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Decoding Therapy-induced Senescence EVs: Proteomic and miRNomic Clues to NSCLC Chemoresistancele

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INTRODUCTION & AIM

Non-small cell lung cancer (NSCLC) accounts for ~85% of lung cancers and remains one of the leading causes of tumor mortality worldwide.[1] Nevertheless, therapy resistance compromises treatment efficacy and long-term survival.[2] Paradoxically, anticancer therapies do not invariably elimante tumor cells; instead, they may trigger therapy-induced senescence (TIS), forcing cancer cells into permanent arrest. These senescent cells remain metabolically active, secreting the senescence-associated secretory phenotype (SASP): a potent cocktail of growth factors, cytokines, and extracellular vesicles (EVs).[2,3] Far from being passive mediators, these EVs emerge as critical regulators of tumor survival, shuttling proteins and miRNAs between cells to enhance resistance and drive progression.[4,5] However, the temporal dynamics of EV cargo during senescence and their mechanistic role in NSCLC chemoresistance remain poorly understood, and predictive biomarkers have not been identified.[6]

We aim to comprehensively characterize the proteomic and miRNomic signatures of senescence-derived EVs and SASP across two critical time windows (6 and 10 days), revealing their contribution to NSCLC chemoresistance and identifying actionable biomarkers and therapeutic targets. Using integrated mass spectrometry-based proteomics and miRNA sequencing, we will map the molecular architecture of senescence-driven intercellular communication underlying chemoresistance.

METHOD

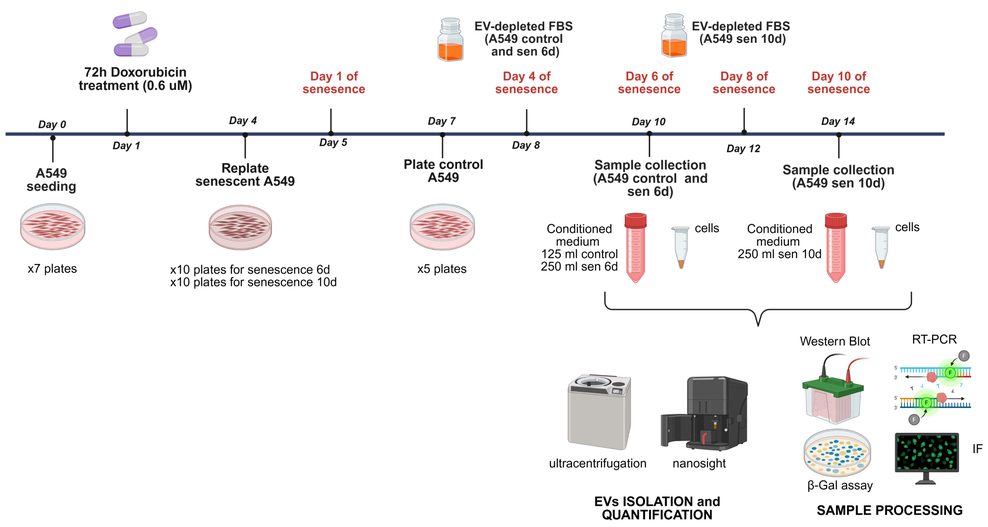


Figure 1. Chemotherapy-induced senescence model. A549 NSCLC cells were treated with Doxorubicin 0.6 uM for 72h to induce senescence. Conditioned media and cells were collected at 6 and 10 days of senescence for EV isolation and characterization, while cells were harvested for senescence validation.

CONCLUSIONS

Our findings demonstrate that therapy-induced senescence profoundly remodels the molecular cargo of extracellular vesicles, establishing a communication network that may influence NSCLC chemoresistance.

