## Thesis Synopsis

# Regulation of expression of p53 and its isoform Δ40p53: Consequences on cellular gene expression

The *TP53* tumour suppressor gene encodes for p53 protein which is the frequently altered gene in most of the cancers. p53 protein is activated in response to different stresses and plays major role in maintaining genome integrity by regulating cell cycle and cell survival. It is known that p53 has twelve isoforms generated using alternate splicing, alternate promoters and translation initiation sites. Out of these isoforms Δ40p53 (also known as ΔN-p53/p47) is the only translational isoform of p53, produced from the same mRNA by using an 'internal ribosome entry site' (IRES). From our laboratory, it has been shown earlier that *p53* mRNA has two IRES elements, IRES1 and IRES2. IRES2 mediated translation of Δ40p53 is maximum during G1-S phase and that of full length p53 (p53FL) by IRES1 is maximum in G2-M phase. p53 gene expression levels are regulated at many levels, including transcription, splicing, mRNA transport, stability and protein translation. The focus of our laboratory is on the translational control of p53. Previous studies from our laboratory has demonstrated PTB (polypyrimidine binding protein), Annexin A2 (ANXA2), PTB-associated splicing factor (PSF) and Death-associated protein 5 (DAP5) to be the crucial ITAFs (IRES trans acting factors) for IRES mediated translation regulation of p53 and Δ40p53 under different stress conditions. PTB binds to the p53 mRNA IRESs and enhances the translation of p53 isoforms by translocating from nucleus to cytoplasm upon doxorubicin-induced DNA damage. ANXA2 and PSF proteins, the other p53 ITAFs, interact with p53 IRESs *ex vivo* in a stress-induced manner, showing greater association with the IRESs upon ER stress (thapsigargin treatment). DAP5 was demonstrated to bind to p53 IRESs and regulate the IRES2 mediated expression of Δ40p53.The tightly regulated p53 in turn regulates different target genes. p53 is also known to regulate miRNAs followed by their respective target genes and hence different cellular outcome. The present study focuses on translational regulation of p53 and its isoform Δ40p53 by binding of proteins and miRNAs at the untranslated regions (UTRs). Further, the effect of the differential expression of these two isoforms on cellular gene expression mediated by miRNA is also studied.

Translation regulation of p53 involves interaction of proteins and microRNAs with the 5'and 3' UTR of *p53* mRNA. Earlier we have shown that PTB and ANXA2 interact

with the 5'UTR of p53 mRNA to regulate its expression. Here we have studied the role of 3'UTR in regulating the expression of the two isoforms, that is, full-length p53 (p53FL) and Δ40p53. We have demonstrated that both PTB and ANXA2 bind to p53 3'UTR and delineated the specific binding regions within 3'UTR that contribute to these interactions. Knockdown of both PTB and ANXA2 led to significant decrease in translation of p53 isoforms., mediated by their interaction to the 5'UTR/3'UTR. Interestingly, we have observed that the addition of p53 3'UTR to the constructs led to a decrease in the expression of both reporter and p53 isoforms, indicating that microRNAs binding to 3'UTR might be playing a role in this regulation. Further, we have explored the role of possible interplay between protein and microRNAs in the 3'UTR mediated translational control of *p53*. Interestingly, PTB showed some overlapping binding regions in the p53 3′UTR with some miRNAs. In order to understand the interplay between PTB and miRNAs different approaches were taken. Firstly, after partial silencing of PTB, increase in the association of Ago-2 complex with p53 mRNA was observed. Secondly, this interplay was also observed under DNA damage (doxorubicin treatment), where PTB is known to be translocated to the cytoplasm from the nucleus. Under DNA damage there was decreased association of p53 mRNA with Ago-2 and vice versa association with PTB. So this increased binding of p53 mRNA with PTB under DNA damage suggests that there is interplay between miRNAs and PTB at the 3′UTR under normal and stress conditions like DNA damage. Interestingly, PTB showed some overlapping binding regions in the p53 3′UTR with miR-1285. In fact, knockdown of miR-1285 as well as expression of p53 3′UTR with mutated miR-1285 binding sites resulted in enhanced association of PTB with the 3′UTR and decreased association with Ago-2, which provides mechanistic insights of this interplay. Furthermore, to understand the physiological relevance, we curated single nucleotide variations (SNVs) in the p53 3′UTR, at the miRNA binding sites from literature. We investigated the effect of these SNVs on the 3′UTR mediated regulation of p53 expression by using reporter gene constructs containing wild type 3′UTR (Fluc-3′UTR WT) or 3′UTRs harbouring individual SNVs (Fluc-3′UTR SNV 93/287/737/806) in H1299 and A549 cells. SNV806 displayed highest reporter activity compared to the WT. Interestingly, *in vitro* experiments in the current study indicates that PTB binding to the 3′UTR with SNV 806 is higher compared to WT 3′UTR, thus suggesting a possibility of miR-1285 and PTB having common binding regions. Taken together, the results provide a plausible molecular basis of how the interplay between miRNAs and the PTB protein at the 3′UTR can play pivotal role in fine tuning the expression of the two p53 isoforms.

