Understanding Immune Tolerance and Escape in Gestational Choriocarcinoma Using Single-Cell Transcriptomics and Organoid Co-Cultures

Comprendre la tolérance immunitaire et l'évasion dans le choriocarcinome gestationnel à l'aide de transcriptomique unicellulaire et de co-cultures organoïdes

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Abstract | Résumé

Gestational choriocarcinoma (GChC) is an aggressive trophoblastic malignancy that displays an unusual degree of immune evasion. Emerging data suggest that GChC hijacks these same tolerance pathways to suppress cytotoxic immune responses. These mechanisms include programmed death receptor 1 (PD-1) and its ligand, programmed death ligand 1 (PD-L1) signaling pathway and human leukocyte antigen G (HLA-G)-mediated Natural killer (NK) cell inhibition. However, the precise cellular interactions and checkpoint circuits responsible for tumour persistence remain poorly defined.

This research proposal aims to integrate a single-cell and organoid-based strategy to identify and functionally validate the immune-tolerance mechanisms that GChC appropriates from the healthy placenta. Single-cell RNA sequencing of archived GChC tumours and term placental tissues will resolve tissue subpopulations and quantify the expression of immune evasive genes. Ligand–receptor inference models will map suppressive communication networks between tumour trophoblasts and maternal immune cells. Spatial multiplex immunofluorescence will confirm the anatomical localization of tolerance markers and identify immune-exclusion zones within the tumour microenvironment. Finally, trophoblast organoid–immune co-culture assays will directly test whether blockade of PD-L1, HLA-G, or other identified pathways restores T-cell and NK-cell activation.

By combining single-cell profiling with functional disruption, this framework aims to define the associated immune-tolerance circuits that enable GChC immune escape. These insights could inform biomarker development and guide selective immunotherapeutic strategies that target tumour- specific tolerance without disrupting normal pregnancy physiology.

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Le choriocarcinome gestationnel (GChC) est une malignité trophoblastique agressive qui présente un degré inhabituel d'évasion immunitaire. Les données émergentes suggèrent que le GChC détourne ces mêmes voies de tolérance pour supprimer les réponses immunitaires cytotoxiques. Ces mécanismes incluent le récepteur de mort programmé 1 (-1) et son ligand, la voie de signalisation du ligand de mort programmé 1 (-L1) ainsi que l'inhibition des cellules tueuses naturelles (NK) médiée par l'antigène leucocytaire humain G (HLA-G). Cependant, les interactions cellulaires précises et les circuits de points de contrôle responsables de la persistance des tumeurs restent mal définis.

Cette proposition de recherche vise à intégrer une stratégie basée sur une seule cellule et un organoïde afin d'identifier et de valider fonctionnellement les mécanismes de tolérance immunitaire que le GChC s'approprie du placenta sain. Le séquençage à ARN unicellulaire des tumeurs GChC archivées et des tissus placentaires à terme résoudra les sous-populations tissulaires et quantifiera l'expression des gènes immunoévasifs. Les modèles d'inférence ligand-récepteur cartographient les réseaux de communication suppressifs entre les trophoblastes tumoraux et les cellules immunitaires maternelles. L'immunofluorescence multiplex spatiale confirmera la localisation anatomique des marqueurs de tolérance et identifiera les zones d'exclusion immunitaire dans le microenvironnement tumoral.

Enfin, les tests de co-culture organoïde–immunité du trophoblaste testeront directement si le blocage de-L1, HLA-G ou d'autres voies identifiées restaure l'activation des lymphocytes T et des cellules NK. En combinant le profilage unicellulaire avec la perturbation fonctionnelle, ce cadre vise à définir les circuits de tolérance immunitaire associés qui permettent l'évasion immunitaire du GChC. Ces connaissances pourraient orienter le développement des biomarqueurs et guider des stratégies immunothérapeutiques sélectives ciblant la tolérance spécifique aux tumeurs sans perturber la physiologie normale de la grossesse.

