Differential reduction in inflammatory mediator production by AML cells using normal human plasma-derived extracellular vesicle subtypes

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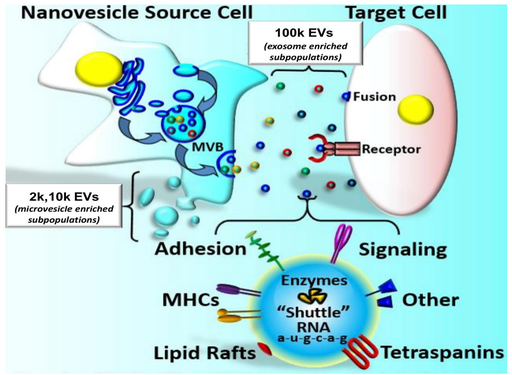
Background & Innovation

Standard differential centrifugation methods used to isolate extracellular vesicles (EVs) from biofluids typically include the application of three consecutive rounds of increasing centrifugal force. This corresponds to approximately 2,000xg (2k), 10,000xg (10k), and 100,000xg (100k). Application of these forces pellets distinct EV subpopulations respectively (1).

Use of cancer patient plasma-derived EVs for diagnostic or therapeutic applications inevitably results in co-isolation of normal EVs. The function of normal EVs is poorly understood in the context of cancer.



Acute monocytic leukemia (AML-M5), a subtype of acute myeloid leukemia (AML), expresses the pro-inflammatory cytokine TNF-α. Autocrine stimulation of AML by TNF-α inhibits inflammatory cell death via the necroptosis pathway resulting in increased survival of clonogenic (less differentiated) AML cells (2). Decreasing the release of TNF-α or other inflammatory mediators should ameliorate systemic inflammatory processes driving AML survival and pain symptoms (3).

Objective

We hypothesize that 2k, 10k, and 100k normal plasma-derived EV types induce different inflammatory responses by AML-M5.

Methods

- Three different types of EVs (2K, 10K, and 100K EVs), were isolated from normal human plasma donors using differential centrifugation. The EVs were characterized by size (dynamic light scattering) and zeta potential (phase-analysis light scattering).
- THP-1 AML-M5 cells, were treated for 24hrs with the three EV types using equal protein mass concentrations.
- ELISAs were used to measure THP-1 production of the pro-inflammatory cytokine TNF-α, and the anti-inflammatory cytokine IL-10.
- RT-qPCR was used to measure gene expression of additional pro- and anti-inflammatory mediators.

Results

EV Size

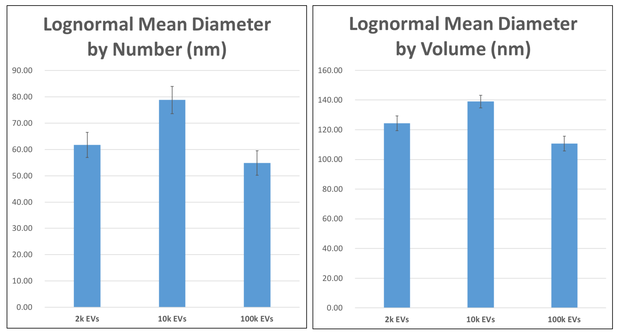
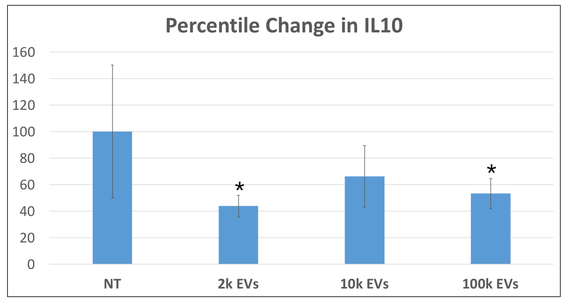


Figure 1. Dynamic light scattering (DLS) was used to determine the size of each EV type. EV sizing was weighted based on particle number to detect smaller EVs and volume to detect larger EVs or EV aggregation. n = 14, error bars = SD.

