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Crispr-Based Modulation of Hla-G and Immune Checkpoint Networks in Triple-Negative Breast Cancer: Emerging Immunogenetic Approaches for Overcoming Tumor Immune Escape

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ABSTRACT: Triple-Negative Breast Cancer (TNBC) is a more aggressive and heterogeneous form of breast cancer that lacks the expression of estrogen receptor, progesterone receptor and HER2, leaving few therapeutic options and an unfavorable prognosis. The ability of tumor cells to escape from the immune system of the host is emerging as an essential mechanism for the progression, metastasis and resistance to treatment of TNBC. Immune checkpoint pathways and HLA-G, a non-classical major histocompatibility complex molecule with strong immunosuppressive effects, are among the most crucial immunoregulatory mechanisms that play part in this process. Aberrant HLA-G expression reduces the ability of natural killer cells, CTLs and APCs to attack and destroy tumors, and allows them to survive and escape the immune system.

The recent development of novel CRISPR-Cas9 applications for the specific immunogenetic modulation of cancer therapy. Gene editing using a CRISPR strategy is a promising method to restore antitumor immunity in TNBC by precisely editing genes linked to immune checkpoint signaling and tumor associated immunosuppressive pathways. Furthermore, combination treatments with a combination of HLA-G and checkpoint inhibitors like PD-1/PD-L1 and CTLA-4 might be more effective for immune-mediated tumor clearance and less prone to resistance.

The immunobiological functions of HLA-G and immune checkpoints in TNBC are reviewed and recent advances in therapeutic approaches with CRISPR technology against these pathways are summarized. In addition, the review reviews the challenges encountered in the delivery, off-target effects, tumour heterogeneity, and clinical safety of these treatments. In summary, immune escape modulation with CRISPR is a swiftly developing approach that may have great promise in precision immunotherapy and ultimately clinical outcomes for TNBC patients.

1. INTRODUCTION

Triple-Negative Breast Cancer is one of the most aggressive and difficult to treat forms of breast cancer and makes up about 15-20% of all breast cancer cases globally. TNBC is unlike the other breast cancer subtypes in that it lacks expression of the following: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2); this severely restricts the number of targeted treatment options. Therefore, patients with TNBC tend to have poorer clinical outcomes, high recurrence rates, rapid metastatic progression and lower overall survival than other breast cancer subtypes. The unique molecular diversity and tumor microenvironment of TNBC adds to the complexity of disease management and further contributes to therapeutic resistance (1,2).

Recent cancer immunology developments have pointed out the pivotal role of tumor immune escape mechanisms in TNBC progression. Tumor cells use several immunoregulatory mechanisms to escape immune surveillance and promote the maintenance of tumor cell proliferation and metastasis. Immune checkpoint signaling pathways are key mediators of immune suppression in the tumor microenvironment among these mechanisms. Under physiological conditions, T-cell activation is kept under check by various immune checkpoints, including PD-1/PD-L1 and CTLA-4, which are frequently used by cancer cells to suppress antitumour immune activity. While there is great potential for immune checkpoint inhibitors to be therapeutically effective in some TNBC patients, therapeutic responses are highly variable, suggesting that there are other immunosuppressive networks that promote resistance (3,4).

HLA-G is a non-classical major histocompatibility complex class I molecule of high immunoinhibitory function that is one of the most significant emerging immunogenetic regulators involved in the immune evasion of TNBC. From the physiological point of view, the role of HLA-G is protective, ensuring maternal–fetal immune tolerance during pregnancy. In contrast, however, aberrant overexpression of HLA-G has been observed in a number of human malignancies, such as TNBC, which leads to inhibition of natural killer (NK) cells, cytotoxic T lymphocytes, dendritic cells, and other immune effector cells. Upregulation of HLA-G has

also been linked with increased tumour aggressiveness, metastatic potential and poor prognosis, highlighting its role as a potential target for cancer immunotherapy (5,1).

