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In silico analysis reveals p53 responsive ribosomal protein and eukaryotic translation initiation factor genes: a route for p53 to influence ribosome assembly and translation

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Abstract
The ribosome is the protein synthesis machinery of cells, which provides them with essential proteins. An increase in ribosomal content and protein synthesis is evidenced in cancers, most notably in cancers with a loss of p53. However, very little is known as to how the p53 or its family influences the ribosome and translation. Interestingly, in this study, a large number of genes encoding ribosomal proteins (RP) and eukaryotic initiation factors (eIFs) that are intrinsic to ribosome assembly and translation initiation, respectively, were found to possess p53 responsive elements (REs) in their regulatory region. To understand whether p53 or its family influences the ribosome, the expression patterns of the RE-containing RP and EIF genes were examined in a p53 family target microarray database. Many RP and EIF gene expressions were found to be deregulated during the p53 or its family gene overexpression. These results indicate that ribosome assembly and its functions respond to p53 activity. The overall influence of p53 was found to be repressive on RP and EIF gene expression, which led the author to hypothesize that p53 controls the formation of ribosomes and the translation of mRNA into proteins. The study brings to light a regulatory pathway through which the p53 family influences the ribosome with great implications on the cell fate.

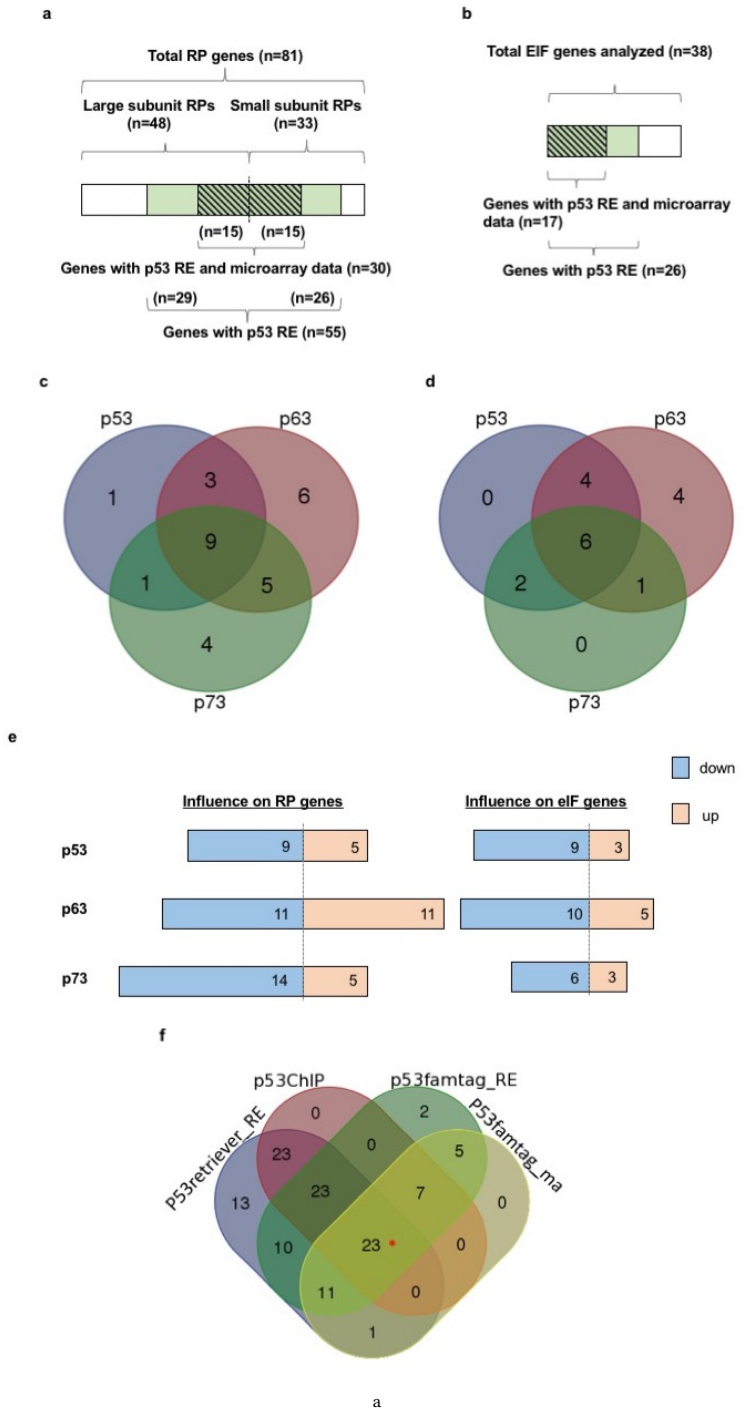
Introduction
Ribosomes are central to cellular processes because of their role in translating mRNA into proteins. The assembly of the ribosome from several ribosomal proteins (RPs) and ribosomal RNAs (rRNA) and its subsequent association with eukaryotic initiation factors (eIFs) and mRNA is a prerequisite for translation [1]. A balanced production of RPs, rRNAs and eIFs influences translation [2] [3] and determines cell fate [4] [5]. Hence, it is important to know how the transcription of ribosomal and translational genes is regulated to ensure sufficient levels of RPs and eIFs for the assembly and function of ribosomes respectively.
