Stress and CXCR2-Dependent Inflammation in Female Reproductive Cancers in Central America: A Proposed Model

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Abstract

Female reproductive cancers remain a global health challenge, disproportionately impacting women in Central America where socioeconomic and healthcare disparities worsen prognoses. Studies have shown that psychosocial stress plays a key role in modulating molecular pathways, ultimately increasing the risk of reproductive cancer. This study aims to elucidate how chronic restraint stress influences female reproductive cancer progression by investigating CXCR2 signaling as a potential mediator, and as relevant to Central American populations experiencing similar stress-related disparities. We used a chronic restraint stress model in male Balb/c mice inoculated with H22 hepatocellular carcinoma cells as a controlled system to investigate CXCR2 signaling dynamics. Complementary analyses of female reproductive tumors in human cohorts provide cross-species insight into stress-associated cancer biology. We observed that stress significantly enhanced tumor expression of CXCR2 and its downstream effector CXCL5, as demonstrated by Western blotting ($p < 0.01$). Western blot analyses also revealed elevated phosphorylated Erk1/2, while CXCR4 and total Erk1/2 remained unchanged. Collectively, this data demonstrates that chronic stress selectively activates the CXCR2–CXCL5 signaling axis and downstream Erk1/2 phosphorylation in the tumor microenvironment, revealing a targeted inflammatory pathway that drives stress-induced tumor progression. In vitro, lentiviral-mediated CXCR2 knockdown in multiple ovarian cancer cell lines confirmed the receptor's role in driving expression of pro-inflammatory chemokines CXCL1 and CXCL8, validated via Western blotting and immunofluorescence. Flow cytometry of CFSE-labeled myeloid-derived suppressor cells (MDSCs) further indicated that chronic stress facilitated MDSC accumulation and migration within tumors, contributing to an immunosuppressive microenvironment. Mechanistically, we found that CXCR2 knockdown impaired NF-κB activation as determined by an electrophoretic mobility shift assay (EMSA), while treatment with the polyphenol ampelopsin dose-dependently reduced nuclear Snail and NF-κB p65 levels. ELISA assays also revealed that Snail selectively modulated secretion of CXCL1 and CXCL2, but not CXCL5, indicating that chemokine expression is controlled by separate mechanisms. These mechanistic insights prompted us to explore whether similar stress-linked inflammatory responses occur in humans. In a parallel human cohort, serum cytokine profiling before and after the Trier Social Stress Test (TSST) showed significant upregulation of IL-5 and IL-27 ($p < 0.05$), demonstrating that short-term psychosocial stress triggers early inflammatory responses. Additionally, transcriptomic analyses of human gynecologic tumor tissues ($n = 439$) revealed that neighborhood disadvantages—but not early trauma—were associated with divergent conserved transcriptional response to adversity (CTRA) signatures, with ER+ and ER- tumors exhibiting differences in inflammation and immune response based on tumor subtype. These results identify CXCR2 as a key mediator linking psychosocial stress to female reproductive cancer progression and highlight its potential as a therapeutic target to address cancer disparities in Central America.

Keywords:
CXCR2 signaling, psychosocial stress, ovarian cancer, tumor microenvironment, Central America, inflammation, NF-κB activation, MDSCs, translational oncology, cancer disparities

1. Introduction

Female reproductive cancers continue to take a devastating toll on women across the world. These deadly diseases strike the core of female biological function. Breast, ovarian, endometrial, cervical, vaginal, and vulvar cancers do not just threaten survival; they reshape entire families. From 2000-2021

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alone, over 3,890,000 women in the United States were diagnosed with female reproductive cancer, with epithelial breast cancers accounting for the majority (2,727,326 cases) of female reproductive cancers, followed by endometrial carcinomas (470,598), epithelial ovarian carcinomas (210,495) and cervical squamous cell carcinomas (131,053) (Surveillance, Epidemiology, and End Results (SEER) Program 2023). Additionally, one in six women in the United States are diagnosed with a female reproductive cancer in their lifetimes (Surveillance, Epidemiology, and End Results (SEER) Program 2023). Collectively, these cancers are diverse, but share dangerous traits: they are often hormonally regulated, inflammation-sensitive, and closely tied to life course factors such as carcinogenic factors and exposure to psychosocial stress (Ezzat 1996; Campaña et al. 2025; Crosswell et al. 2014; Di Sibio et al. 2016; Shen et al. 2025). This unique intersection of biology, gender, and life experience distinguishes female reproductive cancers from other types of cancer, making them especially difficult to study and treat.

The need to better understand and address female reproductive cancers is even more urgent in underserved communities. In low-income countries, including many in Central America, cancer care is constrained by fragile health infrastructure, cultural stigma, and gaps in diagnostic access. The result is one where women are diagnosed too late, treated too little, and counted too loosely in national records, resulting in a gap in diagnosis, treatment, and record-keeping (Goss et al. 2013). This gap in diagnosis and treatment can be quantified in the fact that one in four women get diagnosed with a female reproductive cancer in their lifetime.

Such disparities are not random—they stem from entrenched political, economic, and cultural barriers that limit access to cancer screening and care. Studying Central America helps highlight these challenges and informs more effective interventions (Ezzat 1996; CancerOverTime 2025). Breast cancer incidence in Central American countries such as Costa Rica and Ecuador, for example, has accumulated over 34,000 cases (CancerOverTime 2025). While registry data from Costa Rica and Ecuador report a combined total of 34,578 female reproductive cancer cases between 2000 and 2016 (CancerOverTime 2025), this figure appears significantly lower than expected based on population-level incidence rates. Using age-standardized incidence rates (ASRs) from the Global Cancer Observatory (GCO) and average population estimates for the region (18.8 million), a projection based on ASRs suggests approximately 121,000 cases should have occurred during this 17-year period (CancerOverTime 2025) in these countries. The nearly threefold discrepancy suggests that national cancer registries may underreport cases, have incomplete geographic coverage, and face healthcare access barriers that lead to underdiagnosis (Goss et al. 2013; Campaña et al. 2025). Addressing the crisis of female reproductive cancers requires a dual perspective: one that examines the molecular pathways driving tumor aggression and another that considers the social and structural forces shaping patient prognosis and disease progression.

To address cancer disparities in Central America, we must understand not only who is affected but also how their cancers behave at the cellular level. One of the most critical mechanisms driving the progression of female reproductive cancers is the epithelial-to-mesenchymal transition (EMT), a biological process that enables tumor cells to become more invasive, resistant to treatment, and capable of spreading beyond their original site (Elloul et al. 2010; Rosanò et al. 2011; Yuan et al. 2013). Through EMT, cancer cells gain the ability to break through surrounding tissue, enter the bloodstream or lymphatic system, and seed metastases in distant organs. What makes EMT particularly dangerous is that it is not a permanent switch; it is reversible (Jo et al. 2009). Tumor cells can cycle between epithelial and mesenchymal states, adapting to changing microenvironments or different cell signaling pathways. Such plasticity allows them to hide from immune location, survive chemotherapy, and re-establish tumors even after aggressive treatment (Moody et al. 2005). EMT is especially prevalent in aggressive female reproductive cancers such as epithelial ovarian carcinoma (Liu et al. 2015). Diagnosis often occurs at late stages, when cells have already undergone EMT and begun to disseminate. Certain transcription factors like Snail are known to trigger this process, turning on genes that promote mesenchymal traits and suppress epithelial ones (Liu et al. 2015; Hsu et al. 2013). In under-resourced healthcare systems, late diagnosis is common, and by the time patients receive care, their cancers may already show markers of EMT. For example, in advanced ovarian cancer, tumor cells present in ascites were shown to exhibit EMT characteristics, indicating widespread dissemination and poor prognosis (Elloul et al. 2010). These findings highlight EMT as a crucial target for understanding and addressing disparities in cancer outcomes. By focusing on how cancers evolve, rather than solely when they are detected, we can develop treatments that consider both the timing and nature of disease progression. Importantly, EMT does not occur in isolation, it is often triggered or amplified by inflammatory signaling within the tumor microenvironment. A central player in this process is CXCR2, a chemokine receptor that responds to stress and inflammation by activating pathways that reinforce EMT. Through ligands like IL-8 and CXCL5, CXCR2 promotes a pro-invasive, mesenchymal state in cancer cells, linking immune dysregulation directly to metastatic growth (Yang et al. 2010; De Larco et al. 2001; Lin et al. 2004). Tumors often take over CXCR2 to promote inflammation, remodel tissue, and evade immune destruction. CXCR2 orchestrates the recruitment of neutrophils and myeloid-derived suppressor cells (MDSCs) into the tumor microenvironment through binding with inflammatory ligands like interleukin-8 (IL-8), CXCL1, CXCL2, and CXCL5 (Taki et al. 2018). These MDSCs, rather than attacking the tumor, are repurposed to suppress anti-tumor immune responses, secrete growth factors, and remodel tissue to favor invasion. In many cancers, CXCR2 signaling directly promotes angiogenesis, the development of new blood vessels, ensuring the tumor has access to nutrients and oxygen as it grows (Yang et al. 2010).

