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Small Molecules as Research Tools for Studying the Biology of the tumour suppressor p53

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ABSTRACT

P53 is a potent tumour suppressor that is inactivated in the majority, if not all human cancers. In about 50% of the cases, the gene is mutated and in the rest it is rendered inactive mainly by deregulation of its negative regulators such as Hdm2 and HdmX. Targeting p53 has been shown to be a promising strategy to fight cancer and the first compounds reactivating p53 have reached the stage of clinical trials. In addition to immediate clinical use, compounds that activate p53 are valuable tools to increase the knowledge about fundamental p53 biology.

RITA is an example for such a compound activating p53. We found that it induces decreased protein levels of the negative regulator of p53, HdmX, in a p53-dependent manner. This is mediated by ATM-induced phosphorylation. In addition expression of Wip1 is inhibited. Wip1 depletion is an important event for HdmX decrease, as Wip1 reverses ATM mediated phosphorylation and thus can prevent ATM-induced HdmX decline. The biological significance of HdmX and Wip1 inhibition is highlighted by the fact that a knockdown of either of them enhances cell killing by the p53-activating drugs RITA and nutlin3a.

To get insight into mechanisms of p53 mediated transcriptional regulation, we compared genome-wide chromatin occupancy by p53 upon its activation by three different compounds, RITA, nutlin3a and 5-FU that cause different biological outcomes. We compared genome-wide chromatin occupancy by p53. Surprisingly, the regions that were bound by p53 to the highest extent were the same after all three treatments, despite their different biological outcomes. Comparison of the p53-occupied sites with gene expression changes upon p53 activation by nutlin3a allowed identifying 280 previously unknown target genes. The common p53 binding motif is present much more frequently in the promoter region of induced genes than in that of repressed genes. This suggests different mechanisms for gene induction versus repression by p53, presumably distinguished by the involvement of cofactors. This is in line with our finding that binding sites for cofactors in the proximity of p53 sites are distinct for induced and repressed genes. We identified *AURKA* (Aurora kinase A) as a novel p53-repressed target gene, and found that STAT3 also regulates it, antagonising p53. Finally we found, that expression of our newly identified panel of p53 target genes correlated with the p53 status, the grade of tumours and the long-term survival in a set of 250 breast cancer patients.

Malignant melanomas have very poor prognosis with extremely low long-term survival once the metastatic stage is reached. It has been shown that in 3-dimensional collagen type I matrix that mimics the microenvironment of the human dermis, melanoma cells induce integrin-dependent inactivation of p53, rescuing the cells from apoptosis. Thus, reactivation of p53 might be a promising strategy to kill melanoma cells. To test this, we treated melanoma cells that were grown in 3D conditions with the p53 reactivating compound PRIMA-1^{MET}/APR-246. Indeed, this induced apoptosis in the cells in a p53 dependent manner. Consistently, the growth of melanoma xenografts in mice was suppressed in a p53-dependent manner after PRIMA-1^{MET} treatment.

In summary, this thesis demonstrates examples for both the use of p53 activating compounds as research tools to uncover new details of p53 biology as well as their application for therapy, exemplified by effects of PRIMA-1^{MET} in malignant melanomas *in vitro* and *in vivo*.

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