Replicative senescence in pancreatic beta cells alters extracellular vesicle characteristics

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Background

- Type 2 diabetes (T2D) occurs in people of all ages, and its prevalence increases with age. T2D is characterized by a sustained demand for insulin which likely drives the accumulation of senescent pancreatic β-cells.
- Senescent cells release pro-inflammatory cytokines and extracellular vesicles (EVs). EVs are small, membranous nanoparticles that are critical to intercellular communication. EVs vary in size: small-EVs (sEVs), medium/large (m/lEVs), and cargo.
- Little is known about EVs released from pancreatic β-cells during replicative senescence. We hypothesized that senescent pancreatic β-cells will release more EVs than non-senescent cells and be smaller in size.

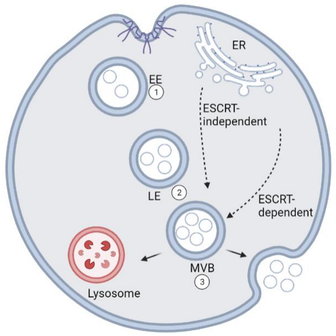

Figure 1. Extracellular vesicle formation. Early endosome (EE) formation occurs by endocytosis, which can be mediated by caveole-dependent processes. During endocytosis, the EE will mature to a late endosome (LE) as it accumulates more cargo in the form of intraluminal vesicles (ILV). ILV cargo can be recruited in an endosomal sorting complex required for transport (ESCRT)-dependent or independent manner. A multivescular body (MVB) is formed as the LE continues to recruit cargo. Eventually, the MVB can either fuse with the lysosome and deposit its contents, or travel to and bind with the plasma membrane, releasing its contents as extracellular vesicles (EVs).

Research Aim

Evaluate differences in biophysical characteristics of pancreatic β-cell derived EVs with replicative senescence.

Methods

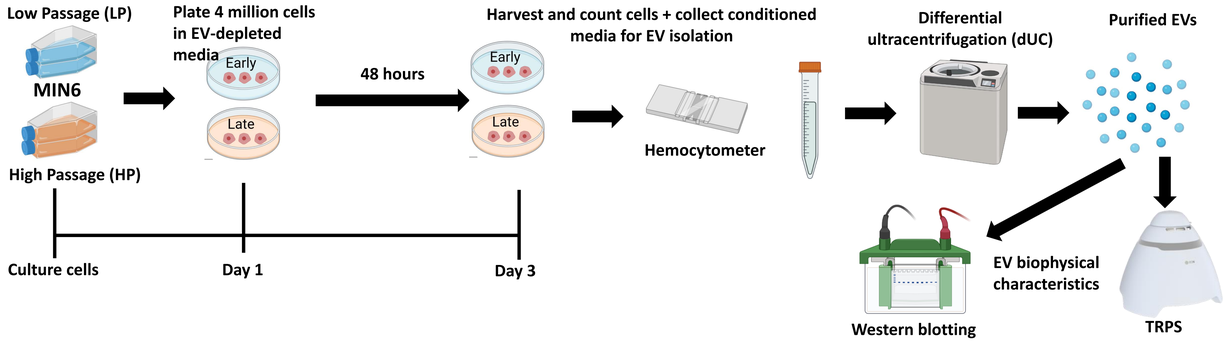

Figure 2. Methods: 4 million Low-passage (LP) and high-passage (HP) murine pancreatic β-cells (MIN6) were plated on day 1 and grown in EV-depleted media for 48hrs. On day 3, media was collected from LP (P22-30) and HP (P50-60) cells, and EVs isolated using differential ultracentrifugation (dUC). Cells were harvested, counted and viability determined using a hemocytometer and trypan blue exclusion, and lysates frozen at -80°C for future analysis of senescence markers. Fresh EVs were characterized for size, concentration, and zeta potential using Tunable Resistive Pulse Sensing (TRPS; N=6). Frozen EVs were lysed and analysed for EV protein markers using Western blotting (N=1).

