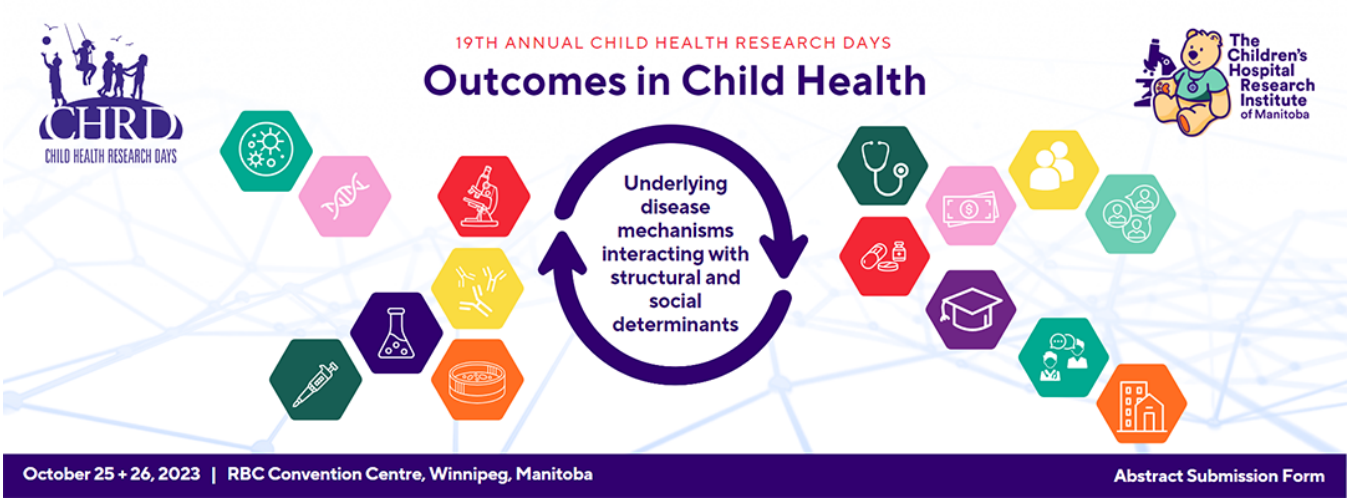

CHRD 2023: Abstract Submission Form

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Presenter Name
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Presenter Status
PhD Student

Research Category
Basic Science

Role in the project
Perform Experiments
Analyze Data
Write Abstract

Title
Changes in extracellular vesicle (EV) subtype and secretion markers with replicative senescence in murine pancreatic beta cells

Background
Type 2 diabetes (T2D) is an age-associated disease characterized by a sustained elevated insulin demand, linked to an accumulation of senescent pancreatic □-cells. Senescent cells release pro-inflammatory cytokines, perpetuating increased inflammation, which can be packaged in extracellular vesicles (EVs). EVs are small, membrane-bound nanoparticles that facilitate cellular communication through biological cargo. EVs are characterized as small (sEVs) and medium/large (m/lEVs) EVs.

Objective
The effect of replicative senescence on the secretion of EVs from pancreatic beta cells is currently unknown.

Methods
Murine pancreatic beta cells (MIN6) at low (LP, P22-30) and high (HP, P54-61) passage were grown in EV-depleted media for 48 hrs (N=6, unless otherwise noted). Differential ultracentrifugation and ultrafiltration were used to isolate EVs from conditioned media. EVs were characterized using tunable resistive pulse sensing. Cells were assessed for viability and baseline senescence (beta-galactosidase). Immunoblotting on cell lysates was used to measure proteins related to EV subtypes and secretion.

Results
sEV concentration was ~23-fold higher in LP-cells (1.18E+09 particles/ml; p=0.0002) and ~16-fold higher

in HP-cells (1.35E+0.9 particles/ml; p<0.0001) vs. m/lEVs in each group. Secretion of m/lEVs was 1.77-fold higher in HP-cells vs. LP-cells (p=0.02). sEV secretion was unchanged between HP vs. LP cells. Average EV size was 9% lower in HP-EVs (113nm) vs. LP-EVs (125nm; p=0.04, N=5), while cell count and viability remained unchanged. Beta-galactosidase staining was ~1.6-fold higher in HP-cells vs. LP-cells (p=0.003, N=3). TSAP6, a regulator of EV secretion, was 68% higher in HP vs. LP cells (p=0.04, N=5), while sEV markers CD9, CD81, TSG101, and ALIX remained unchanged.

Conclusion
Beta cells preferentially release small EVs regardless of passage. HP-cells have higher levels of senescence, and show increased m/lEV release vs. LP-cells, concomitant with increased expression of TSAP6. Differences in EV release with replicative senescence may be linked to enhanced EV secretion in pancreatic beta cells. Future work on EV trafficking within HP-cells is warranted.

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