

55° Congresso AINPeNC Associazione Italiana
Neuropatologia e Neurobiologia Clinica

45° Congresso AIRIC Associazione Italiana
Ricerca Invecchiamento Cerebrale

Bologna, 23-25 Maggio 2019



IMPACT OF LYSOSOMAL STORAGE DISORDERS ON MESENCHYMAL STROMAL CELL BIOLOGY: EVIDENCES FROM AN IN VITRO MODEL OF GAUCHER AND FABRY DISEASES

Marina Melone

*Department of Advanced Medical and Surgical Sciences,
2nd Division of Neurology, Center for Rare Diseases and
InterUniversity Center for Research in Neurosciences,
University of Campania "Luigi Vanvitelli"
marina.melone@unicampania.it*



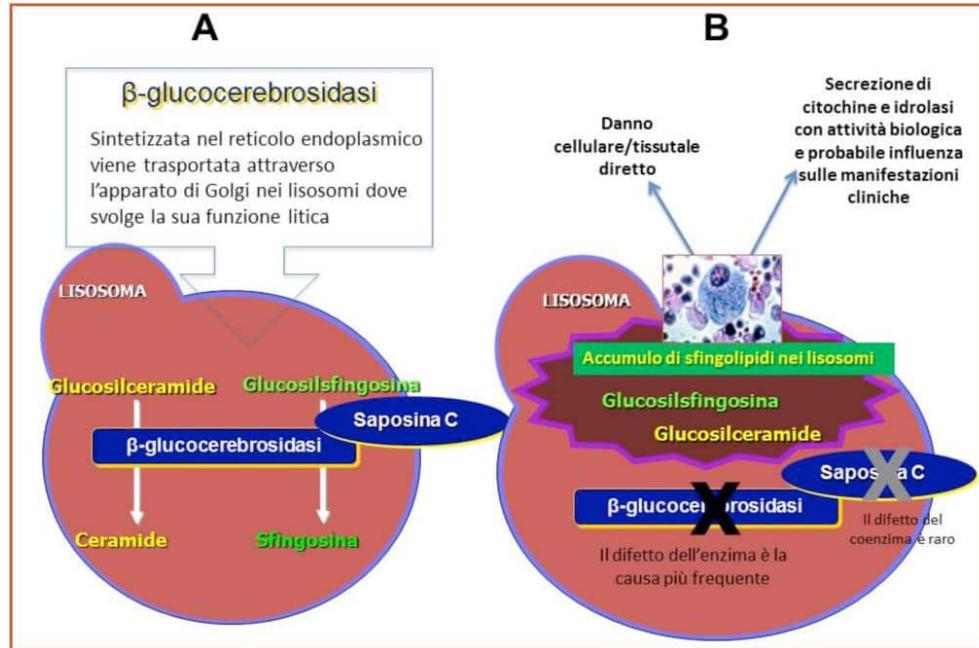
V • Università
degli Studi
• della Campania
Luigi Vanvitelli



Lysosomal storage diseases (LSDs)

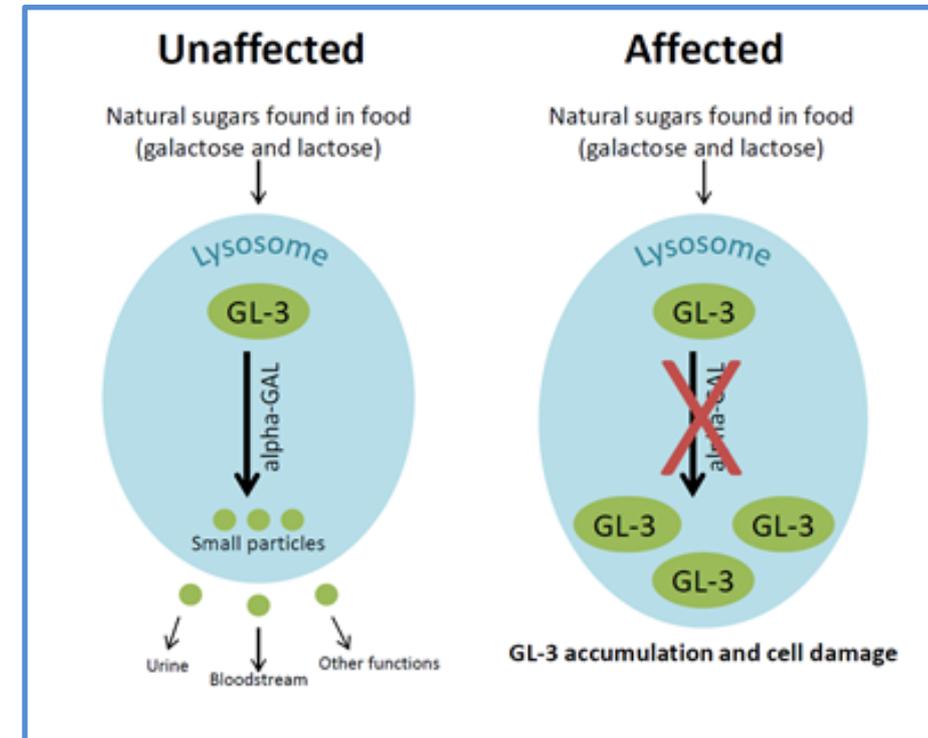
Gaucher disease (GD) and **Fabry disease (FD)** are the most common inherited LSDs, caused by deficiencies in the lysosomal glycosidases glucocerebrosidase (GBA) and alpha-galactosidase (GLA), respectively

