A telomerase deficient model of ageing reveals perturbed immune cell dynamics and mitochondrial function contribute to impaired skeletal muscle regeneration

Helen G. Potts[1], Jose Guijarro Nuez[1,2], Kok Hao Chen[2], Yue Yuan[1] and Robert Knight[1]

1. Centre for Craniofacial and Regenerative Biology, Guy's Campus, Kings College London
2. A*STAR Research Institute, Genome Institute of Singapore

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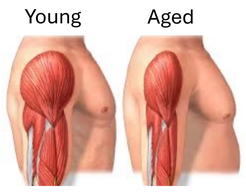
Introduction



Ageing results in a gradual decline in the strength, speed of contraction, and mass of our skeletal muscles[1], Additionally, our capacity for muscle repair and regeneration decreases with age[2]. As a result, older adults experience age-related muscle weakness which significantly reduces quality of life in our later years.

To *improve* and/or *prevent* age-related muscle weakness, it is essential that we understand the cellular and molecular mechanisms that drive ageing phenotypic changes. To date, research has shown that aged skeletal muscle is characterised by decreased muscle stem cell (muSC) activity, with a corresponding perturbed immune response to injury[3]. However, the underlying ageing mechanisms that result in these pathological changes to skeletal muscle are currently unknown.

To study the molecular and cellular changes of ageing, we utilise the telomerase-deficient *(tert)* zebrafish larvae model which displays chronic telomere attrition[4] and has been shown to effectively capture the hallmarks of ageing skeletal muscle.

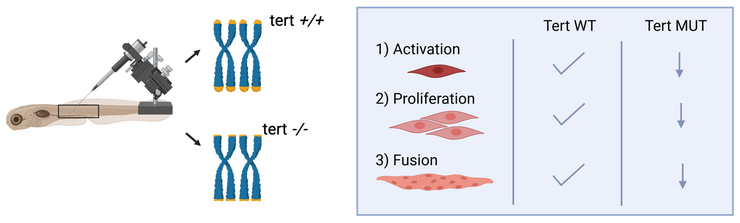


Figure 1: The telomerase deficient zebrafish displays an accelerated ageing phenotype. *The tert larval model successfully recapitulates key hallmarks of ageing during muscle repair such as delayed myofibre regeneration, reduced muSC proliferation and persistent macrophage infiltration following injury[5].*

Aims & Methodology

Hypothesis: Age-related impaired skeletal muscle repair is driven by a failure to resolve inflammation, perturbing muSC responses.

Aim 1 – Characterise the inflammatory response of young *(tert^{WT})* and aged *(tert^{MUT})* larvae to skeletal muscle injury

Aim 2 – Identify age-regulated changes to gene expression dynamics in regenerating muscle

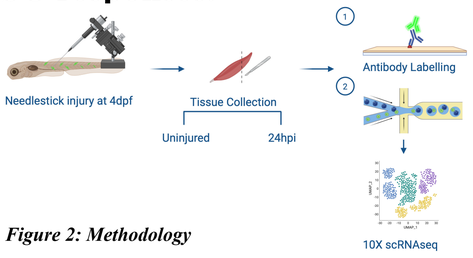


Figure 2: Methodology

Result 1 – Aged *tert* mutants show a significantly perturbed inflammatory response to muscle injury

