Ribonucleotide contamination of genomic DNA in ageing humans and zebrafish

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DNA damage in ageing

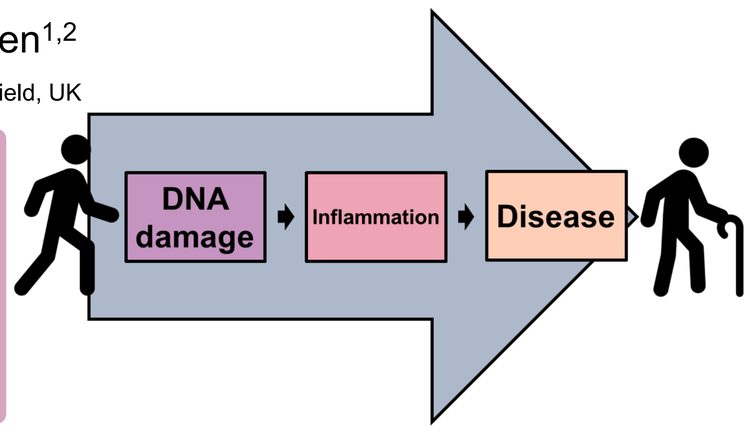
Ageing is broadly defined as the progressive loss of bodily functions that increase the possibility of death. This could be through a decreased ability to handle stress factors such as DNA damage, making genomic instability one of the hallmarks of ageing[1].

Single ribonucleotide insertion in disease

Ribonucleotides can be incorporated into the DNA by accident or by purpose during replication or repair. However, they are more reactive than DNA molecules, and can readily cause strand breakage in nuclear DNA, and likely in the mitochondrial DNA as well. Therefore, they have to be removed by enzymes such as RNaseH2. Loss of RNaseH2 function can cause Aicardi-Goutieres syndrome (AGS), a neurological inflammatory disease[2]. Further, in human fibroblasts it has been shown that there is a significant reduction in RNaseH2 in old humans compared to young[3].

Hypothesis

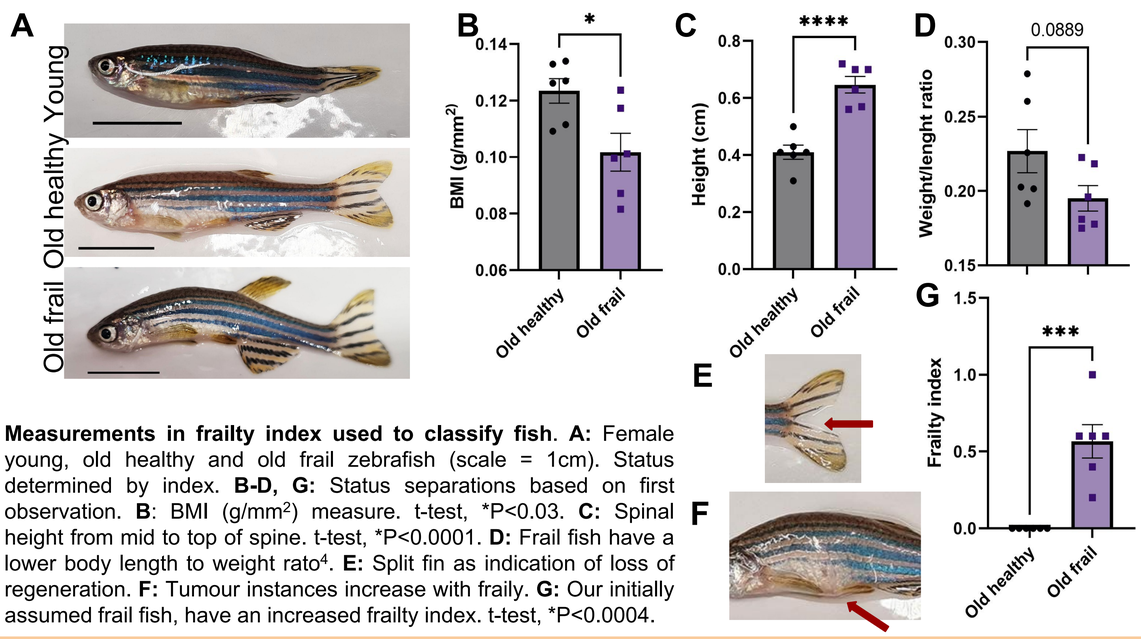
As we age, the ribonucleotide repair pathway declines causing an increase in RNA damage in the DNA. This activates an antiviral immune response causing inflammation, and promoting frailty.



