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21 E 22 DE MARÇO | AUDITÓRIO PRINCIPAL DO CENTRO HOSPITALAR UNIVERSITÁRIO DE COIMBRA | NÚCLEO DE ESTUDANTES DE FARMÁCIA DA ASSOCIAÇÃO ACADÉMICA DE COIMBRA

CCAVADAS LAB NEUROENDOCRINOLOGY AND AGING | CNC CENTER FOR NEUROSCIENCE AND CELL BIOLOGY UNIVERSITY OF COIMBRA PORTUGAL | CIBB CENTRE FOR INNOVATIVE BIOMEDICINE AND BIOTECHNOLOGY | 1290 FACULDADE DE FARMÁCIA UNIVERSIDADE DE COIMBRA | EIT Health | EIT Health Ageing PhD School

LIPODYSTROPHY AS A NOVEL TARGET FOR MITIGATING PREMATURE AGING IN HUTCHINSON-GILFORD SYNDROME PROGERIA

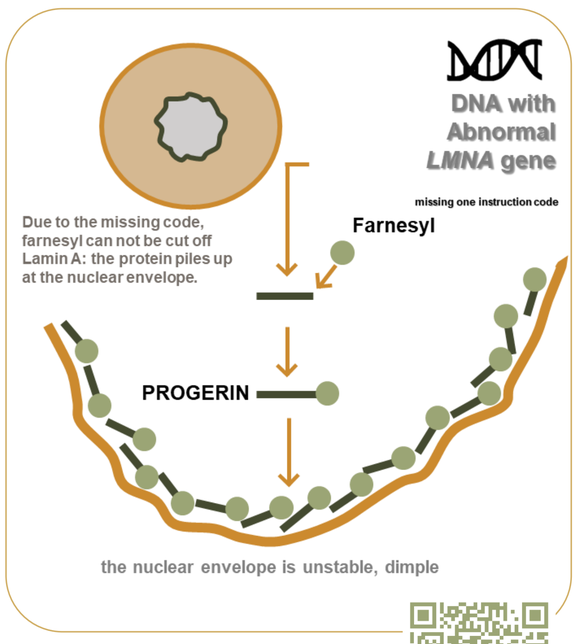
Daniela G. Costa[1,2,3,4]; Marisa Ferreira-Marques[1,2,3]; Cláudia Cavadas[1,2,3]

[1]CNC-UC – Center for Neuroscience and Cell Biology, University of Coimbra, Portugal: [2]CIBB – Center for Innovation in Biomedicine and Biotechnology, University of Coimbra, Portugal; [3]Faculty of Pharmacy, University of Coimbra, Portugal; [4]EIT Health Ageing PhD School

INTRODUCTION

Single point mutation in *LMNA* gene (1824C>T;G608G)[1]

- Rare disease 1:18 millions
- Premature and accelerate aging
- Lifespan: ~14.5 years old



Cardiovascular dysfunction

Metabolic dysfunction

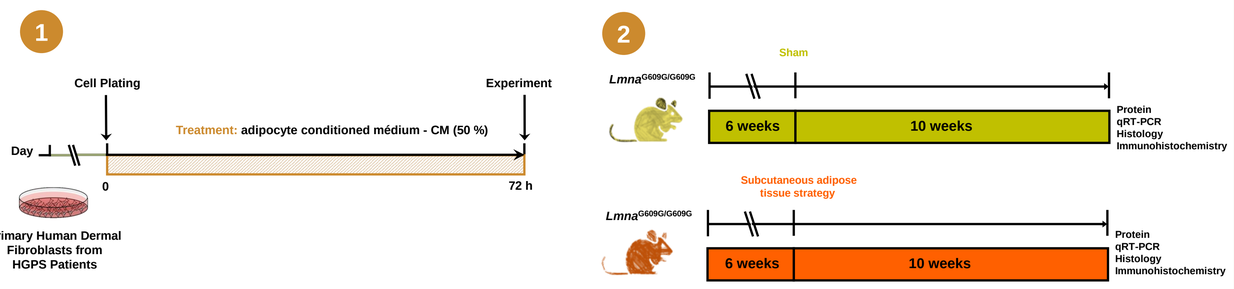
Skin abnormalities

Musculoskeletal dysfunction

LIPODYSTROPHY[2]

AIM: To investigate the therapeutic potential of adipose tissue in mitigating the aging phenotype of Hutchinson-Gilford progeria syndrome (HGPS), by elucidating its effects on i) cellular aging mechanisms; ii) the aging phenotype in an HGPS mouse model (*Lmna*$^{G609G/G609G}$ mice).