Perhaps even more critically, CXCR2 plays a key role in enabling cancer cells to undergo epithelial-to-mesenchymal transition (EMT) (Taki et al. 2018). Activation of the CXCR2

receptor by IL-8 and related ligands shifts cancer cells into a more motile, invasive, and therapy-resistant state. In this way, CXCR2 functions not merely as a passive responder to inflammation, but as an active driver of it. In ovarian cancer, CXCR2 has been shown to promote tumor growth by accelerating cell cycle progression, reducing apoptosis, and encouraging the formation of new vasculature (Yang et al. 2010).

Despite over two decades of research highlighting the role of CXCR2 in tumorigenesis, few studies have focused on this pathway within the context of female reproductive cancers in Central America. This is a significant gap, especially considering the inflammatory burden and chronic stress disproportionately experienced by many women in under-resourced regions (Nsonwu-Anyanwu et al. 2021). CXCR2's inflammatory ligands like IL-8 have been shown to upregulate in breast and ovarian cancers, promoting both invasion and immune evasion (Lokshin et al. 2006). However, the intersection of this pathway with social causes of health remains vastly unknown. Understanding CXCR2's function in these settings could uncover targeted therapies or interventions customized to patients facing both biological and systemic risks. Cancer biology must examine treatments from both their underlying mechanisms and the contexts they take place in. Social conditions such as poverty, trauma, and marginalization do more than shape access to care; they biologically embed themselves within the body, modifying immune systems, hormone signaling, and cellular environments in ways that can accelerate disease (Cunningham et al. 2022). Psychosocial stress, when sustained over time, disrupts cortisol rhythms (Kirschbaum et al. 1993), interferes with the balance of estrogen and progesterone, and generates reactive oxygen species (ROS) that wear down mitochondrial health and DNA integrity (Mahalingaiah y Singh 2014; Santner et al. 1997; De Olivera et al. 2009; Zhao et al. 2016). Recent work using the Trier Social Stress Test (TSST) (Kim et al. 2021) has confirmed that acute psychosocial stress directly elevates systemic oxidative stress, as shown through sensitive biochemical assays that measure the body's overall ability to neutralize oxidants (global reducing capacity). These findings support a causal link between psychological stress and oxidative damage at the cellular level. In Central America, where chronic stressors such as economic instability, violence, and limited healthcare access are widespread, prolonged exposure to these stressors may drive oxidative stress through the stress–oxidative stress axis, potentially contributing to increased cancer vulnerability in already at-risk populations. By understanding how psychosocial environments translate into molecular dysfunction, we can begin to address disparities in female reproductive cancer outcomes across regions historically excluded from mechanistic research. These biological disruptions are especially relevant to cancer progression. In postmenopausal Nigerian women with breast cancer, for example, elevated oxidative stress was associated with activation of the CXCR2 pathway, highlighting how hormonal shifts and chronic inflammation converge to worsen outcomes in marginalized populations (Nsonwu-Anyanwu et al. 2021). Previous studies have shown that increased ROS generation and hormonal shifts in vitro leads to increased cell growth, survival, and tumorigenic potential of cancerous cells (Mahalingaiah y Singh 2014). Increased production of ROS similarly has been shown to contribute to the metastatic potential of human breast cancer cells (Mahalingaiah y Singh 2014). Similarly, chronic psychological stress has been shown to accelerate cellular aging through oxidative damage (Elloul et al. 2010). Murine models further reinforce this link between stress, oxidative signaling, and tumor progression. In one study, repeated restraint stress in mice led to increased ROS production, downregulation of immune receptors, and recruitment of myeloid-derived suppressor cells (MDSCs), creating an immunosuppressive environment that promoted the growth and spreading of tumors (Cao et al. 2021), offering a link between stress and tumor immune evasion. Together, these studies show that the intersection of stress biology and cancer progression is not only real but necessary to address, especially in under-resourced regions like Central America. There have been limited, multifaceted study models, and all face challenges in interpretability. This study aims to comprehensively investigate these complex interactions while ensuring experimental rigor through carefully designed methodologies.

While many studies have explored the molecular pathways driving cancer progression, few have accounted for how chronic social stress, particularly in low- and middle-income countries, may influence tumor behavior. Existing studies tend to center on high-income regions, overlooking the unique stress exposures, healthcare barriers, and sociocultural factors that shape cancer outcomes in low-income populations. In this context, psychosocial stress in Central America driven by systemic poverty, trauma, and limited healthcare access may activate CXCR2 and EMT pathways in breast and ovarian tumors, contributing to worsened prognosis. Uncovering this mechanistic link could help explain elevated cancer mortality in regions where timely screening and intervention remain limited.

To investigate this, the study uses a three-way approach: murine and in vitro models simulate chronic stress and assess tumor responses at the molecular level; human transcriptomic and cytokine data from the Black Women's Health Study provide clinical relevance; and a proposed field-based study in Central America aims to extend these findings to underrepresented, high-stress populations. Together, these components allow for a more comprehensive understanding of the link between psychosocial stress, CXCR2/EMT activation, and tumor progression.

2. Methods

Animal Model Selection

Murine models are essential in cancer research because scientists have access to the full mouse genome. This makes it easier to study how genes are altered in cancer. For example, researchers can map changes in DNA, like copy number variations or mutations, and figure out which genes or pathways are involved in tumor growth. Having the whole genome also helps scientists look closely at important genes, like oncogenes or tumor suppressors, to better understand how cancer develops (Chinwalla et al. 2002).

2.1. Mice Selection and Conditions

Male Balb/c mice were housed in pathogen-free conditions with standard temperature, lighting, and feeding protocols (Cao et al. 2021). H22 hepatocellular carcinoma cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics, and confirmed to be mycoplasma-free, as previously described (Cao et al. 2021).

2.2. Chronic Restraint Stress Procedure

Protocols were adapted from a recent study involving mice (Cao et al. 2021) in order to elucidate the connection between stress and its influences on CXCR2 expression and related signaling pathways in the tumor microenvironment. Male Balb/c mice (5 weeks old) were introduced to feeding conditions for one week before the experiment. Mice were randomly assigned to either a control group housed in their home cages or a chronic restraint stress group. The stress group underwent daily restraint for 2 hours between 9 A.M. and 11 A.M. to minimize circadian rhythm effects. During restraint, mice were placed in well-ventilated 50 mL syringes that restricted movement without causing injury. Control mice were deprived of food and water during the corresponding restraint period. On day 14, 5×10^4 H22 hepatocellular carcinoma cells were subcutaneously injected into the right flank of each mouse. Following tumor inoculation, the chronic restraint stress paradigm continued for an additional 21 days. At the conclusion of the study, all mice were humanely euthanized through cervical dislocation, following approved ethical procedures.

2.3. Tumor Measurements:

Tumor growth was monitored every other day by measuring each tumor's dimensions with a digital caliper as previously described (Cao et al. 2021). To estimate tumor volume, the standard formula was used: volume = (length $\times$ width^2) $\div$ 2.

2.4. Western Blotting Analysis

Protein was extracted from flash-frozen tumor tissues and bone marrow–derived myeloid cell pellets using RIPA lysis buffer (containing protease and phosphatase inhibitors; Beyotime Biotechnology, Shanghai, China) according to standard protocols. Tissue samples were homogenized using a bead mill homogenizer, incubated on ice for 30 minutes, and centrifuged at 12,000 $\times$ g for 15 minutes at 4°C to remove cellular debris. Supernatants were collected, and total protein concentrations were quantified using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, USA), as previously described (Zhang et al. 2015).

Equal amounts of total protein (20–40 μg per lane) were mixed with 5$\times$ SDS loading buffer, denatured at 95°C for 5 minutes, and resolved by SDS-PAGE using 10% or 12% polyacrylamide gels. Proteins were then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Temecula, CA, USA) using a wet transfer system at 100 V for 90 minutes on ice.

Membranes were blocked in 5% non-fat dry milk prepared in Tris-buffered saline with 0.1% Tween-20 (TBST) for 1 hour at room temperature. They were then incubated overnight at 4°C with primary antibodies diluted in 5% BSA/TBST, as previously described (Zhang et al. 2020). The following primary antibodies were used: rabbit anti-CXCR2 (1:1000, Abcam, ab14935), rabbit anti-CXCL5 (1:1000, R&D Systems, AF118), rabbit anti-Erk1/2 (1:1000, Cell Signaling Technology, #9102), rabbit anti-phospho-Erk1/2 (Thr202/Tyr204) (1:1000, Cell Signaling Technology, #9101), mouse anti-GAPDH (1:3000, Proteintech, 60004-1-Ig), and mouse anti-β-actin (1:5000, TransGen Biotech, HC201-01) as previously described (Cao et al. 2021; Zhang et al. 2015; 2020).

Following primary incubation, membranes were washed three times with tris-buffered saline (five minutes each) and incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-rabbit; 1:5000, Jackson ImmunoResearch) for 1 hour at room temperature. After final washes, bands were visualized using enhanced chemiluminescence (ECL) detection reagents (Applygen Technologies, Beijing, China) and imaged on a ChemiDoc MP Imaging System (Bio-Rad) (Zhang et al. 2015).

Densitometric analysis was then performed using ImageJ software (NIH). Relative protein expression was calculated by normalizing band intensities to internal loading controls (GAPDH or β-actin) as previously described (Cao et al. 2021).