Along with these advances, there have been significant advances in genome editing technology that have revolutionized precision oncology. Of these technologies, CRISPR-Cas9 has become a game-changer in targeted genetic modulation because of its specificity, efficiency, and versatility. New opportunities for reprogramming the tumor microenvironment are provided by the precision of CRISPR-based approaches in editing or silencing genes that are involved in tumor progression and immune regulation. CRISPR-based regulation of both immune system checkpoint pathways and HLA-G in the context of TNBC is a potential immunogenetic approach to circumvent the tumor immune escape mechanism and to boost antitumor immunity (6,7).

This review will focus on the comprehensive discussion of the immunobiological role of HLA-G and immune checkpoint networks in TNBC and will critically review the latest advances in the CRISPR-based therapeutic interventions targeting these pathways. In addition, the review discusses challenges, translational hurdles and future directions of integrating CRISPR-driven immunotherapy into next generation precision medicine strategies to treat TNBC (8,5).

2. IMMUNOBIOLOGY OF HLA-G IN TRIPLE-NEGATIVE BREAST CANCER

HLA-G is a non-classical MHC class I molecule that has essential functions in immune tolerance and immunosuppression. Physiologically, HLA-G is expressed primarily in immune-privileged tissues, including the interface between mother and fetus where it is important for the prevention of maternal immune rejection of the fetus. But, in recent years, it has become recognized that elevated expression of HLA-G is found in many cancers, such as Triple-Negative Breast Cancer, pointing to its critical role in tumor immune escape mechanisms (9,5).

In TNBC, an increase in HLA-G expression promotes the formation of a highly immunosuppressive tumor microenvironment, inhibiting both innate and adaptive immune responses. HLA-G interacts with inhibitory receptors on immune cells such as ILT2, ILT4, and KIR2DL4, which result in decreased natural killer (NK) cell cytotoxicity, decreased CD8+ T cell proliferation, impaired dendritic cell (DC) antigen presentation, and induction of regulatory T cells (Tregs) (10,4). All of these effects contribute to tumor survival, immune escape and metastasis.

There are a number of clinical studies that have shown that elevated HLA-G expression has been linked to poor prognosis in patients with TNBC. High levels of HLA-G are often associated with high tumour stage, increased metastatic ability, resistance to treatment and decreased overall survival. In addition, soluble HLA-G molecules found in serum could be used as surrogate markers for the progression of the disease and prediction of response to immunotherapies (11,12).

The many immunoregulatory roles of HLA-G make it an attractive therapeutic target for precision immuno-oncology. Blocking HLA-G-mediated signaling pathways may enhance the ability of immune checkpoint blockade therapies to be more effective and more anti-tumour immune responses in TNBC (13,14).

Table 1. Immunological Functions of HLA-G in Triple-Negative Breast Cancer (15,5,2,1).

Immunological Mechanism	Target Immune Cells	Biological Effect in TNBC	Clinical Impact
Interaction with ILT2/ILT4 receptors	T cells and dendritic cells	Suppression of immune activation	Enhanced immune escape
Inhibition of NK cell cytotoxicity	Natural killer (NK) cells	Reduced tumor cell elimination	Increased tumor survival
Induction of regulatory T cells (Tregs)	CD4+ T lymphocytes	Promotion of immunosuppressive microenvironment	Tumor progression
Reduction of antigen presentation	Dendritic cells	Impaired adaptive immune response	Therapeutic resistance
Expression of soluble HLA-G isoforms	Multiple immune cell populations	Systemic immunosuppression	Poor prognosis and metastasis
Modulation of cytokine secretion	Immune microenvironment	Increased anti-inflammatory signaling	Enhanced tumor aggressiveness

3. IMMUNE CHECKPOINT NETWORKS AND TUMOR IMMUNE ESCAPE IN TNBC

The microenvironment of triple-negative breast cancer is extremely complex and immunologically suppressed, which helps malignant cells to get away with the immune surveillance of the host. The dys-regulation of immune checkpoint networks is one of the main mechanisms underlying this immune evasion and is a critical physiological pathway involved in the regulation of immune homeostasis and the suppression of unwanted immune activation (16,10) . However, in cancer, these pathways are often hijacked by tumor cells to inhibit anti-cancer immune responses and promote the growth of the disease (2).

