Macrophage Polarization Status Influences Macrophage Responsiveness to Lung Cancer Exosome-derived Nanocarriers

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Background & Innovation

Exosomes are cell-derived nanovesicles (1). They relay information between tissue microenvironments. Exosomes are ideally suited for use as therapeutic nanocarriers given their unique biocompatibility and transportation properties (2).

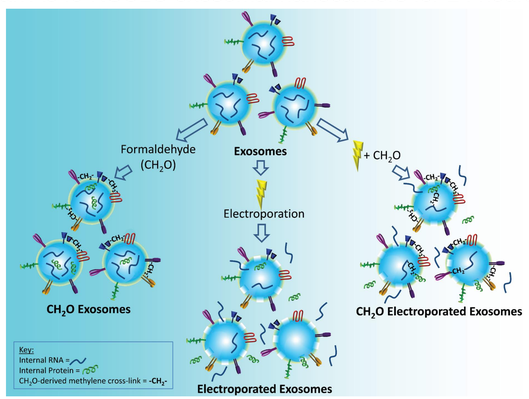
Macrophages (Mϕ), within the monophagocytic system capture and remove foreign nanomedicines, greatly impeding treatment efficacy (3). Mϕs also participate in pro- and anti-tumor processes within non-small cell lung cancer (NSCLC) microenvironments (4). We have been developing formalin-fixed (FF) and electroporated (EP) NSCLC exosome-based immunotherapeutic nanocarriers to antagonize Mϕ pro-tumor functions *in vivo*. However, it is unknown whether the nanocarriers themselves, devoid of immunotherapeutics, influence Mϕ function.

Objective

We tested the hypothesis that changes in Mϕ polarization, in response to FF, EP, or FF EP NSCLC exosomes, depend on the pre-existing Mϕ polarization state.

Methods

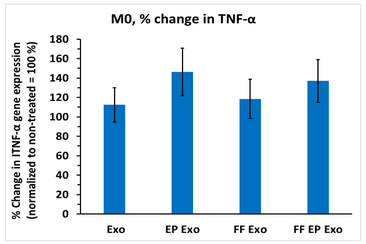
- Human THP-1 (ATCC® TIB-202™) monocytes were converted to Mϕs using phorbol myristate acetate.
- THP-1 Mϕs (M0) were polarized to anti-tumor (M1) and pro-tumor (M2) Mϕs using typical IFN-γ and M-CSF treatment regimens.
- Post polarization, Mϕs were treated with equivalent amounts of formalin-fixed (FF), electroporated (EP), or FF EP modified human A549 NSCLC exosomal nanocarriers for 24 hours.

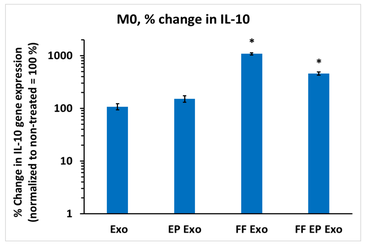


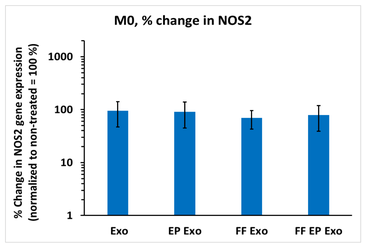
- Subsequently, qRT-PCR was used to assess shifts in Mϕ polarization. Induction of key M1 (TNF-α, NOS2), and M2 (IL-10, ARG1) markers were evaluated.

Results

M0 Macrophages







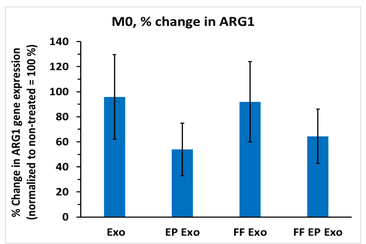
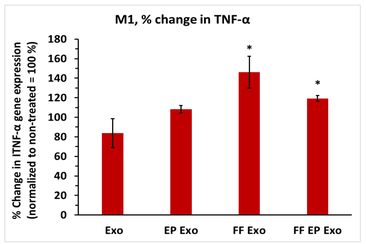
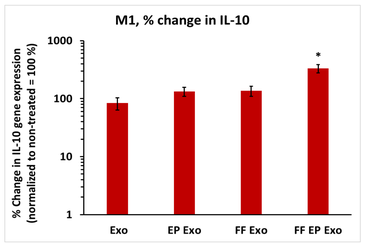
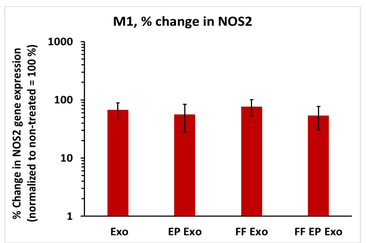


Figure 1. Formalin-fixed (FF) and/or electroporated (EP) NSCLC exosomes (Exo) influence M0 Mϕ polarization status. Error bars = SD (n = 3), * = p value < 0.05 (2-tailed Student's t-test) versus non-treated control (normalized to 100%)

