CHARACTERIZATION OF MYELOID-DERIVED SUPPRESSOR CELLS IN RESPONSE TO RESTRAINT STRESS IN PRECLINICAL MODELS OF OVARIAN CANCER

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Introduction

- Ovarian cancer (OC) is one of the deadliest forms of gynecological malignancies, highlighted by a five-year survival rate of less than 50%.
- Myeloid-Derived Suppressor Cells (MDSC) are immature and immunosuppressive cells that play a key role in the tumor microenvironment (TME).
- Tumor-associated MDSC aid immune evasion and are associated with poor prognosis in cancer patients.
- MDSC have been associated with the dysregulation of several pathways, such as those mediated by Notch.
- The Notch signaling pathway is an important pathway for cell development and proliferation.
- Chronic stress has been shown to increase tumor-associated inflammation and promote immune escape.

Objective

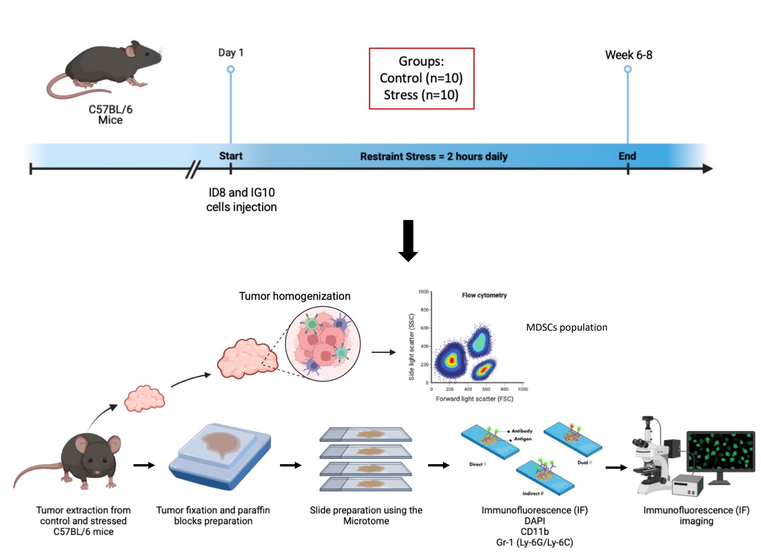
- **Our overall objective** is to determine the role of chronic stress on MDSC infiltration and biology in the OC TME.

Hypothesis

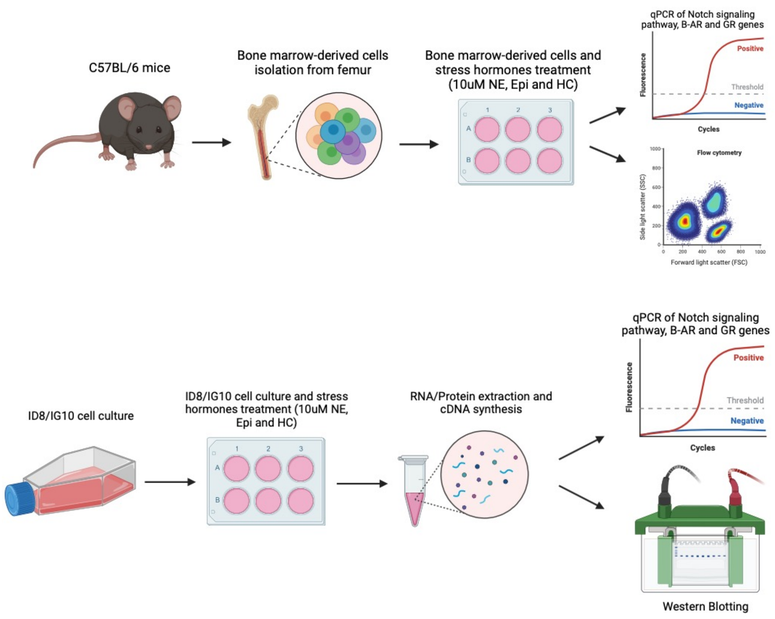
- Chronic stress increases MDSC infiltration into the TME and promotes disease progression by modulating the Notch signaling pathway in ovarian cancer cells.

