Induction of Macrophages by Liver Tumor-Derived Small Extracellular Vesicles

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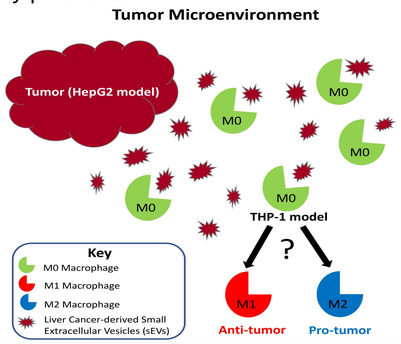
Background & Innovation

Nanoscale tumor-derived small extracellular vesicles (sEVs) promote tumor growth and metastasis via interactions with immune cells (1). Our recent data demonstrate that tumor sEVs induce macrophage (Mϕ) functions (2).

Mϕs are a heterogeneous population of myeloid cells of the immune system involved in a variety of physiological and pathological processes (3). Tumor-associated M1 (proinflammatory) or M2 (immunosuppressive) Mϕs are pivotal in the progression of inflammation-related cancers. Growing evidence shows crosstalk between tumor cells and Mϕs is involved in hepatic tumor progression (4). However, the ability of liver tumor-derived sEVs to influence Mϕ-related pro-tumor inflammatory processes is largely unknown.

Objective

Using the HepG2 liver tumor model we explored the hypothesis that HepG2 sEVs will induce Mϕ polarity, indicative of pro-tumor inflammatory processes.



Methods

- Human THP-1 (ATCC® TIB-202™) and HepG2 cells were cultured in DMEM with 10% FBS media at 37°C and 5% CO2
- Human THP-1 (ATCC® TIB-202™) monocytes were converted to Mϕs using phorbol myristate acetate.
- sEVs were isolated from the HepG2 media via differential ultracentrifugation. sEV concentration (µg protein mass/µl) was measured using a BCA assay (Thermo Scientific).
- Human IL-10 ELISA Max™ Deluxe Set (BioLegend) and Human TNF-α Uncoated ELISA (Thermo Fisher) were used to test cytokine production in treated and untreated cells.
- A PrestoBlue™ Cell Viability Reagent (Thermo Fisher) was used to determine the viability of Mϕs.
- Subsequently, qRT-PCR was used to assess shifts in Mϕ polarization. Induction of key M1 (TNF-α), and M2 (IL-10) markers were evaluated.

Results

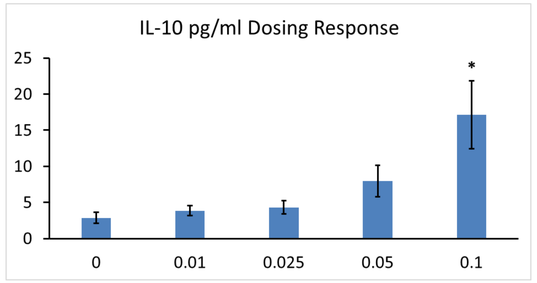


Figure 1: Dose response induction of IL-10 by THP-1 macrophages treated with HepG2 sEVs. IL-10 levels were assessed by ELISA. The x-axis indicates sEV treatment concentration (µg protein mass/µl). Error Bars=S.E.M (n=5). *= p value < 0.05 (ANOVA-Tukey-HSD test) versus NT control.

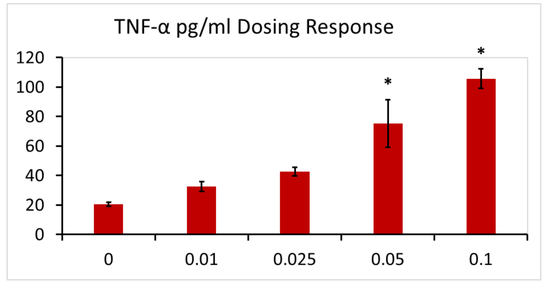


Figure 2: Dose response induction of TNF-α by THP-1 macrophages treated with HepG2 sEVs. TNF-α levels were assessed by ELISA. The x-axis indicates sEV treatment concentration (µg protein mass/µl). Error Bars=S.E.M (n=5). *= p value < 0.05 (ANOVA-Tukey-HSD) versus NT control.

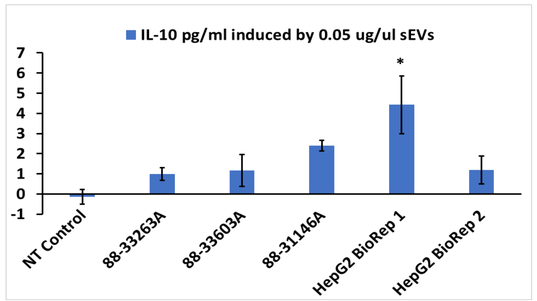


Figure 3: Induction of IL-10 by THP-1 macrophages treated with normal human plasma versus HepG2 sEVs. IL-10 levels were assessed by ELISA. The x-axis indicates sEV treatment types ("88-" codes indicate unique lots of plasma-derived sEVs). Error Bars=S.E.M (n=3). *= p value < 0.05 (ANOVA-Tukey-HSD test) versus NT control.