Gaucher disease (GD)



www.ematologiainprogress.it/malattie-rare-malattia-di-gaucher

Fabry disease (FD)



www.newbornscreening.info/Parents/otherdisorders/Fabry.html



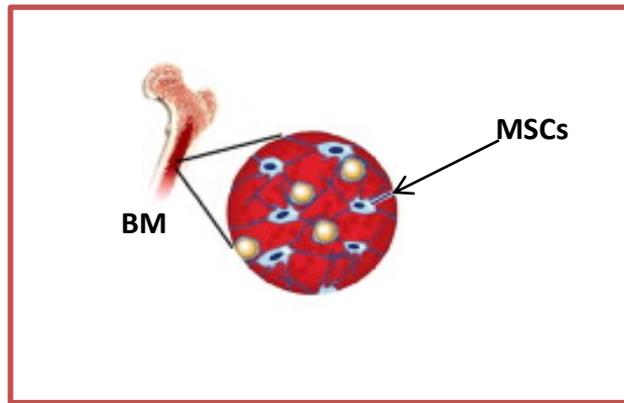
Human stem cells for disease modelling



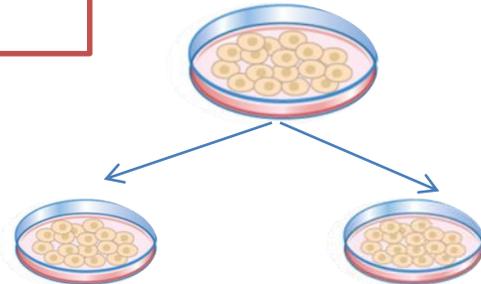
- Given the systemic nature of enzyme deficiency, the stem cell compartment of GD and FD patients can be also affected.
- This can pose profound consequences for an organism's physiology, since stem cells are responsible for maintaining tissue homeostasis and repairing subsequent injuries.

Mesenchymal stromal/stem cells (MSCs)

MSCs isolated from bone marrow (BM)



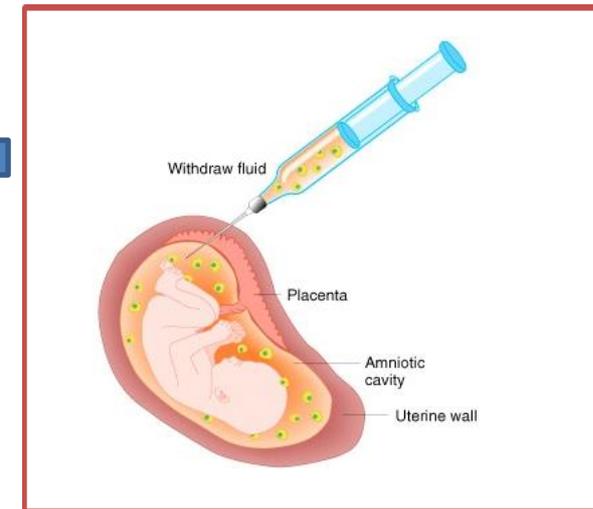
BM-MS culture



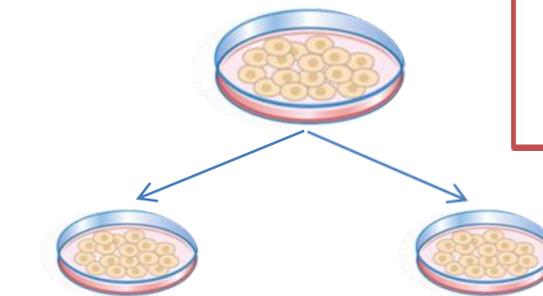
GBA silenced BM-MSCs

GLA silenced BM-MSCs

MSCs isolated from amniotic fluid (AM)



AM-MS culture



GBA silenced AM-MSCs

GLA silenced AM-MSCs

Gene silencing by small interfering RNA



Our experience in using stem cells for disease modelling

[Exp Mol Med](#). 2018 Mar 22;50(3):1. doi: 10.1038/s12276-017-0005-x.

Neural stem cells from a mouse model of Rett syndrome are prone to senescence, show reduced capacity to cope with genotoxic stress, and are impaired in the differentiation process.

[Alessio N¹](#), [Ricciello F²](#), [Squillaro T^{1,3}](#), [Capasso S¹](#), [Del Gaudio S¹](#), [Di Bernardo G¹](#), [Cipollaro M¹](#), [Melone MAB³](#), [Peluso G⁴](#), [Galderisi U^{5,6}](#).

[Cell Cycle](#). 2014;13(3):482-90. doi: 10.4161/cc.27275. Epub 2013 Nov 26.

Silencing of RB1 and RB2/P130 during adipogenesis of bone marrow stromal cells results in dysregulated differentiation.

[Capasso S¹](#), [Alessio N²](#), [Di Bernardo G¹](#), [Cipollaro M¹](#), [Melone MA³](#), [Peluso G⁴](#), [Giordano A⁵](#), [Galderisi U⁶](#).

[Mol Biol Cell](#). 2012 Apr;23(8):1435-45. doi: 10.1091/mbc.E11-09-0784. Epub 2012 Feb 22.

Reduced expression of MECP2 affects cell commitment and maintenance in neurons by triggering senescence: new perspective for Rett syndrome.

[Squillaro T¹](#), [Alessio N](#), [Cipollaro M](#), [Melone MA](#), [Hayek G](#), [Renieri A](#), [Giordano A](#), [Galderisi U](#).

[World J Stem Cells](#). 2019 Mar 26;11(3):180-195. doi: 10.4252/wjsc.v11.i3.180.

Circulating factors present in the sera of naturally skinny people may influence cell commitment and adipocyte differentiation of mesenchymal stromal cells.

[Alessio N¹](#), [Squillaro T²](#), [Monda V³](#), [Peluso G⁴](#), [Monda M³](#), [Melone MA²](#), [Galderisi U¹](#), [Di Bernardo G⁵](#).

[Cancer Biol Ther](#). 2009 Jul;8(13):1300-6.

Genes involved in regulation of stem cell properties: studies on their expression in a small cohort of neuroblastoma patients.