2.5. Isolation and Flow Cytometric Assessment of MDSCs and T-cell Proliferation Using carboxyfluorescein succinimidyl ester (CFSE) Labeling

Spleen and bone marrow cells were collected from the mice's tibia and femur following established protocols (Cao et al. 2021). Red blood cells were removed using a cold lysis buffer. Tumor tissues were minced and enzymatically digested with DNase I and collagenase at 37°C for two hours, then mechanically dissociated and filtered through a 40-μm mesh to obtain single-cell suspensions. Immune cells from tumors were enriched using Lympholyte-M separation according to manufacturer's instructions. Cells from spleen, bone marrow, and tumors were stained with fluorescent antibodies targeting CD11b, Gr-1, Ly6G, Ly6C, CD45, CD4, and CD8 in a buffered solution containing BSA to maintain stability. Myeloid-derived suppressor cells (MDSCs), identified as CD11b$^+$Gr-1$^+$, were sorted with high purity (90–99%) using BD ARIA III or SONY SH800S cell sorters. Separately, splenocytes from healthy, tumor-free male mice (6–8 weeks old) were isolated by mechanical disruption and filtered through a 40-μm strainer. After RBC lysis and triple PBS washes, cell viability exceeded 99% by trypan blue exclusion. Splenocytes were labeled with 2 μM carboxyfluorescein succinimidyl ester (CFSE; BioLegend) for 20 minutes at 37°C, washed with pre-warmed RPMI-1640, and cultured in 96-well plates precoated overnight with 5 μg/mL anti-CD3/CD28 antibodies. Cells were incubated with or without sorted MDSCs for 72 hours in a humidified 5% CO_2 incubator at 37°C. Post-incubation, cells were stained with fluorescent anti-CD3 antibody and analyzed by flow cytometry. T-cell proliferation was assessed by CFSE dilution within the CD3+ population and expressed as the percentage of proliferating cells compared to unstimulated controls.

2.6. Statistical Analysis

Data are presented as mean values plus or minus the standard error of the mean (SEM). For comparisons between two groups, the Student's t-test was used. When comparing more than two groups, one-way analysis of variance (ANOVA) was applied as previously described (Hsu et al. 2013). A p-value less than 0.05 was considered statistically significant.

Mechanistic Study Cancer Cell Lines

Human ovarian cancer cell lines (HEY, OVCA429, SKOV3, SNU251, OVCAR3, and OVCA433) and normal ovarian surface epithelial cells (OSE72 and OSE137) were generously donated by Professor Engda Hagos of Colgate University. All cell lines were authenticated by short tandem repeat (STR) profiling and tested regularly for Mycoplasma contamination. Cells were maintained in RPMI-1640 or DMEM media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified incubator with 5% CO_2 following procedures previously described (Yang et al. 2010). For CXCR2 knockdown experiments, lentiviral transduction was performed using CXCR2-targeted shRNA (CXCR2i) or GFP control shRNA (GFPi), followed by selection with puromycin. Knockdown efficiency was validated by Western blotting and immunofluorescence.

2.7. Preparation of Samples for Enzyme-Linked Immunosorbent Assay (ELISA)

Tumor tissues were weighed, homogenized using a bead homogenizer with sonication in 1× RIPA buffer containing protease and phosphatase inhibitors as previously described (Taki et al. 2018), and centrifuged at 16,000×g for 10 minutes at 4°C. Serum was collected from tumor-bearing mouse blood after clotting on ice for 30 minutes and centrifugation under identical conditions. Protein levels were quantified using sandwich ELISA. Then, mouse CXCL1, CXCL2, and CXCL5 were measured using Quantikine® ELISA kits (R&D Systems) where samples were added to antibody-coated microplates, captured analytes were bound by HRP-conjugated detection antibodies after washing, and color was developed using TMB substrate (stopped to yield yellow) with absorbance read at 450 nm. Human CXCL1 and CXCL2 were measured using ABTS ELISA Development Kits (PeproTech) according to the manufacturer's protocols, while human CXCL5 was quantified using a human Quantikine® ELISA kit following the same TMB-based procedure as described for mouse chemokines.

2.8. M-MDSC Migration and Tumor Infiltration Assay

Bone marrow–derived MDSCs were collected, labeled with either a high or low amount of CFSE dye, mixed equally, and transferred into non-stressed mice that had H22 tumors as previously described (Cao et al. 2021).

2.9. Electrophoretic Mobility Shift Assay

To assess NF-κB activation in tumor samples, we performed an electrophoretic mobility shift assay (EMSA) using nuclear extracts prepared from tumor tissues following established protocols (Yang et al. 2010). Synthetic DNA probes containing the NF-κB consensus binding sequence, along with mutated control sequences, were labeled with radioactive phosphate groups to serve as "hot" probes. Nuclear extracts (10 μg) were first incubated with a nonspecific competitor DNA to reduce background binding, then combined with the labeled probes to allow protein-DNA complexes to form. To confirm the specificity of NF-κB binding, some reactions included an antibody targeting the p65 subunit, which causes a characteristic shift ("supershift") in mobility. The mixtures were run on a non-denaturing polyacrylamide gel to separate free DNA from DNA-protein complexes. After electrophoresis, gels were dried and exposed to film at low temperature to visualize the DNA-protein bands, reflecting NF-κB binding activity. This assay allowed us to quantify the degree of NF-κB transcription factor activation in tumors from women exposed to psychosocial stressors such as war and displacement in Central America, linking stress signaling to cancer progression.

Human Studies

To explore how psychological stress influences immune signaling in humans, methodologies from prior studies were referenced and adapted (Barnard et al. 2024; Annam et al. 2024; Boyle et al. 2023). Blood samples were collected before and after stress induction to assess circulating cytokine levels using multiplex bead-based assays. These clinical protocols illustrate how gene expression and immune signaling pathways such as those involving CXCL1, CXCL2, and CXCL5 can be modulated by real-world psychosocial stressors. In this study, published Trier Social Stress Test (TSST) and survey frameworks were referenced to inform translational parallels between murine restraint stress and human stress-induced chemokine dysregulation.

Following procedures previously outlined (Barnard et al. 2024; Nsonwu-Anyanwu et al. 2021), we investigated the influence of psychosocial stress on gene expression in female reproductive cancers using data from the Black Women's Health Study (BWHS), a large, ongoing prospective cohort study of African American women in the United States. Participants included adult women (ages 18–65) with confirmed diagnoses of cervical, ovarian, uterine, or other gynecologic cancers. Tumor subtype classifications were confirmed through pathology reports where available. Psychosocial stress was evaluated through structured survey instruments administered at multiple time points as part of the BWHS protocol, capturing experiences of early life adversity, neighborhood disadvantage, and chronic life stressors. All participants gave informed consent, and study procedures were approved by relevant institutional review boards.

To be included in the present analysis, participants were required to have available data on at least one of two psychosocial stress exposure domains: (i) early life adversity and/or (ii)

chronic neighborhood-level hardship. Early life adversity was defined as self-reported experiences of maternal loss before age 18, forced family separation, or exposure to domestic violence. Responses were coded dichotomously (”yes” or ”no”). Neighborhood hardship was assessed using a composite index of participant-reported access to essential resources (e.g., healthcare, nutritious food, and clean water), perceived neighborhood safety, and local economic opportunity. This data was superimposed with geocoded census tract information to classify participants' residential environments as ”high” or ”low” disadvantage. For the primary analyses, neighborhood status was based on residence within two years prior to cancer diagnosis. Sensitivity analyses were also conducted to consider residential history four years prior in order to account for cumulative exposure.

2.10. Tissue Collection

For participants diagnosed with reproductive cancers, including ovarian and cervical cancer, we requested formalin-fixed, paraffin-embedded (FFPE) tumor samples from their treating clinics across the United States following previous procedures (Barnard et al. 2024). About half of the individuals contacted gave informed consent to use their tumor tissue for research purposes. Most regional pathology laboratories cooperated with tissue release. In total, archived tumor tissue was obtained from 552 consenting participants. RNA was extracted using the Qiagen AllPrep DNA/RNA FFPE kit at a centralized molecular research facility. Seventeen samples were excluded due to insufficient RNA yield, and another 96 were removed because the RNA quality (as measured by DV200) did not meet sequencing standards. Ultimately, 439 tumor samples were viable for transcriptomic analysis. Of those, 22 were excluded from final analyses due to ambiguous estrogen receptor (ER) status.

2.11. Trier Social Stress Test

In addition to tissue collection, participants underwent the Trier Social Stress Test (TSST), a well-established method for experimentally inducing acute psychosocial stress (Kirschbaum et al. 1993). Following a 30-minute rest in a quiet room, participants were asked to give a five-minute speech after four minutes of preparation, followed by a mental arithmetic task involving serial subtraction out loud. These tasks were conducted in front of two evaluators wearing white lab coats who remained neutral and expressionless throughout the procedure to heighten the stress response. All TSST sessions were conducted at approximately 2 p.m. to control for diurnal variation in cortisol levels. Participants were instructed to abstain from food and drink for at least two hours prior to testing. Blood samples were collected before and after the stress procedure for cytokine and immune profiling.

2.12. Blood Sample Collection

Before the TSST began, a saline-locked venous cannula was inserted into the participant's arm, and a baseline blood sample was taken. To minimize stress from the insertion itself, participants then rested for 20 minutes in a quiet room. Following the TSST, additional blood samples were collected at 60 and 90 minutes from the start of the procedure as previously described (Annam et al. 2024). Each sample was drawn into a 4 mL serum-separating tube and left at room temperature for at least 30 minutes to allow clotting. Samples were then centrifuged at 1300 RCF for 15 minutes at 4 °C. The serum was carefully transferred into cryovials and immediately frozen at -80 °C. Each sample was only thawed once prior to cytokine and immune assays to preserve integrity.