Keywords: Gestational choriocarcinoma, Placental immune tolerance, PD-L1 signaling, HLA-G signaling, Trophoblast organoids, Immune evasion, Single-cell RNA sequencing, Maternal–fetal interface, tumour microenvironment

Background

Gestational choriocarcinoma (GChC) is a rare and highly aggressive trophoblastic cancer that employs many of the same immune-evasion strategies that the healthy placenta uses to protect the fetus from the maternal immune system. This cancer forms because trophoblasts, specialized placental cells that establish the maternal-fetal interface, naturally regulate immune tolerance. This physiological process suppresses or restrains immune response to prevent tissue damage during pregnancy. The formation of the trophoblasts gives the tumour an unusual ability to evade immune detection, which allows for aggressive metastasis. GChC has good prognosis when detected early (5-year survival >80%). However, mortality persists when diagnosed late or in high-risk groups (1). Understanding the immune escape mechanisms exploited by GChC can allow for more targeted therapies to be developed that preserve maternal fertility and reduce chemotherapy exposure. The proposed study aims to map the specific trophoblast–immune interactions that drive immune escape.

The Medawar paradox describes the unique immunological state of pregnancy, since a fetus, which is semi-allogeneic due to paternal genetic contributions, can survive without being rejected by the maternal immune system (2). Reproductive immunology research has highlighted the importance of extravillous trophoblasts in immune tolerance. Extravillous trophoblasts are a subtype of trophoblasts, specialized placental cells that embed the placenta into the uterine wall, which express non-classical MHC molecules such as Human leukocyte antigen G (HLA-G). HLA-G is specialized for Natural killer (NK) cell modulation and interacts with maternal immune receptors which suppresses immune cell proliferation, cytotoxicity, and inflammatory cytokine secretion. HLA-G functions in parallel with immune system pathways such as programmed death receptor 1 (PD-1) and its ligand programmed death ligand 1 (PD-L1), responsible for inhibition of T cell cytotoxic effect, creating a suppressive immune microenvironment (3-4).

Several pathways responsible for placental immune regulation are also associated with GChC progression and tumour immune evasion. In both placental development and cancer progression, PD-L1 and HLA-G are implicated, as they inhibit T cell cytotoxicity and NK activation (5). GChC arises from trophoblasts of a preceding pregnancy, almost always in the uterus. Choriocarcinoma are semi-allogeneic, as they contain paternal DNA and by extension paternal antigens (6). NLRP7, a gene involved in immune regulation, has been identified as a key driver of GChC immune evasion, enhancing PD-L1, HLA-G, and macrophage/T-cell suppression, while silencing it restores immune visibility and reduces tumour burden. This positions NLRP7 as a central tolerance regulator whose downstream circuits warrant targeted mapping (7). Clinically, PD-1 blockades induce durable remissions in drug-resistant gestational trophoblastic neoplasia, emphasizing the centrality of the PD-1/PD-L1 axis in GChC immune escape (8).

Current literature highlights that GChC relies on a coordinated tolerance network rather than any single pathway (9). Previous studies have suggested GChC parallels placental biology. However, these findings derive primarily from bulk or immunohistochemical analyses and do not clarify the cellular circuitry or causal dynamics of immune evasion. These approaches cannot resolve which trophoblastic or immune subpopulations drive tolerance, nor can they reconstruct the ligand–receptor interactions or involved immune-suppressive circuits that sustain tumour persistence. The project addresses the lack of cell-level mechanistic understanding through single-cell resolution mapping and functional co-culture assays. This mechanistic clarity is essential for developing safer fertility-preserving immunotherapies and improving outcomes in advanced or drug-resistant disease.

Research Hypothesis

It is hypothesized that gestational choriocarcinomas take advantage of maternal immune-tolerance pathways, particularly the PD-1/PD-L1 axis and HLA-G-mediated inhibition to suppress cytotoxic and NK cell responses. This creates a microenvironment analogous to the healthy placenta's mechanisms of immune evasion. By mapping immune signaling pathways at a single-cell resolution and functionally testing the blockade of these immune axes in organoid co-cultures, the aim is to identify tolerance circuits that drive choriocarcinoma invasion, proliferation, and persistence.

