ESTABLISHMENT AND CHARACTERIZATION OF A SENESCENCE IN VITRO MODEL OF VISCERAL ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS (vAD-MSC)

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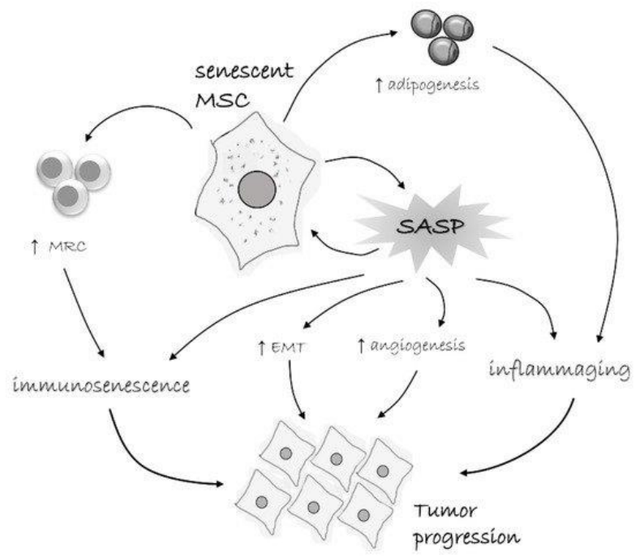
BACKGROUND

In the last few decades, a worldwide progressive extension of the mean lifespan can be observed, especially in less developed countries. However, this extended lifespan is accompanied by an increased prevalence of age-associated diseases, which impair the quality of life. Aging can be considered as the main risk factor for the development of several diseases, such as cardio-vascular disorders, neurodegenerative diseases, and cancer. Senescence is an aged status of cells characterized by an irreversible cell cycle arrest, molecular/morphological alterations and displaying of a senescence-associated secretory phenotype (SASP). AD-MSCs, in particular of visceral AD-MSC (vAD-MSC), are multipotent stem cells that have been studied for their immunomodulatory and regenerative capabilities. However, during senescence, all these features are impaired; instead, it is observed the switching to a pro-inflammatory phenotype, with MSCs secreting several molecules, such as cytokines, adipokines, and hormones that sustain an inflammatory microenvironment, and promote tumor development. In vitro senescence models can help to elucidate these processes, but, since the establishment of a replicative senescence model by extensive culturing of MSCs is time consuming and affected by donor intrinsic factors, a faster and reproducible senescence model of MSCs can improve research.

AIM OF THE STUDY: the establishment and characterization of an in vitro model of oxidative stress-induced senescence of vAD-MSC in order to obtain a stable and reproducible model to investigate the role of vAD-MSC senescence in tumor development and progression.



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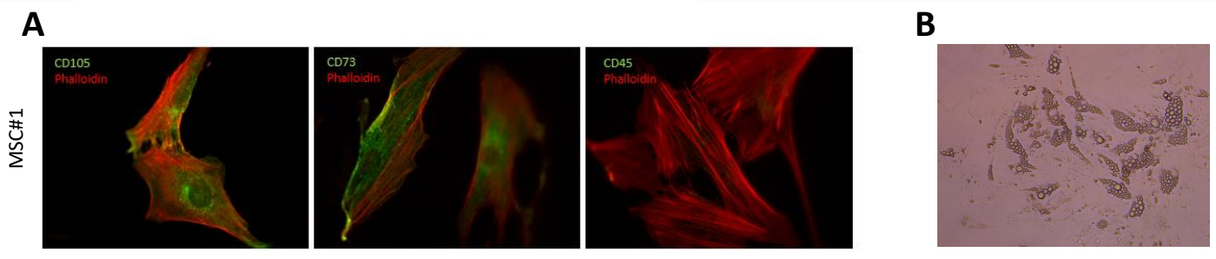
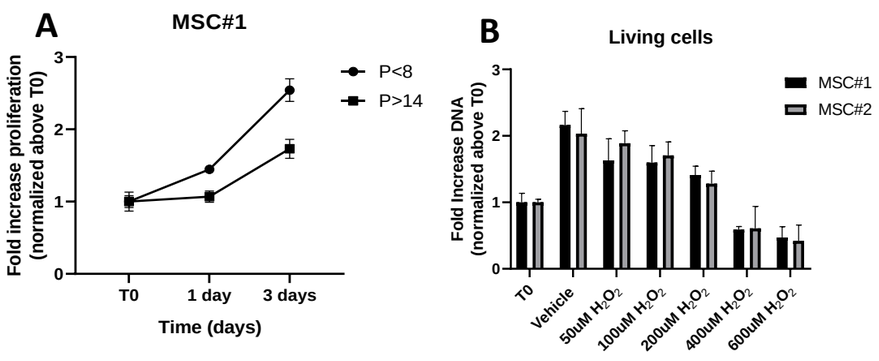
Isolation and characterization of vAD-MSC



