A telomerase deficient model of ageing reveals that age-related changes to immune cell dynamics and metabolism may contribute to impaired skeletal muscle regeneration

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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 captures hallmarks of ageing skeletal muscle.

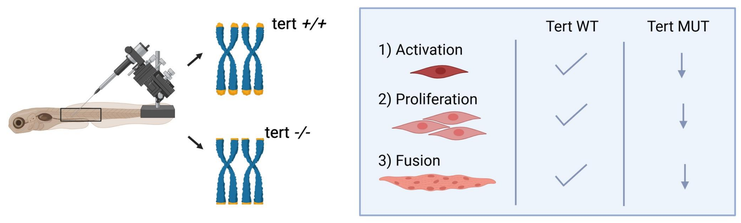


Figure 1: The telomerase deficient zebrafish displays an accelerated ageing phenotype. *The larval model successfully recapitulates key hallmarks of ageing during muscle repair such as delayed myofibre regeneration, reduced muSC proliferation and persistent macrophage infiltration following injury[5]. A unique advantage of the larval model is the ability to conduct in vivo live cell imaging, enabling age-related changes to muSC and macrophage cellular behaviour to be tracked and visualised.*

Aims

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

Aim 1 – Assess the duration of the immune response in aged *tert* mutants
Aim 2 – Define age-related transcriptional changes within muscle tissue
Aim 3 – Explore whether aged muSC behaviour can be improved pharmacologically

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

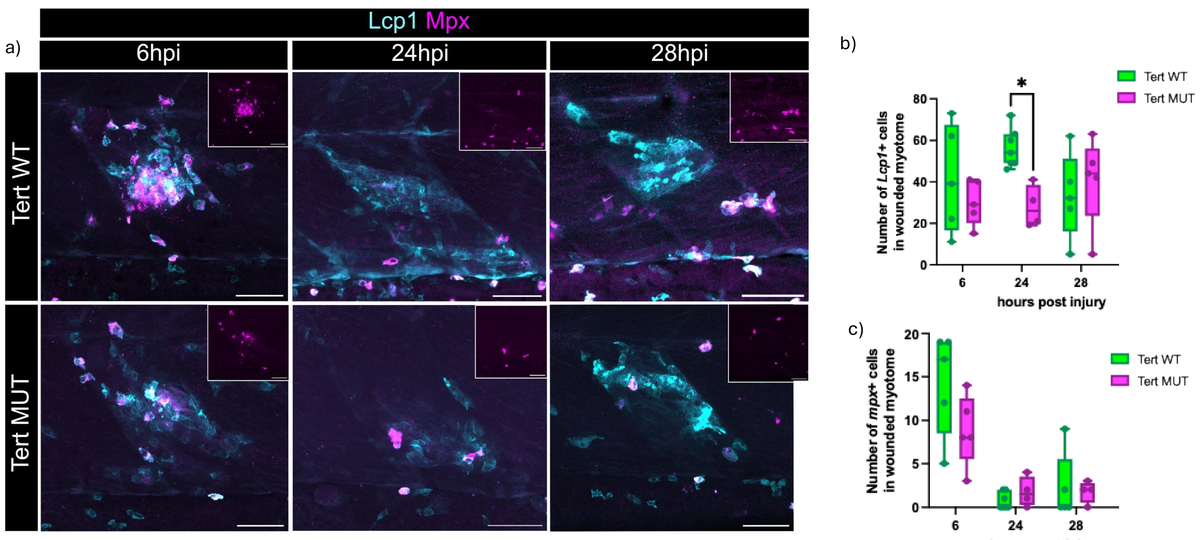


Figure 2: Characterisation of the immune response to needlestick injury in tert muscle revealed that aged tert mutants display an attenuated immune in comparison to wildtype siblings. Critically, the inflammatory neutrophil response fails to rapidly resolve in tert mutants, suggesting a failure to resolve inflammation. *(a) Immunofluorescent Lcp1 (a pan-leukocyte marker) and mpx (a neutrophil marker) staining (scale bars = 50μM). (b&c) Quantification of Lcp1+/ mpx+ cells present in the injured myotomes shows that 'young' terts have a significantly stronger immune response than 'aged' terts. (d&e) Comparison of elevated neutrophils reveals that the neutrophil response remains active within aged tert muscle, suggesting a perturbed inflammatory response. (n=4-6/per condition, 2-way ANOVA with Sidak's multiple comparisons test).*

Result 2 – Age-related inflammation may be driven by changes in metabolism and ROS signalling

