Effect of cancer cell-derived exosome on energy metabolism associated with macrophage activation

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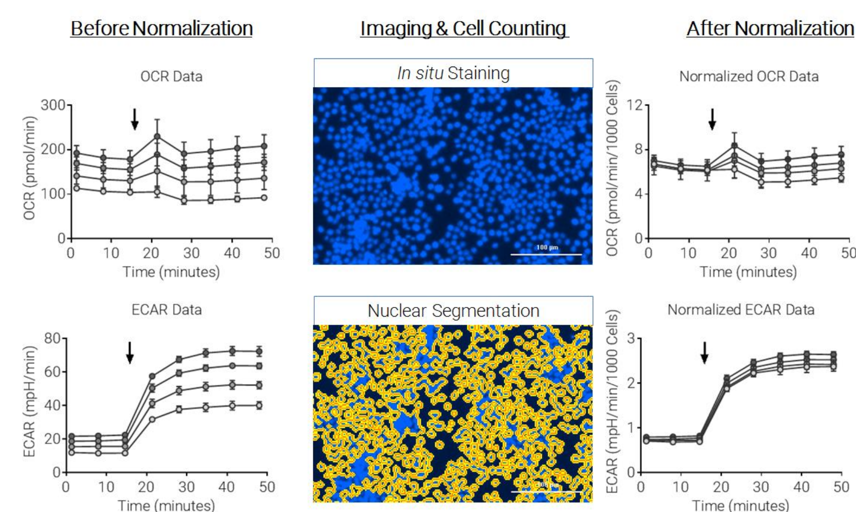
Introduction

- Macrophages show dynamic metabolic changes during the polarization and activation as a crucial tumor microenvironement factor affecting tumor cell survival and progression.
- The metabolic switching from oxidative to glycolytic phenotype is an essential process for inflammatory macrophage activation, and this change can be quantitatively compared by measuring ATP production rates.
- Since macrophage activation is accompanied with proliferation rate changes, the metabolic rates measured by Seahorse XF system should be normalized. A newly developed Agilent Seahorse XF Imaging and Normalization System enables cell number-based data normalization.
- Cancer cell-derived exosome is a newly emerging tumor microenvironmental factor enables cancer cells to communicate with macrophages. Recent researches suggest that exosomes from cancer cells can promote inflammatory macrophage activation.
- *In vitro* activation of RAW264.7 macrophages by injecting lipopolysaccharide (LPS) and/or interferon γ (IFNγ) in a Seahorse XF analyzer enables a real-time monitoring of cellular metabolic changes. The exosome effect on the glycolytic activation of macrophage was examined by using the real-time activation assay protocol.

Approach

XF Data Normalization using Seahorse XF Imaging and Cell Counting Software

Cellular metabolic rates (oxygen consumption rate, OCR; proton efflux rate, PER) and ATP production rates were normalized by the Agilent Seahorse XF Imaging and Normalization System which counts fluorescently-labeled cells by Seahorse XF Imaging and Cell Counting software linked to Cytation 1 (BioTek). Cell permeable Hoechst 33342 was co-injected with the last injection reagent (e.g. 2DG in Glycolytic Rate Assay) to stain nuclei. Fluorescently-labeled cells were imaged and counted right after XF analysis by the software. Captured images and cell counts were imported to normalize XF data in Wave software.



