EXOSOMAL PREPARATIONS TO NEW NANOMEDICINE APPROACHES FOR SENESCENCE-ASSOCIATED DISEASES

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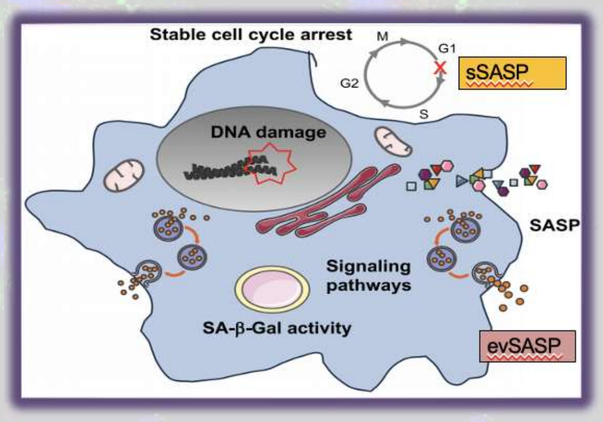
INTRODUCTION: Senescent cells are reported to show increased secretion of small extracellular vesicles (sEVs), characterized by altered protein composition and pro-proliferative function on some cancer cell lines. Previous our data have reported the effect of chronic resveratrol treatment, in the modulation of SASP-factor secretion by senescent Neonatal Human Dermal Fibroblasts (NHDFs). Here, we studied the role of the chronic resveratrol treatment in the modulation of sEVs and the role of sEVs in tumor cell proliferation and invasion.

EXPERIMENTAL: Senescent NHDFs were treated for 5 weeks with resveratrol 5μM (R5). Conditioned media (CM) collected from untreated (CM-sen) and R5 treated (CM-R5) senescent NHDFs were used for sEVs isolation, through two sequential steps of ultracentrifugation. The effect of R5 treatment on the exosome secretion and composition was evaluated by analyzing the size, count rate and expression of specific EVs markers. Cancer cell lines (A375-M6 and MCF7) were incubated for 48 hours with both complete and depleted of sEVs CM-sen and CM-R5 in order to evaluate cell proliferation and invasion.

RESULTS AND DISCUSSION: Our results evidenced that CM-R5 showed a decrease of sEVs secretion, compared to CM-sen; in parallel we demonstrated a reduction of proliferation and invasion activity of A375-M6 and MCF7, incubated with CM-R5 complete with sEVs, compared to CM-sen. These data suggest that R5 treatment modulate the composition of senescent sEVs and exert an anti-tumoral effect on cancer cells.

The role of the R5 treatment in the modulation of senescence markers

a. **Biomarkers of senescence**



b. **SA-β- Gal-staining**

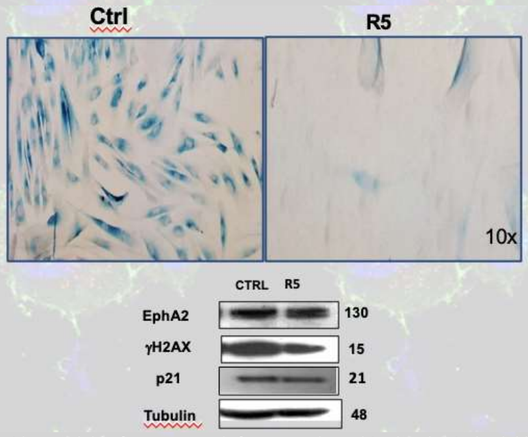


Figure 1. a: Senescence Biomarkers; SA-β-Gal, senescence-associated beta galactosidase; soluble factors, growth factors, released in the classical soluble senescence-associated secretory phenotype (sSASP) model**;** SASP factors include extracellular vesicles (evSASP). **b**: Effect of chronic resveratrol treatment on SA-β-Gal activity and senescence markers evaluated in Western Blotting.