METHODOLOGIES



RESULTS

1

Conditioned medium from adipogenesis delays the hallmarks of aging in HGPS fibroblasts

decreases in progerin accumulation | *decreases extracellular matrix alteration* | *decreases DNA damage*

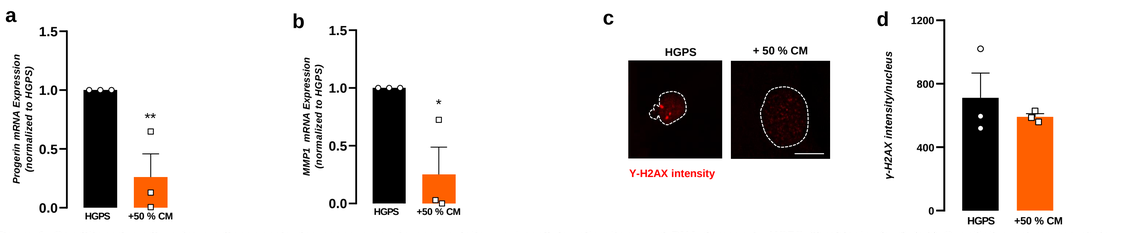


Figure 1. Conditioned medium from adipogenesis decreases progerin accumulation, extracellular alterations and DNA damage in HGPS fibroblasts. (a–d) (a-b) Quantitative polymerase chain reaction analysis mRNA levels of (a) *Progerin and MMP1,* (c-d) Conditioned medium decreased γ-H2AX immunoreactivity. Cells were immunolabeled for γ-H2AX (red). Images are representative of three independent experiments. Scale bar, 20 µm. Data are expressed as the mean ± SEM, at least, three independent experiments, and are expressed as a percentage of HGPS. *$p < 0.05$, **$p < 0.01$, significantly different from HGPS, as determined by Student's *t* test. HGPS, Hutchinson-Gilford progeria syndrome.

rescues nuclear morphology

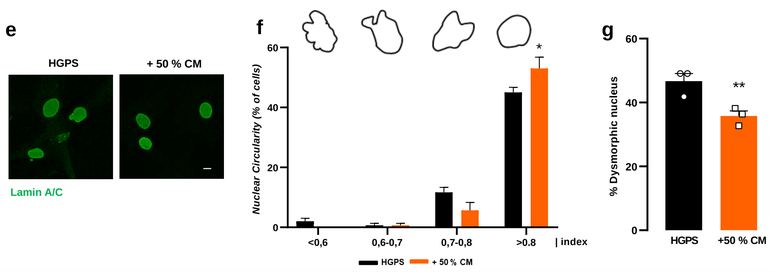


Figure 2. Conditioned medium from adipogenesis rescues nuclear morphology in HGPS fibroblasts. (e–g) (e). Images are representative of three independent experiments. Scale bar, 10 µm. Quantification of the number of misshapen nuclei (f) and nuclear circularity (g) upon conditioned medium. Data are expressed as the mean ± SEM, at least, three independent experiments, and are expressed as a percentage of HGPS. **$p < 0.01$, significantly different from HGPS, as determined by Student's *t* test. HGPS, Hutchinson-Gilford progeria syndrome.

decreases in cellular senescence markers

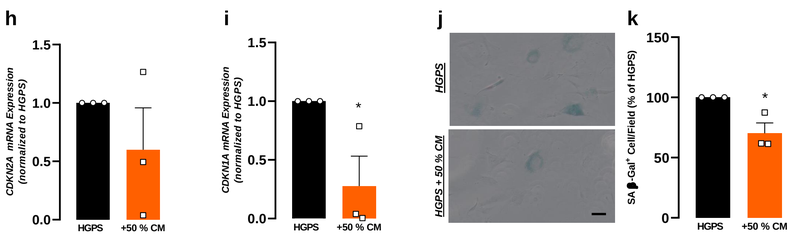


Figure 3. Conditioned medium from adipogenesis mitigates cellular senescence in HGPS fibroblasts. (h-k). (h-i) Quantitative polymerase chain reaction analysis mRNA levels of (h) *CDKN2A and CDKN1A,* (j) Images are representative of three independent experiments. Scale bar, 100 µm. (k) Conditioned medium decreases cellular senescence, as determined by SA-β-Gal activity. Data are expressed as the mean ± SEM, at least, three independent experiments, and are expressed as a percentage of HGPS. *$p < 0.05$, significantly different from HGPS, as determined by Student's *t* test. HGPS, Hutchinson-Gilford progeria syndrome.