In some cancers, the loss of p53 is correlated with an increase in ribosomal content and translation, which suggests that accelerated protein synthesis is a major contributor to oncogenesis during p53 loss [6] [7] [8] [9] [10]. Recently, an experimental knockdown of p53 in zebrafish embryos increased the ribosomal content, but the mechanism through which this is achieved is yet to be known [11]. The transcription factor p53 controls the expression of an exhaustive list of target genes, which are involved in cell cycle arrest and apoptosis, and thus acts as a tumor suppressor that regulates cell proliferation [12] [13]. On the other hand, the p53 family genes (p63 and p73) are known for their role in transcription of genes involved in development and differentiation [14]. The transcriptional targets of p53 family have responsive elements (REs), which are consensus sequences of nucleotides that are usually distributed on promoter or intronic regions. In its tetrameric form, the p53 binds to REs on target genes and influences their transcription. It is noteworthy that p53 REs are distributed throughout rRNA gene cluster (RGC) in the genome (http://p53.iarc.fr/TargetGenes.aspx) and that p53 controls rRNA transcription [15] [16] [17]. Although the rRNA genes have been implicated as p53 target genes, the RP genes or EIF genes as p53 targets have remained unappreciated so far. Therefore, little is known as to how the p53 or its family regulates the ribosome.
In this study, I have systematically probed the RP and EIF genes for the presence of p53 REs. The influence of p53 and its family on the expression of those RP and EIF genes that

have REs is reported here. Overall, the present study indicates that p53 exerts a large-scale influence on ribosome content and function through ribosomal and translational gene regulation.

Objective

The main objective of this study is to determine the influence of the p53 family on ribosomal content and translation.

Figure 1



a

Figure Legend

Figure 1. Influence of p53 on ribosomal and translational genes.