Key findings:

- Validation of TIS induction and proper EV isolation confirmed increased EV release from senescent cells.
- Identification of 239 intracellular DEmiRNAs and 39 EVs, predominantly upregulated.
- Key upregulated DEmiRNAs include miR-483-5p (EV-specific), miR-206, miR-146b-5p, and miR-34a-5p (both compartments), showed the most pronounced alterations at both 6 and 10 days of senescence.
- Enriched miRNAs pathways are related to apoptosis evasion, ECM remodelling, immune modulation, and DNA damage mechanisms, potentially involved in chemoresistance.
- Proteomic profiling identified 1296 proteins (including SERPINEs, FAS, CAV1) shared with ExoCarta/Vesiclepedia databases and 83 novel EV-associated proteins
- Upregulated differential protein pathways are involved in tight junctions, cell communication and p53 signaling pathways.

REFERENCES

1. Kamboj A. et al., Biotech 15 (2025)
2. Di Micco L. et al., Nature rev. (2020)
3. Wang L. et al., Nat Rev Cancer 22 (2022)
4. Prašnikar E. et al., Ageing Research Reviews 66 (2021)
5. Shahi S. et al., Cells (2024)
6.Da Silva Correia A.B.A , et al., Cell Biochem Biophys (2025)

RESULTS & DISCUSSION

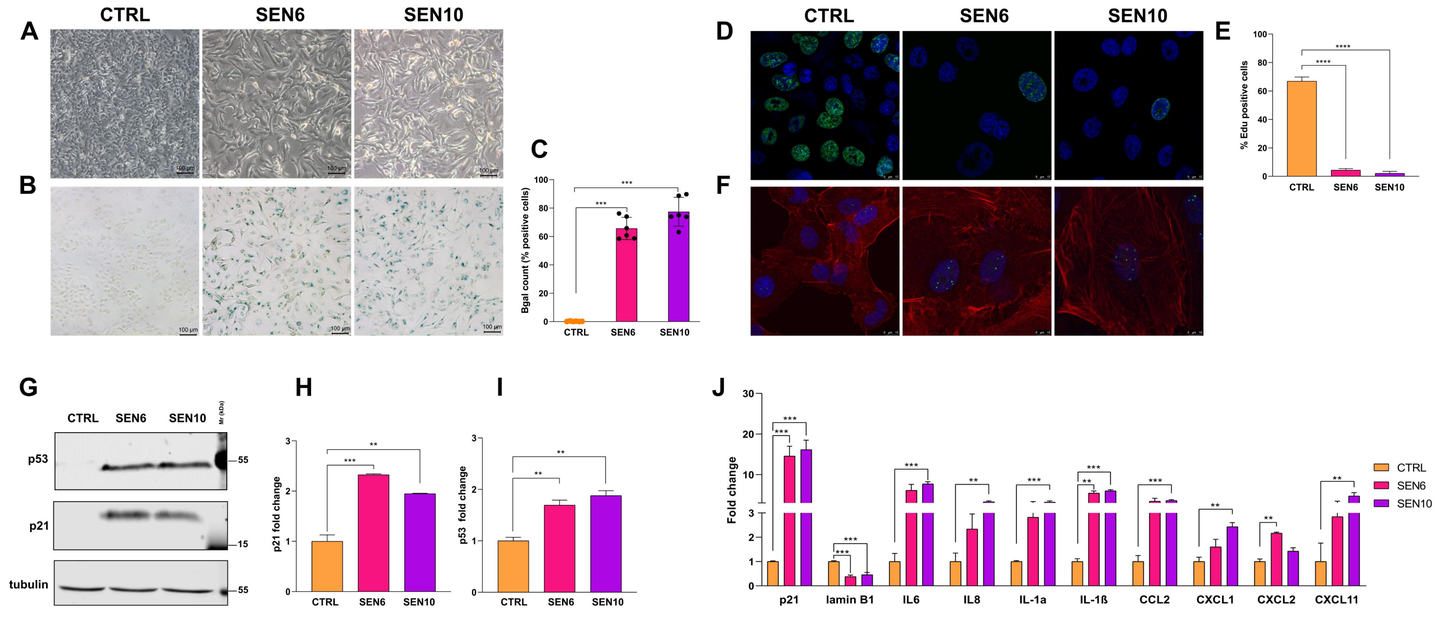
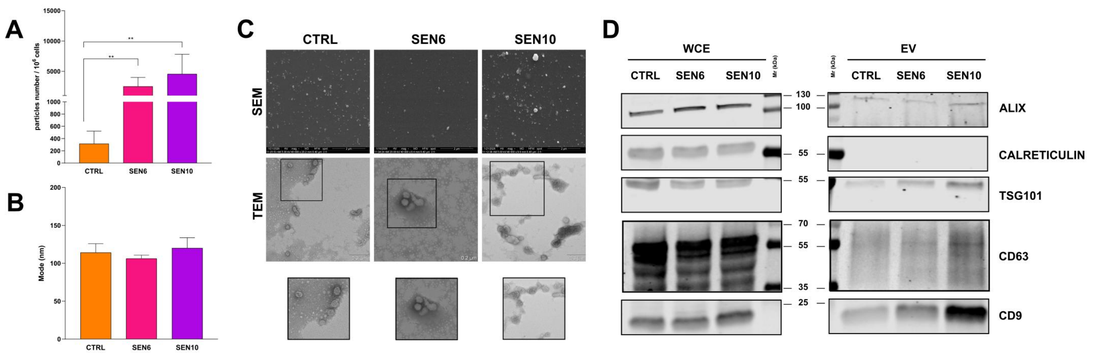