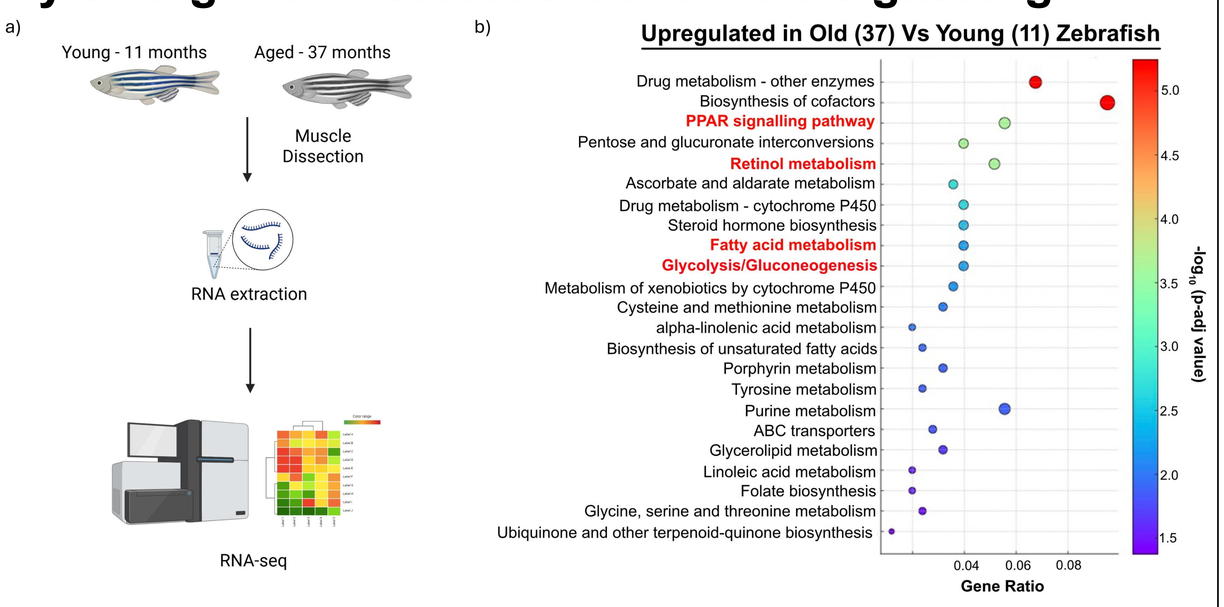


Figure 3: RNAseq data from naturally aged zebrafish demonstrates that ageing is associated with changes in key metabolic pathways that may alter the behaviour of muSCs and leukocytes following skeletal muscle injury. *(a) Schematic demonstrating the collection of sample collection for bulk RNA sequencing (b) Differential gene expression of RNAseq data using differential gene expression and KEGG pathway analysis demonstrates that ageing results in significant changes to the metabolism of glucose and lipids (pathways highlighted in red), with a concurrent upregulation of retinol metabolism, which could indicate increased reactive oxygen species signalling.*

Result 3 – muSC activity following injury can be rescued in aged *tert* mutants through metabolic targeting

