In vivo and *in vitro* cardiac aging: developing of cellular aging models

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INTRODUCTION

Age is one of the most relevant predisposing risk factors for cardiovascular diseases. Aged hearts undergo structural and functional changes that are orchestrated at the molecular level, and this molecular basis is poorly characterized in humans. We study the transcriptome dynamics of the aging human left ventricle (LV) according to both chronological (CA) and biological (BA) age. BA is measured using a transcriptional marker: *CDKN2A* expression, a cell senescence marker. Besides, we aim to establish a cellular model that recapitulates the mechanisms of cardiac aging.

AGE-RELATED FUNCTIONAL PROCESSES

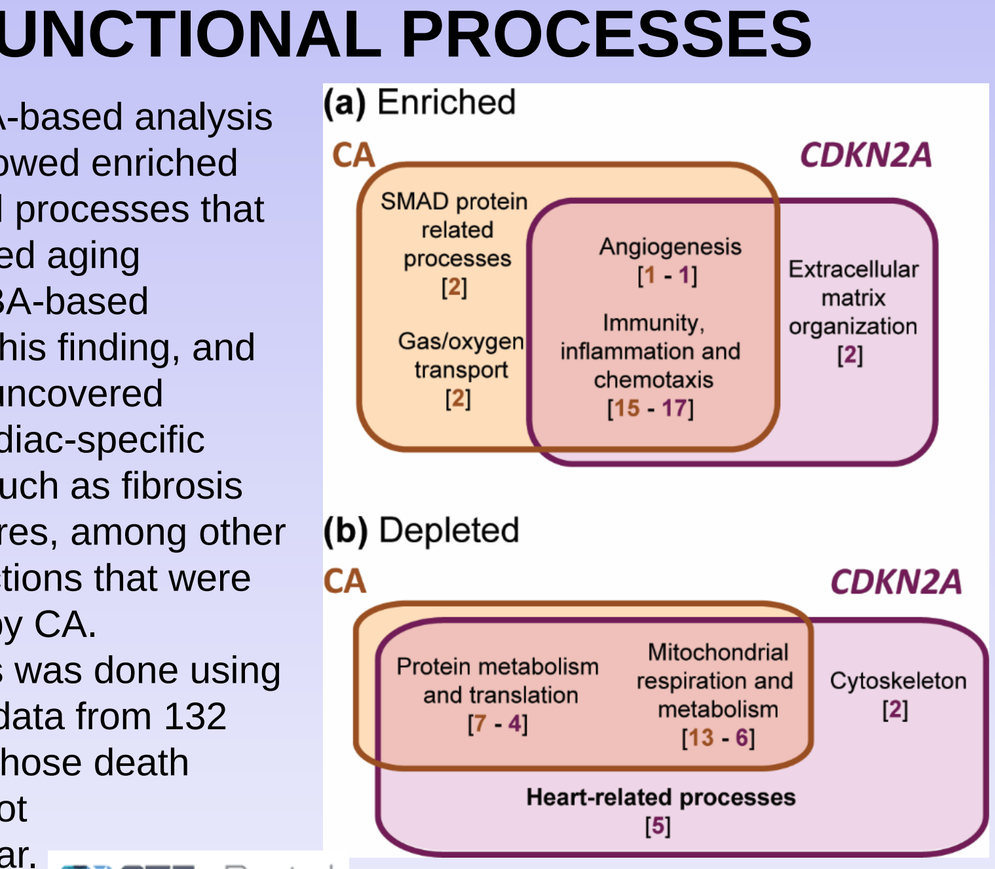

Figure 1. CA-based analysis by GSEA showed enriched and depleted processes that are considered aging landmarks. BA-based reproduced this finding, and additionally uncovered depleted cardiac-specific processes, such as fibrosis related features, among other relevant functions that were undetected by CA.
This analysis was done using LV RNAseq data from 132 individuals whose death cause was not cardiovascular.

GTEx Portal

CARDIAC FIBROSIS IS RELATED TO LV AGING

Fibrosis accumulation with CA or BA was analyzed using histological image processing techniques to determine the relationship of age with structural changes in the LV. The fibrosis amount was significantly correlated with both age markers, but it was stronger with BA.

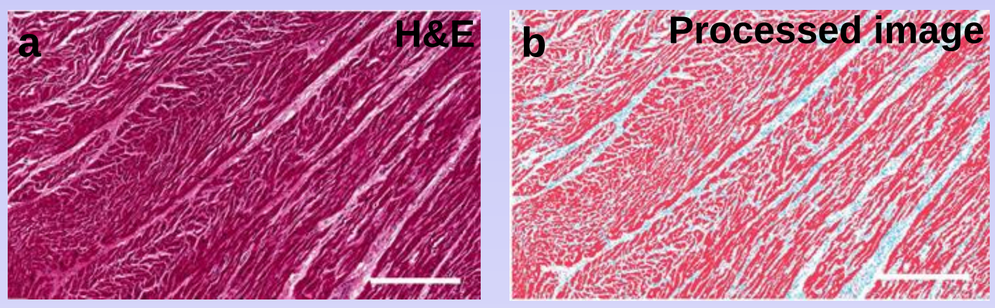

Figure 2. a) Representative human left-ventricle hematoxylin-eosin image from GTEx study. **b)** The corresponding mask of each image depicts myocytes in red and extracellular matrix in blue. Scale bars are 300 µm.

