Extracellular vesicles-based identification of lung fibrosis-associated biomarkers for targeted therapies.

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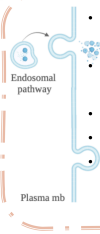
Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic lung disease with a median survival rate of 3 years due to the lack of effective therapies. Its development and progression are partly driven by senescence and clearance of these cells by senolytics have showed significant anti-fibrotic effects. Unfortunately, senolytics are not broadly efficient and showed undesirable side effects *in vivo* which could be overcome by targeted therapies. To identify potential therapeutic targets, we propose the use of extracellular vesicles (EVs) described as a fingerprint of the parental cell.

Background: Extracellular vesicles

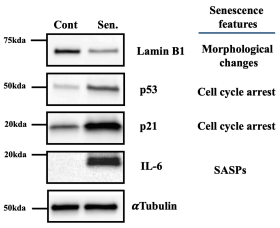


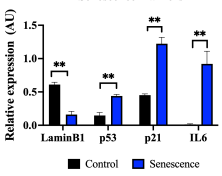
- Lipid bilayer particles released by cells that originate from the endosomal system (exosome) or shed from the plasma membrane (microvesicles).
- EVs could be classified in function of their biogenesis or size.
  Small EVs (sEVs): 0-200 nm *Large EVs* (LEVs): 200-1000 nm
- They carry nucleic acids, lipids, and proteins from their parental cell.
- They are cell messengers with pleiotropic functions in health and diseases.
- Different clinical applications have been described for these particles such as source of diagnostic biomarkers and vehicles to deliver therapeutics. ***Could extracellular vesicles be also used as a discovery platform for therapeutic targets?***

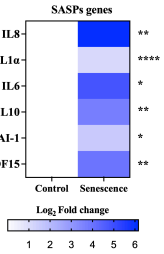
Experimental desing

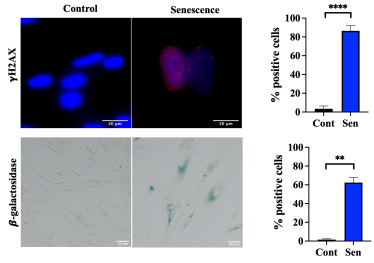
** Chemotherapeutic drug associated with development of lung fibrosis in vivo.*

1. Senescence characterisation



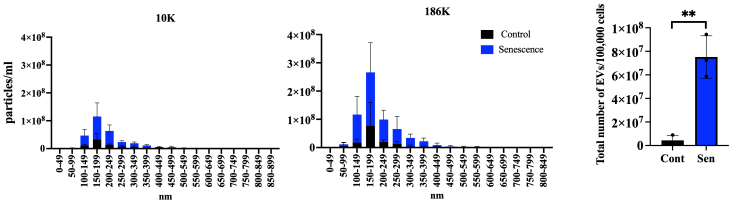






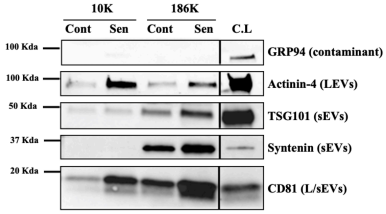
2. Extracellular vesicles characterisation

EVs size distribution & concentration measured by Nanoparticle Tracking Analysis (NTA)



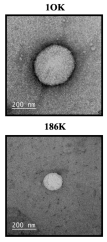
Extracellular vesicles were isolated by successive ultracentrifugation steps at 10,000xg (10k) and 186,000xg (186K). NTA analysis showed that 10k pellet had a wide range of particles sizes with similar concentrations while 186k pellet, was highly enriched in small EVs. In parallel, measurement of particles concentration showed that bleomycin-induced MRC5 cells produced and released an increased number of EVs in comparison to its control.

Large & small EVs markers by Western blot



Isolated EVs were further characterised by western blot. Small EVs associated targets were highly present in the 186k fraction, while the large EVs marker was majorly found on the 10k pellet. This result confirmed the enrichment of sEVs after 186k centrifugation and highlights the major isolation of LEVs on the 10k fraction.