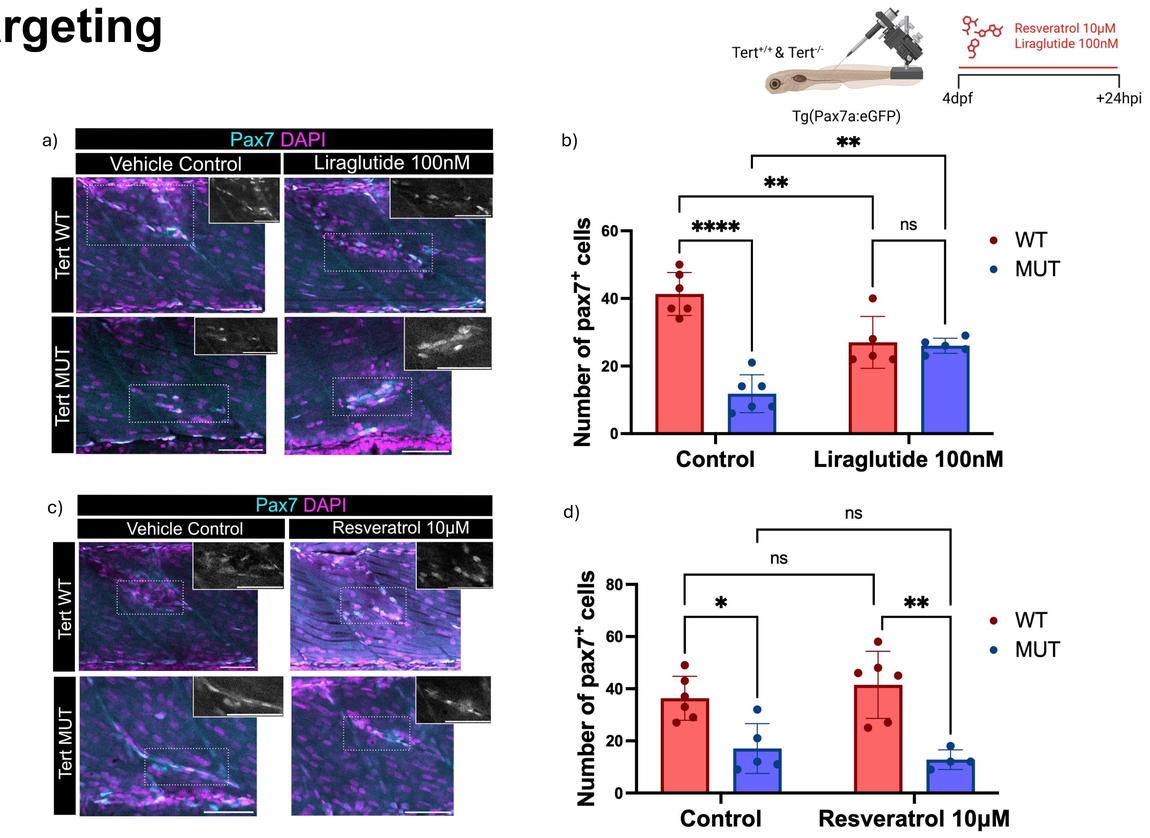


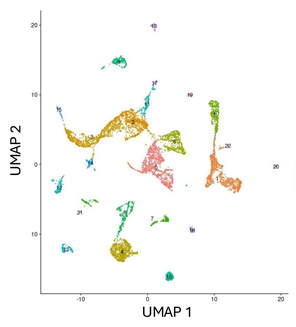
Figure 4: Liraglutide (a GLP-1R agonist) treatment treatment effectively rescues muSC activation following injury in aged tert mutants, whilst Resveratrol (a ROS inhibitor) had no impact on muSC activation *(a&c) Immunofluorescent Pax7 staining of activated muSCs at 24 hours post-injury following Liraglutide/Resveratrol treatment (scale bars = 50μM). (b&d) Quantification of the number of Pax7+ cells present in injured Tert WT and MUT myotomes (n= 4-6/per condition , 2-way ANOVA with Sidak's multiple comparisons test).*

Conclusions

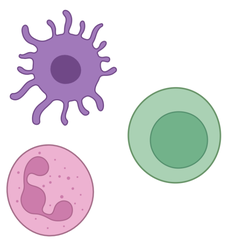
- Ageing *tert* mutants fail to resolve the inflammatory neutrophil response following injury which may drive impaired muscular repair.
- Impaired muSC activation during ageing injury repair can be significantly increased and rescued with Liraglutide, a GLP-1R agonist.
- Liraglutide treatment results in a significant decrease in muSC activation in $tert^{+/+}$ following injury, suggesting that the impact of Liraglutide on skeletal muscle repair is context-specific. This finding *could* point towards a future need for age-dependent patient stratification of Liraglutide treatment.

Future Work

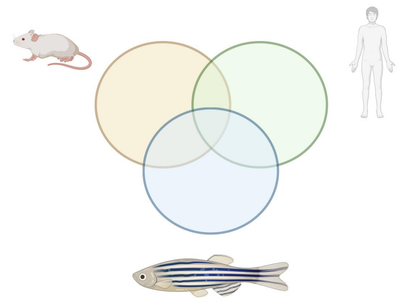
scRNAseq



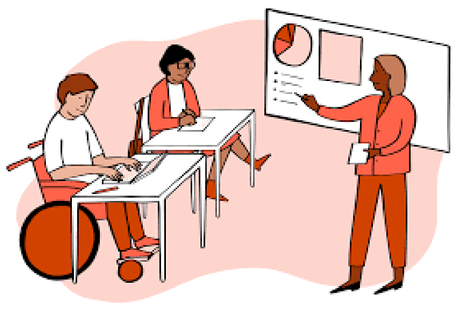
Impact on immune response



Conserved Ageing Signature



Older Adult Involvement



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