ELISA Results



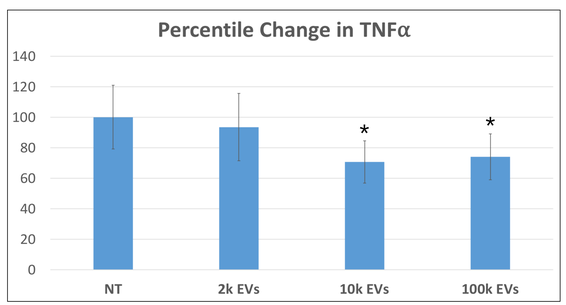


Figure 3 ELISA results for TNFα. and IL-10 relative to cell viability, shown as percentile change from non-treated control (NT). n = 9, error bars = SD, * = $p < 0.05$ and was considered statistically significant. ANOVA and Tukey's HSD test was used to compare EV treated and NT groups.

EV Charge

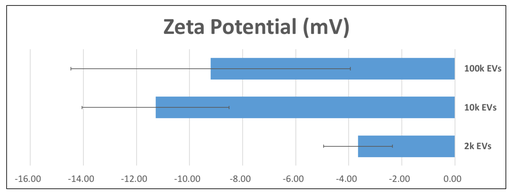


Figure 2. Phase-analysis light scattering (PALS) was used to determine the zeta potential of each EV type. n = 10, error bars = SD.

RT-qPCR Results

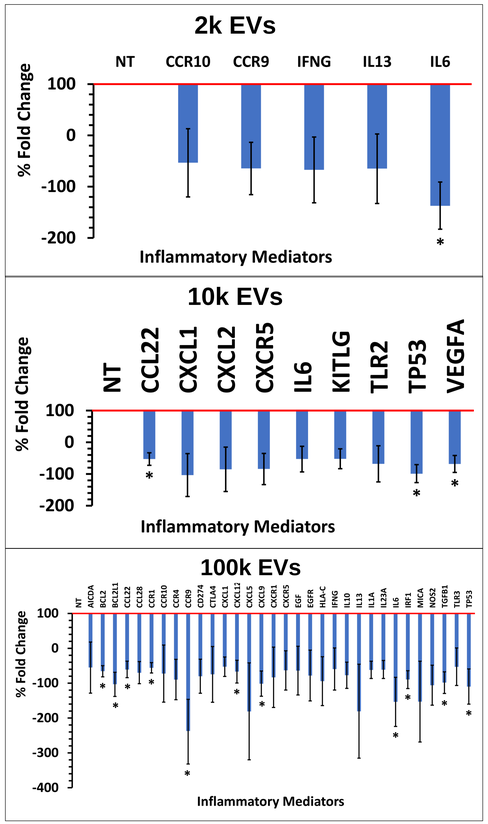


Figure 4. RT-qPCR assessed down regulation of inflammatory mediator gene expression compared to non-treated control (NT). n = 3 replicate arrays, error bars = SD, * = $p < 0.05$ and was considered statistically significant. Student's t-test used to compare EV treated and NT groups.

Summary & Conclusions

ELISA results showed that plasma 2k EVs and 100k EVs decreased IL-10 production by THP-1 cells compared to non-treated cells. The 10k EVs and 100K EVs decreased TNF-α production, while 2k EVs induced no change. Gene expression of a variety of additional inflammatory mediators were also downregulated in THP-1 cells after treatment with any plasma EV type. However, more inflammatory mediators were downregulated by 10k EVs than 2k EVs, and by 100k EVs than 10K EVs. This finding demonstrates general differences in the anti-inflammatory properties of normal plasma EV subtypes.

Normal plasma 10k EVs and 100k EVs are more likely to decrease TNF-α production by AML cells. Future adaptation of normal EVs as nanomedicines could find utility in alleviating systemic inflammation and related symptoms for AML patients.

Significance and Impact

A novel implication to the findings presented herein is that the EVs present in normal human plasma may impart therapeutic effects to AMLs, such as AML-M5, by decreasing autocrine stimulation with TNF-α and other inflammatory mediators. Lower TNF-α release by leukemic cells may attenuate their clonogenicity by making them more susceptible to spontaneous or induced differentiation by IFN-γ (2). In addition, reduction in inflammatory mediators should allow for symptom relief.

Our ongoing investigations are exploring whether autologous versus allogeneic normal EV subtypes induce similar anti-inflammatory effects in AML-M5 cells and macrophages. We are also evaluating what EV components are involved.

References

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Acknowledgements

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