1. Eriksson, M. *et al.* (2003) Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 423, 293-298. 10.1038/nature01629

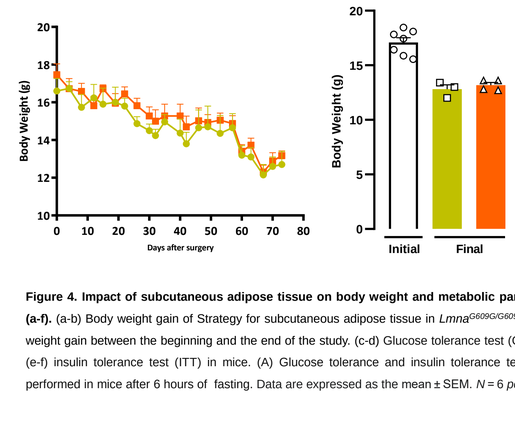
2. Costa, D.G. *et al.* (2023). Lipodystrophy as a target to delay premature aging. *Trends in Endocrinology & Metabolism* .10.1016/j.tem.2023.10.006

RESULTS

2

Impact of subcutaneous adipose tissue on *Lmna*$^{G609G/G609G}$ phenotype

Body Weight



Metabolic parameters

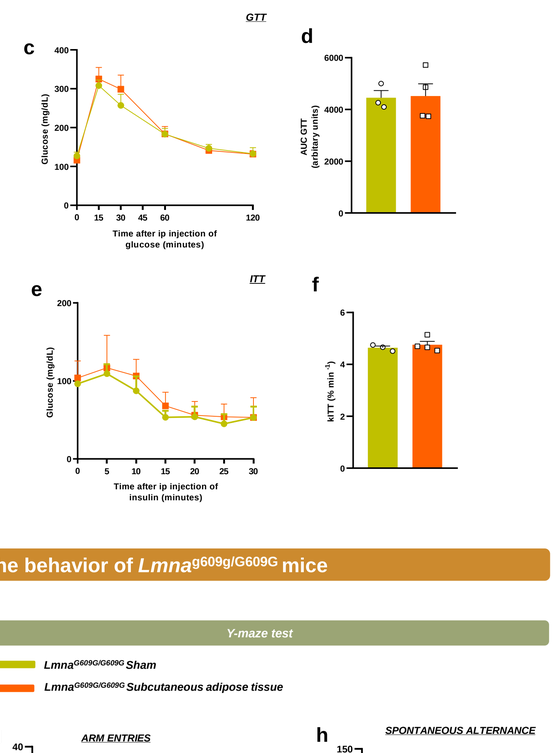
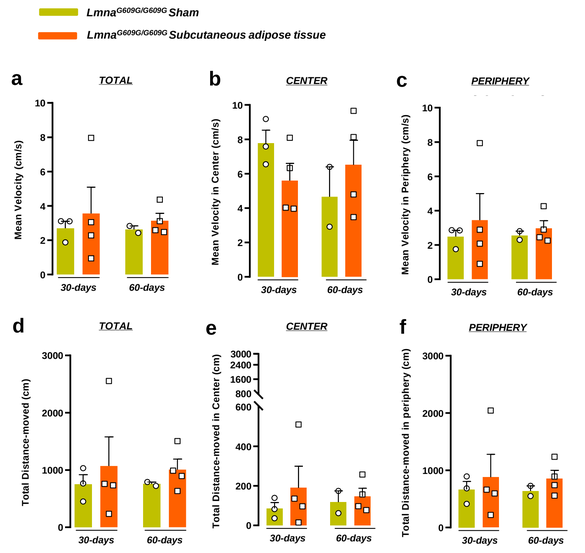


Figure 4. Impact of subcutaneous adipose tissue on body weight and metabolic parameters (a-f). (a-b) Body weight gain of Strategy for subcutaneous adipose tissue in *Lmna*$^{G609G/G609G}$ as the weight gain between the beginning and the end of the study. (c-d) Glucose tolerance test (GTT) and (e-f) insulin tolerance test (ITT) in mice. (A) Glucose tolerance and insulin tolerance tests were performed in mice after 6 hours of fasting. Data are expressed as the mean ± SEM. *N* = 6 *per* group.