[Melone MA¹](#), [Giuliano M](#), [Squillaro T](#), [Alessio N](#), [Casale F](#), [Mattioli E](#), [Cipollaro M](#), [Giordano A](#), [Galderisi U](#).

[J Cell Biochem](#). 2010 Jul 1;110(4):903-9. doi: 10.1002/jcb.22602.

Controlled delivery of the heparan sulfate/FGF-2 complex by a polyelectrolyte scaffold promotes maximal hMSC proliferation and differentiation.

[Calarco A¹](#), [Petillo O](#), [Bosetti M](#), [Torpedine A](#), [Cannas M](#), [Perrone L](#), [Galderisi U](#), [Melone MA](#), [Peluso G](#).

[J Cell Physiol](#). 2018 Nov;233(11):8996-9006. doi: 10.1002/jcp.26845. Epub 2018 Jun 15.

Mesenchymal stromal cells from amniotic fluid are less prone to senescence compared to those obtained from bone marrow: An in vitro study.

[Alessio N¹](#), [Pipino C²](#), [Mandatori D³](#), [Di Tomo P³](#), [Ferone A¹](#), [Marchiso M³](#), [Melone MAB^{4,5}](#), [Peluso G⁶](#), [Pandolfi A²](#), [Galderisi U^{1,5}](#).

GBA and GLA silencing in BM- and AF-MSCs

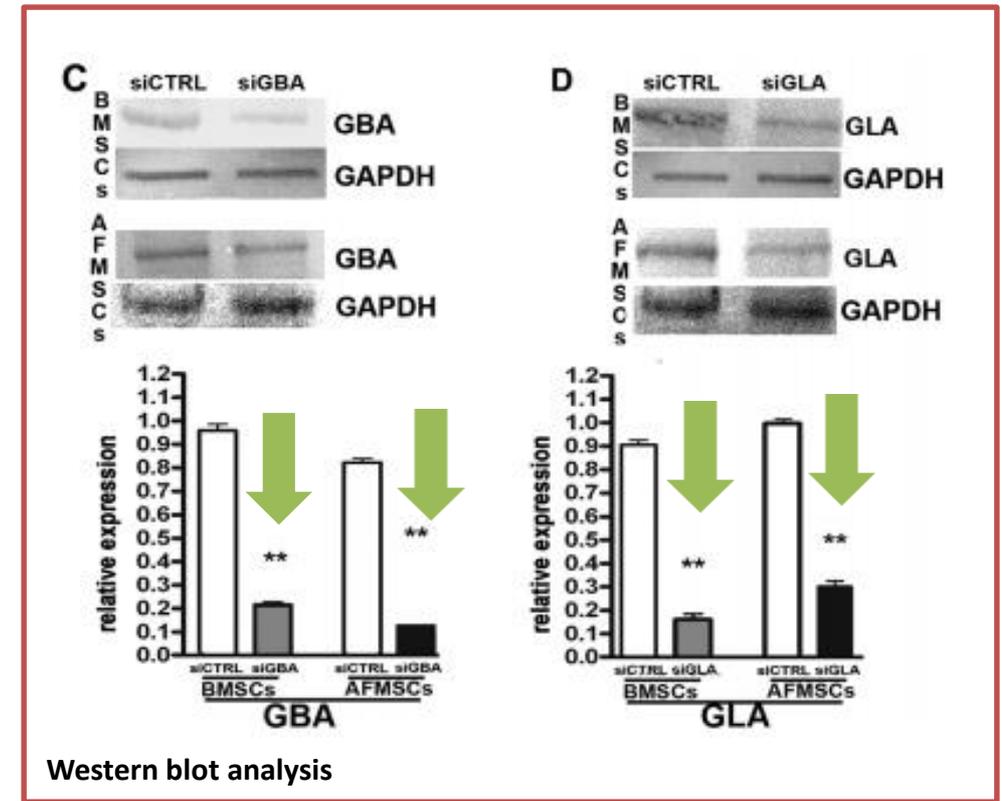
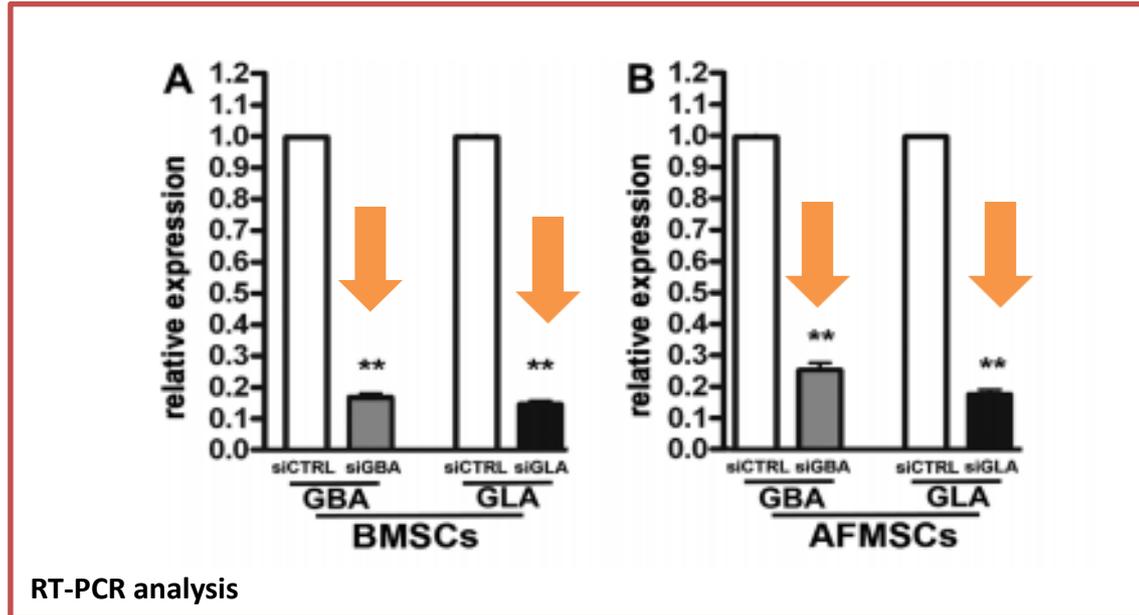


Results

J Cell Physiol. 2017 Dec;232(12):3454-3467. doi: 10.1002/jcp.25807. Epub 2017 Feb 7.