(A) A representative image illustrates a total of 81 RP genes, where 48 RPs are from the large subunit and 33 RPs are from the small subunit of the ribosome. The consensus RE was present in a majority, 55 RP genes, where there are 29 large subunit RPs and 26 small subunit RPs. Among these, the microarray data is available for 30 RPs (bar with slant lines), where 15 RPs belong to the large subunit and 15 RPs belong to the small subunit.
(B) A representative image illustrates a total 38 EIF genes, where 26 EIFs have the consensus RE, but the microarray data was available only for 17 of these EIFs.
(C) A Venn diagram illustrates the influence of p53, p63 and p73 genes on RP gene expression pattern in the microarray data. Here 9 genes respond to p53, p63 and p73; 3 genes respond to p53 and p63; 5 genes respond to p63 and p73 and 1 gene responds to p73 and p53. Some RP genes exclusively respond to p53 (n=1), p63 (n=6) and p73 (n=4).
(D) A Venn diagram illustrates the distribution of the influence of p53, p63 and p73 genes on EIF gene expression pattern in the microarray data. Here 6 genes respond to p53, p63 and p73; 4 genes respond to p53 and p63; 1 gene responds to p63 and p73; 2 genes respond to p73 and p53 and 4 genes respond to p63 alone.
(E) A representative image illustrating the effect of each p53 family on the overall RP gene and EIF gene expression patterns. The numbers inside bars represent the number of genes downregulated in the blue bar or upregulated in the orange bar.
(F) A Venn diagram depicting the overlap across different datasets, as deduced from the supplementary table 1 (p53 retriver derived REs, p53retriver_RE; p53 ChIP-seq data, p53ChIP; p53famtag derived REs, p53famtag_RE and p53famtag derived microarray data, p53famtag_ma) for both RP and EIF genes combined. Total of 30 genes (23 genes that overlap all the 4 datasets, which is indicated by a "*" in red and 7 genes that overlap 3 datasets from p53ChIP, p53famtag_RE list and p53famtag_ma) strongly suggest that their transcription could be regulated directly by p53 binding.

Data collection

List of RP and EIF genes

The list of human RP genes used for the query in this study was derived from RP gene database [http://ribosome.med.miyazaki-u.ac.jp/] [24] and the list of EIF genes for the query was obtained from a review by Spilka et al., 2013 [19], including five more EIFs, the EIF4E2, EIF4E3, EIF4G2, EIF4G3 EIF5A1 and EIF5A2 [17]. The list of genes used for query also contains the 8 RP-like protein genes and 1 EIF-like protein gene, among which the p53 responsive genes with microarray data are highlighted in gray in the supplementary table 1. However, these were not considered in the analysis for figure 1.

Data from p53famtag

Human RP genes and EIF genes were probed for the presence of p53 responsive elements (RE) by using the p53famtag, an open resource tool for search and prediction of p53 family target genes [p53famtag.ba.itb.cnr.it/] [18]. The list of genes obtained above was used as a query in the p53famtag to retrieve information related to the presence of RE and microarray data. Query genes, which have at least one RE in their regulatory region and whose expression is altered at 6 h or at 24 h when a minimum of one p53 family gene is overexpressed, were considered to obtain the microarray data from p53famtag database. The microarray data curated in the p53famtag is from HEK Flp-In T-Rex-293 stable isogenic cell lines overexpressing above mentioned p53 family genes. This database curates only statistically significant fold change data. Therefore, non-statistically significant data, which indicates expression values with PPDE <0.995 were not used in the study. The p53famtag was found to be resourceful in this study because of the provision to detect REs on the genes of interest and availability of corresponding microarray data during various p53 family overexpressing conditions in the same type of cell lines. The TAp63α and ΔNp63α are p53 family members that belong to p63 group and TAp73α and TAp73β are p53 family members that belong to p73 group. Therefore, for a convenient understanding of the data used in the present study, either the TAp63α or ΔNp63α overexpression is referred to as p63, whereas either the TAp73α or TAp73β expression is referred to as p73. However, a detailed data corresponding to each p53 family member overexpressing conditions obtained for the present study from p53famtag database is listed in supplementary table 1.

p53 retriever detected RE data

The p53 retriever detected RE data was collated from a recently published study of whole genome cartography of p53 REs [20]. Enrichment of REs on the RP and EIF genes used for the query in the p53famtag were again checked with the data listed in this recent study. The REs detected by the p53 retriever belong to grades of REs considered as likely binding targets (grade 2, poor p53 binding RE; grade 3, low p53 binding RE and grade 4, moderate p53 binding RE) and unlikely binding targets (grade1).

p53 ChIP-seq data

The p53 ChIP-seq data was collated from a recent study of genome-wide chromatin association of p53 [21]. The ChIP-seq data of p-value <0.05 was considered in this analysis, where <0.05 is indicated by a "*", <0.01 is indicated by a "**", <0.001 is indicated by a "***" and a p-value of very high significance is indicated by p-value written next to "****" (Suppl. Table 1). The data, which meets the above p-value criteria was obtained from any of the 4 different conditions [Mock, nutilin3A, reactivation of p53 and induction of tumor cell apoptosis (RITA) and 5-fluorouracil treatment (5-FU)] from the referenced study [21].