Impact of subcutaneous adipose tissue on the behavior of *Lmna*$^{g609g/G609G}$ mice

Open field test



Y-maze test

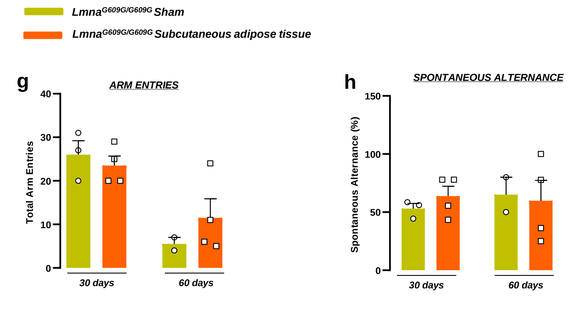
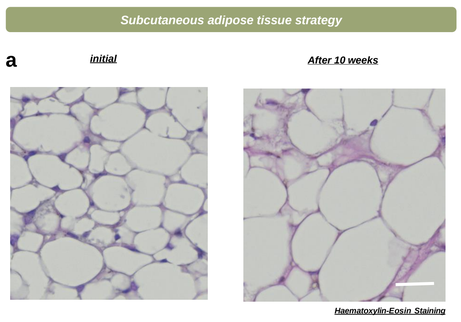


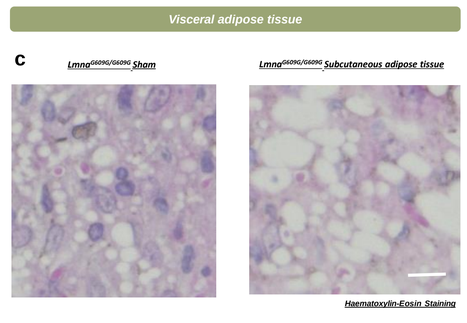
Figure 5. Impact of subcutaneous adipose tissue on behavior (a-h). Locomotor activity and anxiety-like behavior in Open field test: and cognitive performance in the Y-maze (g,h). (a) velocity (b) mean velocity in center area and © mean velocity in periphery area, (d) total distance traveled, (e) total distance in the center area, and (f) total distance in the periphery area. Y-maze: animals were individually placed in the start arm of the apparatus and allowed to explore freely the entire maze. Behavior was video-monitored for a 5 min time period and data are shown as number of total entries (g) and % time spent in the other and novel arms (h). All data are expressed as mean ± SEM. *N* = 6 *per* group.

Impact of subcutaneous adipose tissue on lipodystrophy in *Lmna*$^{g609g/G609G}$ mice

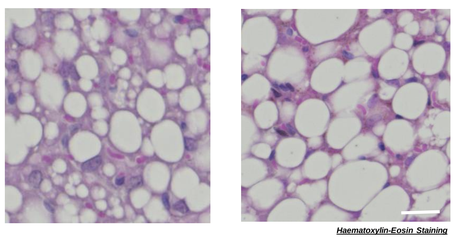
Subcutaneous adipose tissue strategy



Visceral adipose tissue



Subcutaneous adipose tissue



Visceral adipose tissue weight

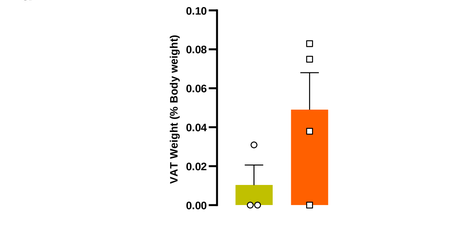


Figure 6. Impact of the strategy on adipose tissue viability and on lipodystrophy (a-d). (a-c) Representative images of hematoxylin–eosin-stained sections of subcutaneous adipose tissue using for strategy (a), subcutaneous adipose tissue of sham- and subcutaneous adipose tissue *Lmna*$^{G609G/G609G}$ mice. Scale bar, 20 µm. (d) Size of the visceral adipose tissue (VAT), expressed as a percentage of % of body weight in 3 months-old sham- and subcutaneous adipose tissue *Lmna*$^{G609G/G609G}$ mice. Data are expressed as the mean ± SEM. *N* = 6 *per* group.

CONCLUSIONS

1. Conditioned medium of adipogenesis attenuates the accumulation of progerin, senescence markers and improves circularity;
2. After 10 weeks, the strategy using subcutaneous adipose tissue contributes to reversing partial lipodystrophy;
3. These preliminary results support that adipose tissue could be a potential therapeutic target to mitigate the progression of premature aging in HGPS.

Contact: Daniela G.Costa | daniela.costa@uc.pt

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