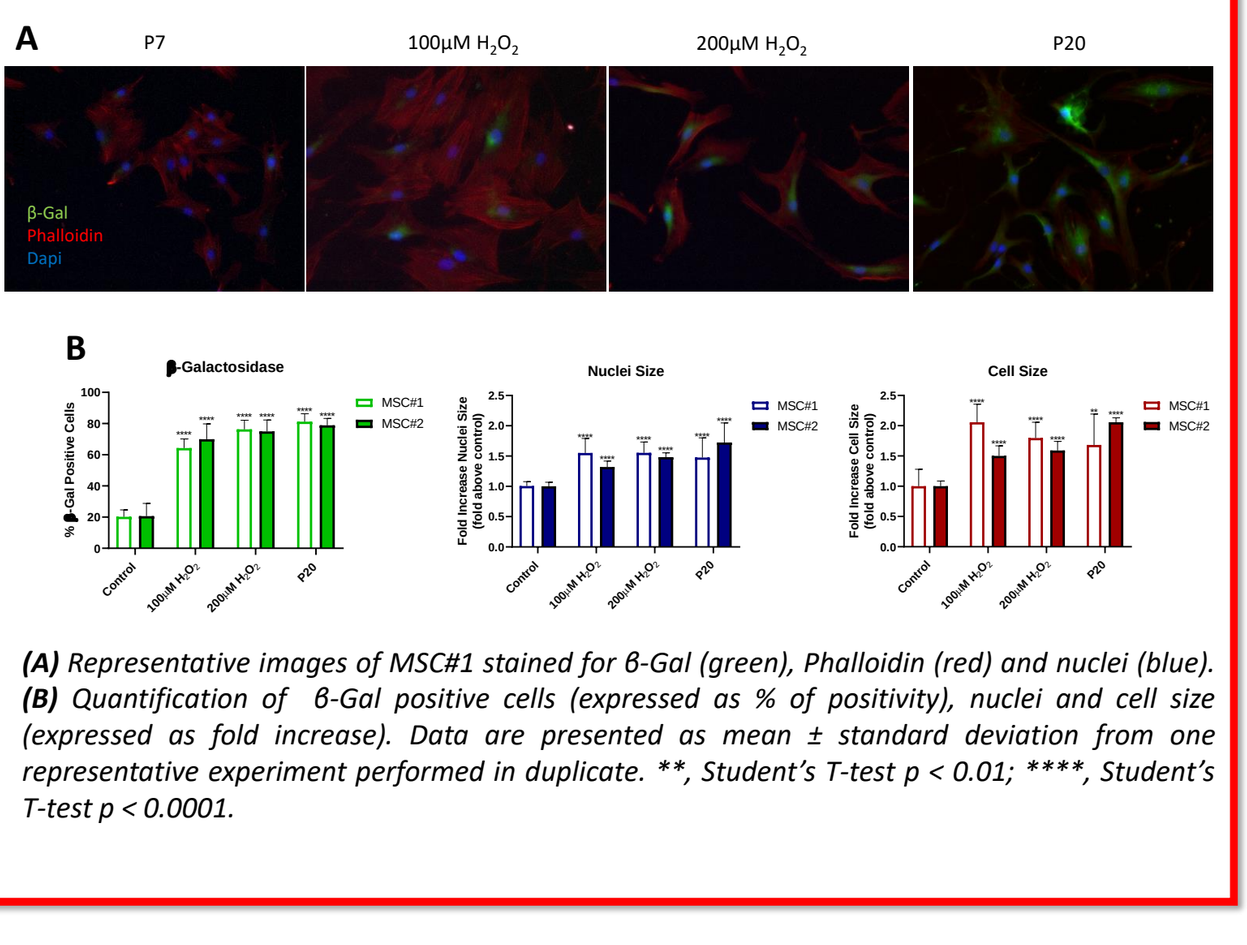
(A) *Stemness of vAD-MSC was confirmed by the positivity of CD105 and CD72 markers and negativity for CD45 marker. Pictures were acquired at fluorescent microscope.* ***(B)*** *Adipogenic potential was evaluated after 3 weeks of differentiation by culturing vAD-MSC with medium containing insulin, dexamathasone, IBMX and rosiglitazione, by observing lipid droplets (LDs) formation. Pictures were acquired at optical microscope.*