2.13. Chemokine/Cytokine Assays

Cytokine and chemokine assays were conducted at the designated immunology core laboratory following the manufacturer's protocol as previously described (Yang et al. 2010; Annam et al. 2024). Serum samples were analyzed in duplicate for key inflammatory markers relevant to female reproductive cancers and stress, including CXCL1, CXCL2, CXCL3, CXCL5, CXCL8, IL-6, TNF-α, and NF-κB. These assays utilized Luminex fluorescent bead-based multiplex technology on a 96-well plate format with the Milliplex MAP panel (MilliporeSigma).

2.14. Immunohistochemistry

Immunohistochemistry was used to detect CXCR2 expression in tumor tissue samples as described previously (Yang et al. 2010). After preparing and rehydrating the tissue sections, antigen retrieval was performed by heating. Sections were then treated to block nonspecific binding and incubated overnight with a CXCR2 antibody. Detection was done using a biotin-streptavidin system, with negative controls replacing the primary antibody. CXCR2 levels were quantified using digital imaging, and expression was analyzed against clinical factors such as stage, age, family history, relapse, and response to chemotherapy. Patients were classified as chemo-sensitive or chemo-resistant based on their response to platinum-based treatment, using relapse timing as the criterion.

2.15. Human Tumor Analysis

For each tumor sample, we calculated conserved transcriptional responses to adversity (CTRA) contrast scores using normalized and log-transformed gene expression data for 52 selected CTRA genes (one gene, IGLL3, was excluded due to missing data) as previously described (Barnard et al. 2024). These included 19 pro-inflammatory genes weighted positively ($^{+}1$), 31 genes involved in antiviral Type I interferon responses weighted negatively (-1), and two antibody-related genes also weighted negatively (-1). Summing these weighted values provided a CTRA contrast score that represented the overall stress-related gene expression profile for each tumor.

2.16. Real-time reverse transcriptase–PCR

In order to assess the correlation between CXCR2 expression and clinical variables, we isolated tumor RNA using TRIzol reagent following standard protocols as previously described

(Hsu et al. 2013). Complementary DNA (cDNA) was synthesized using oligo(dT) primers and reverse transcription reagents according to manufacturer instructions. Quantitative PCR amplification was performed using SYBR Green chemistry on a real-time PCR system, with each sample run in triplicate to ensure reproducibility. Expression levels of target genes were normalized to the housekeeping gene GAPDH, and relative expression was calculated using the $2^{-\Delta\Delta CT}$ method.

3. Results

3.1. Chronic Stress Enhances Tumor Growth

In order to determine whether chronic psychosocial stress influences tumor progression, we subjected mice inoculated with H22 hepatocellular carcinoma cells to a 35-day chronic restraint stress protocol, following previously described methods (Cao et al. 2021). Tumor burden was monitored weekly using caliper measurements.

We found that mice in the stress group exhibited significantly greater tumor growth over time compared to non-stressed controls ($p = 0.01$; Figure 1). This increase in tumor burden over time supports the hypothesis that chronic stress accelerates tumor progression, underscoring the role of stress-related biological pathways in shaping tumor dynamics in vivo.

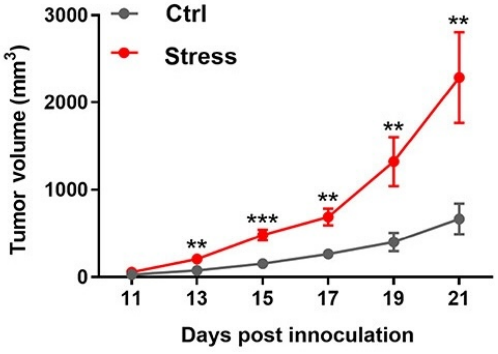

Figure 1

Figure 1. Chronic Restraint Stress Increases Tumor Growth in Mice ($n = 10$ for control group and 11 for stress group, two replicates, one mouse in stress group died on Day 20 due to tumor malignancy). Tumor growth over time was significantly higher in the stress group compared to controls following H22 hepatocellular carcinoma cell inoculation ($p = 0.01$), as measured weekly by caliper-based volume estimation (Cao et al. 2021).

3.2. Stress Activates CXCR2 Signaling in the Tumor Microenvironment

In order to determine whether chronic restraint stress activates pro-inflammatory signaling within the tumor microenvironment, and to further elucidate the connection of stress and increased tumor burden, we performed Western blot analysis on tumor tissues collected from stressed and control mice. Protein bands were detected using enhanced chemiluminescence (ECL) and imaged using a digital gel documentation system. The relative protein expression levels were quantified by densitometric analysis of band intensities using ImageJ software (NIH). For each blot, regions of interest were drawn around protein bands of the expected molecular weight, and the integrated density (area × mean gray value) was measured. Background subtraction was performed using a region of equivalent size in a blank area of the membrane. To control for sample loading variability, target protein expression levels were normalized against internal housekeeping proteins—either β-actin or GAPDH—run on the same membrane as previously described (Cao et al. 2021). Normalized values were then used for statistical comparison between experimental groups. We found that stress exposure significantly elevated CXCL5 protein levels in tumor tissues compared to controls ($p < 0.01$; Figures 2a and 2b), with quantification normalized to β-actin. We also observed a corresponding upregulation of CXCR2 and phosphorylated Erk1/2 (Thr202/Tyr204), while total Erk1/2 and CXCR4 levels remained unchanged (Figures 3a and 3b). This upregulation of CXCR2 and CXCL5 in stressed tissues suggests that chronic stress selectively activates CXCR2-mediated MAPK signaling pathways. To further investigate the functional relevance of CXCR2 signaling, we silenced CXCR2 in ovarian cancer cells using CXCR2-targeted shRNA (CXCR2i) and compared protein expression to cells transduced with GFP control shRNA (GFPi). Western blotting revealed that CXCR2 knockdown reduced levels of downstream chemokines CXCL1 (Gro-1) and CXCL8 (IL-8), confirming CXCR2's role in regulating pro-inflammatory signaling (Figures 4a and 4b), when normalized to β-actin. In parallel, to examine regulation of epithelial–mesenchymal transition (EMT)-associated transcription factors, we treated ovarian cancer cells with increasing concentrations of ampelopsin. We found that nuclear Snail protein expression decreased in a dose-dependent manner, with a marked reduction observed at concentrations exceeding 10 μM (Figures 5a and 5d). The reduction of nuclear Snail protein indicates that Snail, a downstream effector of ERK signaling, is modulated by both CXCR2 activity and ampelopsin exposure.

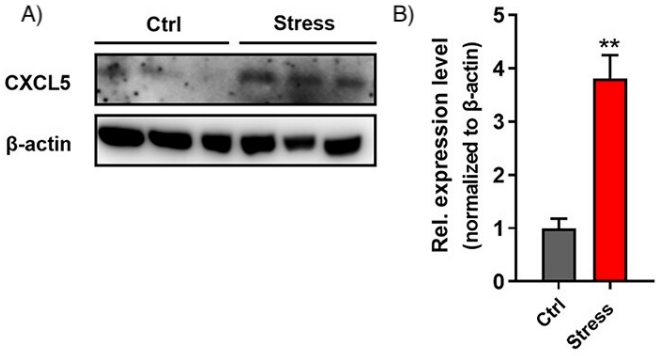

Figure 2

Figure 2: Protein expression of CXCL5 in tumor tissues of control and stressed mice ($n = 3$). (a) Western blotting reveals increased CXCL5 protein levels in tumor tissues from stressed mice when compared to controls. **(b)** Quantification of Western blot band intensity normalized to β-actin confirms a significant increase in CXCL5 expression in the stress group ($p < 0.01$), as described previously (Cao et al. 2021).

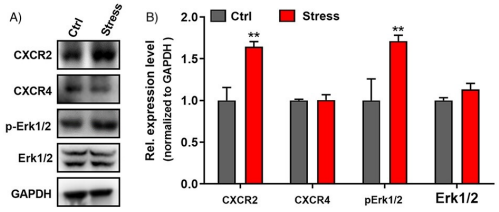

Figure 3

Figure 3: Protein expression of CXCR2, CXCR4, Erk1/2, and phosphorylated Erk1/2 in BM-MDSCs of control and stressed mice ($n = 3$)**. (a)** Western blot analysis showed higher expression of CXCR2 and phosphorylated Erk1/2 in tumors from the stress group compared to controls, while CXCR4 and total Erk1/2 levels remained unchanged; all protein levels were normalized to GAPDH. **(b)** Quantification of band intensities confirmed the statistically significant increase in CXCR2 and phosphorylated Erk1/2 expression in stressed mice ($p < 0.01$), as described previously (Cao et al. 2021).