Methods

A. Restraint Stress Experiment (*In vivo*)



B. Stress Hormones Treatment Experiment (*In vitro*)



Results

Restraint Stress Induces MDSC Infiltration in the Ovarian Tumor Microenvironment

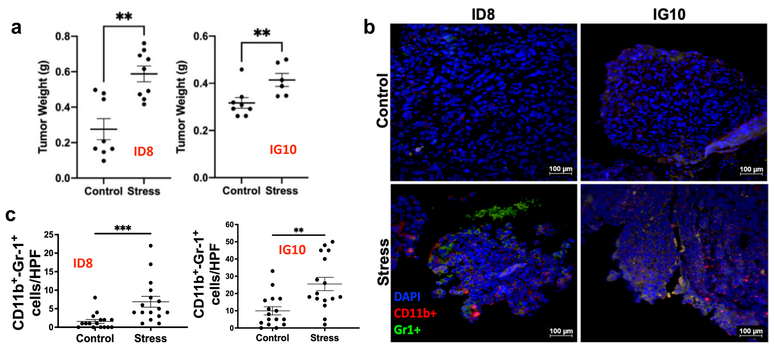


Figure 1. Daily restraint stress promotes tumor growth and MDSCs infiltration in the OC TME. **(a)** Effect of daily restraint stress on ID8 and IG10 tumor growth (** p<0.01) (Aquino, A.N. et al 2022). **(b)** Images of MDSC CD11b+/Gr-1+ cells from ID8 and IG10 tumors. **(c)** Quantification of CD11b+/Gr-1+ cells on ID8 (N=4) (** p<0.01) and IG10 (N=4) (*** p<0.001) tumor samples from mice (N=4) subjected to restraint stress. Statistical analysis was performed using the Mann-Whitney test. Data presented are represented as mean ± SEM.

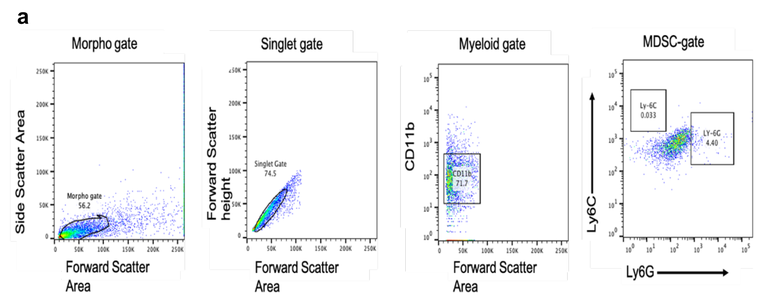


Figure 2. Daily restraint stress promotes PMN-MDSCs infiltration in the OC TME. **(a)** Gating for the myeloid population. **(b)** Quantification of cellular expression of CD11b+ (Myeloid), CD11b+/Ly-6C+ (M-MDSCs) and CD11b+/Ly-6G+ (PMN-MDCSs) cells (* p<0.05) measured by flow cytometry on IG10 tumors (N=6) from mice subjected to restraint stress. Statistical analysis was performed using the Mann-Whitney test. Data presented are represented as mean ± SEM.

Stress Hormones Promotes Polymorphonuclear MDSC (PMN-MDSC) Enrichment

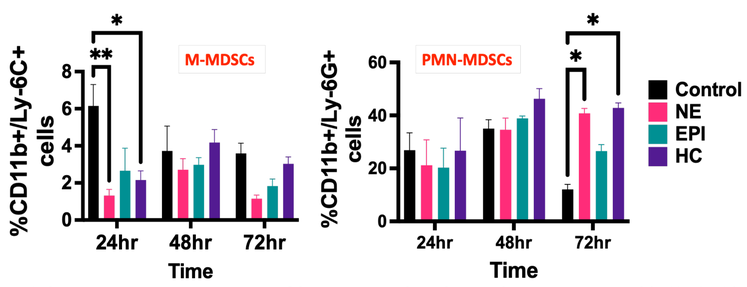


Figure 3. Stress hormones promote PMN-MDSCs enrichment. Quantification of cellular expression of CD11b+/Ly-6C+ (M-MDSCs) (* p<0.05; ** p<0.01) and CD11b+/Ly-6G+ (PMN-MDCSs) (* p<0.05) cells measured by flow cytometry on C57BL/6 bone marrow-derived MDSCs treated with stress hormones for 72hr. Statistical analysis was performed using two-way ANOVA with Tukey multiple comparison correction. Data presented are represented as mean ± SEM of three independent experiments performed in duplicate.

Stress Hormones Upregulate the Notch Signaling Pathway and β-adrenergic/Glucocorticoid Receptor Genes Expression in OC Cells