Experimental Protocol

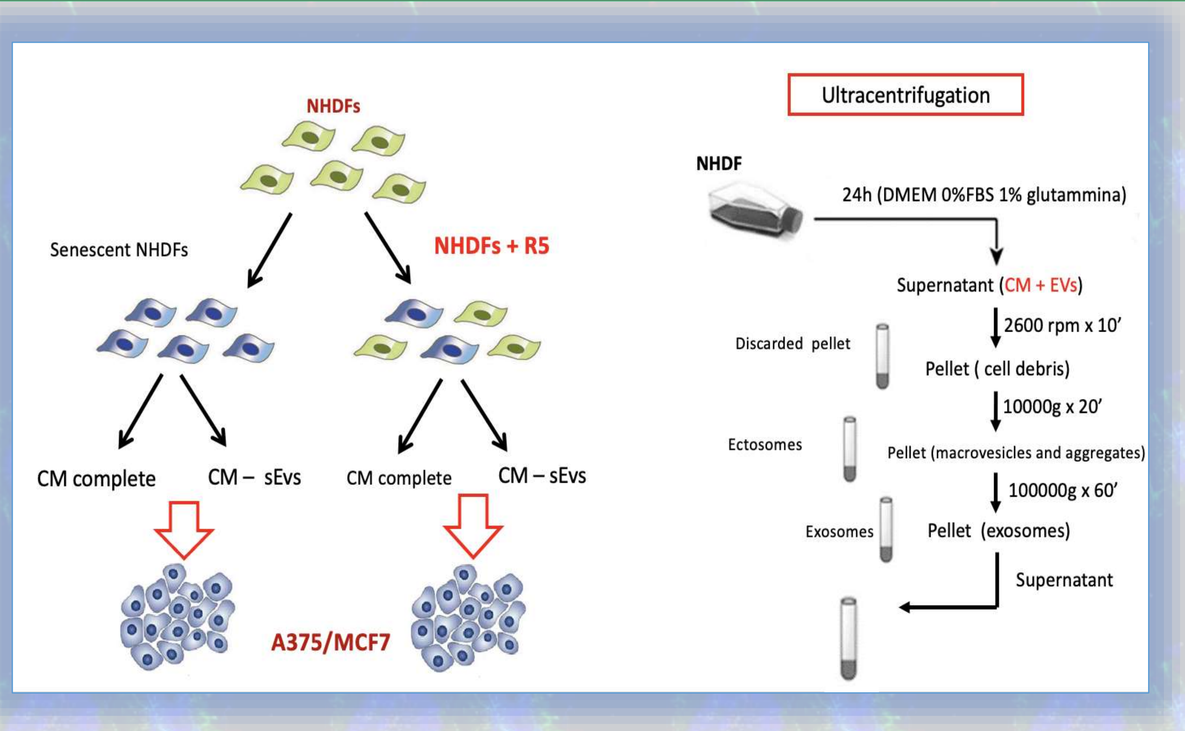


Figure 2: CM-sen and CM-R5 were collected for isolation of sEVs ; sequential steps of ultracentrifugation were performed to isolate sEVs; a first ultracentrifugate (12 000 rpm, for 20 minutes at 4°C) were performed to isolate ectosomes (0.1-1μm). Supernatant was subjected to further ultracentrifugate (30 000 rpm, for 1h at 4°C) for the isolation of exosomes (50-150nm)**.** Cancer cell lines (A375-M6 and MCF7) were seeded at density of 3.0 × 10^4 cells/cm^2; once grown to approximately 60% confluency cells were incubated for 48 hours with unconditioned DMEM plus 2% FBS or CMs-sen and CMs -R5 , both complete and depleted of EVs.

sEVs - TEM Analysis

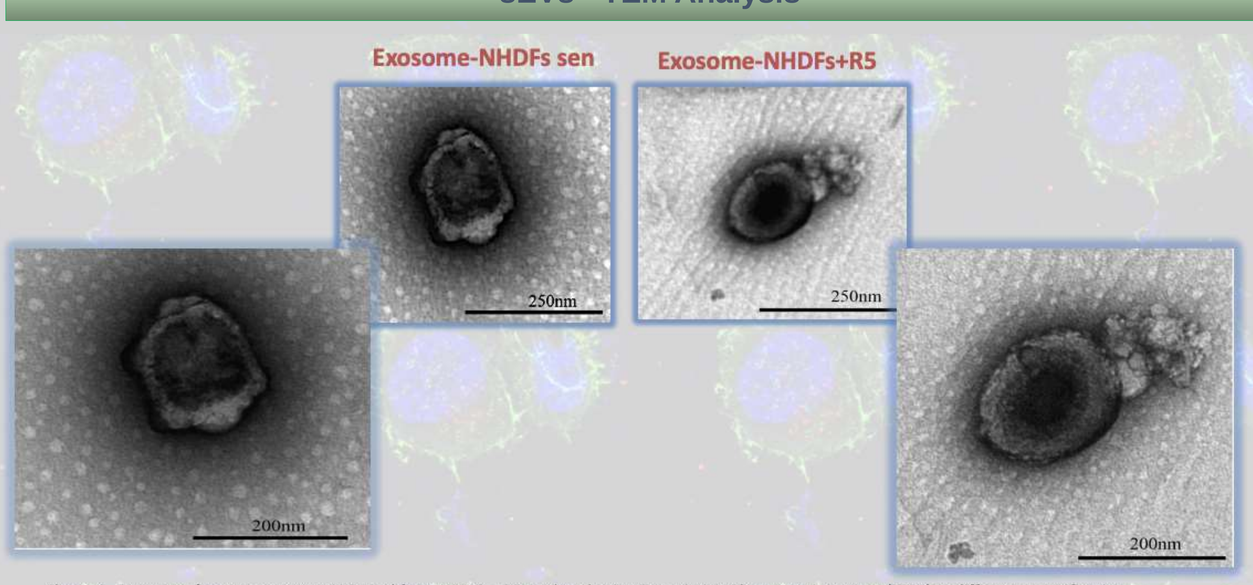


Figure 3. Exosomes from senescent NHDFs and from NHDFs +R5 analyzed using Trasmission Electron Microscopy (TEM) at different magnification.