Note: The Venn diagrams were drawn using http://bioinformatics.psb.ugent.be/webtools/Venn/

Results & Discussion

The RP and EIF genes were examined for the presence of RE, which is a consensus sequence of nucleotides for the binding of p53 or its family of transcription factors (http://p53.iarc.fr/TargetGenes.aspx) [18]. Next, the expression pattern of numerous RP and EIF genes, which possess the RE were then obtained from p53famtag microarray database to understand the influence of p53 and its family on corresponding RPs and eIFs. The data discussed here is from HEK-293 cell lines that individually overexpress the p53 families (p53, p63 and p73) [18].

Influence of the p53 family on RP gene expression

A majority, constituting 2/3 of total RP genes (55 out of 81 genes) were found to have the p53 RE according to the p53famtag (Fig. 1A). Although the data was not available for all RP genes that have REs, the expression profiles could be obtained for 30 RP genes (Fig. 1A and Suppl.Table 1), which constitute above one-half of RP genes that have the RE and above 1/3 of total RP genes. Among these, 9 RP genes (RPL7A, RPL27, RPL28, RPL36A, RPL37, RPL37A, RPS17, RPS25, and RPSA) respond to all the three p53 family gene expression (p53, p63 and p73), whereas 9 other RP genes (RPL9, RPL13, RPL34, RPS15, RPS18, RPS20, RPS23, RPS27A and RPS30) respond to any 2 p53 family gene expression (Fig. 1C and Suppl. Table 1). These two groups together constitute 3/5 of all the RE-containing RP genes that have microarray data and 1/5 of the total RP genes. Here, the presence of uniform expression trends, either only upregulations or only downregulations of RP genes, across different p53 family member overexpression conditions were found (Suppl. Table 1). A majority, 18 out of 20 genes, follow this trend across different p53 family member overexpression conditions, which is an unambiguously genuine indicator of p53 family influence on the ribosome. Two RP genes were found to be exceptions to the above trend (highlighted by yellow in Suppl. Table 1). Besides, several RP genes respond specifically to a certain p53 family only (Fig. 1C). Nevertheless, the p53 family was found to exhibit profound influence on the expression of many RP genes. Among the RP genes with available microarray data, 14 RP genes respond to p53, 24 RP genes respond to p63 and 19 RP genes responded to p73 (Fig. 1E). The influence of p53 and p73 on the RP gene expression was found to be largely repressive, where 9 out of 14 RP genes were downregulated in the p53 overexpressing cell lines and 14 out of 19 RP genes were downregulated in p73 overexpressing cell lines (Fig. 1E and Suppl. Table 1). The observation that p53 represses a large number of RP genes' expression corroborates the hypothesis that loss of p53 gives rise to ribosomal contents in cancer [16] [17] [7] [8]. Meanwhile, it was noted that many RP genes were deregulated by p63 overexpression, where 11 RP genes were downregulated and 11 RP genes were upregulated. The p63 and p73 are p53 family genes that are chiefly involved in development and differentiation processes [14]. Their influence on the ribosome and its function is not yet known. In this study, it was found that the p53 and its family largely exhibit a negative influence on the ribosome content. The comprehensive list of RP genes (Suppl. Table 1) that respond to any p53 family expression retrieved from this analysis indicates that p53 and

its family exerts a greater influence on ribosome structure and assembly than previously conceived.