ATP Production Rate Comparison

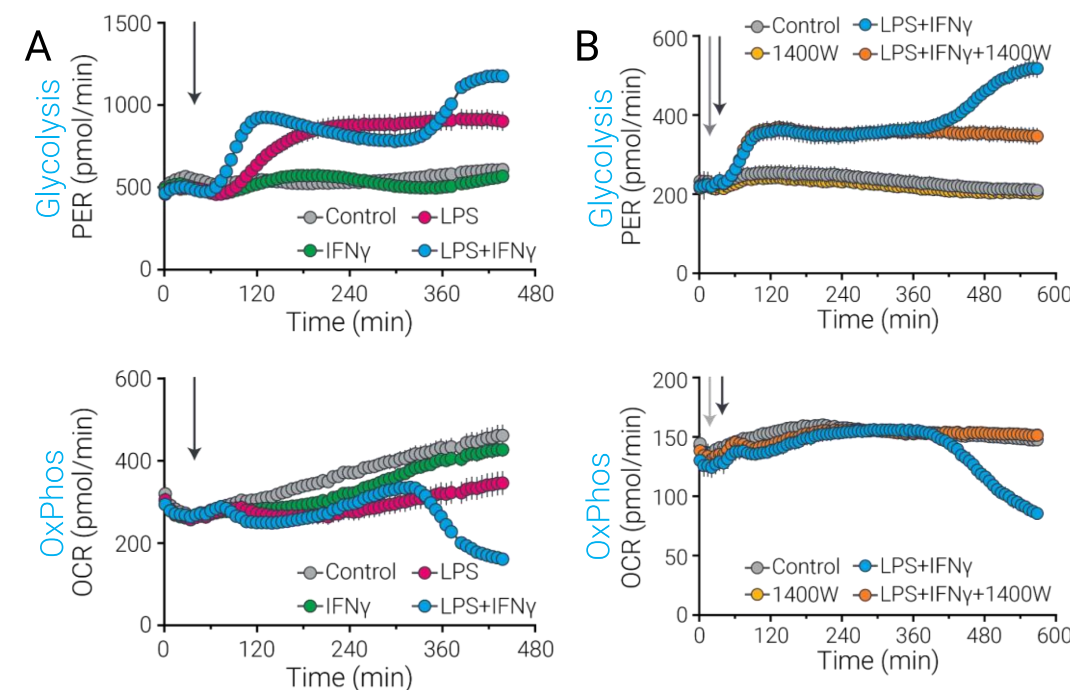
Total ATP production rate is the sum of ATP production rate from glycolysis and mitochondrial oxidative phosphorylation. Using calculations previously validated for the Seahorse XF glycolytic rate assay and based on known reaction stoichiometry, ECAR data can be converted to glycolytic ATP production rate. During oxidative phosphorylation, OCR that drives mitochondrial ATP synthesis can be calculated by addition of oligomycin (ATP synthase inhibitor). To convert ATP-coupled OCR into mitochondrial ATP production rate, we must know the stoichiometry of ATP phosphorylated per atoms of oxygen reduced known as P/O ratio. An average P/O value of 2.75 was validated that accurately represents cell experimental conditions where cells oxidize a mixture of available fuels.

Exosome preparation

Exosome-enriched fractions were isolated from HT-29, MCF7 and SKOV-3 cell lines by using ExoQuick™ Exosome Precipitation Solution (System Biosciences).