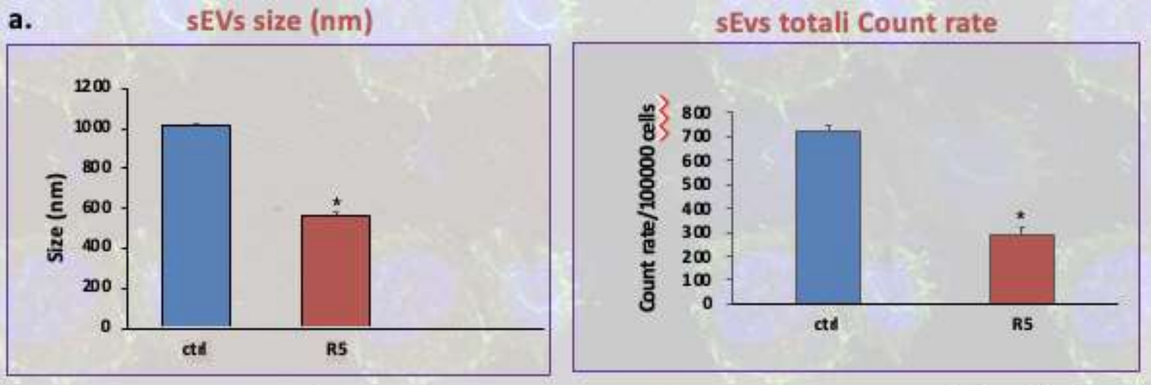
Next experiments

Focus on the investigation of molecular cargo of sEVs from untreated and R5 treated senescent cells. Evaluation of the presence of resveratrol in sEVs collected from R5 treated NHDFs, in order to exploit exosomes as nanovectors for delivery resveratrol on cancer cells to new nanomedicine approaches for cancer and senescence-associated diseases.

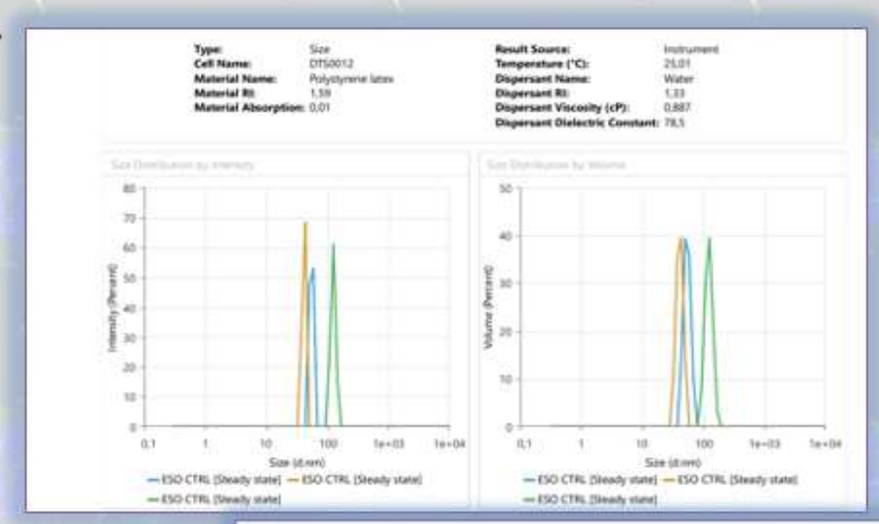
This work was supported by Tuscany Region (Call on Health Bando Ricerca Salute 2018) through Project "THERMINATOR.