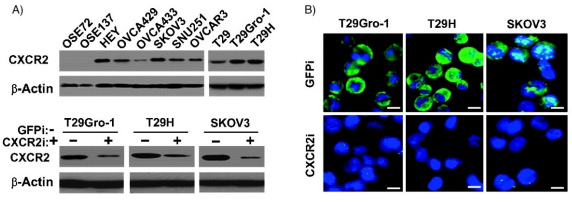

Figure 4

Figure 4: Western blot of multiple ovarian cancer cell lines (HEY, OVCA429, SKOV3, SNU251, OVCAR3, OVCA433) compared to normal ovarian surface epithelial cells (OSE72, OSE137), normalized to β-actin, assessing CXCR2 expression (Yang et al. 2010). (a) CXCR2 was found to be overexpressed in ovarian cancer cell lines relative to normal ovarian surface epithelial cells. Silencing CXCR2 led to decreased secretion of pro-inflammatory chemokines Gro-1 (CXCL1) and IL-8 (CXCL8), indicating its role in promoting inflammatory signaling. **(b)** Immunofluorescence staining demonstrated effective knockdown of CXCR2 in ovarian cancer cells treated with CXCR2-specific shRNA (CXCR2i), evidenced by diminished green fluorescence compared to GFP shRNA control (GFPi) cells.

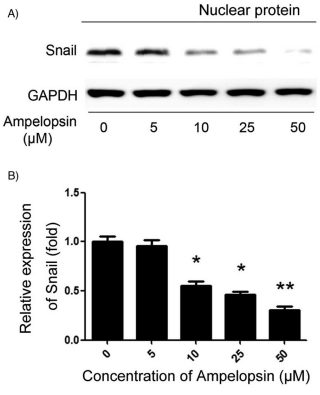

Figure 5

Figure 5: NF-κB Mediates the Regulation of Snail. (a) Treatment with ampelopsin for 12 hours caused a dose-dependent decrease in nuclear Snail protein levels, with a pronounced reduction observed at concentrations above 10 μM. **(b)** Quantification using an electrochemiluminescence (ECL) detection system (Millipore), as described previously (Liu et al. 2015), confirmed significant downregulation of Snail protein between 10 and 25 μg of ampelopsin treatment (Liu et al. 2015).

3.3. CXCR2 Is Overexpressed in Ovarian Cancer and Effectively Silenced by shRNA

In order to determine the relative expression of CXCR2 in ovarian cancer cells versus normal ovarian surface epithelial cells, we performed Western blotting and immunofluorescence staining across a panel of established cell lines. Specifically, CXCR2 protein levels were assessed in six ovarian cancer cell lines (HEY, OVCA429, SKOV3, SNU251, OVCAR3, OVCA433) and compared to two non-malignant ovarian surface epithelial lines (OSE72, OSE137). We found that CXCR2 was consistently overexpressed in all cancer cell lines relative to normal controls (Figure 4a). Protein quantification was conducted via densitometric analysis using ImageJ software, with CXCR2 band intensities normalized to β-actin. These findings confirm that CXCR2 is aberrantly upregulated in malignant ovarian epithelial cells. In order to test whether CXCR2 expression could be silenced effectively, we transduced cancer cell lines with CXCR2-targeted short hairpin RNA (CXCR2i) or a control GFP shRNA (GFPi). Then, we used immunofluorescence to visualize the results. Immunofluorescence revealed a marked reduction in CXCR2 signal intensity in CXCR2i-transduced cells compared to controls, confirming efficient knockdown of CXCR2 at the protein level (Figure 4b). The combined western blotting data and visual immunofluorescence confirmation highlights CXCR2's role as a potential molecular target in ovarian cancer.

3.4. Stress Enhances MDSC Accumulation and Migration in Tumors

In order to investigate whether chronic restraint stress affects the accumulation and migration of myeloid-derived suppressor cells (MDSCs) in tumor-bearing mice, we performed flow cytometry analysis on CFSE-labeled MDSCs isolated from spleen and tumor tissues. MDSCs were harvested from bone marrow, labeled with the fluorescent dye CFSE, and adoptively transferred into mice subjected to chronic stress or control conditions. We found that stressed mice exhibited a significant increase in the number of CFSE-positive MDSCs in both the spleen and tumor tissues compared to controls (Figure 6). Additionally, the decreased fluorescence intensity of CFSE in these cells suggested enhanced infiltration and potential proliferation within these tissues, which likely contributes to the establishment of an immunosuppressive tumor microenvironment. These findings indicate that psychosocial stress promotes the recruitment and expansion of MDSCs, thereby potentially facilitating tumor immune evasion.

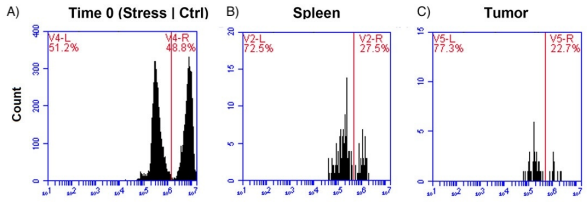

Figure 6

Figure 6: Chronic Restraint Stress Increases MDSC Accumulation and Infiltrative Behavior, as Visualized by Flow Cytometry. (a) At the initial time point, both stressed and control groups display two distinct CFSE fluorescence peaks of similar size and intensity, indicating that MDSCs were equally labeled and have not undergone proliferation or dye dilution prior to transfer. This confirms comparable baseline CFSE staining in both groups before in vivo migration. **(b)** In the spleens of stressed mice, the CFSE peak is noticeably smaller and shifted toward lower fluorescence intensity compared to controls. This reduction in CFSE intensity reflects increased proliferation or dye dilution of MDSCs, suggesting that chronic restraint stress enhances both the accumulation and expansion of these cells within the spleen microenvironment. **(c)** Similarly, tumor tissues from stressed mice show decreased CFSE fluorescence intensity and increased $CFSE^{+}$ cell counts relative to controls. This indicates that stress promotes greater infiltration and possibly local proliferation of bone marrow–derived MDSCs within tumors, contributing to an immunosuppressive microenvironment that may facilitate tumor progression (Cao et al. 2021).

3.5. Snail Modulates CXCL1 and CXCL2, but Not CXCL5

To determine the regulatory role of the transcription factor Snail on chemokine secretion, we conducted ELISA assays measuring CXCL1, CXCL2, and CXCL5 levels following Snail overexpression and knockdown in ovarian cancer cells. We found that Snail overexpression significantly increased the secretion of CXCL1 and CXCL2 at higher concentration ranges (0–2 ng/mL). Conversely, knockdown of Snail resulted in a marked decrease in CXCL1 and CXCL2 levels, particularly at lower concentration ranges (0–200 pg/mL). In contrast, CXCL5 secretion remained unaffected under both Snail overexpression and knockdown conditions (Figure 7). These results suggest that Snail selectively modulates the expression of CXCL1 and CXCL2 in a concentration-dependent manner, while having minimal influence on CXCL5 production.

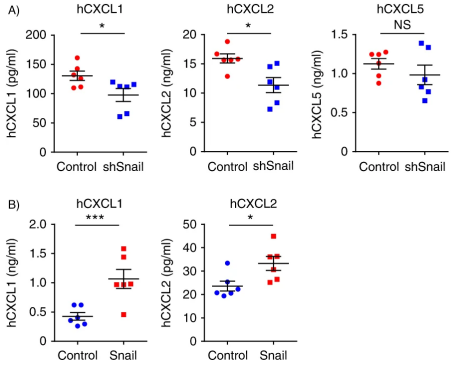

Figure 7

Figure 7: ELISA of cell supernatants of human ovarian cancer cell lines, *a: OVCAR8 and OVCAR8-shSnail, and b: OVCA433 and OVCA433-Snail;* $n = 6$. **(a)** The ELISA analysis demonstrated that in the lower concentration range (0–200 pg/mL), shSnail expression significantly downregulated human CXCL1 and CXCL2 levels compared to control ($p < 0.05$), while CXCL5 levels remained unchanged. **(b)** Conversely, at higher concentrations (0–2 ng/mL), Snail overexpression markedly upregulated CXCL1 ($p < 0.001$) and significantly increased CXCL2 levels relative to control ($p < 0.05$). These findings suggest that Snail differentially regulates CXCL chemokine expression depending on expression level and concentration range, strongly promoting CXCL1 and CXCL2 production while having minimal effect on CXCL5 (Taki et al. 2018).

3.6. CXCR2 Knockdown Reduces NF-κB Binding Activity

In order to assess whether CXCR2 influences NF-κB transcriptional activity in ovarian cancer cells, we performed electrophoretic mobility shift assays (EMSA) using nuclear extracts from CXCR2-silenced (CXCR2i) and control (GFPi) cells. Synthetic oligonucleotide probes containing the NF-κB consensus binding sequence were labeled and incubated with nuclear extracts to detect DNA-protein complex formation. We found that nuclear extracts from GFPi cells exhibited robust NF-κB DNA binding activity, which was further confirmed by a supershift upon addition of anti-p65 antibody. In contrast, CXCR2i nuclear extracts displayed markedly reduced or absent NF-κB binding, suggesting that CXCR2 positively regulates NF-κB signaling (Figure 8). These findings indicate that CXCR2 is necessary for maintaining NF-κB DNA-binding capacity.