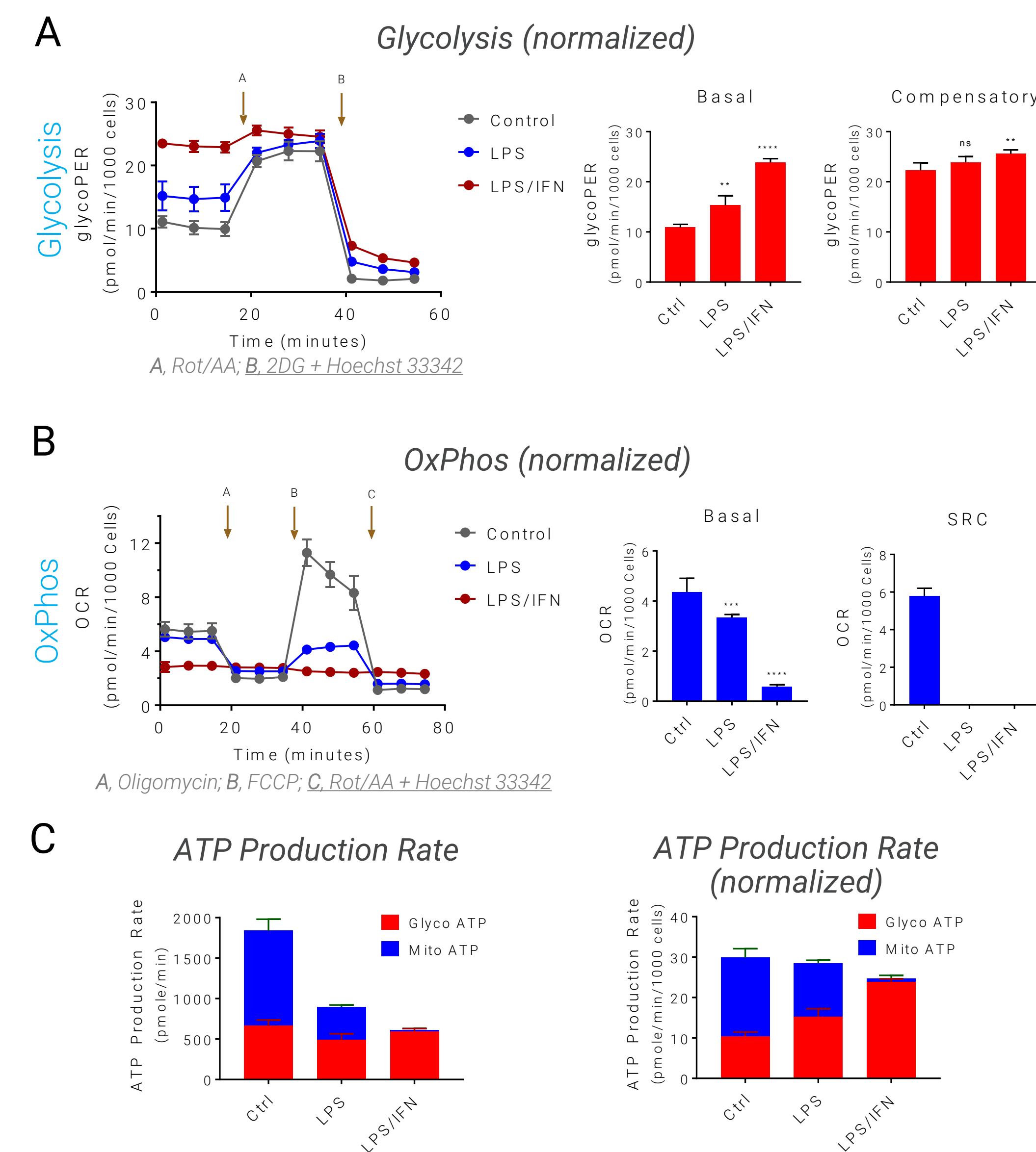
Results and Discussion

Bi-phasic activation of macrophages



A. RAW264.7 cells were stimulated by injection of LPS, IFNγ or both, and metabolic responses were measured for an extended period (> 6 h) after the stimulation. Data shown are mean ± SD, n=4 technical replicates. B. Assay medium with or without iNOS inhibitor (1400W, *gray arrow*) was injected prior to LPS and IFNγ (*black arrow*). Only the secondary changes in PER and OCR were completely blocked by the inhibitor. Data shown are mean ± SD, n=6 technical replicates.

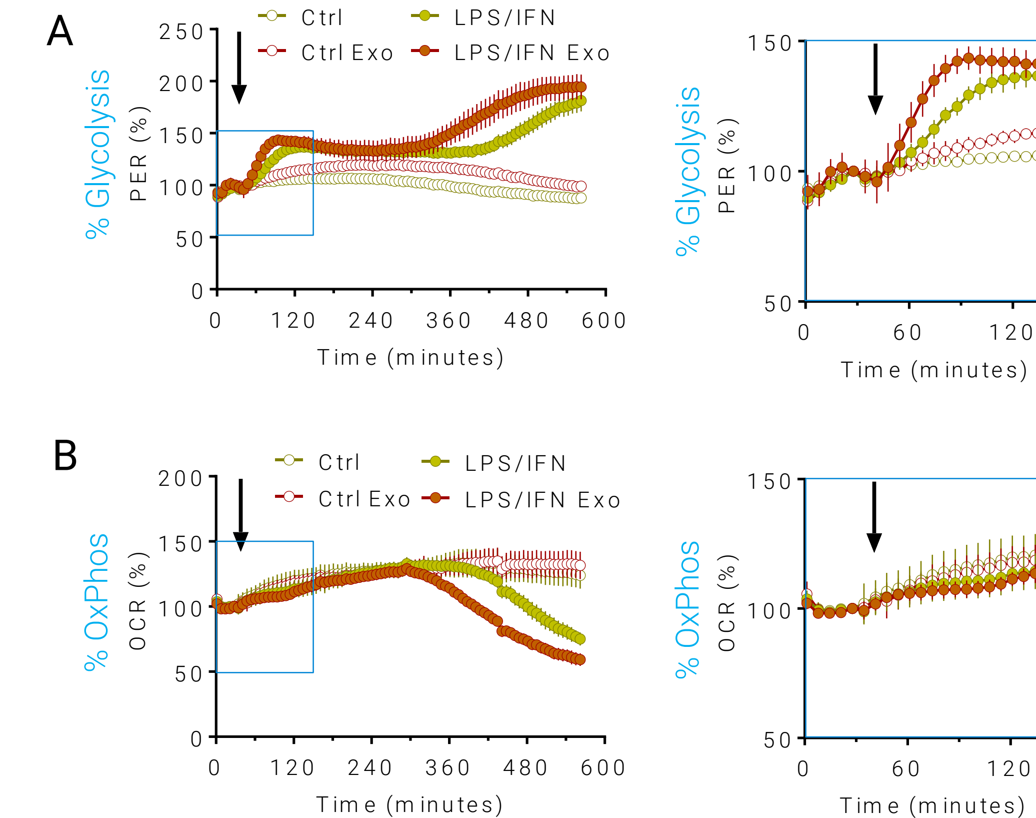
Activation-associated changes in cellular energy metabolism