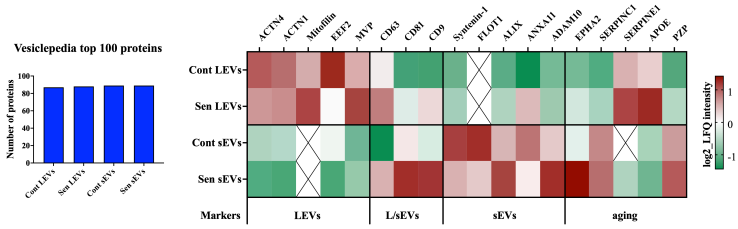
EVs morphology by TEM*



** Transmision electron microscopy*

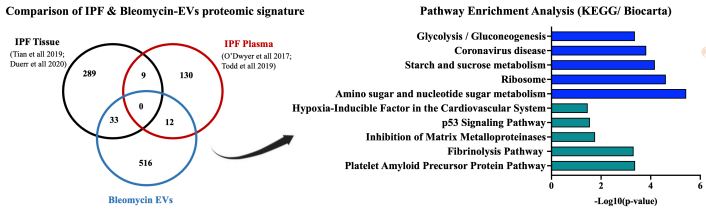
3. Extracellular vesicles proteomic analysis

Validation EVs Mass Spectrometry data



Quantified EV proteins from MRC5 cells overlap with approximately 80% of those listed in the *vesiclepedia* top 100 most abundant EV proteins. Intensity levels of EV markers confirmed the enrichment of LEVs and sEVs after 10k and 186k centrifugation, respectively. This data is in line with our previous results and further confirm the optimal isolation of EVs by ultracentrifugation. In addition, previously EV markers associated to senescence and aging have been found upregulated on senescence EVs further validating the induction of senescence by bleomycin.

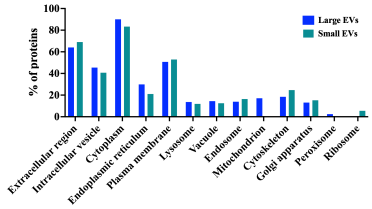
Relevance of Bleomycin-MRC5 model in IPF



Comparison analysis of IPF human tissue and plasma proteome with the Bleomycin-EVs enriched proteins shows a common proteome signature between IPF and bleomycin model. Pathway enrichment analysis of these common proteins identified pathways related to fibrosis and senescence highlighting the relevance of this model in the IPF context. Interestingly, this proteomic signature seems to be associated with coronavirus which pathogenesis seems to be partly mediated by senescence and EVs.

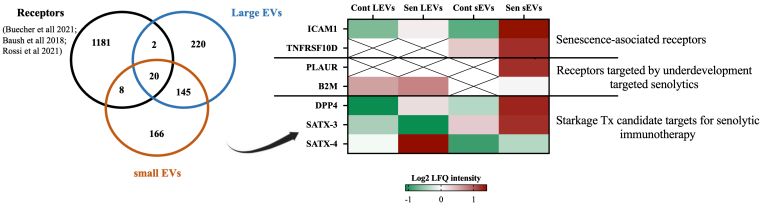
Extracellular vesicles as a source for therapeutic targets

GO cellular compartment of bleomycin enriched EVs proteins



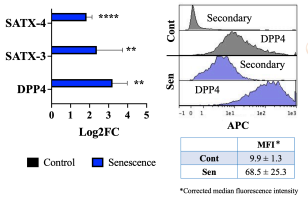
GO cellular compartment analysis revealed that 50.7 and 52.7% of bleomycin-enriched proteins from LEVs and sEVs respectively, were located at the plasma membrane.

Identification of receptors in bleomycin-EVs



A database containing 1211 recpetors was created to figure out wether any of those plasma proteins were plasmatic receptors. The results showed that at least 14% and 16% of the plasma proteins were receptors located at LEVs and sEVs, respectively. Interestingly, among those receptors were found PLAUR and B2M, two plasmatics receptors already validated as optimal target candidates for targeted senolytic therapy. Moreover, reported senescence associated receptors were also identified on bleomycin-EVs. Interesting potential therapeutic targets were also discovered.

Starkage Tx targets: Expression in Bleomycin-MRC5 cells.



Starkage Tx targets expression were validated at genomic (PCR) and protein (FACs) level in senescence MRC5 cells. All the targets were upregulated on senescence cells confirming the results provide by EVs.

4. Conclusions

- Bleomycin-induced senescence cells is a relevant cell model to explore therapeutics targets for IPF.
- This preliminary data highlighted Extracellular vesicles as an optimal source of therapeutic targets for senescence cells. This data led to the development of a platform named **ExoCise™** which objective is identifying posible therapeutics targets for senolytic immunothepies against IPF and other age-related diseases.