The PD-1/PD-L1 Pathway is one of the most widely studied pathways in TNBC. In TNBC, programmed death-ligand 1 (PD-L1) is often highly expressed on the surface of both tumor cells and immune cells, and can bind to the programmed death receptor 1 (PD-1) found on the surface of activated T lymphocytes. This interaction causes T cells to become exhausted, reducing their proliferation, cytokine production, and killing activity and leading to decreased tumor clearance. Likewise, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a negative regulator of the early activation of T cells that competes with co-stimulatory receptors, thereby dampening down adaptive immunity to tumour cells (17,18).

In recent years, evidence has emerged that immune checkpoint pathways in TNBC operate in a complex regulatory network as opposed to independent signaling pathways. The tumor microenvironment is characterized by deep immunosuppression as a result of crosstalk among PD-1/PD-L1, CTLA-4, TIM-3, LAG-3, and others. Furthermore, together with checkpoint pathways, the immunoinhibitory molecule HLA-G is demonstrated to contribute to immune escape and therapeutic resistance (19).

In a small number of patients with TNBC, immune checkpoint inhibitors (ICIs) have been found to be effective, but others have only a partial response or become resistant. This variability is mainly due to the heterogeneity of the tumor, activation of compensatory checkpoints and the ongoing immunosuppressive signaling. Therefore, knowledge of the molecular mechanisms of checkpoint networks is crucial for the creation of next-generation combination immunotherapies and individualized treatment approaches for TNBC (20,21).

4. CRISPR TECHNOLOGY AS AN IMMUNOGENETIC TOOL IN CANCER THERAPY

CRISPR-Cas9 has transformed the molecular oncology field by introducing a molecular genome editing platform that is very precise, highly efficient and programmable to modify specific DNA sequences. CRISPR-Cas9 technology was adapted from bacteria and archaea, and can be used for targeted gene editing using guide RNA (gRNA) molecules that target complementary genomic regions with the Cas9 nuclease. The system has quickly become a formidable immunogenetic tool in cancer research and therapy, as it is a powerful system enabling the manipulation of genes involved in cancer progression, immune evasion and therapeutic resistance (22,23).

Cancer Immunotherapy: CRISPR technology is unparalleled in its ability to selectively target pathways that regulate immune responses, such as the immune checkpoint pathway, cytokine signaling and tumor associated antigens, in order to enhance antitumor immune responses. The use of gene knockout techniques for inhibitory molecules like PD-1, PD-L1 and CTLA-4 has shown to be effective in restoring the function of T-cells and enhancing the effectiveness of immune-mediated tumor clearance. Moreover, engineering of immune cells—such as chimeric antigen receptor T (CAR-T) cells—with CRISPR technology has demonstrated great therapeutic potential, with improved tumor specificity and decreased immune exhaustion (24,11).

The use of CRISPR-based strategies is especially attractive in the context of Triple-Negative Breast Cancer, where there are few established or traditional targeted therapies. The technology allows to modulate directly immunosuppressive regulators including HLA-G and other immune checkpoint networks for tumor immune escape. Even with these developments, there are still a number of problems to be addressed: off-target mutations, delivery system limitations and long-term safety and ethical issues. However, CRISPR technology remains a game-changing approach to precision oncology immunotherapies of the future (25,15).

Table 2. Major Applications of CRISPR Technology in Cancer Immunotherapy (26,27).