Impact of lysosomal storage disorders on biology of mesenchymal stem cells: Evidences from in vitro silencing of glucocerebrosidase (GBA) and alpha-galactosidase A (GLA) enzymes.

Squillaro T^{1,2}, Antonucci J³, Alessio N¹, Esposito A¹, Cipollaro M¹, Melone MAB⁴, Peluso G², Stuppia L³, Galderisi U¹.

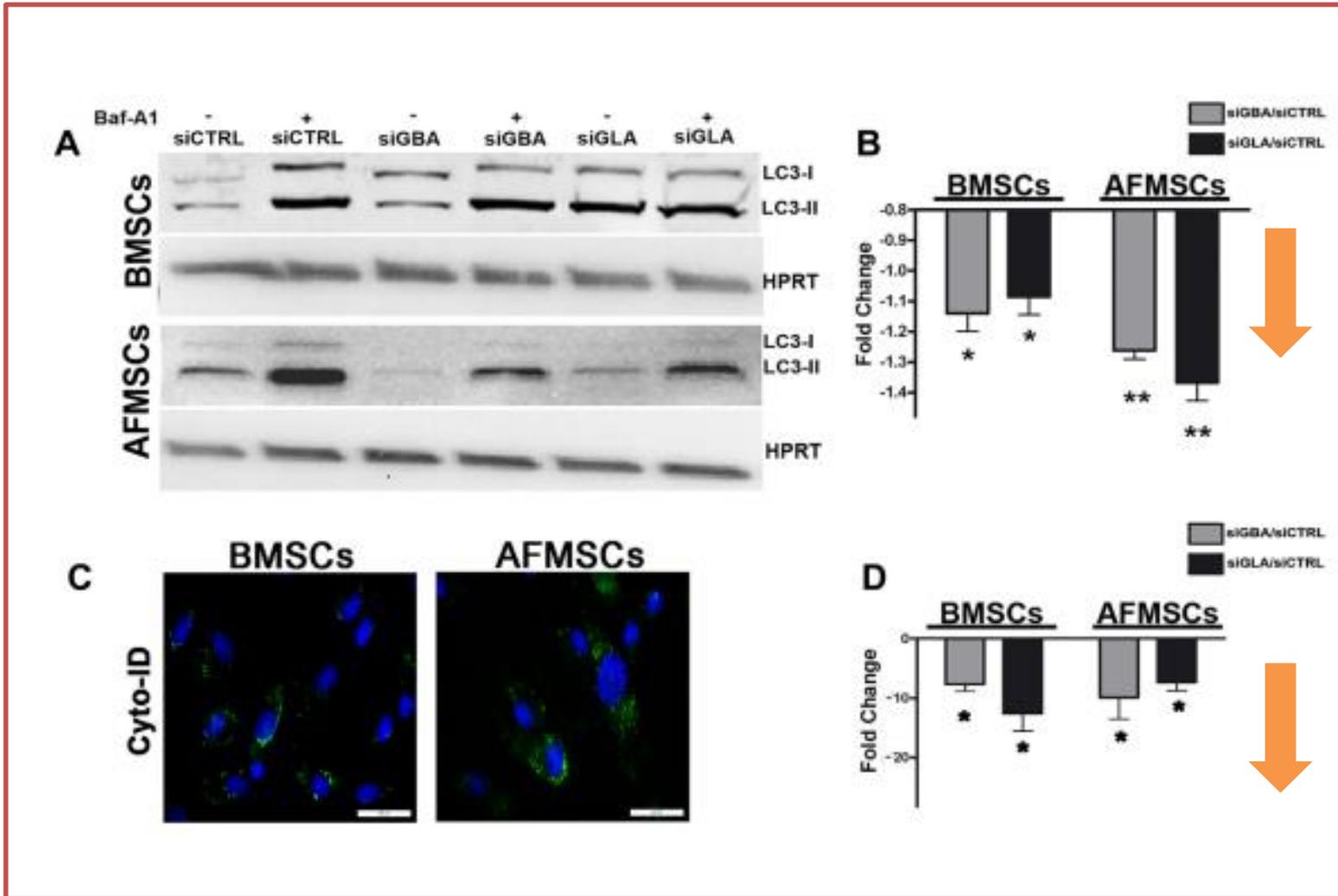


➤ GBA- and GLA-siRNAs were **effective in silencing and decreasing target mRNAs and proteins** for both cell cultures