Aims for the project

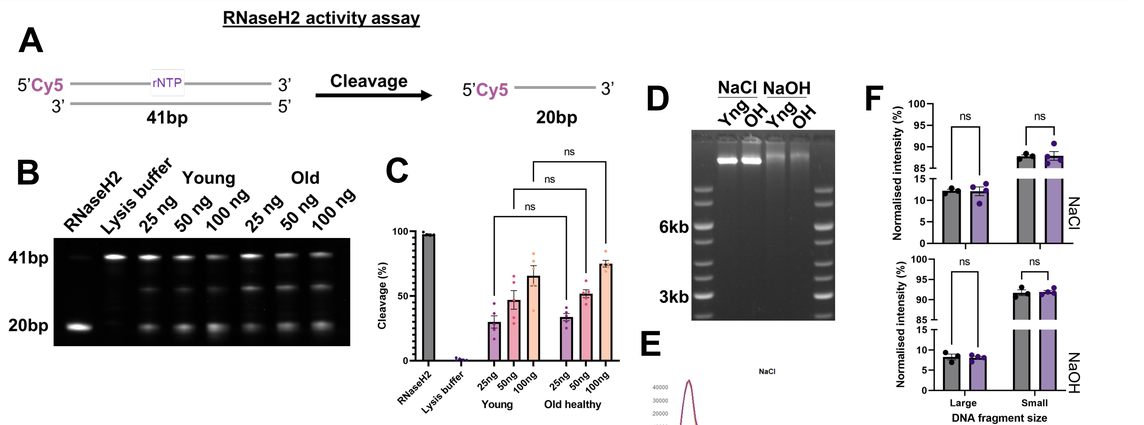
- Explore the role of ribonucleotide damage in ageing and frailty using zebrafish models, and data and blood samples from the CARE75+ study of frailty.
- Explore ribonucleotide removal in mitochondrial DNA in relation to ageing markers

1) Classifications of healthy and frail fish



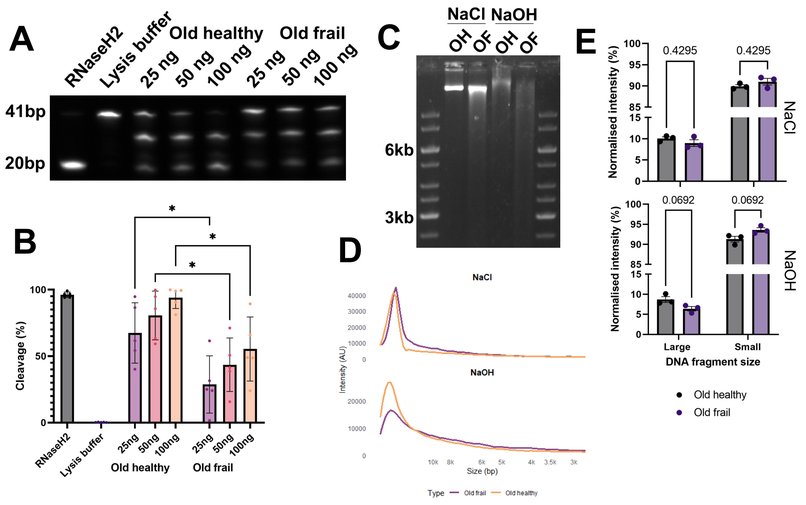
Measurements in frailty index used to classify fish. **A:** Female young, old healthy and old frail zebrafish (scale = 1cm). Status determined by index. **B-D, G:** Status separations based on first observation. **B**: BMI (g/mm^2) measure. t-test, *P<0.03. **C:** Spinal height from mid to top of spine. t-test, *P<0.0001. **D:** Frail fish have a lower body length to weight rato[4]. **E:** Split fin as indication of loss of regeneration. **F:** Tumour instances increase with frailty. **G:** Our initially assumed frail fish, have an increased frailty index. t-test, *P<0.0004.

2) No change in RNaseH2 activity in young and old fish



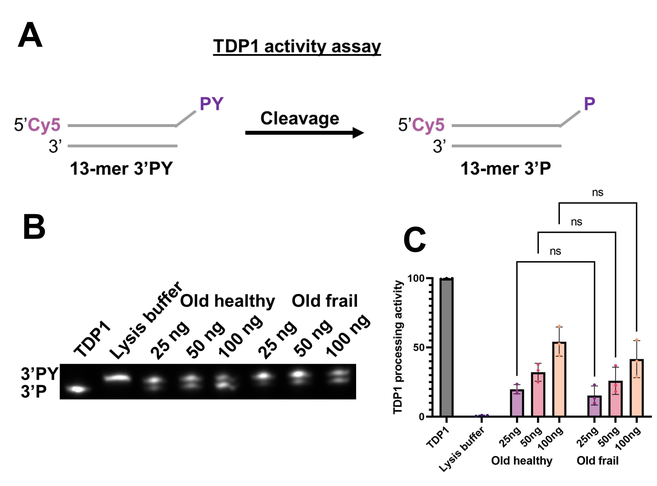
A: Protein lysate was incubated with Cy5 tagged dsDNA with a single ribonucleotide. **B:** No difference in ribonucleotide cleavage between young and old fish (fin protein). **C:** Quantification of cleavage product. One-Way ANOVA, (n=5). **D:** Alkaline assay showing no difference in DNA fragmentation after alkaline treatment. (Yng = young, OH = old healthy). **E:** Quantification of DNA fragment sizes. **F:** No significant difference in area under the curve for control or treated DNA. t-test (n=3).