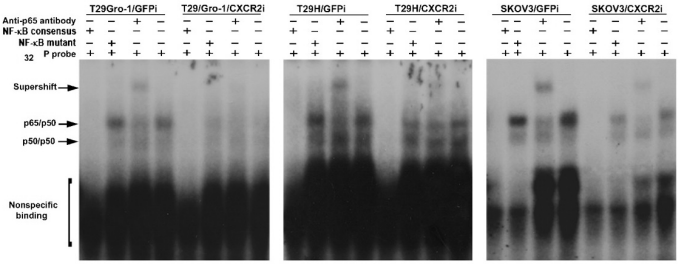

Figure 8

Figure 8: CXCR2 Knockdown Reduces NF-κB DNA Binding Activity in Ovarian Cancer Cells Electrophoretic mobility shift assay (EMSA) shows reduced NF-κB DNA binding activity following stable CXCR2 knockdown (CXCR2i) in ovarian cancer cells. Control cells (GFPi) displayed strong NF-κB binding, confirmed by a supershift with an anti-p65 antibody. In contrast, CXCR2i samples showed weaker or absent bands, indicating diminished p65 interaction with the NF-κB consensus sequence. These findings suggest that CXCR2 plays a key regulatory role in maintaining NF-κB transcriptional activity in ovarian cancer (Yang et al. 2010).

3.7. Psychosocial Stress Elevates IL-5 and IL-27 Levels in Human Serum

In order to examine the inflammatory response to acute psychosocial stress in humans, we measured circulating cytokine levels before and after participants underwent the Trier Social

Stress Test (TSST). First, blood samples were collected at baseline and at defined post-stress intervals, and serum was isolated for cytokine profiling. We found that participants exposed to the TSST exhibited significantly elevated concentrations of IL-5 and IL-27 compared to non-stressed controls ($p < 0.05$; Figure 9). These cytokines were quantified using Luminex-based multiplex bead assays, with each sample analyzed in duplicate to ensure accuracy.

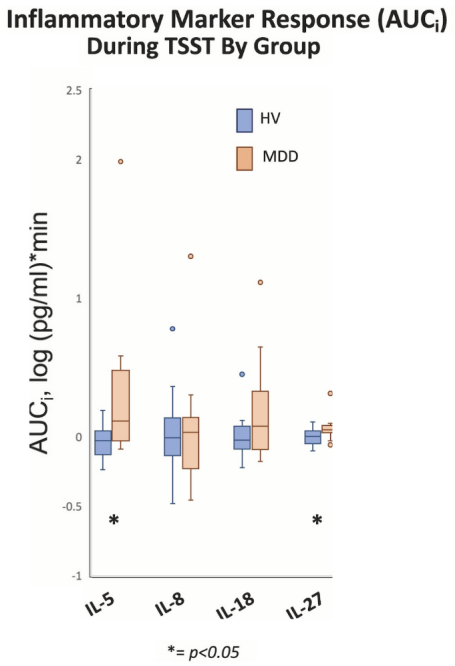

Figure 9

Figure 9. Measures of serum inflammatory markers in response to the Trier Social Stress Test in participants with major depressive disorder (MDD) and healthy volunteers (HV). Box-and-whisker plots showing the concentrations of IL-5 and IL-27 (log pg/mL) in serum samples collected during the Trier Social Stress Test (TSST), comparing stressed and control groups. Both IL-5 and IL-27 were significantly upregulated in the stress group ($p < 0.05$), indicating an enhanced inflammatory response triggered by acute psychosocial stress (Annam et al. 2024).

3.8. Transcriptional Responses to Adversity in Tumors

In order to assess how psychosocial adversity shapes gene expression in female reproductive tumors, we analyzed transcriptomic profiles using the Conserved Transcriptional Response to Adversity (CTRA) framework. Tumor RNA was extracted from formalin-fixed, paraffin-embedded (FFPE) samples, and CTRA scores were computed based on the expression of 53 genes involved in inflammation, antiviral response, and antibody production. Participants were stratified by early life trauma exposure and by residence in high- or low-disadvantage neighborhoods, as previously defined. We found no significant differences in overall CTRA expression between early trauma-exposed and non-exposed patients, irrespective of estrogen receptor (ER) status. However, when stratified by neighborhood disadvantage, distinct transcriptional adaptations emerged (Figure 10). ER$^+$ tumors from high-disadvantage areas demonstrated marked downregulation of pro-inflammatory and Type I interferon-related genes. In contrast, ER$^-$ tumors exhibited significant upregulation of both pro-inflammatory genes and those associated with humoral immune responses. These findings suggest subtype-specific transcriptional adaptations to chronic environmental adversity.

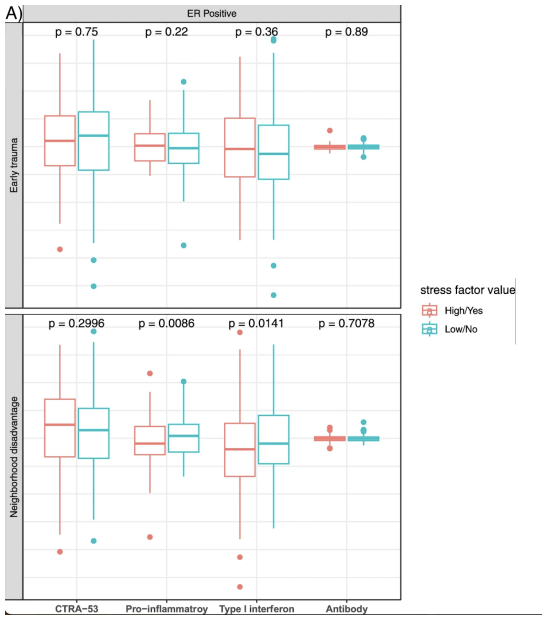

Figure 10

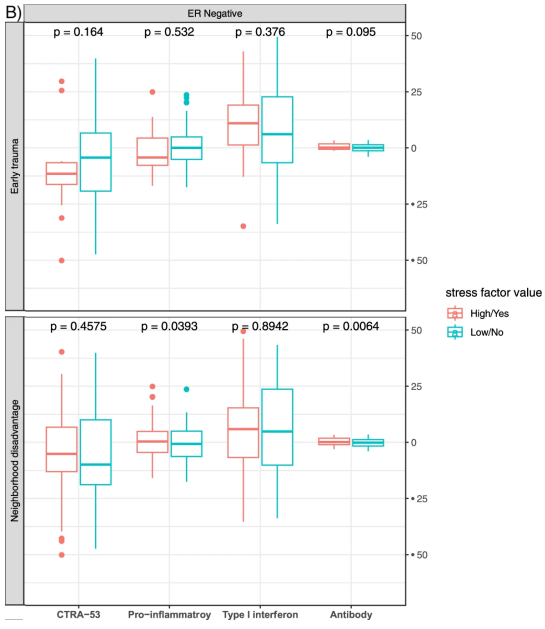

Figure 10

Figure 10. Box-and-whisker plots depicting Conserved Transcriptional Response to Adversity (CTRA) gene expression in ER$^+$ and ER$^-$ tumor samples, stratified by early trauma exposure and neighborhood disadvantage. (a) ER$^+$ tumors showed significant downregulation of pro-inflammatory

($p = 0.0086$) and Type I interferon genes ($p = 0.0141$) in patients from high-disadvantage neighborhoods, with no significant differences ($p < 0.05$) observed in CTRA expression or antibody-related genes from high neighborhood disadvantage groups. Additionally, no ER^+ tumors showed significant downregulation ($p < 0.05$) from early trauma groups, regardless of stress factor value. **(b)** ER^- tumors exhibited significant upregulation of pro-inflammatory ($p = 0.0393$) and antibody-related genes ($p = 0.0064$) from high neighborhood disadvantaged groups, with no significant change in CTRA or Type I interferon gene expression. Additionally, no ER^- tumors showed significant ($p < 0.05$) downregulation from early trauma groups, regardless of stress factor value (Barnard et al. 2024).

4. Discussion

This study investigates the impact of psychosocial stress on female reproductive cancers through a multifaceted approach. We first used a murine model of hepatocellular carcinoma to examine how chronic stress influences tumor growth and immune signaling in vivo. Building on these findings, studies in ovarian cancer cell lines explored the molecular pathways mediating stress-related tumor progression, focusing on CXCR2 signaling and its downstream effects. Finally, we extended our investigation to a clinical group of women with breast cancer in the Black Women's Health Study, assessing how real-world psychosocial stressors relate to immune profiles and tumor gene expression. This multifaceted approach bridged experimental models with human disease. Our study found that chronic psychosocial stress promotes tumor progression in a murine model of hepatocellular carcinoma, shown by significantly increased tumor volume in stressed mice when we compared them to control mice. By quantifying molecular analyses through techniques such as western blots, we revealed that stress enhances CXCR2 signaling in the tumor microenvironment, with elevated CXCL5, CXCR2, and phosphorylated Erk1/2 levels observed in tumors from stressed animals. Silencing CXCR2 in ovarian cancer cells reduced the expression of downstream pro-inflammatory chemokines CXCL1 and CXCL8, highlighting CXCR2's role in regulating tumor-promoting inflammation. Furthermore, CXCR2 was found to be upregulated in multiple ovarian cancer cell lines relative to normal ovarian epithelial cells, with shRNA-mediated knockdown decreasing CXCR2 protein expression. Stress also increased the accumulation and migration of myeloid-derived suppressor cells in tumor and spleen tissues, suggesting enhanced immunosuppression within the tumor microenvironment. At the transcriptional level, the Snail transcription factor selectively modulated chemokine secretion, significantly upregulating CXCL1 and CXCL2 levels while not affecting CXCL5. CXCR2 knockdown impaired NF-κB DNA-binding activity in ovarian cancer cells. Treatment with the polyphenol ampelopsin reduced nuclear NF-κB subunit p65 and Snail levels in a dose-dependent manner, showing potential pathways for modulating stress-associated signaling. Moving these findings to human subjects, serum cytokine analysis during an acute psychosocial stress test demonstrated that stressed participants presented significantly elevated levels of IL-5 and IL-27. This directly correlates a strong systemic inflammatory response to psychosocial stress. Studies reporting on postmenopausal women in Nigeria showed that transcriptomic profiling of tumors from women with female reproductive cancers revealed no difference in overall stress-related gene expression (CTRA) by early trauma exposure. However, neighborhood disadvantage was associated with subtype-specific transcriptional changes: ER-positive tumors from high-disadvantage areas exhibited downregulation of pro-inflammatory and interferon response genes, whereas ER-negative tumors showed upregulation of pro-inflammatory and antibody-related gene expression.