Extensive culturing and H_2O_2 impair vAD-MSC proliferation



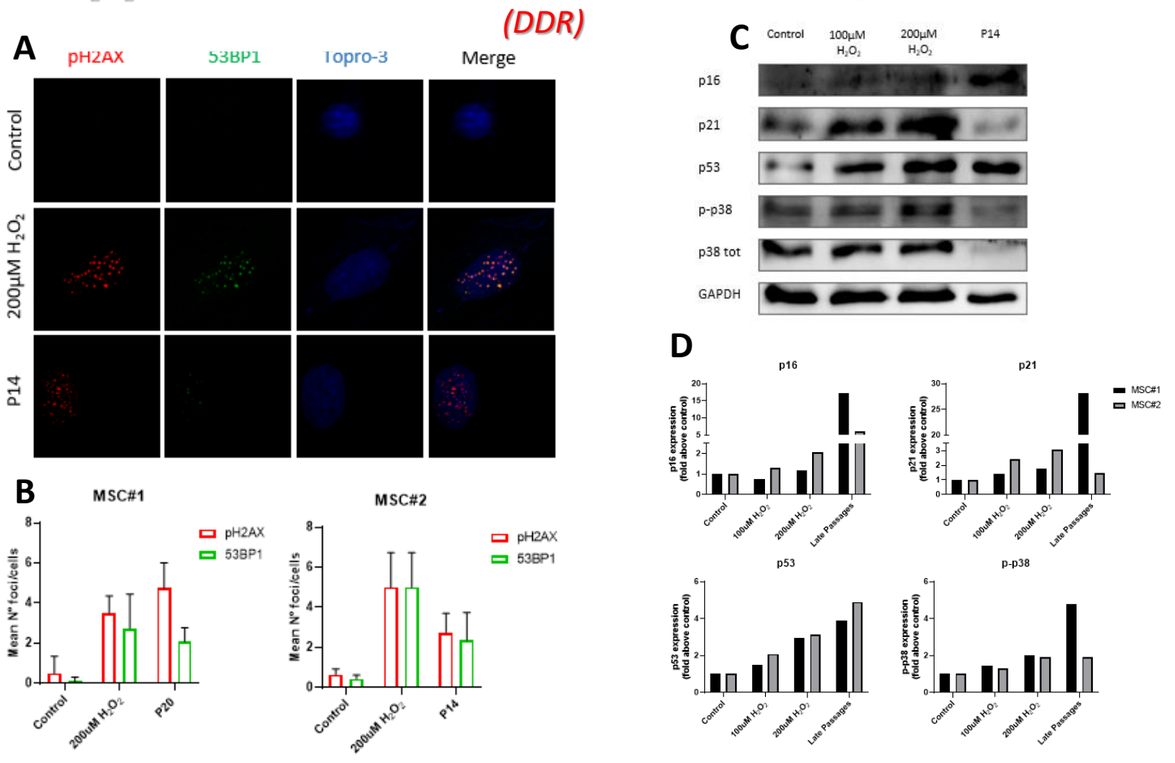
(A) *vAD-MSC proliferation capability was evaluated through DNA measurement.* ***(B)*** *H_2O_2 cytotoxicity was evaluated through dose-response. Cell were treated for 2h with H_2O_2 and then cultured for 48h.*