Human cancer cells are often associated with a widespread decrease in miRNAs, which shows a crosstalk between p53 tumour suppressor pathway and miRNA regulation system. It is known that the p53 isoforms are differentially regulated and their differential expression leads to differential downstream target gene expressions, which can be a direct regulation or regulation mediated by miRNAs. The transcription of some pri-miRNAs is regulated by p53 through binding to consensus sites in their promoters. Also, p53 modulates miRNA processing through interaction with Drosha-p68 complex. miRNAs can target various transcripts, so they are involved in diverse processes such as cellular differentiation, metabolism and cell proliferation. Role of full length p53 in controlling expression of miRNAs is established but the role of Δ40p53 in regulating the p53 responsive miRNAs was not known. So here we have investigated the effect of differential expression of the two isoforms individually or both the isoforms together on the downstream miRNAs. Out of several differentially regulated miRNAs some miRNAs were found to be commonly regulated by both the isoforms individually and with their combination. However, out of these some were unique to the individual isoform. In order to understand the functions of Δ40p53 alone the miRNAs either uniquely regulated or the ones which showed the maximum fold change under Δ40p53 expression were short-listed for further investigation. Results from the microarray of the miRNA showed that there are different miRNAs whose expressions are regulated under Δ40p53 expression. The network analysis for Δ40p53 revealed its involvement in different cellular functions. Δ40p53 upregulated miRNAs which are involved in pathways viz. cell cycle regulation, apoptosis, cell proliferation, senescence etc. Interestingly Δ40p53 showed antagonistic regulation of miR-186-5p as compared to the dataset obtained with either p53 alone or the combination of both the isoforms. Also this miRNA is known to be involved in cell proliferation, senescence and cell cycle arrest pathways. Hence we pursued miR-186-5p for further characterization under Δ40p53 expression. In this study we focused on miR-186-5p mediated effect of Δ40p53 in cell proliferation. One of the established targets of miR-186-5p is *YY1*, which is also known to be involved in cell proliferation. Our results showed significant decrease in *YY1* mRNA levels under the expression of Δ40p53. Further assays with anti-miR-186 established the interdependence of Δ40p53−miR-186-5p−YY1−cell proliferation. This unravels that Δ40p53 can also regulate cellular fate independent of p53FL.

Furthermore, p53 and Δ40p53 are known to be deregulated in different stress conditions like DNA damage, endoplasmic reticulum stress, oncogene-induced senescence and cancer. We have explored the effect of nutrient-deprivation mediated translational regulation of p53 mRNA using glucose depletion as a model system. We found scaffold/matrix attachment region-binding protein 1 (SMAR1), a predominantly nuclear protein is abundant in the cytoplasm under glucose deprivation. SMAR1 knockdown decreased p53 IRES activity in normal conditions and under glucose deprivation. We also observed concomitant effect of SMAR1 knockdown on the p53 and Δ40p53 target genes involved in cell-cycle arrest, metabolism and apoptosis. In addition, rescue experiments *ex vivo* shows that the induction of p53 isoform levels on nutrient deprivation is reversible and also their targets show similar reversal in their mRNA levels. This study provides a physiological insight into the regulation of this critical tumour suppressor in nutrient starvation and also the downstream transcriptional targets. Interestingly, we also observed that SMAR1 can interact with 3'UTR with binding sites common to PTB, which results in interplay between the two proteins *in vitro*. Individual knockdown of these proteins decreased the p53 expression but silencing of both proteins together showed further decreases in the expression. These results suggest yet another interplay involving PTB with SMAR1 at the 3'UTR under different stress conditions.

Taken together this study unfolds complex mechanisms by which p53 and Δ40p53 are regulated in different stress conditions; DNA damage and glucose starvation by proteins like PTB and SMAR1 respectively. This study also indicates that for the fine tuning of the regulation of p53 isoforms interplay between protein and miRNAs is required. Differential expression of miRNAs under p53 isoforms expression tells us the importance of these isoforms in regulation of miRNAs. It also contributes to the novel role of Δ40p53 in regulating miRNAs independent of p53 and hence consequent changes in cellular fate.