Rationale

The proposal framework is designed as an ethically robust way to safely study immune evasion in pregnancy without human experimentation. GChC provides a unique model to study the mechanisms of immune tolerance. The approach aims to identify checkpoint pathways that maintain immune tolerance and identify pathological pathways establishing a comparative immune-tolerance profile of the healthy placenta and GChC. This will demonstrate whether PD-1 and HLA-G blockages restore immune activation and inform potential targets for safe immunotherapy in pregnancy-related cancers. The design combines a discovery phase using single-cell RNA sequencing with a validation phase through immunofluorescence and organoid co-culture assays. Based on single-cell RNA sequencing data, pathways showing strong gene expression differences will be identified during the discovery phase and subsequently validated through downstream experiments.

Methods

Tissue and single-cell preparation

Individual cells from archived GChC tumours and healthy term placentas are isolated via enzymatic dissociation to compare their cellular composition and microenvironments at single-cell resolution. These will be obtained through ethically approved

biobank collaborations. Healthy term placenta will be defined as tissue collected from uncomplicated pregnancies delivered at term (≥37 weeks gestation), with no evidence of infection. Inclusion criteria will consist of well-preserved samples with sufficient cellular integrity, while degraded cells will be excluded. For frozen specimens where enzymatic dissociation may not be applicable, single-nucleus RNA sequencing may be used for high-resolution cell-specific transcriptomic analysis. Each cell's transcriptome will be characterized through single cell preparations to allow the tumour microenvironment to be dissected into its individual components.

Single-cell RNA sequencing (scRNA-seq) and data processing
Transcriptomic data will be analyzed to identify distinct cell states, differentially expressed tolerance genes, and immunomodulatory trophoblast subpopulations. Differential gene expression across cell types will be assessed. Quality control filtering will be applied to remove low-quality cells, ensuring reliable downstream analysis. As a result, cells will be clustered based on gene-expression similarity to identify cell types and states. scRNA-seq is a technique that measures gene expression at the individual cell level, enabling identification of cellular subtypes and signaling pathways indicating which trophoblast subpopulations are immunomodulatory. These bioinformatic tools will be used determine whether the tumour differentially expresses immune checkpoint molecules or cytokines. By comparing GChC with healthy placenta tissue, differences in the magnitude and regulation of shared tolerance pathways will be determined.

Ligand–receptor and cell–cell interaction inference
Immune system evasion relies on cell communication which involves the computational prediction of cell–cell communication based on matching ligands in one cell type with receptors in another. To map immunosuppressive signaling, ligand–receptor pair activities between trophoblastic and immune cells will be quantified using CellChat and NicheNet. Communication patterns will be computationally predicted based on the expression of receptor-ligand gene pairs and will be used to provide insight into the active signaling pathways as well as targets for therapeutic intervention.

Validation and imaging
Spatial localization of tolerance markers will be confirmed via immunofluorescence with fluorophore-conjugated antibodies targeting PD-L1, PD-1, and HLA-G in both tumour samples and healthy tissue. This analysis will assess co-localization and confirm whether transcriptome data translates to functional protein expression within the tissue microenvironment.

Organoid–immune co-culture assays
3D trophoblast organoids derived from patient stem or progenitor cells will be developed to model the tumour environment *in vitro* (10). These organoids will be paired with matching immune cells to recreate the tumour–immune environment *in vitro*. By selectively blocking pathways such as PD-L1 or HLA-G, immune cell reactivation will be assessed to determine the functional role of these pathways in tumour immune evasion, complementing the descriptive data from single-cell sequencing. While organoid-to-organoid variability represents a limitation, the use of matched tumour–placental pairs will be put in place to reduce this effect.

Limitations

Archived gestational choriocarcinoma samples may influence cell integrity, which can introduce variability in the data set. Inter-patient differences may also impact the consistency of pathway expression and influence downstream analyses. Furthermore, the use of lab-grown 3D organoids represents an *in vitro* model that cannot fully capture the complexity of the *in vivo* tumour environment.

