The Regulation of the tumour suppressor p53 by ribosome biogenesis

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Aims:

Using RNAi to knockdown factors specific to the various stages of ribosome biogenesis we tried to answer the following:

1.Do late stage ribosome biogenesis defects result in p53 activation?

2. If activation occurs is this dependant on 5S RNP?

Introduction:

Shwachman-Diamond syndrome, 5q Syndrome and Diamond Blackfan anaemia are genetic diseases associated with ribosome, machines in cell that synthesis proteins, dysfunctions which occur during their production and cause activation of p53, a tumour suppressor. It has recently been shown by the Watkins lab that a large subunit assembly intermediate, 5S RNP (RPL5, RPL11 and the 5S rRNA) binds to and blocks the p53-suppressor MDM2 causing p53 activation when ribosome biogenesis is defective. At the moment it is unclear whether p53 is induced at all stages of ribosomal defects and if it were to is it using the same pathway?

Acknowledgements

Many thanks to Vacation Scolerships for funding this experience and to the Watkins lab for supporting me throughout my project.

Methods:

1. **Cell Culture:**
U20S cells are grown in DMEM medium containing 10% Fetal Calf Serum FCS, Penicillin/Streptomycin 10mg/ml at 37^{o}C and 5% CO_2.

2. Tr**ansfecrtion with siRNA:**
Lipofetamine RNAiMAX used to transfect siRNA (final concentration 20μM), into US02 cells, incubated for 48 hours before harvesting.

3. **Western Blotting:**
Protein samples were separated by SDS acrylamide gel electrophoresis and then transferred to a nitrocellulose membrane. After incubation with protein-specific antibodies, the signal was developed using ECL solution and Amersham Hyperfilm. Quantified using ImageQuant TL software, normalise results to the levels of Karyopherin and control sample.

References

1.Sloan, KE. Bohnsack, MT. Watkins, NJ. 2013. The 5S RNP couples p53 homeostasis to ribosome biogenesis and nucleolar stress. *Cell Reports.* 17, 5, (1), 237-47.

2.Freed, EF. Bleichert, F. Dutca, LM. Baserga, SJ. 2010. When ribosomes go bad: disease of ribosome biogenesis. Molecular BioSystems. 6, 481-493.

Results:

These figures show the results of both the single and double knockdowns:

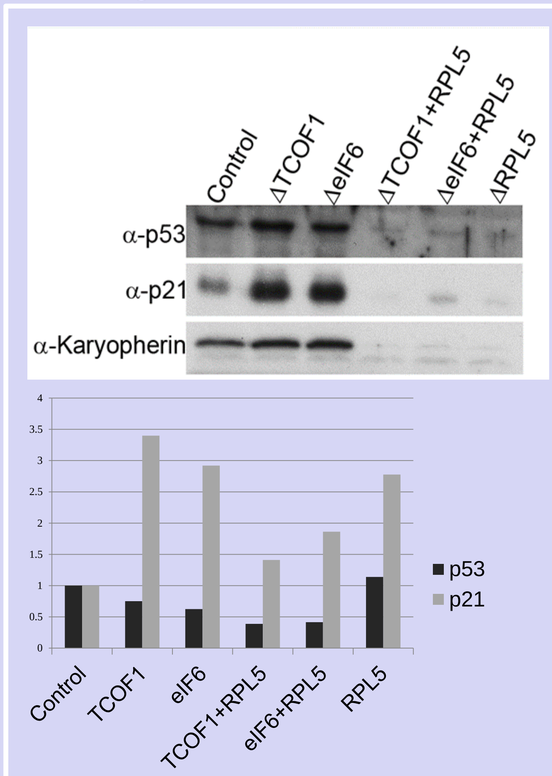

Figure 1. TCOF1 and eIF6 knockdown and double knockdown with RPL5 showing induction of p53 and p21.

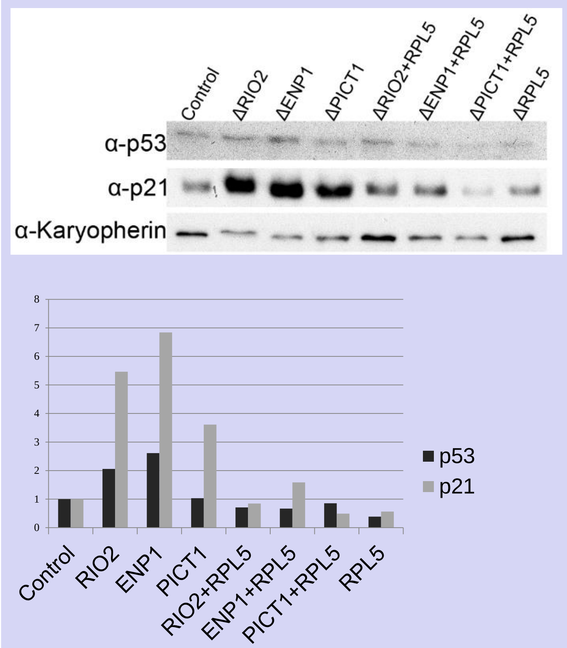

Figure 2. RIO2, ENP1, PICT1 knockdowns and double knockdowns with RPL5 giving induction of p53 and p21.

Discussion and Conclusions:

The preliminary experiments showed a general increase in p53 and p21 levels in the initial single knockdown, suggesting that there is in fact a p53 induction with late stage ribosome biogenesis as shown in the above figures.
However, also seen in both figures is that when RPL5 was co-depleted I saw a significant decrease in the levels of p53 and p21. Due to this decrease we propose that the late stage ribosome defects are in fact using the same pathway, through 5S RNP to induce p53.