Evaluating Effect of Trophoblast Extracellular Vesicles on Macrophage

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Introduction

- The maternal immune system must balance tolerance towards the fetus while protecting against infections.
- Trophoblast cells of the placenta play a crucial role in facilitating essential interactions between the maternal and fetal systems during pregnancy.
- EVs serve as key mediators of intercellular communication by transferring bioactive molecules such as proteins, lipids, and nucleic acids to recipient cells [3].
- Within the maternal-fetal interface, trophoblast-derived EVs have shown potential as significant modulators of immune responses.
- This project investigates changes to macrophage phenotype following treatment with EVs derived from JAR, a choriocarcinoma cell line.

Materials and Method

JAR Cell Culture & EV Isolation

•JAR cells were cultured under sterile conditions in RPMI 1640 medium and maintained at 37°C with 5% CO_2.

•Extracellular vesicles (EVs) were isolated from conditioned media using ultrafiltration and Total Exosome Reagent, then quantified using the Pierce BCA Assay.

THP-1 Differentiation & EV Treatment

•THP-1 monocytes were differentiated into M0 macrophages using 50 ng/mL PMA over 48 hours.

•Differentiated macrophages were treated with JAR-derived EVs (30 µg/mL) at 2, 6, and 24-hour intervals to assess time-dependent effects on phenotype.

Phenotypic Analysis & Study Implications

•. The treatment was conducted at multiple time points, including 2 hours, 6 hours, and 24 hours, to assess the time-dependent effects of EV uptake on macrophage phenotype.

•Fluorescence microscopy and antibody labeling were used to evaluate macrophage phenotype changes post-EV treatment

Results

This study evaluated the impact of JAR-derived extracellular vesicles on macrophage phenotype, evaluated with confocal microscopy and quantitative image analysis. Here, we polarized THP-1 cells towards an M0 phenotype and treated the cells with JAR EVs at a dose of 15ug/mL for 2 and 6 hours. The cells were then stained for pan-macrophage marker CD11b and M2 phenotype marker CD206. Changes in CD206 were observed as a result of treatment for both 2-hour and 6-hour time points.

Phenotypic Changes in Macrophages: Fluorescence imaging analysis revealed expression of CD11b and CD206 markers, both in non-treated and treated conditions, with a reduction in CD206 with treatment. Further study is required to quantify changes in marker expression

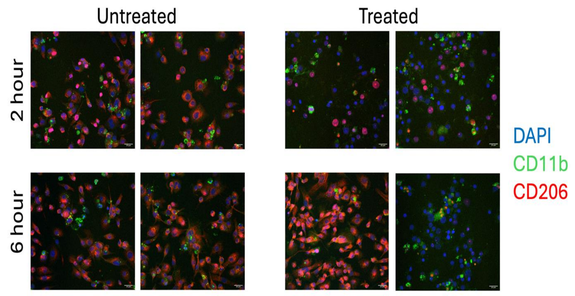

Fig 1: Immunofluorescence staining of macrophages under untreated and treated conditions at 2-hour and 6-hour time points

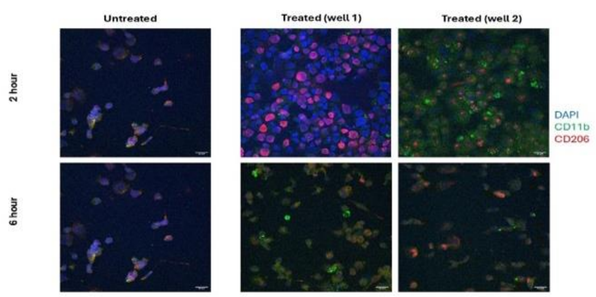

Fig 2: Immunofluorescence staining of macrophages under untreated and treated conditions at 2-hour and 6-hour time points

Results

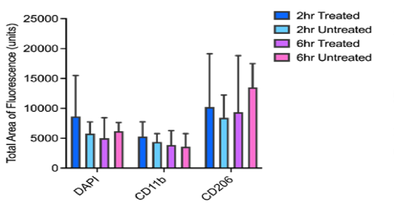

Fig 3: Relative expression levels of antibody markers in THP-1-derived macrophages

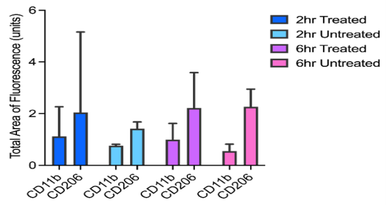

Fig 4: Expression of M1 and M2 macrophage polarization markers in THP-1-derived macrophages following treatment

A comprehensive analysis is performed to evaluate macrophage polarization. The results revealed significant, context-dependent effects. Notably, all experimental groups and periods consistently exhibited CD11b expression, indicating that macrophage identification was maintained regardless of the treatment condition.

Discussion

•Investigated the effect of trophoblast-derived extracellular vesicles (EVs) on macrophage phenotype.

•Observed a reduction in CD206 expression (M2 marker) at both the 2-hour and 6-hour time points.

•The downregulation of CD206 suggests suppression of anti-inflammatory (M2) polarization.

•CD11b expression remained consistent across all groups, confirming macrophage identity.

•It highlights the immunomodulatory role of trophoblast-derived EVs in influencing macrophage behavior.

Conclusion

This study shows that extracellular vesicles (EVs) from trophoblast representative JAR choriocarcinoma cell line can modulate macrophage phenotype by reducing CD206 expression post treatment. The downregulation of CD206 and sustained expression of CD11b suggest that macrophage identity persists while polarization may shift away from M2 state characterised by a high CD206 expression. These findings highlight the immunoregulatory potential of trophoblast EVs on macrophages and their role in shaping immune responses at the maternal-fetal interface although further analysis is required to understand the changes in cell phenotypes

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