CRISPR Application	Therapeutic Target	Immunological Outcome	Potential Benefit in TNBC
PD-1 gene knockout	T lymphocytes	Restoration of T-cell activity	Enhanced antitumor immunity
PD-L1 suppression	Tumor cells	Reduced immune inhibition	Increased immune recognition
HLA-G gene modulation	Immunosuppressive pathways	Decreased immune escape	Improved therapeutic response
CAR-T cell engineering	Tumor-associated antigens	Enhanced cytotoxic specificity	Better tumor targeting
Cytokine pathway editing	Immune signaling molecules	Amplified immune activation	Strengthened immune microenvironment
Multiplex genome editing	Multiple checkpoint genes	Simultaneous pathway modulation	Reduced therapeutic resistance

5. CRISPR-BASED MODULATION OF HLA-G AND IMMUNE CHECKPOINTS IN TNBC

CRISPR-Cas9 has found a promising application in cancer immunotherapy to overcome immune resistance in Triple-Negative Breast Cancer. Given the aggressive biological behavior and limited therapeutic options of TNBC, much attention has been focused on the genetic modification of immune pathways that facilitate tumor immune escape. Of these, combined targeting of both HLA-G and immune checkpoint molecules is a very innovative immunogenetic strategy with a significant therapeutic potential (28,29).

Crispr-Based Modulation of Hla-G and Immune Checkpoint Networks in Triple-Negative Breast Cancer: Emerging Immunogenetic Approaches for Overcoming Tumor Immune Escape

The goal of disrupting HLA-G expression by CRISPR is to restore antitumor immune surveillance by decreasing inhibitory signals given to immune effector cells. Experimental studies have shown that the absence of HLA-G increases the ability of natural killer (NK) cells and of cytotoxic T lymphocytes to kill tumour cells. In addition, the inhibition of HLA-G signaling could reduce the recruitment and activation of regulatory T cells (Tregs), thus altering the tumor microenvironment from an immunosuppressive to an immunoreactive state. The results indicate that targeting HLA-G may be a powerful approach to enhance the immune clearance of TNBC (30,31).

At the same time, preliminary results have been demonstrated in preclinical models for immune checkpoint pathways, including PD-1/PD-L1 and CTLA-4, with CRISPR-based editing. PD-L1 gene knockout approaches in tumor cells could alleviate the immune inhibitory process, while PD-1 gene knockout approaches in T cells could block T-cell exhaustion and sustain antitumor activity. Importantly, recent investigations have shown that the combination of the modulation of both HLA-G and checkpoint pathways could yield synergistic immunotherapeutic effects by blocking multiple immune escape pathways simultaneously.

Another application of CRISPR technology is to modify immune cells for effective antitumor properties. CRISPR modified CAR-T cells and engineered NK cells have shown increased resistance to the tumor microenvironment immunosuppressive signals, persistence, and specificity. This could be especially beneficial in TNBC, where tumor heterogeneity and immune resistance frequently hinder the effectiveness of traditional immunotherapies (32,33).

While all these improvements have been made, there are a number of translation challenges that still need to be addressed. One of the most critical challenges that remain is efficient delivery of CRISPR components, especially in solid tumors like TNBC. Furthermore, issues of off-target genome modification, unintended immune activation and long-term genomic stability should be explored before use in clinical settings can become commonplace. However, ethical issues and regulatory hurdles are other important challenges that will affect clinical use of CRISPR-based therapies going forward (34,35).

However, with the ability to precisely reprogram tumor-associated immune pathways, CRISPR technology has the potential to be a revolutionary platform in precision oncology. The future integration of the modulation of HLA-G gene by crisper, along with immune checkpoint blockade (ICB), nanotechnology-based delivery systems and personalized immunotherapy, could offer new avenues to enhancing therapeutic results and to overcoming immune escape in TNBC patients (Figure 1) (36).