M1 Macrophages







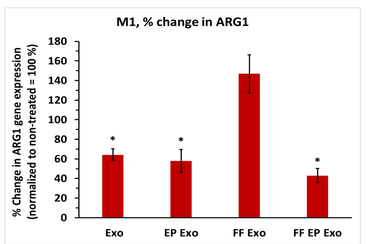
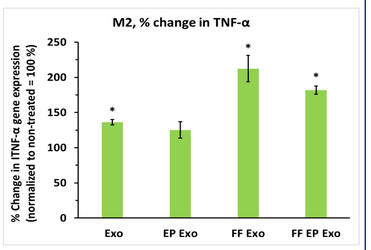
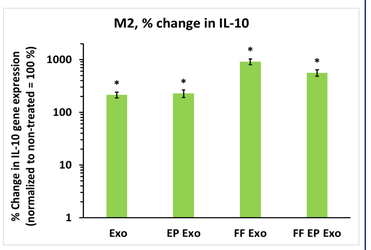
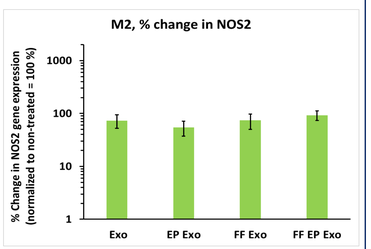


Figure 2. Formalin-fixed (FF) and/or electroporated (EP) NSCLC exosomes (Exo) influence M1 Mϕ polarization status. Error bars = SD (n = 3), * = p value < 0.05 (2-tailed Student's t-test) versus non-treated control (normalized to 100%)

M2 Macrophages







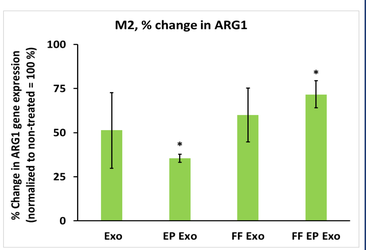


Figure 3. Formalin-fixed (FF) and/or electroporated (EP) NSCLC exosomes (Exo) influence M2 Mϕ polarization status. Error bars = SD (n = 3), * = p value < 0.05 (2-tailed Student's t-test) versus non-treated control (normalized to 100%)

Summary & Conclusions

Macrophage pre-polarization status:	M0	M0	M0	M0	M1	M1	M1	M1	M2	M2	M2	M2
Modified exosome treatment --->	Exo	FF Exo	EP Exo	FF EP Exo	Exo	FF Exo	EP Exo	FF EP Exo	Exo	FF Exo	EP Exo	FF EP Exo
TNF-α (M1 cytokine)	↑	↑	↑	↑	↓	↑	↑	↑	↑	↑	↑	↑
NOS2 (M1 enzyme)	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
IL-10 (M2 Cytokine)	↑	↑	↑	↑	↓	↑	↑	↑	↑	↑	↑	↑
ARG1 (M2 enzyme)	↓	↓	↓	↓	↓	↓	↑	↓	↓	↓	↓	↓
Ratio TNF-α / IL-10:	= 1.0	= 1.0	< 1	< 1	= 1.0	< 1	> 1	< 1	< 1	< 1	< 1	< 1
Polarity Shift --->	None	None	M2	M2	None	M2	M1	M2	M2	M2	M2	M2
Ratio NOS2 / ARG1:	= 1.0	> 1	< 1	> 1	= 1.0	= 1.0	< 1	> 1	> 1	> 1	> 1	> 1
Polarity Shift --->	None	M1	M2	M1	None	None	M2	M1	M1	M1	M1	M1
Trending Combined Polarity Shift --->	None	M1	M2	Mix	None	M2	Mix	Mix	Mix	Mix	Mix	Mix

The results demonstrate no shift in M0 Mϕ polarization status following exposure to natural A549 NSCLC exosomes (Exo). However, treatment with FF Exo skewed M0 polarization toward M1. In contrast, treatment with EP Exo shifted M0 status toward M2. Treatment with FF EP Exo shifted M0 status toward a mixed (M1/M2) phenotype.

An overall different pattern was observed for M1 Mϕs. Treatment of M1Mϕs with natural A549 NSCLC Exo, similar to M0 Mϕs, resulted in no shift in M1 polarization status. Yet, unlike M0 Mϕ results, all M1 and M2 cytokine and enzyme markers were reduced. In further contrast to M0 Mϕs, exposure of M1Mϕs to FF Exo resulted in M2 polarization, and exposure to EP Exo or FF EP Exo resulted in a mixed (M1/M2) phenotype.

Finally, treatment of M2 Mϕs with Exo, FF Exo, EP Exo, or FF EP Exo resulted in mixed (M1/M2) polarization. Collectively, the results demonstrate that Mϕ polarization status, influences Mϕ responsiveness to natural, FF and/or EP modified NSCLC exosomes.

Significance and Impact

A novel implication to the findings presented herein is that processes used to convert exosomes into nanocarriers could impart unforeseen functional properties to the nanocarriers. This in turn could influence the efficacy of therapeutic cargo. Development of protocols to screen the functional effects of such conversion processes might be incorporated into good manufacturing practices for exosome-based nanomedicines. Future investigations will explore the mechanism(s) driving EP and FF-modified exosome influences on Mϕ polarity using additional markers, and determine whether other tumor exosome and Mϕ types produce similar results.

References

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Acknowledgements

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