Nano-intervention to stop therapy-induced cellular senescence.

Agata Henschke[1], Bartosz Grześkowiak [1], Angelika Mielcarek [1], Patrick Perrigue [1], Kaja Jaskot [1], Olena Ivashchenko [1], Celina Sánchez Cerviño [2,3], Emerson Coy[1], Sergio Moya[3]

[1]NanoBioMedical Centre, Adam Mickiewicz University, Wszechnicy Piastowskiej 3, 61-614, Poznan, Poland
[2]Biomedical Polymers Division/Research Institute of Materials Science and Technology, INTEMA (UNMdP-CONICET)/Argentina
[3]Soft Matter Nanotechnology, Centre for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Paseo Miramon 182 C, 20014 Donostia-San Sebastian, Spain

agata.henschke@amu.edu.pl
coyeme@amu.edu.pl

Cellular senescence is a phenomenon that causes genetic, epigenetic, metabolic, and structural changes to original cells, causing cell cycle arrest and prevents them from growing. It is naturally caused by telomere shortening, when cells are unable to divide anymore, and is an inherent part of our lives. In general, this mechanism is a protector from over proliferation, what makes this a tumor-suppressive mechanism [1]. Senescent induction is an effect of various factors, such as epigenetic stress, proteotoxic stress, oxidative stress, telomere damage and DNA damage [2]. Such stressful environment may lead to accumulation of those cells in tissues which are a source of inflammation or tissue disfunction and causes many age-related diseases. Chemotherapy is one of the sources to induce cellular senescence but after induction it is ineffective for those cells making them apoptosis-resistant. It is caused by their growth arrest, while chemotherapeutics are mostly working on highly proliferative cells such as cancer cells. Moreover, Senescent Associated Secretory Phenotype (SASP), which is a very characteristic feature of senescent cells, containing cytokines chemokines, growth factors and proteases, plays a crucial role in tumorigenesis and inflammation [1]. In summary, it is necessary to use drugs that are working selectively, which are senolytics, causing induction of apoptosis on senescent cells (senolysis). Increase of cancer senescent cells after chemotherapy is a considerable problem, as those cells can reenter cell cycle to become cancer stem cells and leads to cancer relapse. The idea is to introduce additional therapy – senotherapy – to eliminate remaining senescent cells [3-5]. This study focused on preparing the protocol of therapy-induced senescence (TIS) with doxorubicin on two cell lines – WI38 (fibroblasts) and A549 (cancer cells). To confirm the effectiveness of the method experiments with senescence markers were conducted, such as evaluation of β-galactosidase level and proliferation of the cells. Followed by investigation of its morphology on confocal microscopy to prove the size changes which were additionally corroborated by the change in complexity as measured by flow cytometry. Next, liposomes were prepared encapsulating the senolytic drug, Fisetin, and administrated to senescent cells. Liposomes were characterized CryoSEM and DLS. Cell uptake of liposomes with encapsulated Fisetin was studied by flow cytometry. Cell viability studies after administration of liposomes were conducted by IN Cell Analyzer, using Live/Dead Assay. This investigation showed that therapy-induced senescence by doxorubicin is greatly effective and senotherapy through fisetin encapsluated in liposomes is highly potential.

Acknowledgements
The financial support of the National Science Centre of Poland under the OPUS-17 grant (2019/33/B/ST5/01495) is gratefully acknowledged.

References

[1] Chambers, C. R., Ritchie, S., Pereira, B. A., & Timpson, P. (2021). Overcoming the senescence-associated secretory phenotype (SASP): a complex mechanism of resistance in the treatment of cancer. In Molecular Oncology (Vol. 15, Issue 12, pp. 3242–3255). John Wiley and Sons Ltd.

[2] Childs, B. G., Durik, M., Baker, D. J., & van Deursen, J. M. (2015). Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nature Medicine, 21(12), 1424–1435.

[3] Zampetidis, C. P., Papantonis, A., & Gorgoulis, V. G. (2022). Escape from senescence: revisiting cancer therapeutic strategies. In Molecular and Cellular Oncology (Vol. 9, Issue 1). Taylor and Francis Ltd.

[4] Perrigue, P. M., Rakoczy, M., Pawlicka, K. P., Belter, A., Giel-Pietraszuk, M., Naskręt-Barciszewska, M., Barciszewski, J., & Figlerowicz, M. (2020). Cancer Stem Cell-Inducing Media Activates Senescence Reprogramming in Fibroblasts. Cancers, 12(7), 1745.