Autophagy detection assays



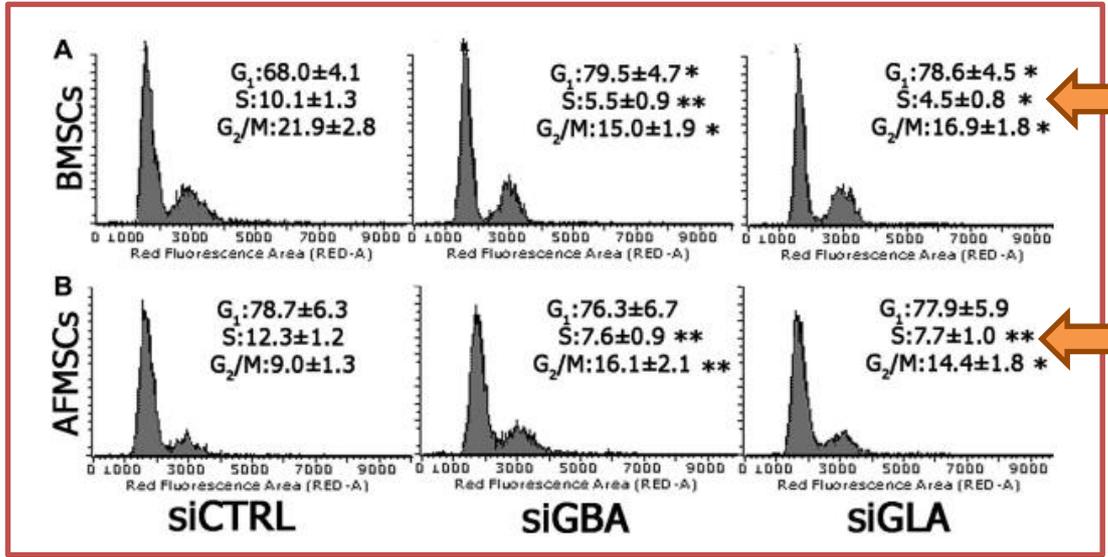
Results



➤ GBA and GLA silencing induced a reduction of autophagic flux in both MSC cultures

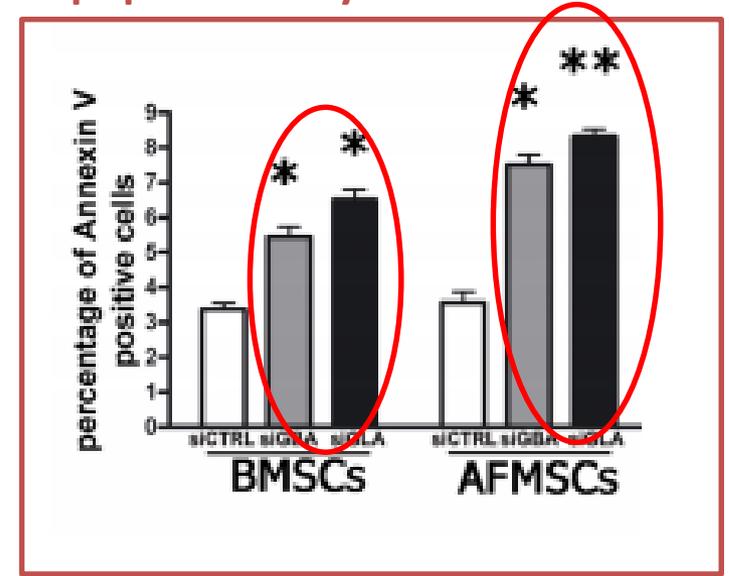
➤ The percentage of cells expressing active autophagic vacuoles was significantly decreased in both MSC cultures

Cell cycle analysis



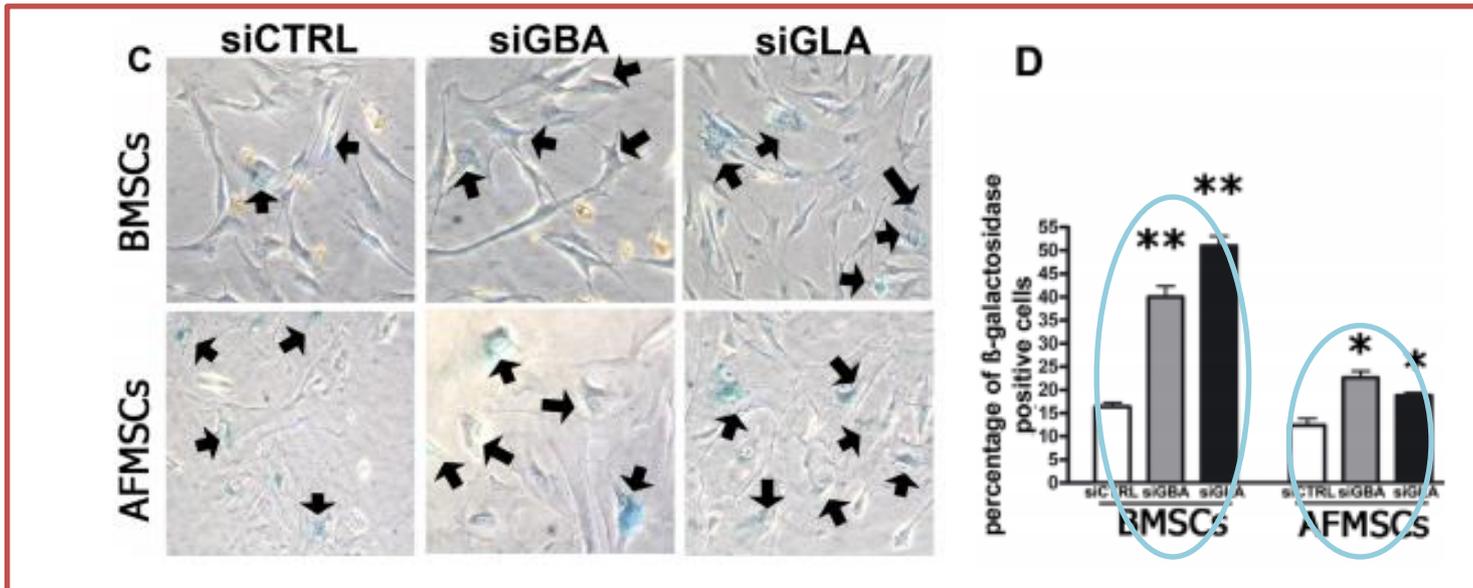
➤ **Significant reduction of S phase cells** in both BM- and AF-MSC silenced cultures

Apoptosis analysis



➤ **Significant increase of apoptotic cell percentage** in both BM- and AF-MSC silenced culture