5. Perspectives

- Identified targets will be validated in IPF human samples.
- Extracellular vesicles will be isolated from plasma and tissue samples derived from IPF patients to continue stregnthening the value of the **ExoCise™** platform.

Identification of senescent cell surface markers as immunotherapeutic targets

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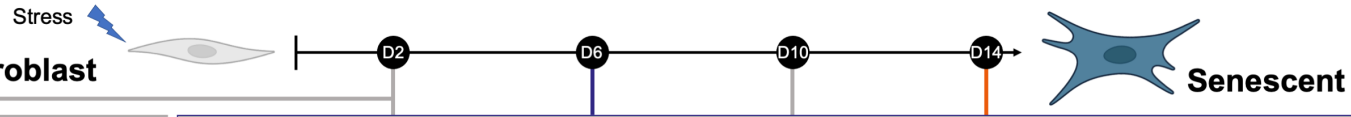
What we do? Since senescence is linked to aging and the occurrence of age-related pathologies, we aim to identify potential markers of senescent cells that can be specifically targeted by immunotherapy.

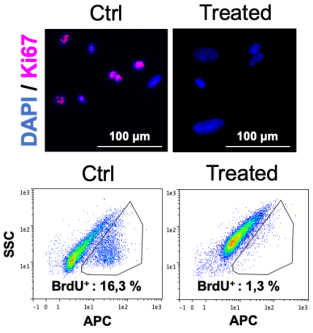
How we do? We combine omics and cell-based approaches to define cell surface senescence markers for immunotherapy development.

What we got? We identified novel markers that are highly expressed at the cell surface of a genotoxic stress-induced premature senescence model of human fibroblasts.

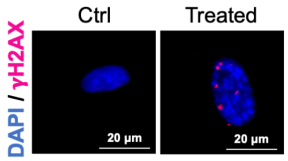


1. Inhibition of proliferation

Downregulation of Ki67 expression and BrdU incorporation

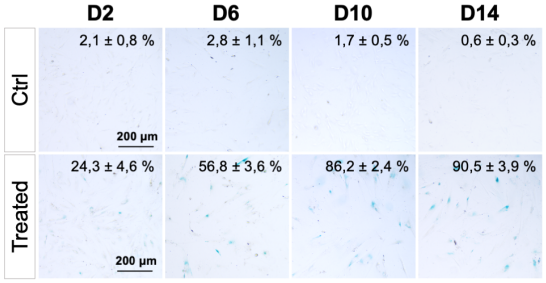


2. Induction of DNA damage

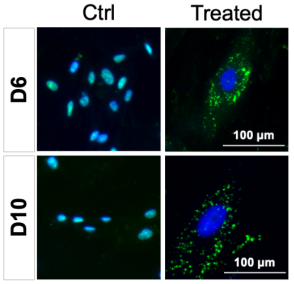


3. Appearance of senescence phenotype

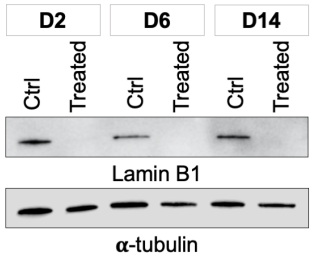
Increased β-Galactosidase activity



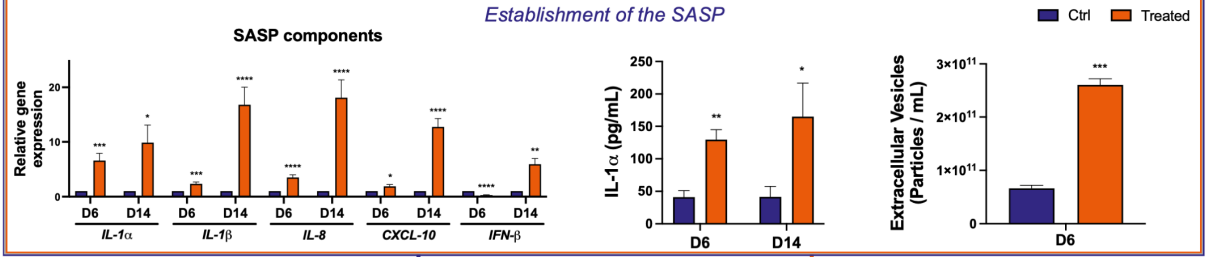
Nuclear/cytoplasmic translocation of HMGB1