Influence of p53 family on EIF gene expression

The initiation of translation, which involves the eIFs, is a rate-limited step in protein synthesis that provides necessary proteins for cell cycle, growth, development and differentiation [4] [7] [17] [19]. A large fraction, constituting 2/3 of total EIF genes (26 out of 38 EIF genes) have the p53 RE according to the p53famtag (Fig. 1B). The microarray data could be accessed for 17 out of the 26 EIF genes that have p53 RE, which constitute nearly 2/3 of EIF genes that have RE and also constitute 2/5 of total EIF genes (Fig. 1B). Among these, 12 EIF genes respond to p53, 15 EIF genes respond to p63 and 9 EIF genes respond to p73 (Fig. 1D and E; Suppl. Table 1). 6 out of 17 EIF genes (EIF2S3, EIF3D, EIF4B, EIF5, EIF5A1 and EIF5A2) respond to the expression of all three p53 families (p53, p63 and p73) (Fig. 1D and Suppl. Table 1). Excluding few EIF genes (EIF3E2, EIF3E3, EIF3I and EIFG2), the rest respond to any two p53 family genes (Suppl. Table 1). Among these genes, which respond to any two or more p53 family genes, the expression trend for any given EIF gene was similar across different p53 family overexpressing conditions (indicated by orange and blue color rows in Suppl. Table 1). Here, a majority, 12 out of 13 genes, follows this trend, which is unambiguously a genuine indicator that p53 family influences the translation initiation. An exception to this trend, the EIF3A, was repressed by p53 but enhanced by p63. However, the similarity in the expression trends of large numbers of EIF genes, across different p53 family overexpression, indicates a strong influence of p53 family on these translation initiation factors. Interestingly, a majority, 9 out of 12 EIF genes that have p53 RE and respond to p53 overexpression, were found to be downregulated (Fig. 1E and Suppl. Table 1). The influence of p63 and p73 on the overall EIF gene expression was also found to be repressive, where 10 out of 15 EIF genes were downregulated in the p63 overexpressing cell lines and 6 out of 9 EIF genes were downregulated in p73 overexpressing cell lines (Suppl. Table 1). Altogether, the EIF genes were found to be largely repressed by p53 and its family (Fig. 1E and Suppl. Table 1). These results strongly suggest that p53 and its family controls the initiation of protein synthesis by negatively regulating the expression of EIF genes, which are involved in translation initiation.

It is established that many of these EIFs, which are downregulated by p53 family in this study, are in fact overexpressed in several types of cancers: eIF2α is overexpressed in bronchioloalveolar carcinoma, gastrointestinal carcinoma and melanocytic neoplasms; eIF3A is overexpressed in small cell lung cancer, squamous cell carcinoma, adenocarcinoma, breast cancer, gastric carcinoma, colorectal cancer and melanocytic neoplasms; eIF3D is overexpressed in gastric carcinoma; eIF3I is overexpressed in hepatocellular carcinoma; eIF5 is overexpressed in ovarian serous carcinoma and eIF5A2 is overexpressed in ovarian cancer, colorectal cancer and bladder cancer [19] [17]. However, the p53 status in these cancers and the role of EIFs in causing these cancers is not clear. Therefore, in future studies, it would be interesting to probe whether the p53 depletion in these cancers causes aberrant EIF transcription, which increases the protein synthesis rates that eventually leads to oncogenesis. The EIFs that respond to p53 in this analysis (Suppl. Table 1) are highly essential factors in translation initiation [19]. Factors belonging to all the EIF complexes except EIF6 were found to be influenced by the p53 status (Suppl. Table 1), which demonstrates the extensive influence of p53 on the initiation of mRNA translation. By following these observations, it can be proposed that the negative regulation of these EIFs as a consequence of p53 activation decreases the initiation of protein synthesis.