Figure 2. Characterization of TIS in A549 cell line. A-B) Representative images showing morphological changes **(A)** and SA-β-gal staining **(B)** in control (ctrl) and senescent A549 cells at 6 and 10 days, with quantification of SA-β-gal–positive cells **(C)**. **D-E)** Immunofluorescence analysis of EdU incorporation in ctrl and senescent cells, with relative quantification **(E)**. **F)** γ-H2A.X foci detection in ctrl and senescent cells. **G-I)** Western blot analysis of p21 and p53 protein levels normalized to tubulin, with relative fold-change quantification **(H-I)**. **J)** qPCR analysis of senescence-associated markers.

Figure 3. Validation of isolated EVs. A-B) Concentration per 10^6 cells **(A)** and size distribution **(B)** of EVs isolated from control (ctrl) and senescent cells (6 and 10 days), assessed by nanoparticle tracking analysis (NTA). **C)** Representative TEM and SEM images of isolated EVs. **D)** Western blot validation of Calreticulin and EV markers in total cell extracts and isolated EVs.



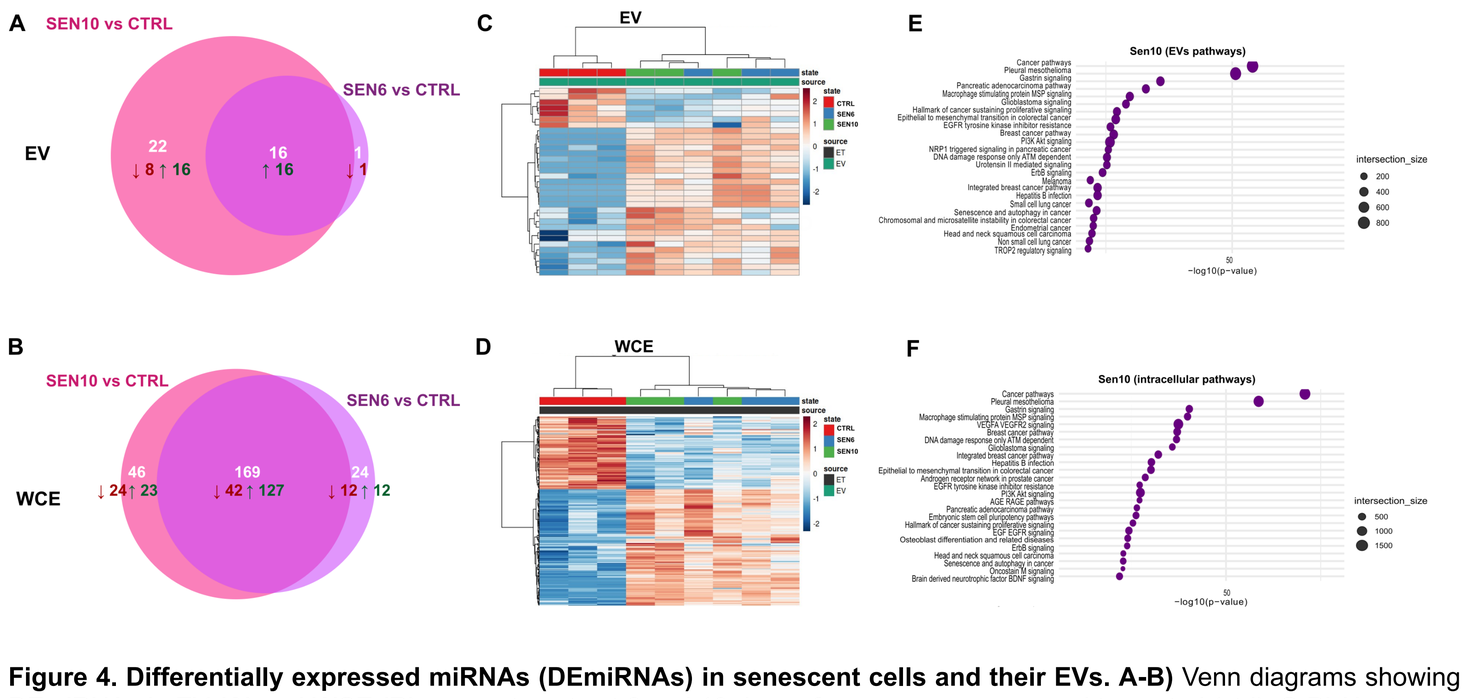


Figure 4. Differentially expressed miRNAs (DEmiRNAs) in senescent cells and their EVs. A-B) Venn diagrams showing DEmiRNAs in EV **(A)** and WCE **(B)** compartments at 6 and 10 days of senescence compared to control (ctrl). **C-D)** Heatmaps displaying expression patterns of the most significant DEmiRNAs in EV **(C)** and WCE **(D)** compartments across conditions, with colour intensity representing log2 fold-change values. **E-F)** Bubble plots of enriched biological pathways from target gene analysis of sen10 DEmiRNAs (WikiPathways) in intracellular **(E)** and WCE **(F)** compartments.

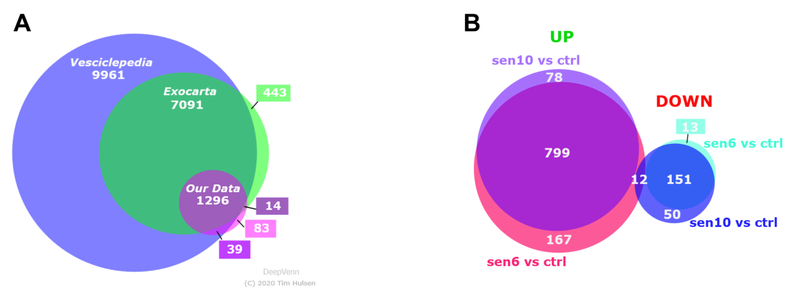


Figure 5. Proteomic EVs profiling during senescence. A) Venn diagrams showing overlap between differentially expressed EVs proteins (DEPs) of our dataset and Vesiclepedia and Exocarta. **B)** Venn diagrams illustrating the distribution of upregulated and downregulated DEPs in EVs at 6 and 10 days of senescence compared to control.

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