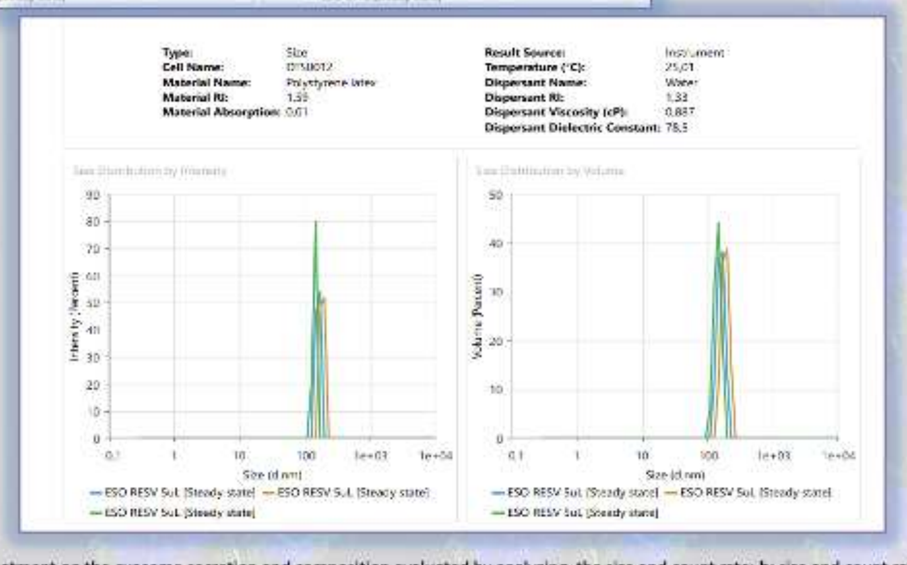
The effect of R5 treatment in the modulation of sEVs

a.



b.



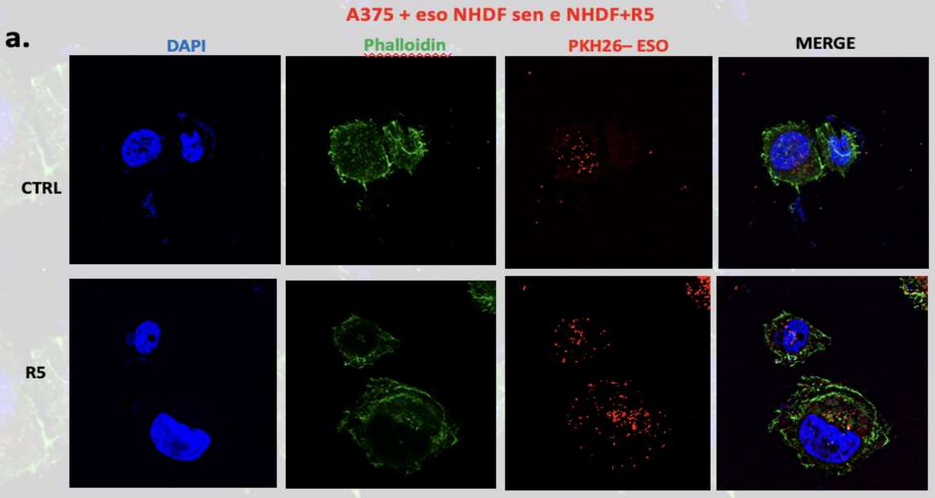


* p<0.05 vs sEVs-Ctrl

Figure 4. a: Effect of R5 treatment on the exosome secretion and composition evaluated by analyzing the size and count rate; **b:** size and count rate distribution of sEVs evaluated by Dynamic Light Scattering (DLS) ; * shows statistical significance (p< 0.05) compared to sEVs Ctrl.

The role of sEVs in tumor cell proliferation and invasion

a.



b.

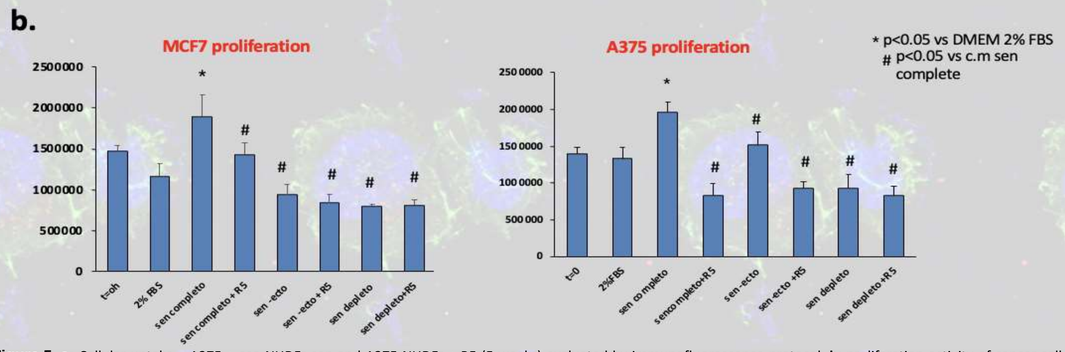


Figure 5. a: Cellular uptake – A375 + eso NHDFs sen and A375 NHDFs + R5 (5 weeks) evaluated by immunofluorescence protocol; **b:** proliferation activity of cancer cell lines (A375 and MCF7) incubated for 48 hours with both complete and depleted of sEV CM-sen and CM-R5; * shows statistical significance (p< 0.05) compared DMEM 2% FBS; # shows statistical significance (p< 0.05) compared to CM sen complete of sEVs.