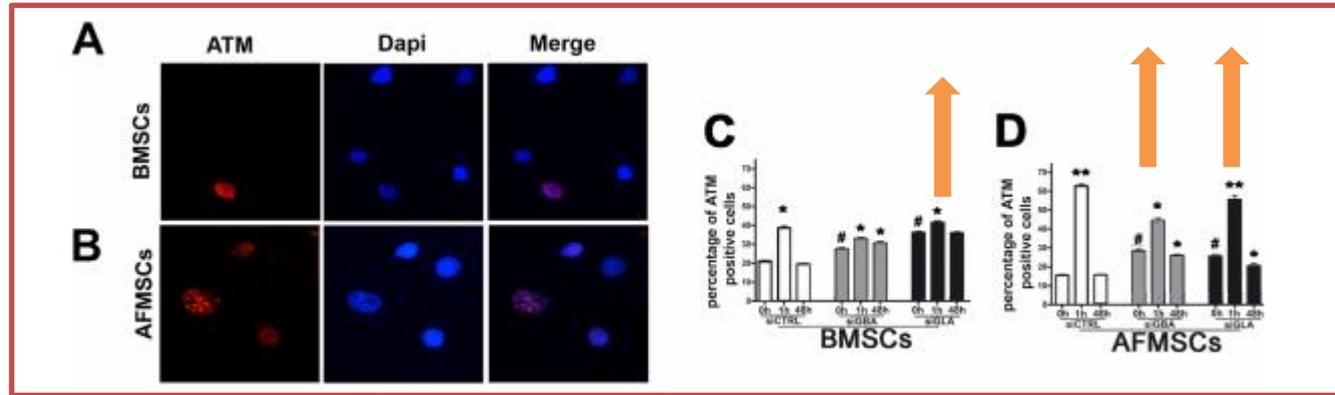
Senescence analysis



➤ **Significant increase of senescent cell percentage** in both BM- and AF-MSC silenced culture

Evaluation of DNA damage and repair

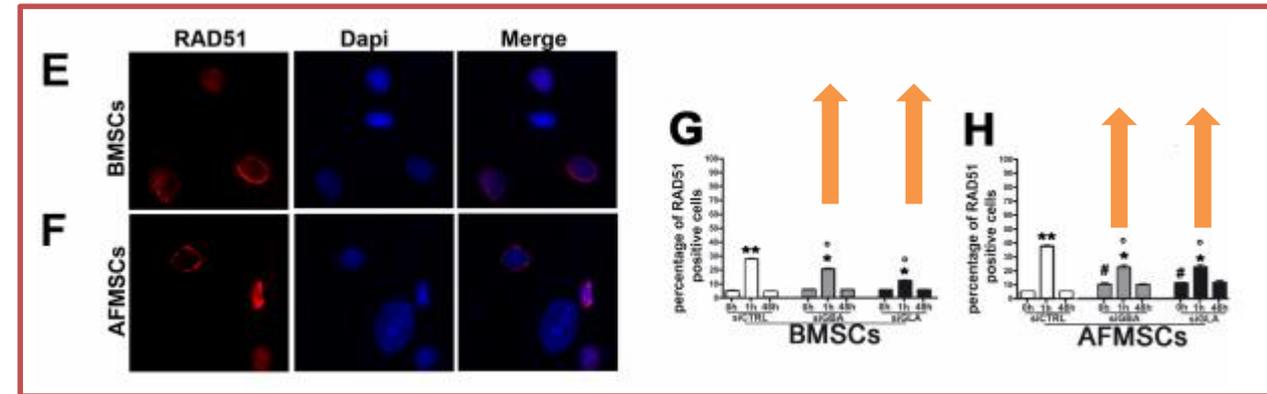
Results



➤ Increase of ATM-positive cells in basal conditions in both silenced MSC cultures

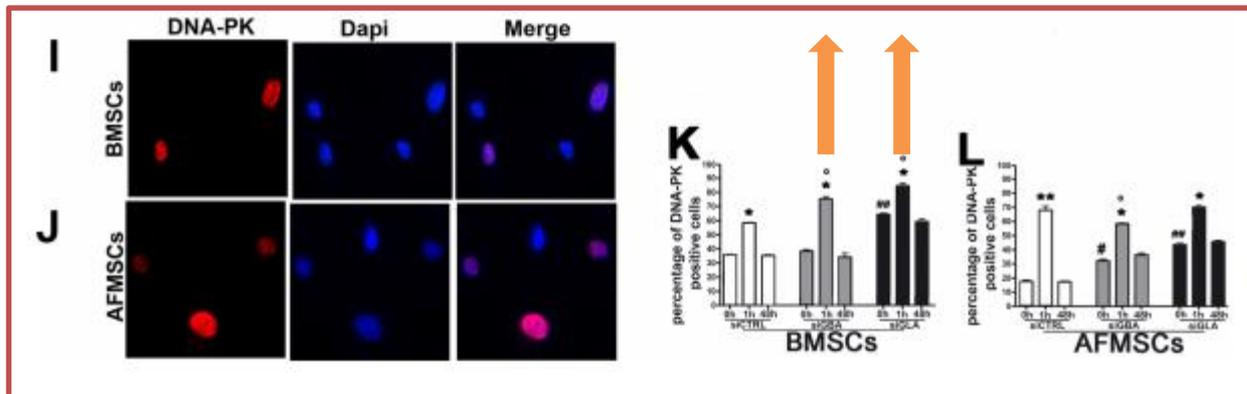
➤ The phenomenon was more remarkable in BM-MSC siGLA and AF-MSC siGLA and siGLA 1hr following H2O2 treatment