[5] Saleh, T., Tyutyunyk-Massey, L., Murray, G. F., Alotaibi, M. R., Kawale, A. S., Elsayed, Z., Henderson, S. C., Yakovlev, V., Elmore, L. W., Toor, A., Harada, H., Reed, J., Landry, J. W., & Gewirtz, D. A. (2019). Tumor cell escape from therapy-induced senescence. Biochemical Pharmacology, 162, 202–212. https://doi.org/10.1016/j.bcp.2018.12.013

Figures

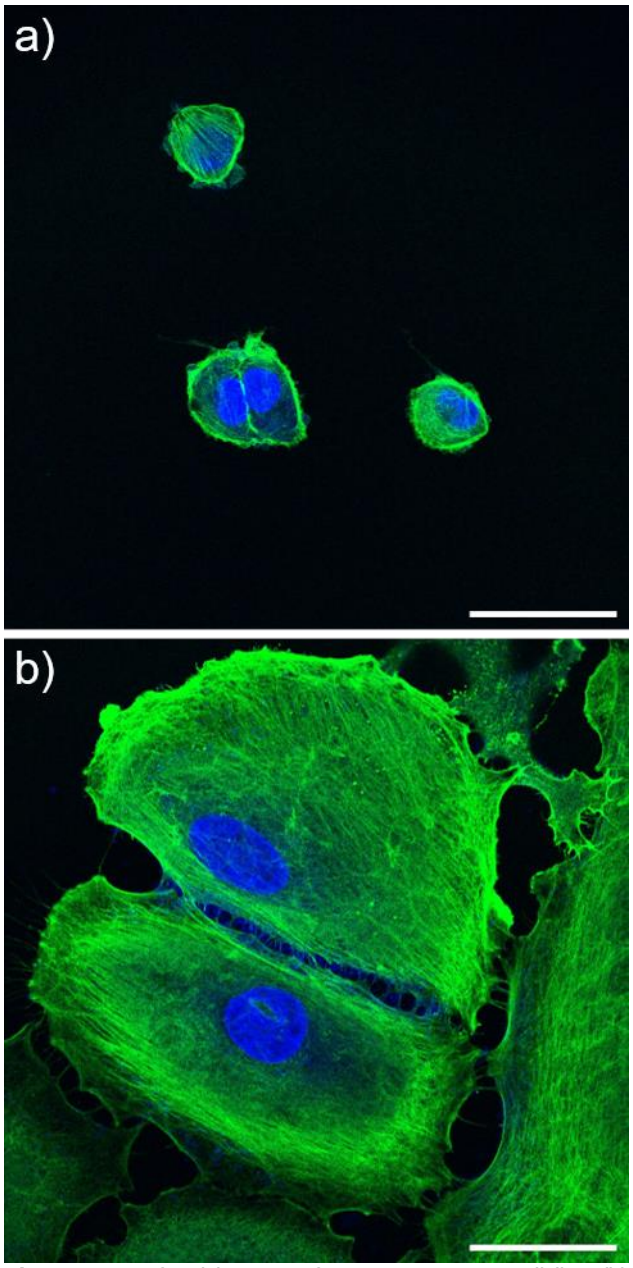

Figure 1. Confocal images of senescent A549 cell line (b) compared to control, non-senescent cells (a). Scale = 50 µm

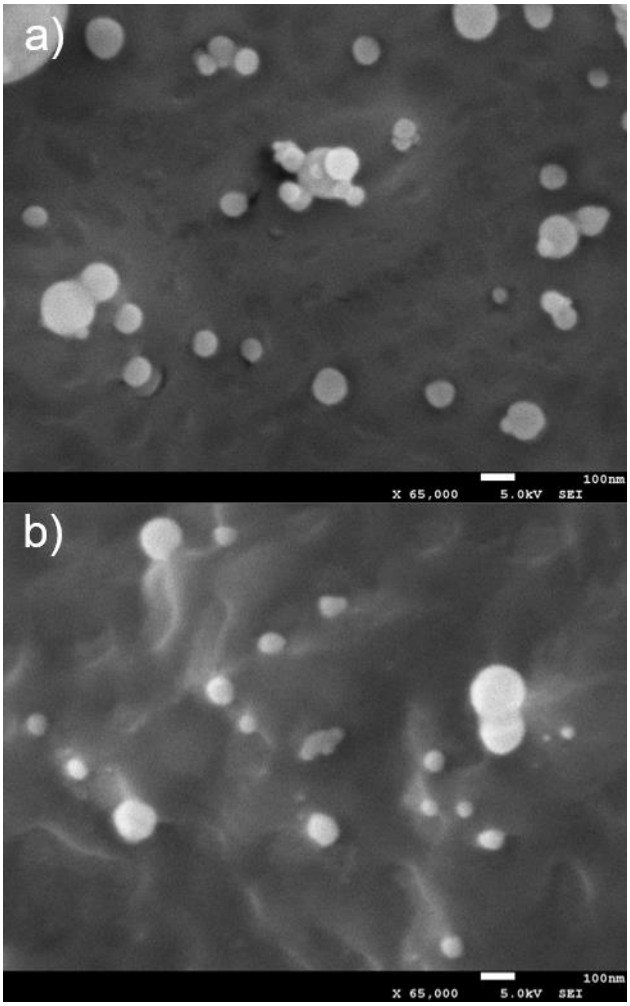

Figure 2. CryoSEM images of liposomes without Fisetin (a) and with Fisetin (b). Scale = 100 nm