



**Karolinska  
Institutet**

**Institutionen för Mikrobiologi, Tumörbiologi och Cellbiologi**

# **Modulation of p53's transcriptional function by small molecules**

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorsexamen vid Karolinska  
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av

**Fedor Nikulenkov**

*Huvudhandledare:*

Professor Galina Selivanova  
Karolinska Institutet  
Institutionen för Microbiologi,  
Tumörbiologi och Cellbiologi (MTC)

*Bihandledare:*

Professor Lars-Gunnar Larsson  
Karolinska Institutet  
Institutionen för Microbiologi,  
Tumörbiologi och Cellbiologi (MTC)

*Fakultetsopponent:*

Professor Moshe Oren  
Weizmann Institute,  
Department of Molecular  
Cell Biology

*Betygsnämnd:*

Associate Professor Annika Wallberg  
Karolinska Institutet  
Institutet för miljömedicin (IMM)

Professor Rolf Ohlsson

Karolinska Institutet  
Institutionen för Microbiologi,  
Tumörbiologi och Cellbiologi (MTC)

Professor Thomas Helleday

Stockholm University  
Institutionen för genetik, mikrobiologi och  
toxikologi (GMT)

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## ABSTRACT

p53 tumour suppressor is a transcriptional factor which induces apoptosis or growth arrest in response to stress thus eliminating damaged cells. p53 function is frequently abrogated in tumours either via inactivation mutations in the TP53 gene or by elevated activity of p53 negative regulators HDM2 and HDMX. Therefore application of small molecules that reactivate p53 function is a promising strategy for anti-cancer therapy. In addition, small molecules can serve as valuable research tool to study p53 biology.

This thesis is focused on the studies of p53 transcriptional response induced by small molecules and the molecular mechanisms contributing to the induction of apoptosis by p53. Using chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) we compared genome-wide DNA binding profile of p53 activated by three different small molecules RITA, 5-FU and Nutlin-3a, causing various biological outcomes in breast carcinoma MCF7 cell line. We found that the major pattern of p53 binding to DNA does not depend on the mechanism of p53 activation or p53-induced cell fate. Surprisingly, we demonstrated that the majority of sites bound by p53 are located far away from transcription starting sites (TSS), thus making unclear their functional role. Comparison of p53 DNA binding sites in vicinity to TSS with changes in gene expression using microarray analysis revealed 280 novel p53 target genes. While the majority of p53 transactivated genes shared classical p53 consensus motif, we found it only in a few repressed genes, suggesting different mechanism of p53 transrepression. We validated several novel p53 target genes, including AURKA gene which is negatively regulated by p53. In addition, we showed that STAT3 transcription factor antagonizes p53-mediated regulation of several target genes, including AURKA. We demonstrated that the expression level of novel p53 target genes correlates with p53 status, tumour grade and survival in 265 breast cancer patients.

Investigation of molecular mechanisms of p53-mediated apoptosis upon RITA treatment revealed that in addition to activation of pro-apoptotic targets, p53 inhibited the expression of several crucial oncogenes. Thus, we showed that inhibition of several oncogenic and pro-survival factors, including c-Myc and Mcl-1, on mRNA and protein levels critically contributes to robust induction of apoptosis. We found that in contrast to p53-mediated transactivation, transrepression is more tightly regulated by HDM2 and depends on the ratio of p53 and HDM2 bound to gene promoters.

We found that RITA-activated p53 mediates a decrease in expression and protein stability of its negative regulator HDMX. Impaired stability of HDMX is caused by ATM-mediated phosphorylation of HDMX. In turn, the elevated activity of ATM correlates with depletion of p53 target gene Wip1 phosphatase that inhibits ATM. We demonstrated that the depletion of either HDMX or Wip1 enhances growth suppressive effects of p53-reactivating molecules RITA and Nutlin3a.

Our data showed that RITA inhibits glycolytic enzymes in p53-dependent manner. We found that p53 binds to DNA in vicinity from TSS of the metabolic genes and represses their transcription. Our data suggests that SP1 is a p53 transcriptional cofactor contributing to p53-mediated transrepression of several metabolic genes. Importantly, we showed that the block of glycolysis amplifies induction of apoptosis in cancer cells upon RITA treatment.

In conclusion, our data contribute to a deeper understanding of transcriptional response induced by p53, along with the identification of novel p53 target genes. Our studies revealed new targets of pharmacologically activated p53 which significantly increase the robustness of p53-mediated apoptosis.