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Thesis title: Plasma Membrane Damage-Dependent Senescent Cells Accelerate Wound Healing In Vitro via Soluble Molecules and Increased Extracellular Vesicles

Research aim:

Cellular senescence is a sustained state of cell cycle arrest associated with many age-related diseases. There are several subtypes of cellular senescence including DNA damage response-dependent senescence (DDR-Sen). The plasma membrane damage-dependent senescence (PMD-Sen) subtype is more recently discovered. This study was conducted to identify the protein cargo of extracellular vesicles (EVs) from these cells to analyze the paracrine signaling potential and find markers of PMD-Sen cells.

Material and methods:

The normal human fibroblast cell strain WI-38 was used to prepare senescent cells by DNA damage and plasma membrane damage. The conditioned medium of senescent cells was collected and EVs were isolated by differential centrifugation, filtration, and ultracentrifugation. The production of EVs between PMD-Sen and DDR-Sen cells was compared using tunable resistive pulse sensing. The EVs from both senescence subtypes and proliferating cells were washed using centrifugal concentrators and then used for data-independent acquisition mass spectrometry. The protein content of PMD-Sen EVs was compared to that of EVs from proliferating cells and DDR-Sen cells to identify upregulated proteins and markers of PMD-Sen. PMD-Sen and DDR-Sen cells were also used in coculture experiments to assay the effect of soluble factors on proliferating fibroblasts in a scratch assay.

Result:

PMD-Sen cells showed greater production of EVs per cell than DDR-Sen cells. When performing coculture experiments wherein small EVs and soluble factors were transferred to proliferating cells, PMD-Sen cell coculture led to greater migration in recipient cells than DDR-Sen coculture. Depletion of EVs from PMD-Sen conditioned media reduced the effect, suggesting an important role for EVs in the paracrine signaling. The majority of EV proteins identified by mass spectrometry were shared by PMD-Sen and DDR-Sen; however, some proteins upregulated in PMD-Sen cell EVs compared to proliferating cell EVs were not upregulated in DDR-Sen cell EVs and some proteins were specifically identified in PMD-Sen cell EVs, leading to a set of markers of PMD-Sen.

Conclusion:

Plasma membrane damage of normal human fibroblasts can result in PMD-Sen with increased EV production. The protein cargo of EVs from PMD-Sen cells is distinct from that of DDR-Sen cell EVs, and the EVs contribute to paracrine signaling that includes promotion of wound healing in vitro.