An uncharacterized function of human P53 protein in transcription elongation

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Ph.D. thesis summary

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Szeged
2017

Introduction

In eukaryotic cells, RNA polymerase II (RNAPII) is responsible for the transcription of protein coding genes and several small nuclear RNAs. The C-terminal domain (CTD) of RPB1, the biggest subunit of the RNAPII, is highly conserved amongst eukaryotes. It consists of a Tyr-Ser-Pro-Thr-Ser-Pro-Ser heptapeptide repeats. The phosphorylation state of Ser and Thr amino acids is dynamically changed during transcription catalyzed by kinase and phosphatase enzymes. At transcription initiation, the CDK-7 subunit of TFIIH complex phosphorylates the Ser5 amino acids, while in the beginning of transcription elongation, the CDK-9 subunit of P-TEFb complex phosphorylates the Ser2 amino acids of the CTD heptapeptides. Several types of stress, among them DNA breaks result in transcription blockage. In these situations, the cell should rearrange its macromolecular processes to allow access for DNA repair factors. In the processes of transcription-coupled DNA repair, transcription-coupled nucleotide excision repair (TC-NER) is involved. A possible outcome of a transcription block could be the polyubiquitylation-mediated degradation of RPB1, which helps the NER factors access the sites of DNA lesions.

A major regulator of events following DNA damage, is P53, which can activate different signaling pathways, thereby facilitating the restoration of the broken DNA or upon serious damages, activating apoptotic pathways.

The p53 protein has been reported to function during transcription elongation by binding to the elongating RNAPII. In *Saccharomyces cerevisiae,* it has been shown that P53 associates with coding regions of RNAPII-transcribed genes, which are not direct targets of it. In this heterologous system, the human P53 expressed in yeast interacts with yRNAPII through its DNA-binding domain. In addition, previous data from our laboratory showed that Dmp53 (*Drosophila melanogaster* p53) was localized at transcriptionally active regions on the *Drosophila* polytene chromosomes and that the phosphorylation state of RPB1 CTD influenced its localization. In accordance with these observations, we setup a model, that the P53 and the transcriptionally active RNAPII co-traverse on the transcriptionally active DNA regions. Upon transcription elongation block, P53 has a role in the ubiquitin-dependent proteasomal degradation of RNAPII.

These observations highlight a mechanism by which transcription blockage that occurs due to different types of DNA damage could be resolved. In my thesis, I present the experimental data supporting this model.

Aims

- Studying the changes in P53 and RNAPII protein level and their subcellular localization upon transcriptional elongation block

- Investigating whether the P53 is present at transcriptionally active gene regions

- Analyzing the binding of P53 and RNAPII at gene regions, which are not direct targets of it under normal conditions and upon transcriptional elongation block

- Studying the interaction between P53 and RNAPII under normal conditions and upon transcriptional elongation block

Methods

- Tripan blue staining to investigate the toxicity of ActD on U2OS cells
- ActD treatment on U2OS cells to block the transcription elongation
- MG132 treatment on U2OS cells to block the proteasome
- Calpain V inhibitor treatment on U2OS cells
- Immunocytochemistry staining to study the subcellular localization of P53 and RPB1; and also the co-localization of P53 and γH2AX, S2P RPB1 and γH2AX, S5P RPB1 and γH2AX
- Western blot analysis to detect the changes in P53 and RPB1 protein levels upon ActD treatment
- Analysis on previously reported ChIP-seq data to reveal whether the P53 and RPB1 bind to the same gene regions
- Chromatin immunoprecipitation experiments to study whether the P53 binding could be observed at transcriptionally active gene regions, which are not direct targets of it
- Co-immunoprecipitation to examine the interaction between P53 and RPB1
- siRNA transfection for P53 silencing to study the role of P53 in the ubiquitylation of S2P RPB1 upon transcription elongation block

- Pull-down of poly-ubiquitylated proteins to determine that the ActD induced transcriptional block has any effects on the ubiquitylation of RPB1
- Propidium-iodid staining for FACS analysis to investigate whether ActD has any effects on the cell cycle of U2OS cells

Results

1. Following DNA damage P53 acting at checkpoints could arrest cell cycle progression and could also activate apoptosis. In order to examine whether ActD induced transcription blockage resulted in cell cycle arrest of programmed cell death we treated the cells with low (5 nM) or high (200 nM) concentration of ActD and we checked the cell cycle defect with cell sorting. Following 200 nM ActD treatment, we detected a decreased cell number in G1 phase and an increased cell number in G2 phase, while we did not observe changes in the S phase. The accumulation of cells in the G2 phase suggests a cell cycle arrest in G2 phase, which explains the reduction in the cell number in G1 phase.

2. ActD treatment resulted in increased level of P53 and decreased level of RPB1. We found that the total P53 level was increased both upon 6 and 24 h of 5 and 200 nM ActD treatments. On the contrary, we detected Ser15-phosphorylated P53 only upon 200 nM ActD treatment, which supports that this post-translationally modified form of P53 is present only following transcription elongation blockage induced DNA double-strand breaks. On the other hand, RPB1, as well as the S5- and the S2-phosphorylated RPB1 levels were reduced mainly 24 h after high concentration of ActD treatment. These

results indicate that P53 is stabilized, while RPB1 may be degraded upon transcription elongation blockage.