Ethical Consideration

Direct patient contact and the collection of biological samples can raise ethical concerns. To address these concerns, this study will use archived human tissues obtained through approved biobank collaborations, avoiding direct patient involvement and maintaining compliance with ethical guidelines.

Conclusions

The proposed study will establish the first cellular-resolution mechanistic mapping of immune tolerance in gestational choriocarcinoma. By integrating transcriptomic data with functional organoid assays, the immunological checkpoints and ligand-receptor circuits responsible for immune suppression will be identified. These insights may guide the development of selective immunotherapies that restore anti-tumour immunity without disrupting reproductive health. This approach is not only relevant to gestational choriocarcinoma but may also be extended to other trophoblastic cancers that share similar immune-evasion pathways.

Editorial Conflict of Interest Statement

Ishaan S. Goswami and Zoha Fatima are members of the OSURJ editorial team. Both authors were fully recused from all aspects of the editorial process for this manuscript, including reviewer selection, peer review, and final decision-making. The manuscript was handled independently by other members of the editorial board.

References

1. C. M. Tarney, C. Tian, E. R. Craig, B. A. Crothers, J. K. Chan, G. D. Gist, N. W. Bateman, T. P. Conrads, C. A. Hamilton, G. L. Maxwell, K. M. Darcy, Relative Effects of Age, Race, and Stage on Mortality in Gestational Choriocarcinoma. International Journal of Gynecological Cancer 28, 338–345 (2017).

2. V. Male, Medawar and the immunological paradox of pregnancy: in context. Oxford Open Immunology doi:https://doi.org/10.1093/oxfimm/iqaa006 (2022).
3. B. Zhuang, J. Shang, Y. Yao, HLA-G: An Important Mediator of Maternal-Fetal Immune-Tolerance. Frontiers in Immunology 12, 744324 (2021).
4. M. G. Petroff, S. L. Nguyen, S. H. Ahn, Fetal-placental antigens and the maternal immune system: Reproductive immunology comes of age. Immunological Reviews 308, 25–39 (2022).
5. H. Pang, L. D Lei, Y. Guo, Y. Yu, T. Liu, Y. Liu, T. Chen, C. Fan, Three categories of similarities between the placenta and cancer that can aid cancer treatment: Cells, the microenvironment, and metabolites. Frontiers in Oncology 12, 977618 (2022).
6. H. Cheng, L. Zong, S. Yu, J. Chen, X. Wan, Y. Xiang, J. Yang, Expression of the immune targets in tumor-infiltrating immunocytes of gestational trophoblastic neoplasia. Pathology oncology research 29, 1610918 (2023).
7. D. Reynaud, R. A. Nahed, N. Lemaitre, P. Bolze, W. Traboulsi, F. Sergent, C. Battail, O. Filhol, V. Sapin, H. Boufettal, P. Hoffmann, T. Aboussaouira, P. Murthi, R. Slim, M. Benharouga, N. Alfaidy, NLRP7 Promotes Choriocarcinoma Growth and Progression through the Establishment of an Immunosuppressive Microenvironment. Cancers 13, 2999 (2021).
8. L. Hennah, M. Seckl, E. Ghorani, Novel approaches to managing gestational trophoblastic tumors in the age of immunotherapy. International journal of gynecological cancer 33, 414–419 (2023).
9. N. Sharma, R. Kundal, V. Kaushal, Immunobiology and immunotherapy of gestational trophoblastic disease. Gynecology and Obstetrics Clinical Medicine 2, 76–81 (2022).
10. M. Y. Turco, L. Gardner, R. G. Kay, R. S. Hamilton, M. Prater, M. S. Hollinshead, A. McWhinnie, L. Esposito, R. Fernando, H. Skelton, F. Reimann, F. M. Gribble, A. Sharkey, S. G. E. Marsh, S. O'Rahilly, M. Hemberger, G. J. Burton, A. Moffett, Trophoblast organoids as a model for maternal–fetal interactions during human placentation. Nature 564, 263–267 (2018).