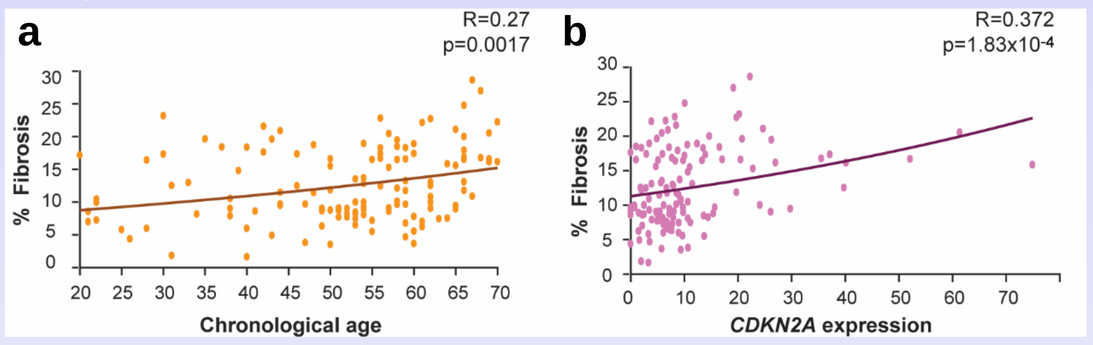

Figure 3. Correlation of fibrosis vs CA (**a**) or BA (**b**). Dots indicate individual data, while the line represents the fitted function. Spearman correlation coefficient (Rho) and p-values are shown.

CELLULAR AGING MODEL

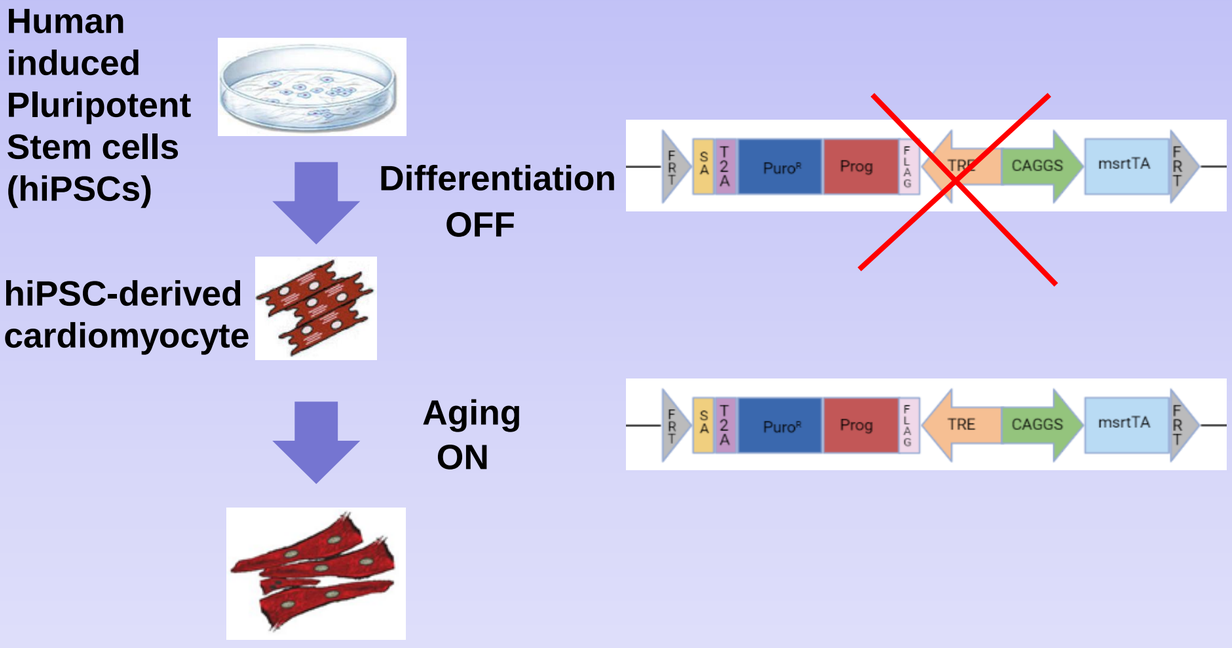

Figure 4. Our hiPSCs have integrated in the genome an inducible cassete to express progerin, a mutated form of LMNA, which is responsible of Hutchinson-Gilford progeria syndrome. Progerin expression is induced with 0.5 mg/ml or 1mg/ml after cardiomyocyte commitment and its maintained for 6 days.