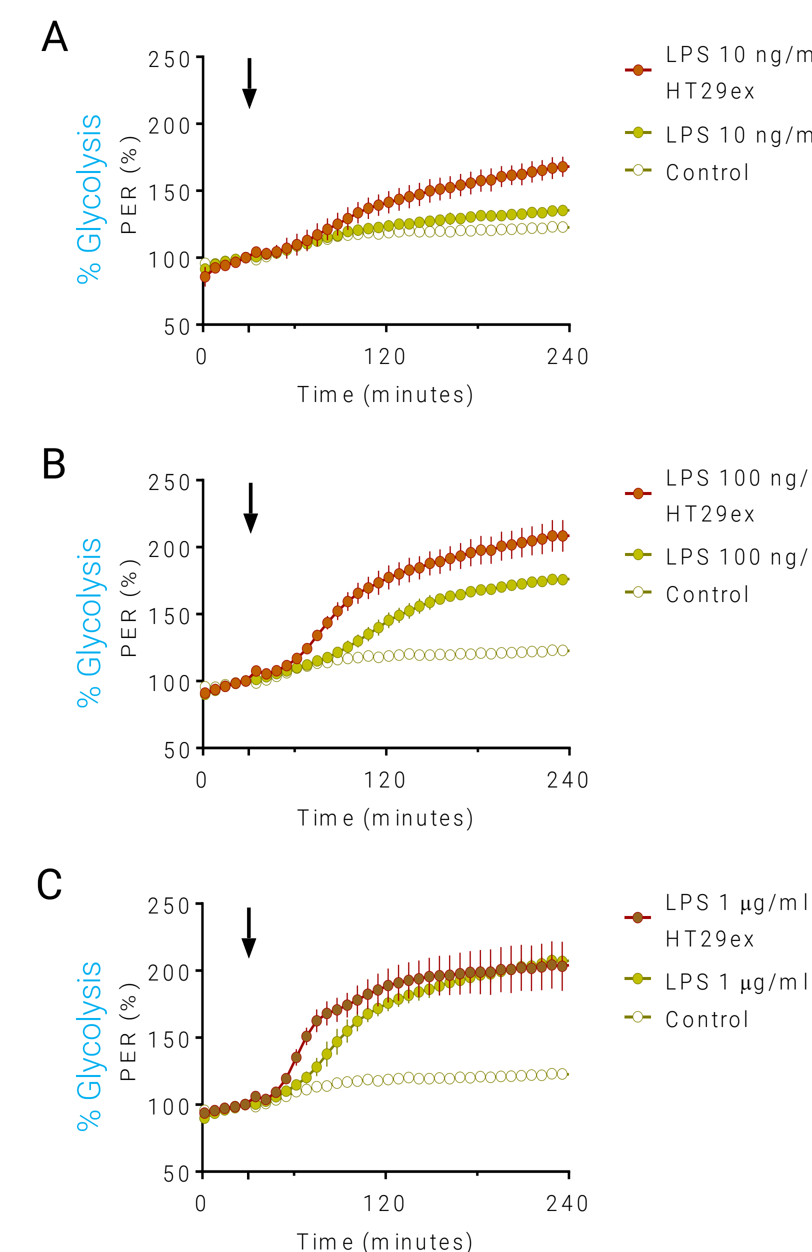
A and B. Changes in glycolysis and mitochondrial respiration upon macrophage activation were measured by Seahorse XF glycolytic rate assay (A) and by XF cell mito stress test (B) and all data were normalized using cell number count by Agilent XF Imaging and Cell Counting software. C. The ATP production rates were calculated using data from A and B. Normalized data show metabolic switch to highly glycolytic phenotype upon the activation. The total ATP production rates per cells were maintained stably even with severe suppression of mitochondrial respiration. Data shown are mean ± SD, n=6 technical replicates.