Molecular and immune changes observed in stressed mouse models, ovarian cancer cell lines, Nigerian breast tumor analyses, and studies of Black women's health consistently demonstrated that chronic psychosocial stress, including displacement, economic hardship, systemic inequity, and community adversity, can drive inflammatory signaling and shape tumor behavior in female reproductive cancers, particularly among women in Central America (Fontvieille et al. 2022). These stressors activate systemic inflammatory pathways and dysregulate immune and hormonal balance, as evidenced by elevated pro-inflammatory cytokines and altered chemokine signaling such as increased CXCR2 activity within the tumor microenvironment (Kavandi et al. 2012; Boyle et al. 2023; Subat et al. 2019). Chronic stress was shown to promote tumor progression by enhancing the recruitment and expansion of immunosuppressive cells like myeloid-derived suppressor cells, which has been shown to impair anti-tumor immunity (Hsu et al. 2013). Moreover, prolonged stress-related signaling activated transcriptional factors such as NF-κB and Snail, which both drove the epithelial-to-mesenchymal transition (EMT), and were shown to facilitate cancer cell invasion and metastasis (as visualized in Figure 11) (Moody et al. 2005; Taki et al. 2018; Hsu et al. 2013). Prolonged stress has been directly linked to economic hardship and community-level adversity, which further compound its effects by limiting access to healthcare and increasing psychosocial burdens, thereby perpetuating a cycle of chronic stress and tumor-promoting inflammation (Shen et al. 2025; Nsonwu-Anyanwu et al. 2021). Together, these factors biologically contributed to more aggressive cancer phenotypes and poorer outcomes in this vulnerable population (DeGuzman et al. 2017). It is necessary to examine the broader implications of psychosocial stress on female reproductive cancer outcomes in Central America under a lens of cultural sensitivity, social determinants, and systemic healthcare disparities. Women throughout the region face stressors such as poverty, food insecurity, displacement due to violence, conflict, community-level adversity, and immigration related stress, all of which can activate biological stress pathways that accelerate tumor progression, immune suppression, and treatment resistance (Crosswell et al. 2014). CXCR2, as identified in this study, is one such pathway. Its activation by stress-related chemokines like CXCL5 and its role in promoting inflammation, epithelial-to-mesenchymal transition, and myeloid-derived suppressor cell recruitment directly links chronic adversity to more aggressive tumors and poorer therapeutic outcomes (Cao et al. 2021; Nsonwu-Anyanwu et al. 2021; Taki et al. 2018; Elloul et al. 2010).

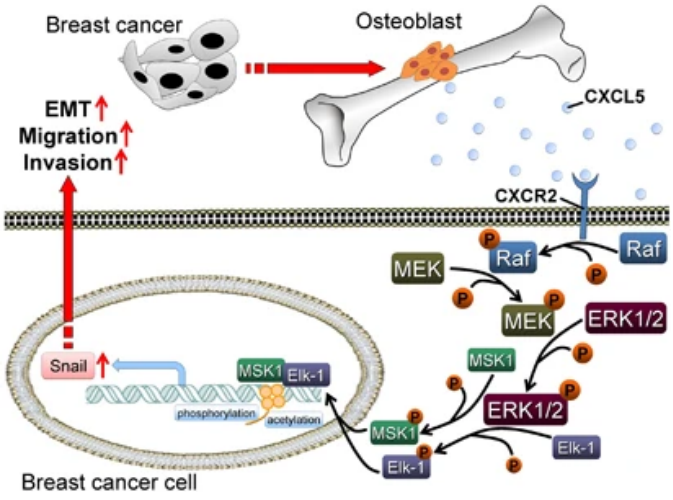

Figure 11: Osteoblast-derived CXCL5 promotes breast cancer cell migration, invasion, and epithelial-mesenchymal transition (EMT) through activation of the CXCR2 signaling pathway. CXCL5, secreted by osteoblasts in the bone microenvironment, binds to CXCR2 receptors on the surface of breast cancer cells, initiating a downstream signaling cascade. This cascade involves phosphorylation of Raf, MEK, and ERK1/2, which subsequently activates MSK1 and transcription factor Elk-1. The activated Elk-1 promotes upregulation of Snail, a key EMT regulator. Elevated Snail expression leads to increased EMT, cell migration, and invasion, contributing to breast cancer metastasis, particularly toward bone tissue (Hsu et al. 2013)

.

Several limitations should be considered when interpreting the findings of this study in relation to their connection to the broader Central American population. First, although the combined sample size across murine and human models was substantial, stratification by state, cancer subtype and trauma exposure resulted in smaller subgroups, limiting statistical power in some analyses. This may constrain the robustness of transcriptomic comparisons, particularly in ER- tumors from high-hardship regions. Second, the Central American region is socioeconomically and culturally unique, with each country possessing distinct health systems, cancer surveillance infrastructure, and histories of political instability (Di Sibio et al. 2016). By aggregating data across seven countries, the study may mask country-specific stressors or systemic barriers, potentially limiting the precision of regional inferences. We propose a model that can be adapted to any country. Third, reliance on self-reported psychosocial exposures, including early life adversity and community-level hardship, introduces possible recall bias in how participants interpret and report stress. Therefore, these data remain inherently limited in establishing direct causality. While the Balb/c mouse model burdened with H22 hepatocellular carcinoma cells provided valuable mechanistic insights into CXCR2 signaling in response to stress, it represented a distinct cancer type from the ovarian or breast cancers studied in humans. Consequently, findings from such controlled animal experiments may not fully reflect the complex tumor biology and multifaceted effects of chronic socially mediated stressors such as displacement, poverty, and gender-based violence that uniquely shape human female reproductive cancers. Likewise, tumor tissue samples used for transcriptomic analysis were collected from public hospitals and universities, which may disproportionately represent women with access to formal healthcare systems, potentially under representing rural or marginalized populations without access to formal healthcare systems. Fourth, while the Trier Social Stress Test (TSST) allowed controlled assessment of cytokine responses to acute stress, it may not fully simulate the long-term or cumulative nature of adversity experienced by many participants. Moreover, the study design was cross-sectional and did not include longitudinal tracking of survival or treatment responses, limiting the ability to link observed immune and transcriptional changes to clinical outcomes. Finally, while this study did not include direct experimental data gathered from Central American populations, it relied on prior studies that either modeled conditions similar to those experienced in Central America or were conducted within the region to help bridge this research gap. The current work builds upon and extends a growing body of literature demonstrating that chronic psychosocial stress affects specific inflammatory chemokines, which contribute to tumor progression in female reproductive cancers. Together, these factors highlight the need for future studies incorporating larger groups studied over a long period of time, country-specific analyses, and expanded access to tissue samples from underrepresented populations to better understand the relationship between chronic psychosocial stress and cancer outcomes in Central America.

In response to this need, the present study investigates the molecular mechanisms by which stress can biologically embed within the tumor microenvironment. This study builds upon and expands existing evidence showing that chronic psychosocial stress influences specific inflammatory chemokines involved in tumor progression in female reproductive cancers. Research from around the world has shown that stress-activated pathways, including NF-κB, CXCR2, and downstream chemokines like CXCL1, CXCL2, and CXCL5, play pivotal roles in promoting inflammation, epithelial-to-mesenchymal transition, and immune evasion in breast and ovarian tumors (Kavandi et al. 2012; Yang et al. 2010). While much of the existing research on stress-related molecular changes in female reproductive cancers has been conducted in high-income areas, this study examines tumor samples from Black women alongside parallel university-derived samples, using murine models to mechanistically investigate the influence of psychosocial stress on tumor progression. Although the United States is classified as a high-income country, significant socioeconomic disparities persist, and many communities experience chronic stressors similar to those in lower-income regions. By focusing on CXCR2 signaling pathways, our findings shed light on how these stress-related mechanisms may contribute to tumor biology within these underserved populations. Moreover, the molecular insights gained here have important implications for understanding female reproductive cancers in Central America, a region where comparable psychosocial stressors and health disparities exist but remain largely underrepresented in research. Notably, we observed elevated CXCR2 expression and conserved transcriptional responses to adversity (CTRA) in tumors from patients experiencing community-level hardship or early life trauma, supporting prior evidence that social stress can pro-

mote systemic immune suppression and inflammatory signaling. There were identified associations between psychosocial stress and breast cancer gene expression, including altered inflammatory signaling (Barnard et al. 2024). It was also demonstrated that acute stress modulates immune and reward processing pathways through inflammatory mechanisms (Boyle et al. 2023). Similarly, patients with major depressive disorder were found to exhibit dysregulated cytokine responses to psychosocial stress, highlighting how chronic psychological stress can exacerbate immune dysfunction(Annam et al. 2024). Together, these findings align with CTRA expression patterns observed in this study, indicating how chronic environmental stressors may mechanistically embed in the tumor microenvironment through inflammatory and immunosuppressive signaling pathways. However, emerging studies offer conflicting evidence regarding the regulation of CXCR2 under stress. A recent study showed that stress disrupts the immune response to aggressive tumors by downregulating CXCR2 expression (Cao et al. 2021). In contrast, a study (Nsonwu-Anyanwu et al. 2021) concluded that high levels of oxidative stress upregulate the CXCR2 pathway, correlating with poor prognosis in postmenopausal Nigerian women with breast cancer. These findings raise important questions about how psychosocial and oxidative stress intersect to modulate CXCR2 signaling, particularly across diverse racial, environmental, and hormonal contexts. The current study, which found increased CXCR2 expression in stressed murine and human tumor samples, aligns with the latter model, suggesting that stress-linked inflammatory environments may activate chemokine signaling cascades in ways that promote immune evasion and metastasis. This discrepancy underscores the urgent need for further research into the CXCR2 axis in female reproductive cancers, especially in globally underserved populations.