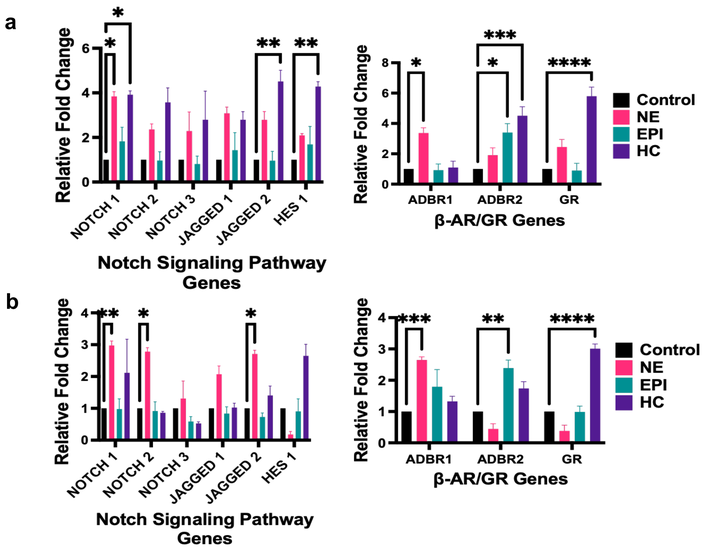


Figure 4. Stress hormones upregulate the Notch signaling pathway, β-adrenergic/glucocorticoid receptors genes in ovarian cancer cells. Notch signaling pathway, b-adrenergic and glucocorticoid receptors gene expression after stress hormones treatment on ID8 at 12 hours **(a)**(* p<0.05, ** p<0.01, *** p<0.001) and IG10 cell at 6 hours **(b)** (* p<0.05, ** p<0.01, *** p<0.001) measured using qPCR. Statistical analysis was performed using two-way ANOVA with Tukey multiple comparison correction. Data presented are represented as mean ± SEM of three independent experiments performed in duplicate.

Results

Stress Hormones Modulate the Expression and Activity of Notch Signaling Pathway Proteins in OC Cells

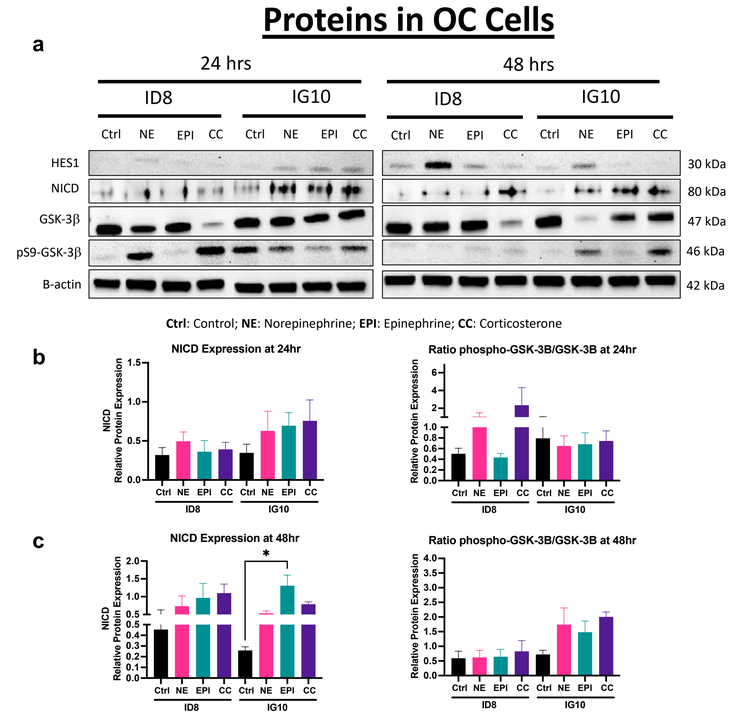


Figure 5. Stress hormones modulate the expression of the Notch signaling pathway in OC cells. **(a)** Notch signaling pathway protein expression in OC cells (ID8 and IG10) treated with stress hormones at 24hrs and 48hrs. Western blot quantification and analysis for Notch signaling pathway protein expression for OC cells at 24hrs **(b)** and 48hrs (*p< 0.05) **(c)**. Statistical analysis was performed using the Kruskal-Wallis test. Data presented are represented as mean ± SEM of three independent experiments.

Conclusions

- Our data suggest high infiltration of MDSC into the TME in two OC syngeneic mouse models (ID8 and IG10) upon exposure to restraint stress.
- Stress hormones significantly sustained the PMN-MDSCs population compared with the control group.
- Stress hormones significantly upregulate the expression of members of the Notch signaling pathway and β-adrenergic/glucocorticoid receptors genes in ID8 and IG10 OC cells.
- WB showed that stress hormones could induce the expression of proteins associated with the Notch signaling pathway in OC cells.
- Notch signaling pathway dysregulation could be a key link between stress-induced MDSC infiltration and OC progression.
- Our results provide the first insights for understanding the role of MDSC in OC and the impact of life stressors on the tumor microenvironment.

Future Directions

- Continue characterizing the effects of stress hormones in MDSCs biology in the ovarian TME.
- Characterize the expression of the Notch signaling pathway, β-adrenergic/glucocorticoid receptors genes in MDSCs in response to stress hormones.
- Measure protein expression of Notch 1, Jagged 2, AKT, and SGK1.
- Perform an MDSC and OC cell co-culture to measure Notch signaling pathway dysregulation.

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