manner in a variety of cell lines that harbor different hot spot p53 mutations. Several known p53 target genes changed their expression in mutant p53-expressing cells upon RITA treatment. Inhibition of mutant p53 prevented RITA-induced effects, suggesting the observed transcriptional response and cell death are dependent on mutant p53.

In summary, our findings demonstrate, that p53 reactivated by the small molecule RITA induces ablation of oncogenic pathways crucial for the survival of cancer cells. RITA acts via an allosteric mechanism and restores the function of both wt and mutant p53.

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