H_2O_2 induces senescence in vAD-MSC



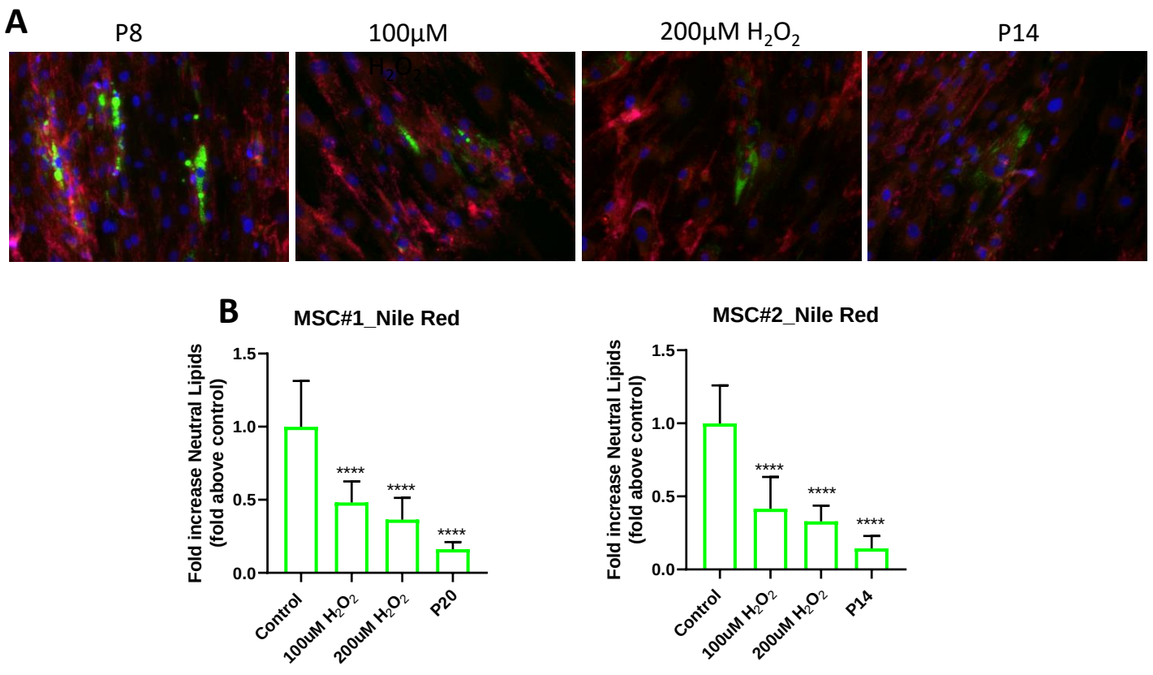
(A) *Representative images of MSC#1 stained for β-Gal (green), Phalloidin (red) and nuclei (blue).* ***(B)*** *Quantification of β-Gal positive cells (expressed as % of positivity), nuclei and cell size (expressed as fold increase). Data are presented as mean ± standard deviation from one representative experiment performed in duplicate. **, Student's T-test $p < 0.01$; ****, Student's T-test $p < 0.0001$.*

H_2O_2 initiates double-strand break (DSB) and DNA damage response (DDR)