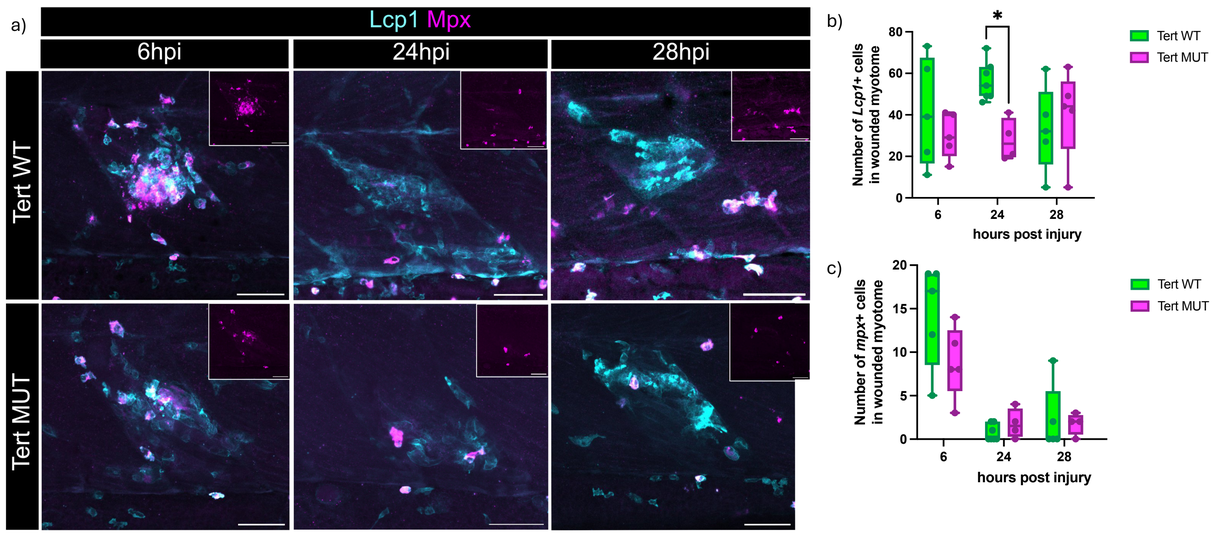


Figure 3: Characterisation of the immune response to injury in tert muscle reveals that aged tert mutants display an attenuated immune in comparison to wildtype siblings. Critically, the inflammatory neutrophil response fails to rapidly resolve in tert mutants, suggesting a failure of inflammation resolution.
(a) Immunofluorescent Lcp1 (a pan-leukocyte marker) and mpx (a neutrophil marker) staining at 6-, 24- and 28 hours post-injury (hpi, scale bars = 50μM).
(b&c) Quantification of Lcp1^{+}/ mpx^{+} cells present in injured myotomes demonstrates that young terts$^{(WT)}$ have a significantly stronger immune response in comparison to aged terts$^{(MUT)}$ at 24 hpi.
(d&e) Comparison of mpx+ cells present in injured vs uninjured myotomes reveals that the aged tert neutrophil response is prolonged and remains active at 24hpi and 28hpi.
Statistical testing: 2-way ANOVA with Sidak's multiple comparisons test, n=4-6.

Result 2 – Young and aged *tert* muscle display significant differences in cellular composition