3. Our results also demonstrated that P53 co-localizes with S2- and S5-phosphorylated (S2P and S5P) forms of RPB1 upon high concentration of ActD treatment. Interestingly, 24 h after high concentration of ActD treatment, S2P and S5P RPB1 co-localize with P53 at discrete nuclear foci, suggesting strong S2P RPB1-P53 and S5P RPB1-P53 interactions at locations where the DNA damage caused transcription blockage. We found that neither γH2AX and S2P RPB1 nor γH2AX and S5P RPB1 co-localized supporting the hypothesis that transcription is inhibited where DNA repair takes place. After 6 and 24 h ActD treatment, γH2AX and p53 co-localize in discrete nuclear foci, marking the sites where active DNA damage repair takes place. We found that P53 interacts both with the initiating (S5P) and elongating form (S2P) of RPB1 under normal conditions and also upon ActD treatment. We concluded that the DNA damage response may result in complete transcriptional silencing at the DNA break sites.

4. Our results indicate that P53 binds to transcriptionally active gene regions upon transcription elongation blockage. By the re-analysis of already existing ChIP-seq data, we could classify genes into three distinct clusters depended on the occupancy of P53 at their different regions. Cluster 1 consists

of the group of genes that show high P53 occupancy at the transcription start site. Cluster 2 genes show elevated P53 binding along the transcribed units. In case of Cluster 3, P53 could not be detected. These data suggest the existence of a correlation between P53 occupancy and gene expression level: P53 localizes on those genes, which show higher expression rate. Since ChIP-seq data indicated that P53 was localized at specific genes, we validated the binding of P53 at specific gene regions (promoter, gene body and 3'UTR) of selected groups of genes, which included: *ActB* (Cluster 1), *Cdk12* (Cluster 1), *Brat1* (Cluster 1) and *Sdcbp* (Cluster 2). At most of the examined regions of randomly chosen 4 non-direct P53 target genes, P53 occupancy was increased upon 6 and 24 h transcription elongation blockage. On the other hand, RPB1 occupancy was reduced 6 h after ActD treatment and then restored after 24 h. *ActB*, however, behaved differently since P53 and RPB1 occupancies showed similar distributions on this gene.

5. We also investigated whether P53 played role in the removal of RNAPII by regulating its ubiquitylation. 6 h after ActD treatment, the level of the ubiquitylated RPB1 was remarkably increased, while 24 h after the treatment, the amount of ubiquitylated RPB1 was reduced compared to the 6 h samples in the presence of P53. We detected a decreased

amount of ubiquitylated S2P RPB1 following 6 and 24 h of transcription elongation blockage in cases when P53 expression was silenced. Additionally, in the absence of P53 S2P RPB1 level was higher 24 h after ActD treatment, than it was in the presence of P53 under similar condition. These results highlight a mechanism by which the ubiquitylation of S2P RPB1 is somehow mediated by the P53 protein upon transcription elongation blockage. In accord with the results of the chromatin immunoprecipitation experiment, these data suggest that 6 h after transcription elongation blockage induction P53 plays a role in the ubiquitylation of the stalling RNAPII to allow access for the repair factors to the damaged DNA.

6. By further experiments we attempted to reveal whether RPB1 ubiquitylation implicates its proteasomal degradation upon transcription elongation blockage. We treated the cells with MG132 proteasome inhibitor and measured the RPB1 protein level upon ActD treatment. We found that the levels of both RPB1 and its transcriptionally active S2P and S5P forms remained nearly unchanged upon ActD treatment. In contrast to that, the protein levels of P53 and also its Ser-15 phosphorylated form were reduced 24 h after ActD and MG132 treatment. These results support a hypothesis that upon stress conditions RPB1 is degraded by the 26S proteasome. On the other hand, the reduction in P53 protein level during proteasome inhibition

could be the result of a proteasome-independent degradation of P53 protein. We hypothesized that P53 was degraded by Calpains and tested the change in P53 protein level upon ActD-induced transcription blockage in the presence of Calpain inhibitor. We found that the P53 turn-over was unaffected since neither the unmodified nor the Ser-15 phosphorylated P53 could be detected 24 h after the treatments. Based on these findings, the reduced P53 protein level observed upon proteasome inhibitor treatment is not the consequence of its degradation by Calpain proteases, rather a still unknown mechanism could influence the P53 protein level in the cells in case of proteasome inhibition.

In summary, our data highlight that P53 can be localized to different regions of transcriptionally active genes, which are not direct targets of it. P53 can presumably bind to those regions through its interaction with RNAPII. Our results indicate that P53 has a role in transcription elongation during transcription blockage. We also showed that P53 plays role in the ubiquitin-dependent proteasomal degradation of RPB1 upon transcription elongation blockage. Consequently, our results suggest that upon transcription elongation blockage, P53 has a role in the removal of the stalled RPB1 from the chromatin to allow access for the repair factors to the damaged DNA. The results of my

PhD work therefore provide a better understanding of the feed-back mechanisms by which cells can resolve the transcription blockage upon different stress conditions.

Publications

MTMT azonosító: 10049721

Article related to this Ph.D thesis:

Human p53 interacts with the elongating RNAPII complex and is required for the release of actinomycin D induced transcription blockage, **Barbara N. Borsos**, Ildikó Huliák, Hajnalka Majoros, Zsuzsanna Ujfaludi, Ákos Gyenis, Peter Pukler, Imre M. Boros, Tibor Pankotai, *Sci. Rep.* **7**, 40960; doi: 10.1038/srep40960 (2017)
***SCIENTIFIC REPORTS* 7:** p. 40960. (2017)
IF: 5,228

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Total impact factor: 15,345

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DNS-hibák és ami mögöttük van. Borsos B, Majoros H, Újfaludi Zs, Páhi Z, Pankotai T, ÉLET ÉS TUDOMÁNY LXXI:(6) pp. 180-182. (2016)
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