3) Decrease in RNaseH2 activity in old frail fish



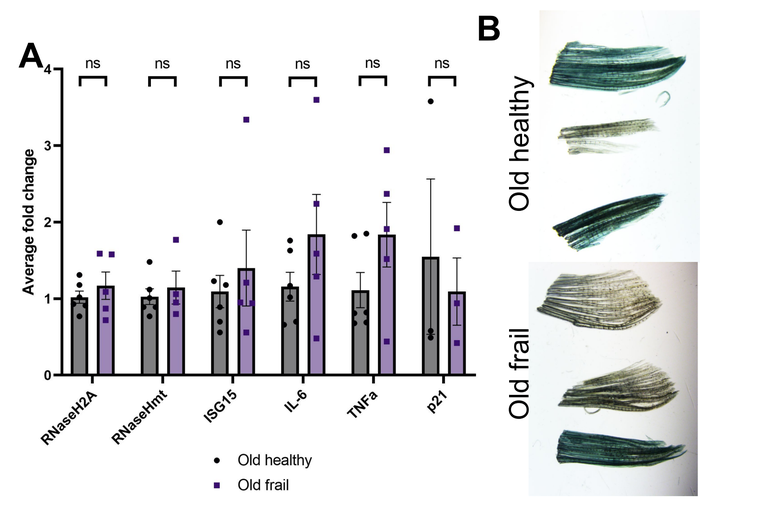
A: Old frail fish have a decreased ability to cleave ribonucleotides compared to old healthy fish (brain protein). **B:** Quantification of cleavage product. One-Way ANOVA, *P<0.02, (n=5). **C:** Alkaline assay showing increase in DNA fragmentation after alkaline treatment in old frail fish. (OH = old healthy, OF = old frail). **D:** Quantification of DNA fragment sizes. **E:** Trend showing decrease in large DNA fragments and increase in smaller DNA fragments in old frail fish upon alkaline treatment. t-test (n=3).

4) TDP1 activity is not affected with RNaseH2 activity



TDP1 is an enzyme involved in resolving top1-ccs on the DNA. This mechanism is not affected with frailty indicating RNaseH2 specificity. A: Protein lysate was incubated with Cy5 tagged dsDNA with a 3'phosphotyrosyl. **B:** No difference in TDP1 activity between young and old fish (brain protein). **C:** Quantification of TDP1 cleavage product. One-Way ANOVA, (n=3).

5) No change in RNaseH2 RNA level or inflammation



Decreased RNaseH2 activity in embryos cause increased inflammation. However, this might not be the case for frail old fish. A: qPCR of RNaseH2A and RNaseHmt show no difference in RNA level. No significant difference in inflammatory markers ISG15, IL-6, TNFα. No difference in senescence marker p21. t-test (n=6). **B:** β-galactosidase staining of pectoral fins support the no difference in p21 RNA expression showing no clear separation of senescence between the two statuses.

Conclusions

Genomic instability is one of the hallmarks of ageing. RNaseH2 is vital in the development, and a lack of RNaseH2 in zebrafish embryos lead to increased DNA damage, genome instability and inflammation.

There is a decrease in RNaseH2 with age in human fibroblasts, however, this does not seem to be the case for general ageing in zebrafish when considering our investigation. Although, when investigating healthy and unhealthy ageing, RNaseH2 activity does decrease, and this seems to be specific to RnaseH2 as the enzymatic activity of TDP1 is not affected. RNA levels of RNaseH2 is not affected, suggesting the decrease in activity might be at protein level. Potentially, it could be a decrease in RNA damage signalling, resulting in decreased repair.

Future work

- We have started to cluster disease progression in the CARE75+ study of community dwelling frail people over the age of 75, and aim to analyse the results in relation to the involvement of DNA damage in ageing and frailty.
- Further, we aim to carry on the same RnaseH2 investigations in human blood plasma samples from the CARE75+ study to investigate what the role of RNA incorporation in DNA is in ageing and frailty in humans.
- Lastly, we have a zebrafish knockout line for mitochondrial RNaseHmt which we aim to characterise in relation to ageing markers.

Contact

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References

1. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. 2023. Hallmarks of aging: An expanding universe. Cell, 186, 243-278.
2. Sparks JL, Chon H, Cerritelli SM, Kunkel TA, Johansson E, Crouch RJ, Burgers PM. RNase H2-initiated ribonucleotide excision repair. Mol Cell. 2012 Sep 28;47(6):980-6. doi: 10.1016/j.molcel.2012.06.035. Epub 2012 Aug 2. PMID: 22864116; PMCID: PMC3470915
3. Storci, G., De Carolis, S., Papi, A. et al. Genomic stability, anti-inflammatory phenotype, and up-regulation of the RNAseH2 in cells from centenarians. Cell Death Differ 26, 1845–1858 (2019). https://doi.org/10.1038/s41418-018-0255-8
4. Carneiro MC, Henriques CM, Nabais J, Ferreira T, Carvalho T, Ferreira MG. Short Telomeres in Key Tissues Initiate Local and Systemic Aging in Zebrafish. PLoS Genet. 2016 Jan 20;12(1):e1005798. doi: 10.1371/journal.pgen.1005798. PMID: 26789415; PMCID: PMC4720274.