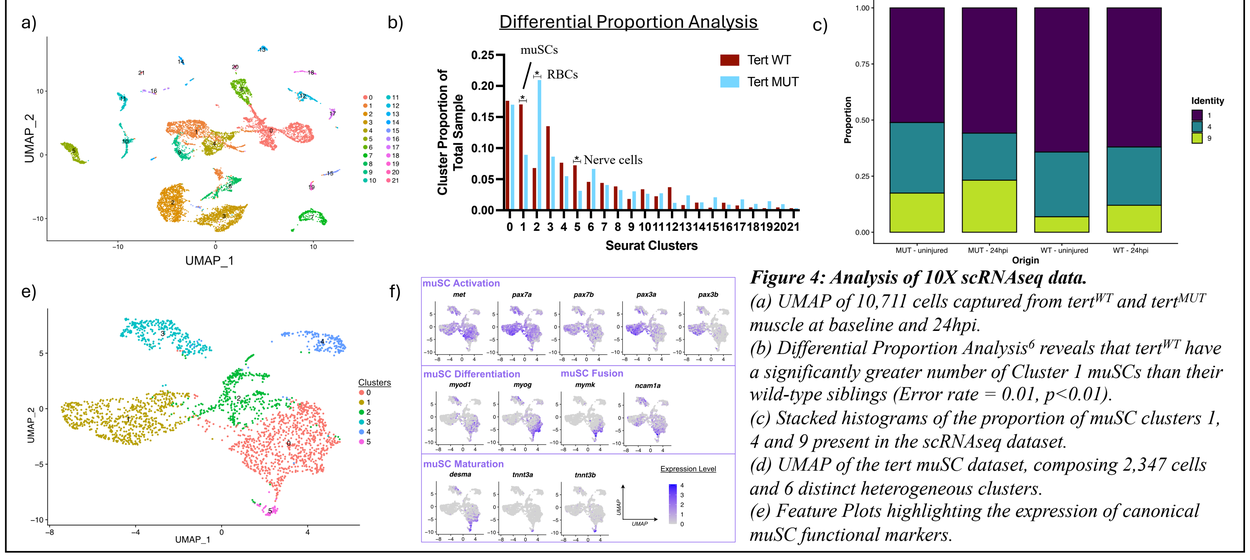


Figure 4: Analysis of 10X scRNAseq data.
(a) UMAP of 10,711 cells captured from tert^{WT} and tert^{MUT} muscle at baseline and 24hpi.
(b) Differential Proportion Analysis[6] reveals that tert^{WT} have a significantly greater number of Cluster 1 muSCs than their wild-type siblings (Error rate = 0.01, p<0.01).
(c) Stacked histograms of the proportion of muSC clusters 1, 4 and 9 present in the scRNAseq dataset.
(d) UMAP of the tert muSC dataset, composing 2,347 cells and 6 distinct heterogeneous clusters.
(e) Feature Plots highlighting the expression of canonical muSC functional markers.