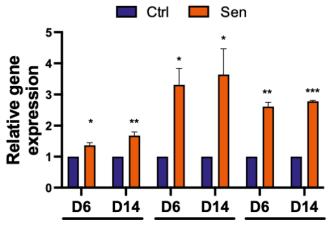
Downregulation of Lamin B1

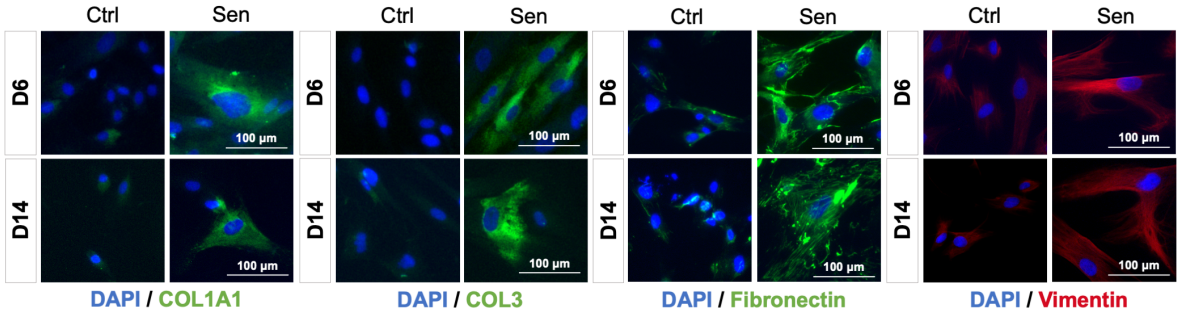


Establishment of the SASP

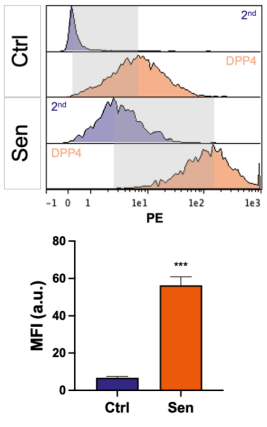


4. Upregulation of central mediators of tissue remodeling and fibrosis



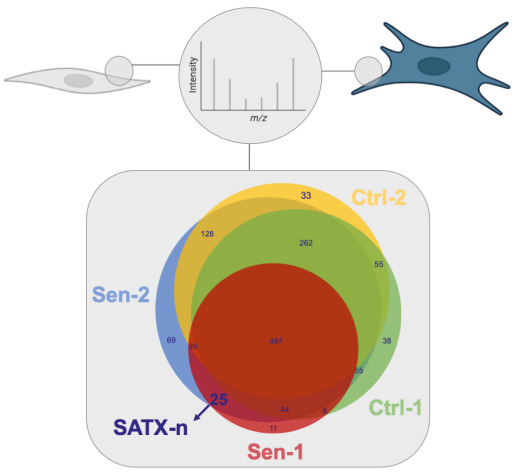


5. Upregulation of dipeptidyl peptidase-4 (DPP4)

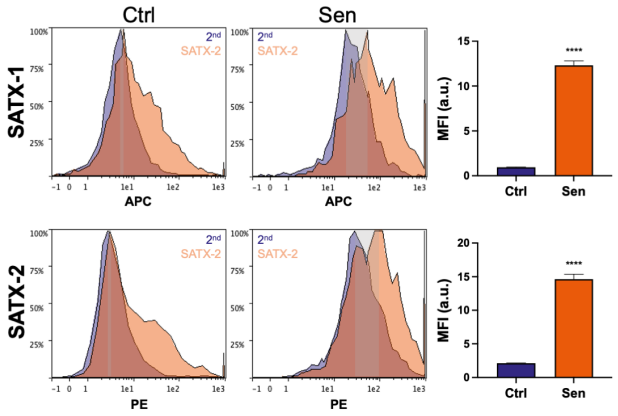


Note: DPP4 was also upregulated in replicative and oxidative-stress induced senescence models

6. Identification of novel cell surface markers



Target validation of identified cell surface markers



Take home message

- Given its expression in different senescence models, DPP4 can be considered as a senescence marker.
- Novel cell surface markers, together with DPP4, define targetable senescence signatures.

Next step

- Validate identified targets in clinical samples and design potential immunotherapy.

Acknowledgement: We thank Dr. Müge Ogrunc for the technical advices