Results

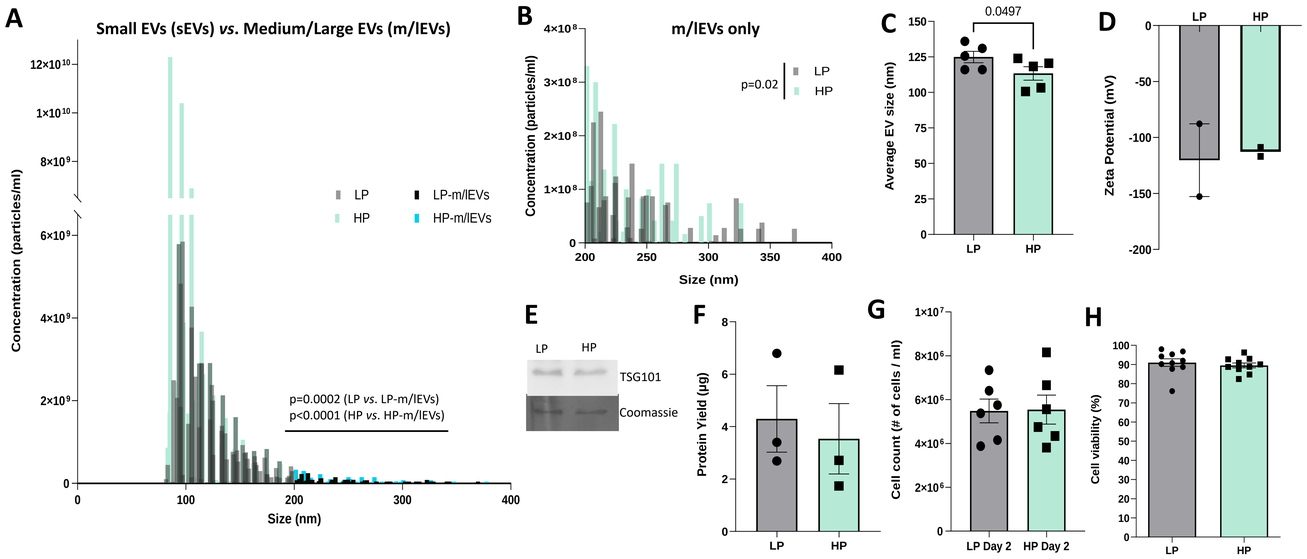

Figure 3. Results: small EV (sEV) concentration was **(A)** ~23-fold higher in LP-cells (1.18E+09 particles/ml; *p=0.0002, N=5) and ~16-fold higher in HP-cells (1.35E+0.9 particles/ml; *p<0.0001, N=5) *vs*. m/lEVs in each group respectively, illustrating a preponderance of sEV release from cells irrespective of passage. **(B)** Comparing between passages, secretion of m/lEVs was 1.77-fold higher in HP-EVs *vs*. LP-EVs (*p=0.02, N=5). **(C)** Average EV size was 9% lower in HP-EVs (113nm) *vs*. LP-EVs (125nm; *p=0.0497, N=5), and **(D)** zeta potential was unchanged (N=2). **(E)** Small EV marker TSG101 expression appeared to increase in HP-EVs vs. LP-EVs (1.4-fold-higher in HP-EVs (N=1)) but requires further testing. **(F)** EV protein yield was unchanged between groups (N=3). **(G-H)** Cell count and viability remained unchanged across groups (N=10) on the day of cell harvest.

Conclusion

- MIN6 cells, regardless of passage, release more sEVs compared to m/lEVs.
- With replicative senescence (HP cells), average EV size is reduced in MIN6 pancreatic ß-cells.
- With replicative senescence (i.e., in HP cells), MIN6 cells also release 1.77-fold more m/lEVs compared to LP cells.
- Zeta potential and EV protein yield remains unchanged with replicative senescence. Cell count and viability show no difference between HP and LP MIN6 cells.

Overall, there is increased EV release, in particular more sEV release, with replicative senescence in murine pancreatic ß-cells.

Future Directions

- Fully characterize EVs from LP *vs*. HP cells and confirm cellular senescence is activated in HP cells.
- Determine the upstream pathways regulating enhanced sEV biogenesis and/or release with replicative senescence.
- Elucidate the functional effects of MIN6 pancreatic ß-cells EVs with replicative senescence.

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