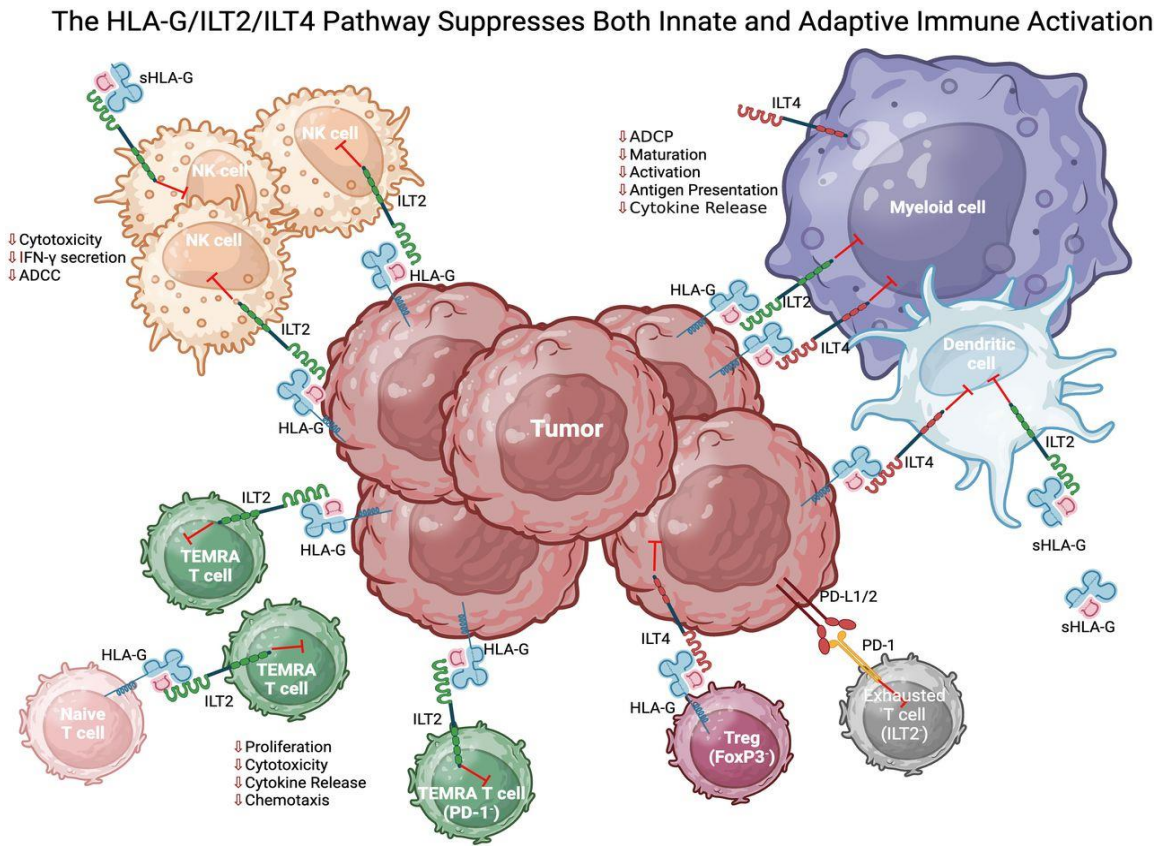

Figure 1. Mechanistic view of the HLA-G/ILT2/ILT4 immunosuppressive axis in the tumor microenvironment. Tumor-derived HLA-G acts as an inhibitor by binding to the receptors ILT2 and ILT4 on immune cells, which in turn negatively regulates CD8+ T cell activation, negatively regulates NK cell cytotoxicity and effector functions, and induces a tolerogenic NK phenotype to favor tumor immune escape.

6. CURRENT CHALLENGES, SAFETY CONCERNS, AND FUTURE PERSPECTIVES

Although significant advances have been made in using CRISPR-Cas9 in cancer immunotherapy, there are still a number of scientific and clinical hurdles that pose a challenge to its widespread use in Triple-Negative Breast Cancer. An important issue is the possibility of off-target genome editing, which is the occurrence of unintended modification of non-target genes, and can lead to genomic instability and/or malignant transformation. It is therefore important that high specificity of editing is maintained to augment the safety profile of CRISPR-based therapeutic approaches (37,38).