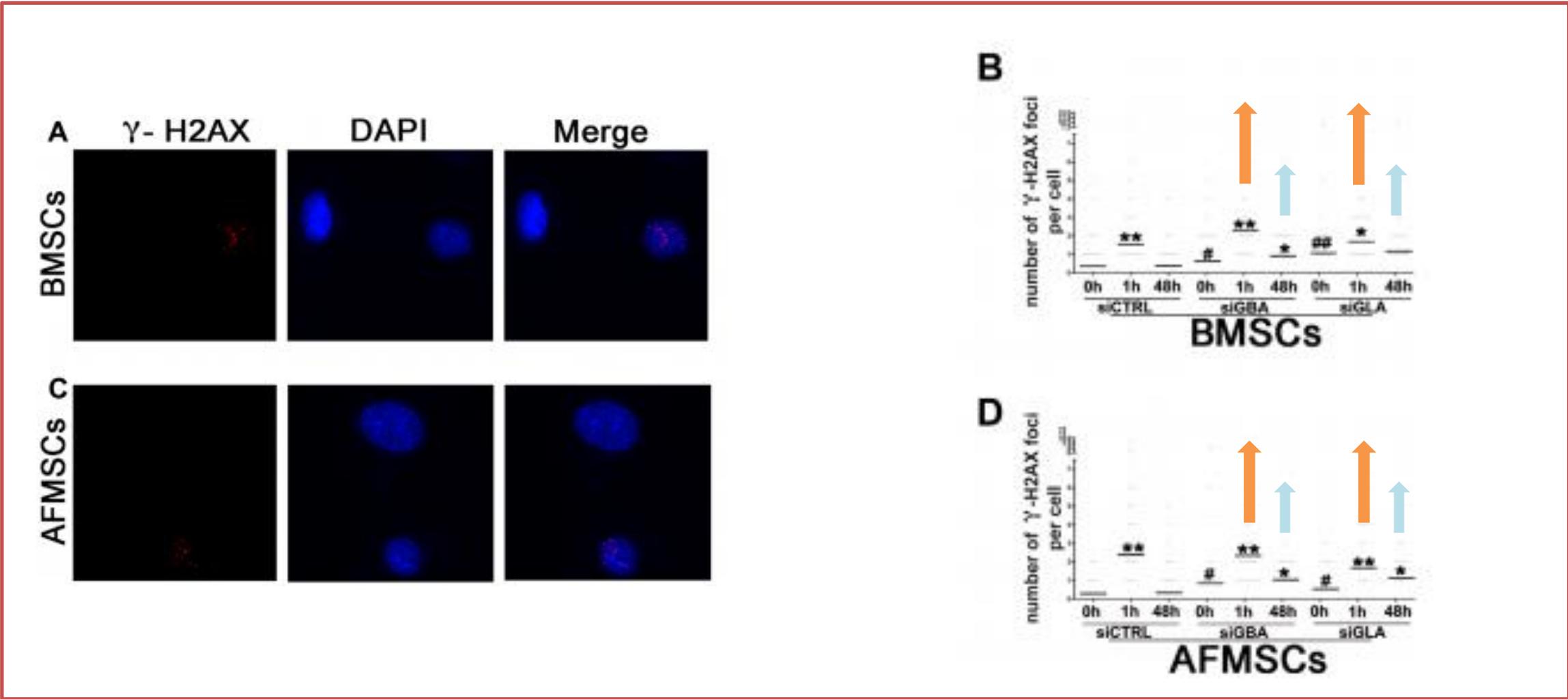
- Increase of **RAD51-positive cells** in basal conditions in both silenced MSC cultures
- Significant increase in the number of **RAD51** positive cells for both silenced cell cultures 1 hr following H2O2 treatment



➤ Increase of DNAPK-positive cells in basal conditions in both silenced MSC cultures

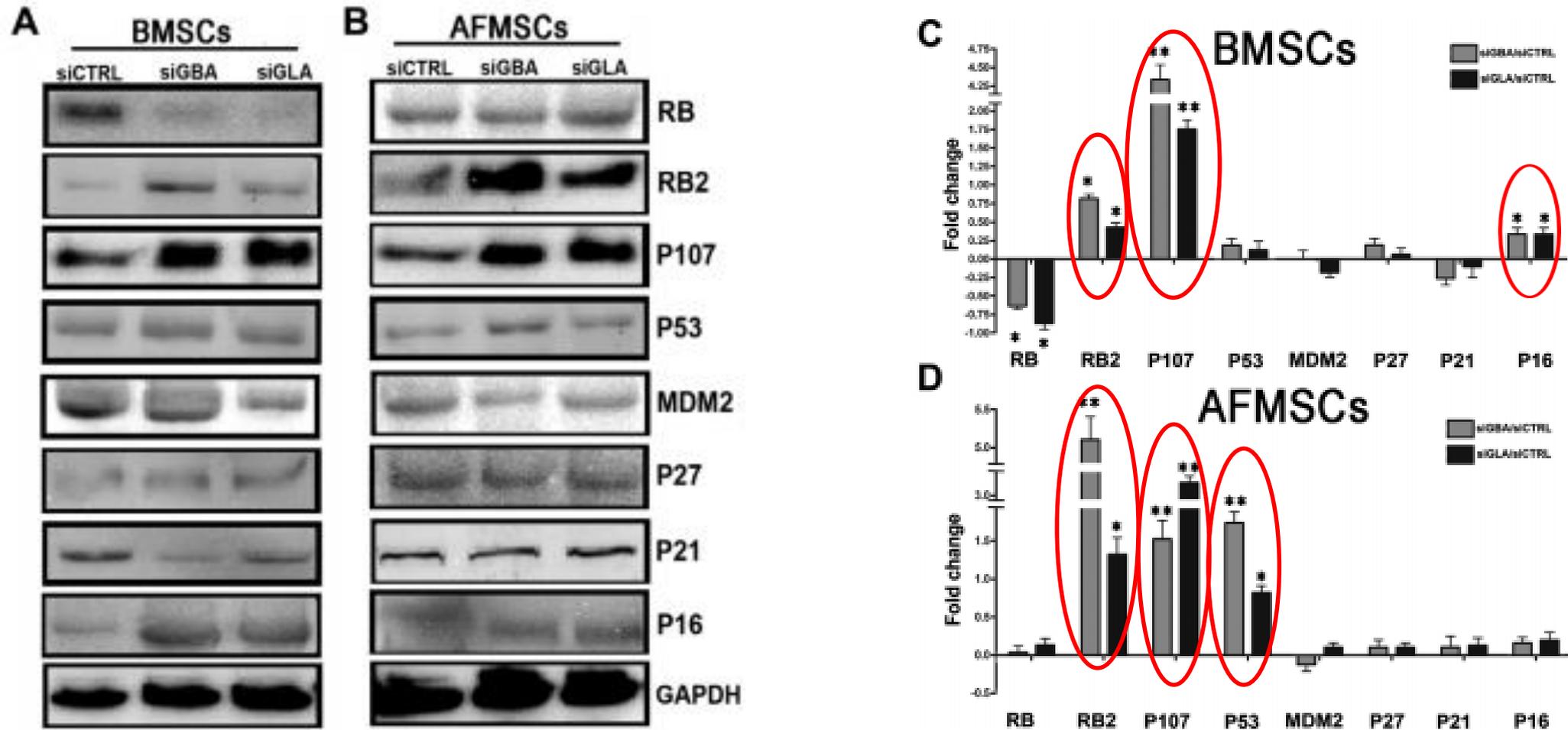
➤ Increase of DNAPK-positive cells in basal conditions and in silenced BMSCs treated for 1 hr with H2O2