Result 3 – Ageing drives a unique transcriptional signature in *tert* muSCs

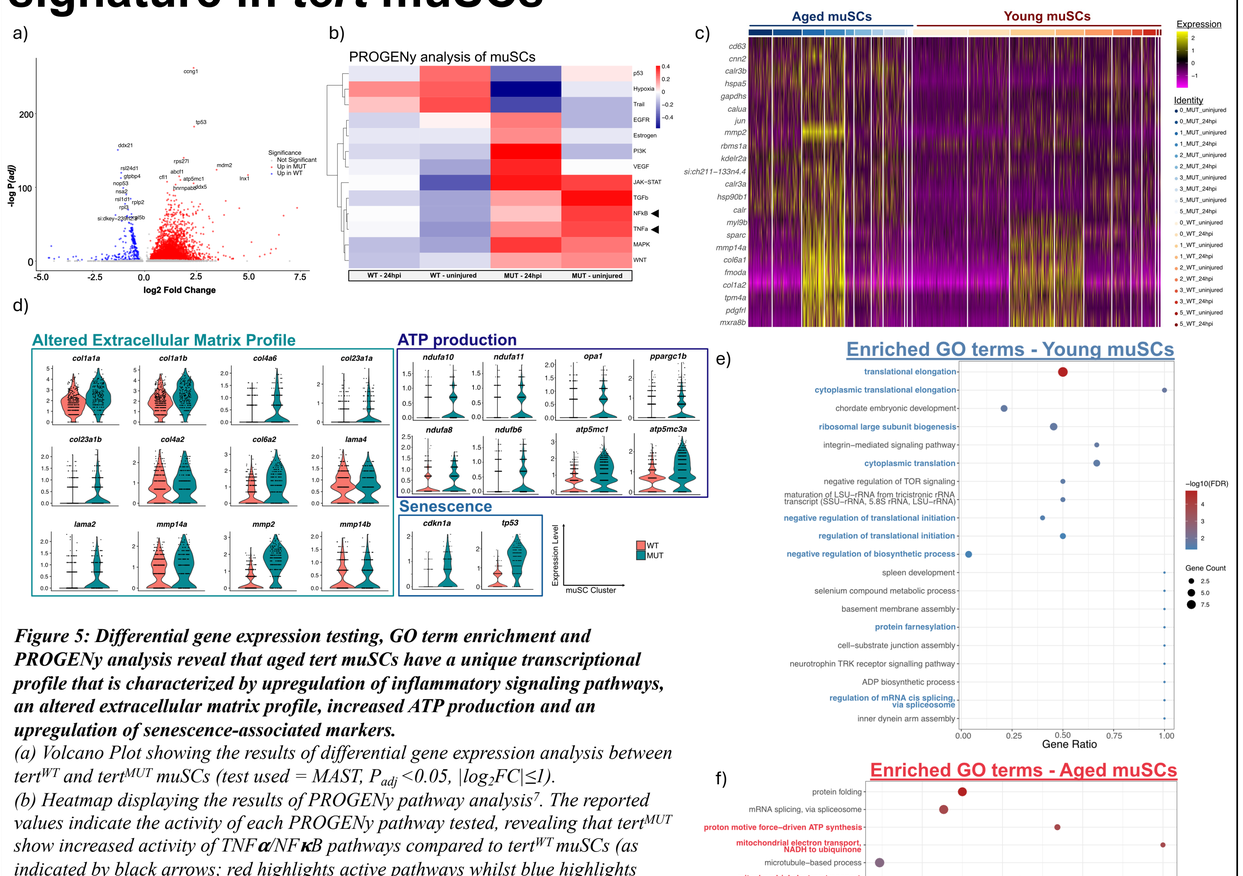


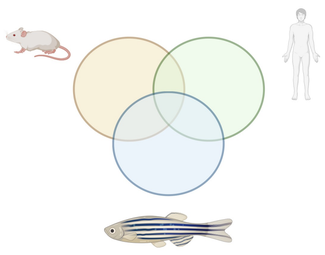
Figure 5: Differential gene expression testing, GO term enrichment and PROGENy analysis reveal that aged tert muSCs have a unique transcriptional profile that is characterized by upregulation of inflammatory signaling pathways, an altered extracellular matrix profile, increased ATP production and an upregulation of senescence-associated markers.
(a) Volcano Plot showing the results of differential gene expression analysis between tert^{WT} and tert^{MUT} muSCs (test used = MAST, P_{adj} <0.05, $|log_2FC| \leq 1$).
(b) Heatmap displaying the results of PROGENy pathway analysis[7]. The reported values indicate the activity of each PROGENy pathway tested, revealing that tert^{MUT} show increased activity of TNFα/NFκB pathways compared to tert^{WT} muSCs (as indicated by black arrows; red highlights active pathways whilst blue highlights inactive pathways).
(c) Heatmap plotting the expression of the top TNFα and NFκB genes annotated in the PROGENy pathways, revealing that aged muSCs show an increased expression of inflammatory genes across muSC clusters compared to young muSCs. This result suggests that ageing drives upregulation of inflammation in muSCs.
(d) Violin Plots comparing the expression of genes in tert^{WT} and tert^{MUT} muSCs, demonstrating that aged tert muSCs show specific upregulation of genes involved in ECM formation, ATP production and senescence.
(e&f) Dot Plots displaying the top GO terms (biological processes) enriched in young (e) and aged (f) tert muSCS. Young muSCs show an enrichment for protein translation, suggesting a greater rate of protein turnover and homeostasis. In comparison, aged muSCs show an enrichment for mitochondrial processes.

Conclusions

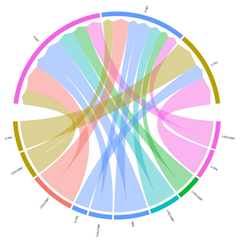
- Ageing *tert* mutants fail to resolve the inflammatory neutrophil response following injury which may drive impaired muscle repair.
- The transcriptional signature of ageing *tert* muSCs recapitulates human ageing signatures, with a comparison of key pathways highlighting mitochondrial dysfunction as a conserved mechanism of ageing.
- Differential Proportion Analysis of regenerating muscle suggests that ageing drives intrinsic changes to *tert* muSC populations, with *tert* mutants displaying a significant change in muSC composition at baseline. This finding could indicate that ageing drives intrinsic muSC changes which impair their ability to respond to injury, resulting in an impaired regenerative response.

Future Work

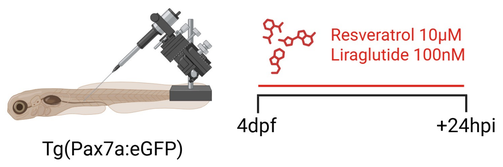
Conserved Ageing Signature



muSC-mϕ Interactions



Therapeutic Screen



Participatory Research with Older Adults

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