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Introduction

Historically, all ribosomes within a cell or organism were assumed to be identical. They were viewed as molecular machines which indifferently synthesize whichever protein is encoded by the mRNA they currently contain. In recent years, however, evidence for heterogeneity among ribosomes accumulated. Depending on their composition, so called “specialized ribosomes” have been demonstrated to preferentially translate distinct subpools of mRNAs. Thus, the ribosome itself can act as a level of regulation of gene expression. In this work, we aim to investigate 1) whether specialized ribosomes arise during cellular senescence, 2) to characterize changes in the composition of ribosomes derived from senescent cells and 3) to establish structure-function relationships for these “senescent ribosomes”.

Induction of human dermal fibroblast senescence by doxorubicin exposure

Presence of senescent cells *in vivo* is associated with several age-related changes in the skin and other organs. Using human dermal fibroblasts (HDFs) we have established protocols to induce and confirm the senescent state of cells *in vitro*.

Nucleoli expand during cellular senescence

Nucleoli are sub-nuclear organelles and the sites of rDNA transcription and co-transcriptional processing of rRNA precursors. We and others recorded that nucleoli expand during cellular senescence. Quantification of images revealed a significant increase in nucleolar compared to nuclear area, suggesting potential alterations in ribosome biogenesis.

Senescent fibroblasts show elevated global translation

To test global protein translation which might be influenced by alterations in ribosome number and composition, we employed pulse-labelling of cells with O-propargyl puromycin (OPP). Indeed, senescent cells from three different donors showed elevated global translation levels compared to quiescent cells. Next, we aimed to elucidate why senescent cells upregulate bulk translation.

Maintenance of the senescence-associated secretory phenotype (SASP) requires increased protein production

Senescent cells secrete high levels of inflammatory cytokines and other factors, which cause damage to surrounding tissues. JAK/STAT inhibition suppresses this secretory phenotype and also blunts the upregulation of global translation in senescent cells.

Purified ribosomes from senescent cells show elevated translation compared to their quiescent counterparts

2'-O-ribose methylation sites on ribosomal RNA partially differ between cellular states

Differential rRNA methylation might explain the functional heterogeneity of ribosomes. To test this hypothesis, we quantified methylation levels of rRNA by Ribometh-seq. Thereby, we identified several differentially methylated sites in senescent compared to proliferating and quiescent cells. For most of the sites, almost complete methylation was detected in all three cellular states. However, we also identified several positions to be hypo- or hyper-methylated in quiescent and senescent cells in comparison to proliferating cells.

Summary

- Senescent human dermal fibroblasts show expanded nucleoli and higher global protein synthesis compared to quiescent cells, which is likely due to the synthesis of SASP-components.
- 2'-O-Methylation patterns change only moderately between cellular states. However, certain sites appear hypomethylated in proliferating (28S: G1303, G4588; 18S: C797, G867, C1272, G1447) and senescent cells (28S: C1327, C3680) compared to quiescent cells.
- Taken together, our data suggest that even subtle modifications of the ribosomal RNA might have profound and very specific effects on cellular physiology and contribute to the functional heterogeneity of ribosomes.