To verify the reliability of this data, it was compared with two separate datasets, the REs detected by another tool, known as p53 retriever [20], and a p53 ChIP-seq data [21] (Fig. 1F representing the data from Suppl. Table 1). From this, it could be deduced that p53 REs are indeed enriched on 105 out of 119 genes (74 RP and 31 EIF genes) as detected by p53 retriever [20] and that p53 binds to at least 76 genes (51 RP and 25 EIF genes). Many RP and eIF genes, in which REs were detected by using p53famtag and p53 retriever, bind p53 in ChIP-seq data (Suppl. Table 1; [21]). Among these p53 binding genes, 30 genes (19 RP genes and 11 EIF genes) have valid microarray data from p53famtag database indicating that their transcription responds to p53. In fact, microarray data is available

for a total of 47 genes used in this study (Fig. 1F), out of which 30 genes bind p53 as said above, which strongly indicates that they are direct targets of p53 and the rest are either indirectly regulated under the control of direct p53 targets, or their ChIP data is yet to be explored systematically. Among these 30 genes, 23 genes (12 RP genes and 11 EIF genes) overlap all the datasets discussed in the study and 7 genes overlap with all datasets except p53 retriever-derived RE (Fig. 1F). So far, all these data together corroborate the hypothesis that p53 transcriptionally controls the expression of many RP and EIF genes to influence the protein synthesis.

Considering the role played by p53 in determining the cell fate, it is not surprising that p53 exerts an influence on the most critical aspect of cell sustenance, the ribosome and translation. However, very few reports suggest that p53 impacts the cells through ribosome and translation. This is said to be achieved by influencing rRNA transcription and rRNA methylation that affects ribosome assembly and translation [10] [16] [17]. Beyond these aspects, the extent of p53 influence on the ribosome or translation is not known. Toward this, the present study provides enough *in silico* evidence that p53 regulates a large number of RP and eIF expression, which are involved in ribosome assembly and translation initiation, mostly in a repressive manner. The presence of RE on these genes and the available p53 ChIP-seq data further indicate that they are most likely to be influenced by p53 family's direct interaction with these genes. In any case, these observations explain why cancers with a loss of p53 have high ribosomal content and protein synthesis rates. Most importantly, this study corroborates the ongoing efforts to target the ribosome and translation in cancer therapy [22] [23], wherein this strategy might prove beneficial in cancers with a loss of p53. To completely comprehend the impact of the master regulator p53 on cells, it becomes necessary to unravel how p53 manipulates the assembly and function of a critical organelle, such as the ribosome. This *in silico* study determined that p53 and its family moderates the ribosome and translation via RPs and eIFs. This data is an important step that can pave the way for several studies on pathways that converge onto ribosomes from p53 or its family and diverge from ribosomes to impact the cells.

Conclusions

RPs are structural components of the ribosome and eIFs are factors that initiate protein synthesis. A large number of RP and EIF genes that have the p53 RE were found to be repressed by overexpression of p53 or its family genes. This leads me to propose that p53 exerts an influence on ribosome by negatively modulating the availability of RPs and eIFs for ribosome assembly and translation respectively. This study is expected to ensue a broader understanding of aspects that govern how p53 restrains the ribosome to determine cell fate.

Limitations

The main limitation of the study is the *in silico* nature of data, which requires further experimental validation. However, meticulously following the influence of p53 family on each gene in the list might require several separate studies, which is a daunting task. Therefore, despite the *in silico* nature of data presented here, it provides a full-scale snapshot of ribosomal gene regulation during p53 activity that serves as a reference point for future studies. Another limitation of this study is that the influence of p53 family loss on the same set of genes is not analyzed due to a lack of such data in the same database. Finally, it has to be mentioned that these data are obtained from 3 independent studies. Also, microarray and ChIP-seq dataset are from different cell lines. Despite this constraint, there is a high overlap of a significant number of genes across all datasets, which further corroborates the hypothesis under study.

Additional Information

Methods

Data collection

List of RP and EIF genes

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Note: The Venn diagrams were drawn using http://bioinformatics.psb.ugent.be/webtools/Venn/

Supplementary Material

Please see https://sciencematters.io/articles/201603000013.

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Ethics Statement

Not applicable.

Citations

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