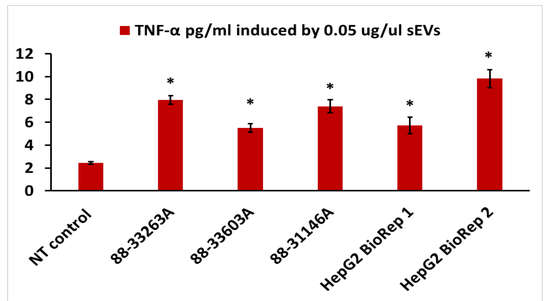


Figure 4: Induction of TNF-α by THP-1 macrophages treated with normal human plasma versus HepG2 sEVs. TNF-α levels were assessed by ELISA. The x-axis indicates sEV treatment types ("88-" codes indicate unique lots of plasma-derived sEVs). Error Bars=S.E.M (n=3). *= p value < 0.05 (ANOVA-Tukey-HSD test) versus NT control.

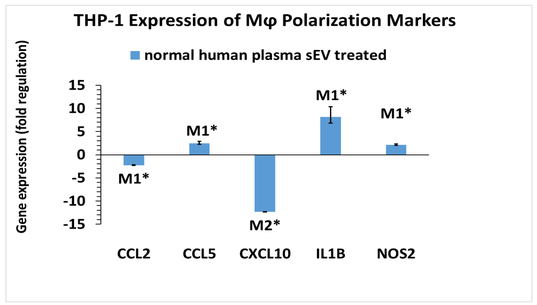


Figure 5: Fold regulation (RT-qPCR) in M1 and M2 markers of macrophage polarization induced by normal human plasma-derived sEVs (0.05 µg protein mass/µl). Error Bars= S.E.M.(n=3) *= p value < 0.05 (2-tailed Student's t-test) versus NT control.

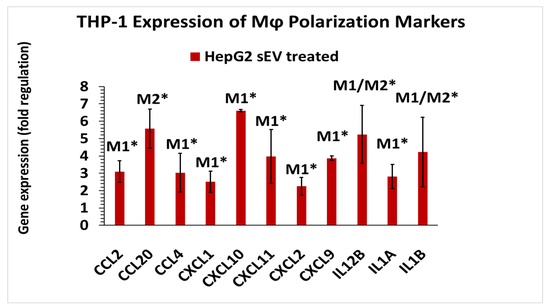


Figure 6: Fold regulation (RT-qPCR) in M1 and M2 markers of macrophage polarization induced by HepG2 sEVs (0.05 µg protein mass/µl). Error Bars= S.E.M.(n=3) *= p value < 0.05 (2-tailed Student's t-test) versus NT control.

Summary & Conclusions

ELISA dose response curves were constructed for IL-10 (**Fig. 1**) and TNF-α (**Fig. 2**) to guide the selection of an appropriate sEV concentration to use for subsequent experiments. Assessment of the immunosuppressive M2 cytokine IL-10 revealed that a dose of 0.1 µg/µl significantly upregulated IL-10 production by THP-1 Mϕs (**Fig. 1**). However, upregulation of the proinflammatory M1 cytokine TNF-α only required 0.05 µg/µl (**Fig. 2**). To conserve samples and avoid competing signals between overlapping TNF-α and IL-10 pathways (5,6), we opted to perform the remaining experiments using the 0.05 µg/µl dose.

Subsequent ELISA experiments demonstrated that one independent batch of HepG2 sEVs significantly induced IL-10 (**Fig.3**) at the 0.05 µg/µl dose, while a second batch did not (**Fig.3**) serving to highlight batch to batch variations. In contrast, normal plasma (np) sEVs did not increase IL-10 production (**Fig. 3**). TNF-α production was significantly induced by all np and HepG2 sEV lots and batches tested (**Fig.4**).

Use of RT-qPCR to evaluate induction of M1 or M2 Mϕ polarization markers was consistent with ELISA data. Np sEVs (**Fig. 5**) induced less polarization markers than HepG2 sEVs (**Fig. 6**). Both types of sEVs favored induction of M1 polarization factors with HepG2's also trending toward induction of a few mixed M1/M2 and M2 markers. This is consistent with our previous melanoma sEV findings (2).

Significance and Impact

The long-term goals are 1. To establish whether liver tumor-derived sEVs influence liver-associated Mϕ pro-tumor processes, and 2. Determine whether liver tumor-derived sEVs might be tuned to serve as personalized immunotherapeutic vaccines routed through liver-associated Mϕs. To achieve these goals, additional functional and sEV nanocarrier modification studies will be required.

References

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Acknowledgements

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