At a mechanistic level, sustained stress was found to accelerate biological aging and tissue degeneration through oxidative damage, relating to processes that may underlie the upregulation of pro-inflammatory chemokines observed in this study (Epel et al. 2004). These oxidative pathways offer a plausible link between chronic environmental adversity and the aggressive tumor profiles frequently seen in patients from disadvantaged settings. Moreover, this work aligns with studies from Latin America that document how structural violence, poverty, and gender-based discrimination intersect to limit women's access to timely cancer care, compounding biological vulnerabilities with systemic exclusion (Di Sibio et al. 2016). For example, breast and gynecologic cancers are underreported in national registries across Costa Rica and Ecuador, despite modeled projections suggesting nearly triple the number of recorded cases. This points to a substantial gap in surveillance and early diagnosis, particularly in disadvantaged communities. This underdiagnosis likely contributes to advanced-stage discovery and worsened prognosis. At the same time, contrasts emerge in the literature. Some global studies suggest that ER^+ tumors may be less susceptible to stress-related immunosuppression due to hormonal regulation buffering systemic inflammation (Barnard et al. 2024), where stratified analysis revealed unique pro-inflammatory gene activation in ER- tumors from high disadvantage areas (Barnard et al. 2024). This suggests a context-dependent interaction between social adversity, tumor subtype, and immune signaling, underscoring the need for more geographically diverse, subtype-specific cancer research. By integrating molecular data from murine models with transcriptomic and cytokine profiles from tumor samples collected across diverse U.S. populations, including individuals exposed to chronic psychosocial stress, this study presents a cross-species framework for understanding how adversity shapes female cancer biology. While grounded in U.S.-based cohorts, these findings have broader relevance for under-resourced regions such as Central America, where structural inequities and chronic stress may similarly contribute to tumor progression through conserved inflammatory and oxidative mechanisms. It furthers current understanding by linking immune changes to both environmental adversity and chemokine-driven signaling mechanisms, findings that may inform more equitable, stress-sensitive cancer diagnostics and interventions in the region.

5. Future Directions: A Targeted Murine Study of CXCR2 in Ovarian Cancer under Psychosocial Stress

Building on existing evidence linking chronic psychosocial stress to tumor progression through CXCR2 signaling and immune modulation, there remains a critical gap in understanding these mechanisms specifically in female reproductive cancers, particularly within underserved populations such as Central American women exposed to social adversity. To address this, we propose a targeted murine study designed to mechanistically investigate how chronic restraint stress influences CXCR2-driven tumor growth, immune suppression, and metastatic potential in female reproductive cancers.

In this proposed study, female C57BL/6 mice aged 6 to 8 weeks will be subjected to established chronic restraint stress protocols (Cao et al. 2021), with daily 2-hour restraint periods timed to minimize circadian confounds. Mice will receive intraperitoneal injections of ID8 cells engineered to express the mCherry fluorescent reporter, enabling longitudinal non-invasive fluorescence imaging to track tumor progression over time (van den Pol et al. 2021).

At predetermined time points, tumor tissues and relevant organs will be harvested for comprehensive molecular and cellular analyses. Western blotting will quantify expression levels of CXCR2 and key downstream effectors including phosphorylated Erk1/2 and Snail, confirming activation of pathways implicated in tumor progression. Immunohistochemistry will allow spatial localization of CXCR2 within tumor tissues and characterization of infiltrating immune cells, particularly myeloid-derived suppressor cells (MDSCs). Flow cytometry will provide quantitative assessment and phenotypic profiling of MDSCs in both tumor and spleen, elucidating the impact of stress on immune cell recruitment and expansion. Concurrently, enzyme-linked immunosorbent assays (ELISAs) will quantify circulating and tumor-associated chemokines such as CXCL1, CXCL2, and CXCL5, providing insight into the chemokine milieu driven by CXCR2 signaling. Electrophoretic mobility shift assays (EMSA)

will evaluate NF-κB DNA-binding activity, serving as a functional readout of stress-induced inflammatory transcriptional programs.

Translationally, these murine studies will be complemented by analyses of tumor samples collected from women diagnosed with female reproductive cancers and exposed to chronic psychosocial stress in Central America. Transcriptomic profiling focusing on CXCR2 and conserved transcriptional response to adversity (CTRA) genes will help establish parallels between murine models and human disease. Additionally, acute psychosocial stress responses will be modeled in human participants using the Trier Social Stress Test (TSST), with serial blood sampling for multiplex cytokine and chemokine assays to characterize inflammatory signaling changes in real time.

Based on current literature, we expect chronic restraint stress to significantly increase the tumor burden in mice, as measured by enhanced mCherry fluorescence and tumor volume. Tumors from stressed animals are anticipated to exhibit upregulated CXCR2 and CXCL5 expression, along with increased phosphorylation of Erk1/2 and elevated Snail protein levels. We also expect increased accumulation and migration of MDSCs to the tumor microenvironment, contributing to immunosuppression and tumor progression. Functional assays such as EMSA are likely to demonstrate enhanced NF-κB activity in stressed tumors, linking CXCR2 signaling to inflammatory transcriptional regulation. Human data are expected to reflect stress-associated increases in pro-inflammatory cytokines such as IL-5 and IL-27, with transcriptomic shifts in CTRA gene expression correlating with psychosocial adversity exposure.

Collectively, this integrative approach will fill a critical gap by elucidating the mechanistic pathways through which psychosocial stress modulates CXCR2 signaling and tumor-immune interactions in female reproductive cancers. This work will provide foundational evidence for developing targeted therapies aimed at mitigating stress-driven tumor progression in vulnerable populations.

6. Conclusion

Female reproductive cancers do not unfold separately from the conditions in which women live. In regions like Central America, tumors are shaped not only by genetic and hormonal factors, but also by stressors rooted in poverty, trauma, systemic inequity, and war. These are too often invisible in cancer research. Consistent with the study's hypothesis, chronic restraint stress significantly increased tumor burden in H22 hepatocellular carcinoma-bearing mice, with stressed mice exhibiting accelerated tumor growth over time compared to controls ($p = 0.01$). At the molecular level, stress exposure induced upregulation of the CXCR2 axis within the tumor microenvironment, including a marked increase in CXCL5 expression ($p < 0.01$), elevated CXCR2 protein levels, and activation of downstream p-Erk1/2 signaling. Functional knockdown of CXCR2 in ovarian cancer cells suppressed the expression of key pro-inflammatory chemokines (CXCL1 and IL-8) and disrupted NF-κB binding activity, suggesting that CXCR2 mediates inflammation-linked tumor progression under stress. Additionally, flow cytometry confirmed enhanced accumulation of myeloid-derived suppressor cells (MDSCs) in tumors from stressed animals, reinforcing the pathway's immunosuppressive impact. Parallel human data further supported this link. Serum analysis during the Trier Social Stress Test revealed significantly elevated IL-5 and IL-27 levels ($p < 0.05$), aligning with the inflammatory profile observed in murine models. Importantly, transcriptional profiling of tumors from patients in high-disadvantage neighborhoods revealed a stress-sensitive divergence: while ER^+ tumors downregulated inflammatory genes, ER- tumors exhibited significant upregulation of pro-inflammatory transcripts, consistent with CTRA activation under social stress conditions. These findings provide both mechanistic and translational evidence that psychosocial stress can biologically embed within the tumor microenvironment, with CXCR2 emerging as a key mediator of this effect. These results demonstrate that chronic psychosocial stress promotes female reproductive cancer progression by activating the CXCR2-driven inflammatory and immunosuppressive pathways within the tumor microenvironment. This mechanistic insight highlights CXCR2 as a potential therapeutic target to mitigate stress-associated cancer progression and underscores the biological impact of social stress on tumor behavior in patients. Although this study is limited by its sample scope and regional focus, it presents a replicable framework for future interdisciplinary inquiry. Moving forward, studies should expand upon this model by incorporating longitudinal tracking, validated stress biomarkers, and culturally responsive cancer screening tools. Understanding how stress interfaces with tumor biology in low-income settings is not only scientifically necessary–it is ethically urgent. Only through such inclusive research can we move toward more equitable cancer outcomes worldwide.

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