One of the other challenges is the efficient delivery of the CRISPR components to tumor tissues. Targeted delivery of drugs to solid tumors, like TNBC, is difficult owing to the presence of dense stromal barriers and to the high cellular heterogeneity of these structures. Currently, various systems are being explored to enhance the delivery efficiency, reducing the toxicity and immunogenicity of the materials.Viral vectors, lipid nanoparticles, and polymer-based systems are being investigated to enhance the delivery efficiency and reduce the toxicity and immunogenicity of the material. Moreover, in cases of tumour heterogeneity, different subpopulations of tumour cells may be responding to genome-editing interventions and checkpoint modulation in different ways (40,15).

Safety issues also involve immune-related side effects such as abnormal activation of the immune system and immune-related toxicities after engineered immune cell therapy, such as from the cytokines. In addition, there are ethical and regulatory issues that are still under debate nationally and internationally regarding permanent genome modification (41,42).

The future in this area is based on the coupling of the CRISPR technology with precision medicine, AI and multi-omics to improve the precision of patient-specific therapy. HLA-G and simultaneous targeting of several immune checkpoint pathways might provide greater therapeutic efficiency and minimize resistance mechanisms. Further preclinical and clinical research is therefore needed to bring immunogenetic strategies to the clinic using CRISPR technology for the treatment of TNBC patients (43,17).

Table 3. Major Challenges and Future Directions of CRISPR-Based Immunotherapy in TNBC (44,45).

Challenge	Description	Potential Solution	Future Perspective
Off-target genome editing	Unintended DNA modifications in non-target genes	Development of high-fidelity Cas systems	Improved genomic safety
Delivery limitations	Inefficient transport into solid tumors	Nanoparticle and viral vector optimization	Enhanced tumor-specific targeting
Tumor heterogeneity	Variable genetic and immunological profiles	Personalized therapeutic design	Precision oncology applications
Immune-related toxicity	Excessive immune activation and cytokine release	Controlled gene editing strategies	Safer immunotherapeutic protocols
Therapeutic resistance	Activation of compensatory immune pathways	Combination immunotherapy approaches	Durable clinical responses
Ethical and regulatory concerns	Risks associated with permanent genome modification	International clinical guidelines	Responsible clinical implementation

7. CONCLUSION

With its molecular heterogeneity, few targeted therapy options, and robust ability to evade the immune system, Triple-Negative Breast Cancer is one of the most aggressive and therapeutic resistant types of breast cancer. The tumor immune escape mechanisms are increasingly recognized as key to TNBC progression and resistance to therapy. Immune checkpoint pathways and HLA-G are among the most relevant immunoregulatory pathways that play a key role in the establishment of an immunosuppressive tumor microenvironment that dampens the tumor response.

Recent development of CRISPR-Cas9 has opened the door for precision immunotherapy by the ability to specifically modify genes involved in immune regulation and tumour progression. CRISPR technology has shown promising preclinical potential in targeting the tumor-associated immunosuppression, boosting the activity of T cells and NK cells, and restoring immune surveillance by targeting HLA-G and immune checkpoint pathways. Moreover, when combined with other therapeutic strategies such as immune checkpoint inhibitor (ICI) therapies and engineered immune cell therapies, the therapeutic potential of CRISPR technology may prove to be synergistic and thus overcome therapeutic resistance in TNBC.

However, there are still a number of translational hurdles to be overcome, such as off-target genome editing, delivery system limitations, tumor heterogenicity, and long-term safety concerns. Continued progress in the precision of genome editing, delivery mechanisms using nanotechnology, and the development of personalized therapeutic design will be necessary to solve these problems. Moreover, additional clinical trials are needed for assessing the safety and effectiveness of immunogenetic interventions based on CRISPR in humans.

In total, the modulation of HLA-G and immune checkpoint pathways using CRISPR has emerged as a fast developing and auspicious method in contemporary oncology. The potential for precision medicine, genome engineering, and immunogenetics to be integrated in the future may greatly enhance the success of therapeutic products and offer hope for those suffering from TNBC.

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