GENE EXPRESSION PROFILING OF AGED iCM

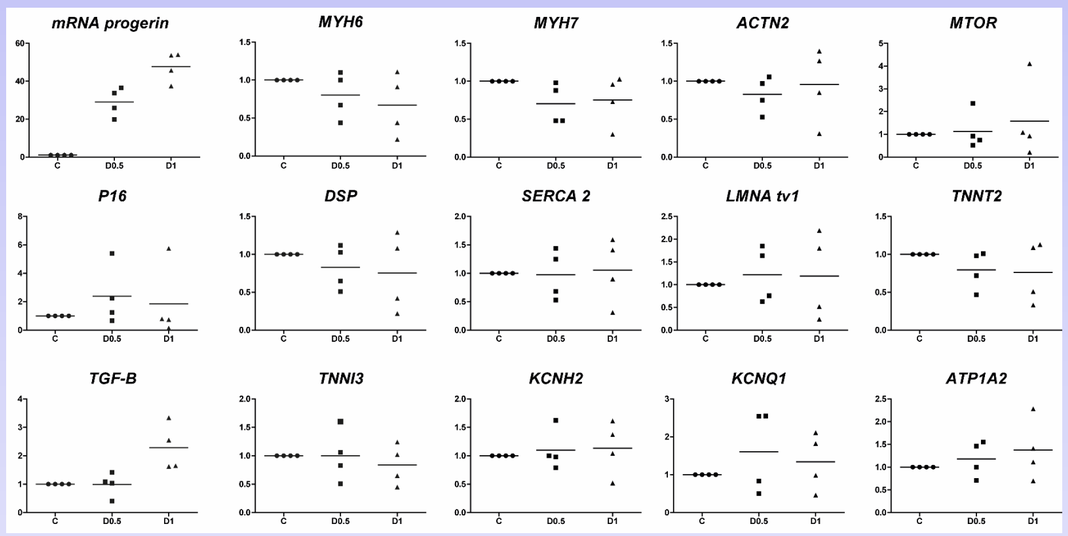

Figure 5. After induction, there is a significant increase in progerin mRNA. In response, the median of *MYH6, MYH7, DSP, TNNI3, ACTN2 and TNNT2* genes tend to decrease with at least one concentration of doxycycline, while *LMNA, P16, TGF-β, MTOR, KCNQ1, KCNH2 or ATP1A2* present an increasing tendency. *SERCA2* appears to be unaffected by the induction. (n=4).

FUNCTIONAL CHARACTERIZATION

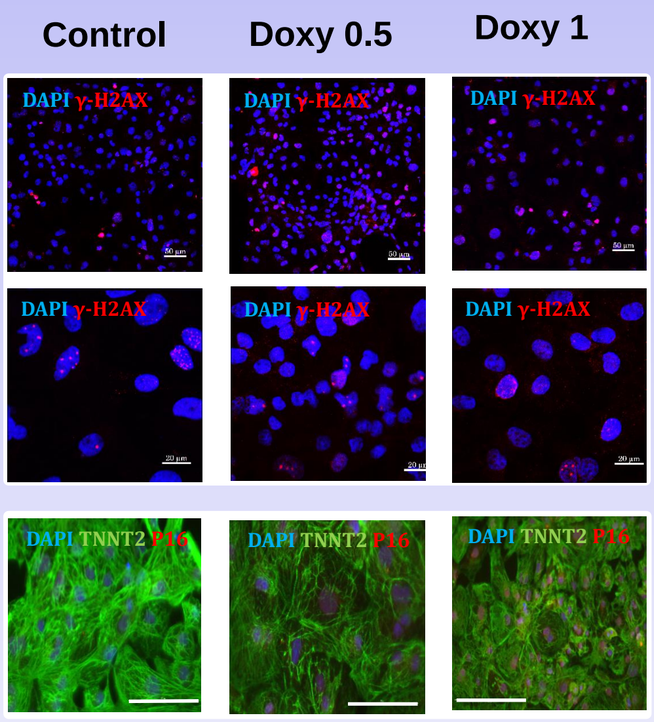

Figure 6. **a)** An apparent increase in *γ-H2AX* foci is observed with respect to the uninduced control situation. This would indicate an increase in genomic damage. There are signs of structural damage to the nucleus with enhanced folding and blebbing (n=1). **b)** For *P16*, the number of positive nuclei are also increased in the inducted conditions (n=1).

CONCLUSIONS

Both the transcriptomic changes associated with functional processes and the relation with cardiac fibrosis in the human LV are better explained by BA than CA. Our human *in vitro* model of biological aging has the potential to replicate some of the *in vivo* age associated hallmarks.

FUNDING SOURCES

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