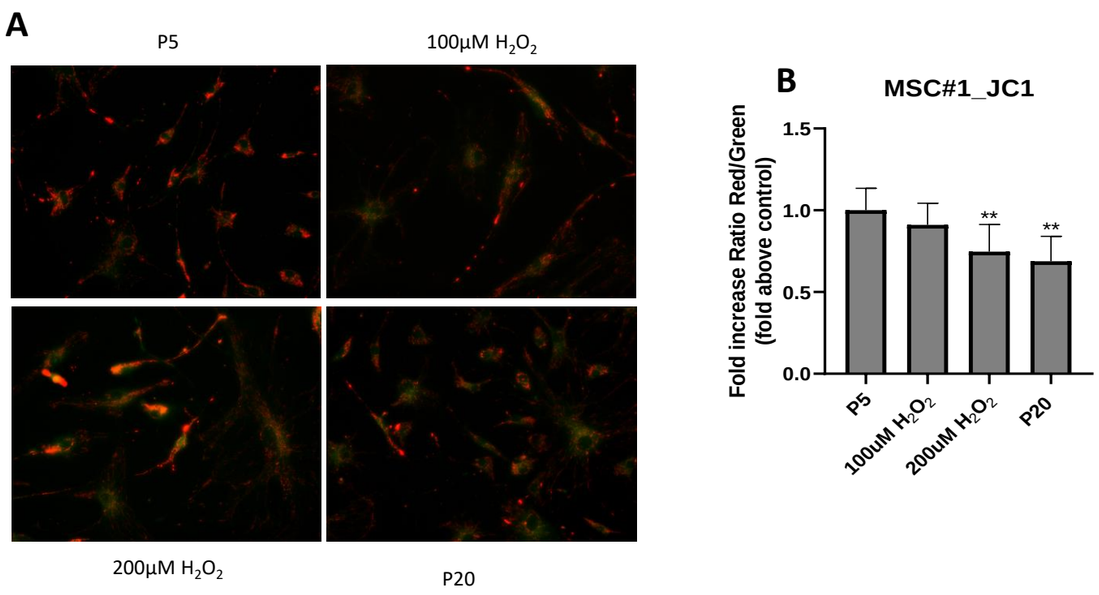
(A) *Representative images of pH2AX and 53BP1 foci formation induced by both induced and replicative senescence in MSC#2.* ***(B)*** *Graphs showing the activation of pH2AX and 53BP1 as mean number of foci/cells.* ***(C)*** *Western Blot performed of AD-MSC#1 after 48h from treatment with H_2O_2.* ***(D)*** *Graphs showing densitometry expressed as fold increase relative to control. Cells with passage <8 were used as control for both cell lines.*

H_2O_2 treatment reduces adipogenic differentiation



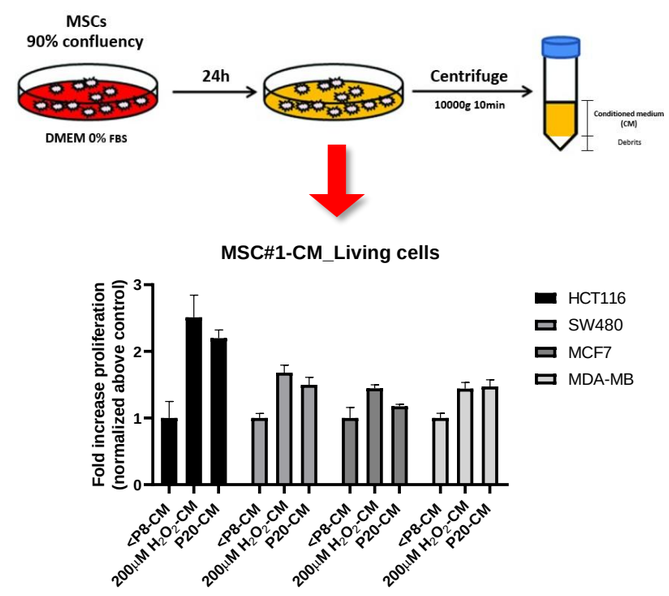
(A) *Representative images of MSC#2 LDs formation after 2 weeks induction of adipogenesis. Photos were acquired at fluorescent microscope. Nile Red was used to stain LDs (green)* ***(B)*** *Quantification of LDs mean fluorescence. Data are presented as mean ± standard deviation from an experiment performed in duplicate. **, Student's T test $p < 0.01$; ****, Student's T-test $p < 0.0001$.*

Senescence induction promotes mithocondrial membrane depolarization



(A) *Images of MSC#1 stained for JC1 acquired at fluorescent microscope.* ***(B)*** *Quantification of mithocondrial membrane polarization expressed as ratio between red and green signals. Data are presented as mean ± standard deviation from one representative experiment performed in duplicate. **, Student's T-test $p < 0.01$; ****, Student's T-test $p < 0.0001$.*

Senescent MSC-CM promotes cancer cells proliferation



Graphs show a significant increase of cancer cell lines viability upon exposure to conditioned medium collected from replicative- and oxidative stress- induced senescent cells compared to control (represented by conditioned medium collected from AD-MSCs at passage <8).

CONCLUSIONS

In conclusion, we established a reproducible model of oxidative stress-induced senescence for vAD-MSCs. This model will be employed for studying senescent vAD-MSCs secretome and its role in cancer, as well for the understanding of the molecular processes involved in vAD-MSCs senescence, adipose tissue aging and its functional impairment.