Results and Discussion

HT29 cell-derived exosome-enriched fraction potentiates macrophage activation



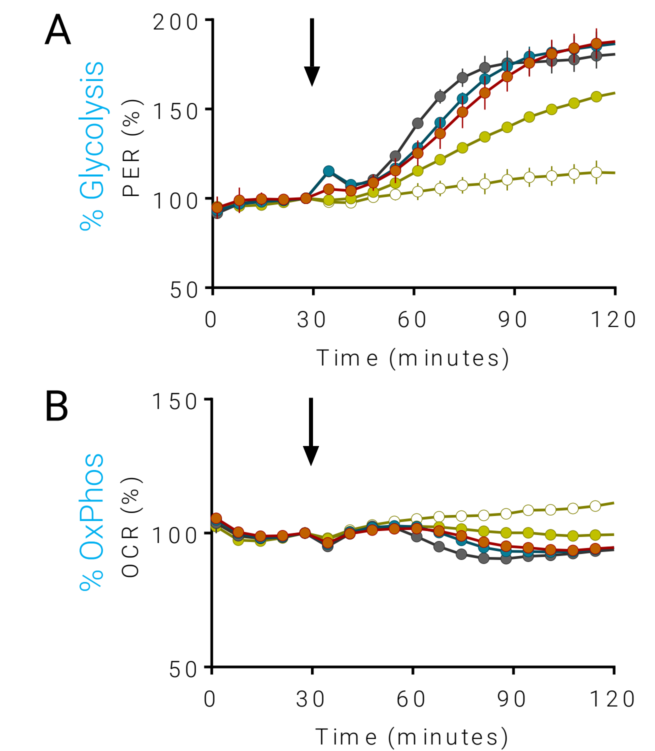
RAW264.7 macrophages were activated by LPS/IFNγ (arrow) with or without co-injection with exosome-enriched fraction isolated from conditioned medium of HT-29 cells. A. The activation-associated glycolytic rate increase was accelerated by exosome fraction co-injection. B. Mitochondria respiration was slightly downregulated but the change was less significant compared to glycolytic activation. Data shown are mean ± SD, n=6 technical replicates.



HT-29 cell-derived exosome-enriched fraction potentiates glycolytic activation by low-dose LPS (arrow; A, 10 ng/ml; B, 100 ng/ml; C, 1 µg/ml). It makes the response faster and stronger. Data shown are mean ± SD, n=6 technical replicates.

Results and Discussion

Difference in exosome effect between cancer cell type



Effect of exosome-enriched fractions from three different cancer cell lines (HT-29, MCF-7 and SKOV-3) on glycolytic activation were compared. The exosome concentration was adjusted by protein quantification. A. Exosome-enriched fractions from all three cell lines potentiated glycolytic activation by LPS. B. Mitochondria respiration was slightly downregulated further by exosome injection. Data shown are mean ± SD, n=6 technical replicates.

Conclusions

- *In vitro* macrophage activation is tightly associated with the oxidative-to-glycolytic switching in metabolic phenotype mediated by bi-phasic responses; immediate early (< 1h) and prolonged (> 6h), which makes cellular ATP energy production more depend on glycolysis.
- LPS/IFNγ co-stimulation suppresses mitochondrial function severely through iNOS signaling and cells turns to rely on glycolysis fully for the ATP energy production.
- Data normalization is critical for quantitative comparison of metabolic phenotype changes. Normalized results showed that the total ATP production rate per cell is stably maintained even with significant downregulation of mitochondria.
- Exosome-enriched fraction derived from cancer cells potentiates LPS-induced glycolytic activation by accelerating activation-associated immediate early metabolic responses. More study is required for the target and mechanism (e.g. mitochondrial downregulation).

References

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- Chow et al. (2015) Macrophage immunomodulation by breast cancer-derived exosomes requires Toll-like receptor 2-mediated activation of NF-kB. Sci. Rep. 4:5750
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