- Both silenced BM- and AF-MSC showed a significant augmentation of cells with **a high number of gamma-H2AX foci** after 1 hr that persisted even **48 hr after H2O2 administration**

Gene expression analysis of RB- and P53-related pathways



- Following GBA and GLA silencing, cell cycle arrest and senescence can occur via RB2/p130-P16INK4A and RB2/p130-P53 pathways in BMSCs and AFMSCs, respectively



Conclusions



✓ Our data suggest that BMSCs and AFMSCs with reduced GBA or GLA activity **are prone to apoptosis and senescence due to autophagy and DNA repair impairment.**

✓ Our results pave the way for further analysis, given that **the senescence of the MSC compartment could profoundly affect tissue and organ physiology** in GD and FD patients.

✓ We demonstrated that **AFMSCs can represent a novel source of stem cells for modeling human genetic diseases**



- ✓ We aim to determine whether antisenescence treatments, such as **mTOR inhibition** and/or the use of **natural bioactive molecules with anti-oxidant property (polyphenols)**, can **ameliorate patients' symptoms or decelerate the progression of the diseases**, if not both.

[J Cell Physiol](#). 2019 May;234(5):5807-5826. doi: 10.1002/jcp.27506. Epub 2018 Oct 14.

Metabolic syndrome, Mediterranean diet, and polyphenols: Evidence and perspectives.

Finicelli M¹, Squillaro T², Di Cristo F³, Di Salle A¹, Melone MAB^{2,4}, Galderisi U^{5,4}, Peluso G¹.

[J Cell Physiol](#). 2018 May;233(5):3955-3967. doi: 10.1002/jcp.26170. Epub 2017 Sep 28.

Adult-onset brain tumors and neurodegeneration: Are polyphenols protective?

Squillaro T¹, Schettino C¹, Sampaolo S¹, Galderisi U², Di Iorio G¹, Giordano A^{3,4}, Melone MAB^{1,3}.

[PLoS One](#). 2015 Mar 18;10(3):e0118864. doi: 10.1371/journal.pone.0118864. eCollection 2015.

Ruta graveolens L. induces death of glioblastoma cells and neural progenitors, but not of neurons, via ERK 1/2 and AKT activation.

Gentile MT¹, Ciniglia C¹, Reccia MG¹, Volpicelli F², Gatti M³, Thellung S³, Florio T³, Melone MA⁴, Colucci-D'Amato L⁵.

[Neurochem Int](#). 2018 Jul;117:174-187. doi: 10.1016/j.neuint.2017.05.013. Epub 2017 May 19.

Resveratrol protects neuronal-like cells expressing mutant Huntingtin from dopamine toxicity by rescuing ATG4-mediated autophagosome formation.

Vidoni C¹, Secomandi E¹, Castiglioni A¹, Melone MAB², Isidoro C³.

[Biochem Pharmacol](#). 2018 Aug;154:303-317. doi: 10.1016/j.bcp.2018.05.016. Epub 2018 May 24.

Nano-delivery systems for encapsulation of dietary polyphenols: An experimental approach for neurodegenerative diseases and brain tumors.

Squillaro T¹, Cimini A², Peluso G³, Giordano A⁴, Melone MAB⁵.

[Nutrients](#). 2017 Jul 21;9(7). pii: E783. doi: 10.3390/nu9070783.

Synergistic Interplay between Curcumin and Polyphenol-Rich Foods in the Mediterranean Diet: Therapeutic Prospects for Neurofibromatosis 1 Patients.

Esposito T¹, Schettino C², Polverino P³, Allocca S⁴, Adelfi L⁵, D'Amico A⁶, Capaldo G⁷, Varriale B⁸, Di Salle A⁹, Peluso G¹⁰, Sorrentino G¹¹, Lus G¹², Sampaolo S¹³, Di Iorio G¹⁴, Melone MAB¹⁵.

Acknowledgements



Tiziana Squillaro



Marina Melone
II Division of Neurology & CIRN
University of Campania Luigi Vanvitelli
marina.melone@unicampania.it



Università
degli Studi
della Campania
Luigi Vanvitelli



Umberto Galderisi
and his group



Antonio Giordano
and his group



Liborio Stuppia
and his group



Gianfranco Peluso
and his group



CNR-IRET

