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MINI-REVIEWS

Sex Differences in Glioblastoma Immunotherapy Response

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Abstract
Glioblastoma (GBM), the most common primary malignant brain tumor, remains difficult to treat and shares phenotypes, including an aberrant immune response, with other neurological disorders. Understanding the cellular and molecular mechanisms underlying this pathological immune response remains a priority, particularly as standard of care for advanced cancers evolves to include immunotherapies, which have yet to show strong clinical efficacy in GBM. Epidemiological evidence supports a sex difference in GBM, with increased prevalence in males, and recent studies identified differences between males and females ranging from genetic aberrations to cellular programs. Sex differences have also been identified in immune response, and in this mini-review, we present these differences to highlight potential sex-specific cellular and molecular mechanisms that underly GBM growth and response to immunotherapies. These sex differences offer an opportunity to understand GBM pathogenesis and extend beyond GBM to other tumors and neurological disorders to inform the development of next-generation therapies.

Keywords Sex differences · Glioma · Immune system · Immunotherapy

Introduction

Glioblastoma (GBM) remains the most common primary malignant brain tumor and, despite aggressive multi-modal therapies including surgical resection and concomitant radiation and chemotherapy, is associated with a poor prognosis. Overall survival has not increased dramatically over the last 30 years, and identifying targetable cellular and molecular mechanisms in GBM remains a priority. While targeted pathway therapies, anti-angiogenic approaches, and now immunotherapies (including immune checkpoint inhibitors (ICIs), vaccines, and chimeric antigen receptor (CAR) T cell therapy) have shown efficacy in advanced cancers, results from large-scale GBM clinical trials continue to be negative (Brahm et al., 2020). This underscores the need to better understand GBM pathogenesis, including the role of a potently suppressive immune microenvironment and may contribute to immunotherapy resistance. In GBM, males have an increased prevalence and poorer outcome (Ostrom et al., 2018). These sex differences are an emerging area of interest, and studies have identified sex-specific genetic aberrations and sex differences in cellular programs, including increased cancer stem cell signaling in samples from males with GBM, and magnetic resonance imaging patterns (Beig et al., 2020). In addition, it is well established that there is a profound sex difference in immune responses, with females having a more active immune system than males (Klein & Flanagan, 2016), suggesting the role of immune system in sexual dimorphism in GBM pathogenesis. In this mini-review, we highlight these sex differences in the context of GBM, the immune system, and immunotherapy response with the goal of identifying potential sex-specific mechanisms that can be leveraged for the generation of more effective therapies (Fig. 1). While GBM is used as a paradigm for this discussion, these differences have implications for other advanced cancers and neurological disorders.

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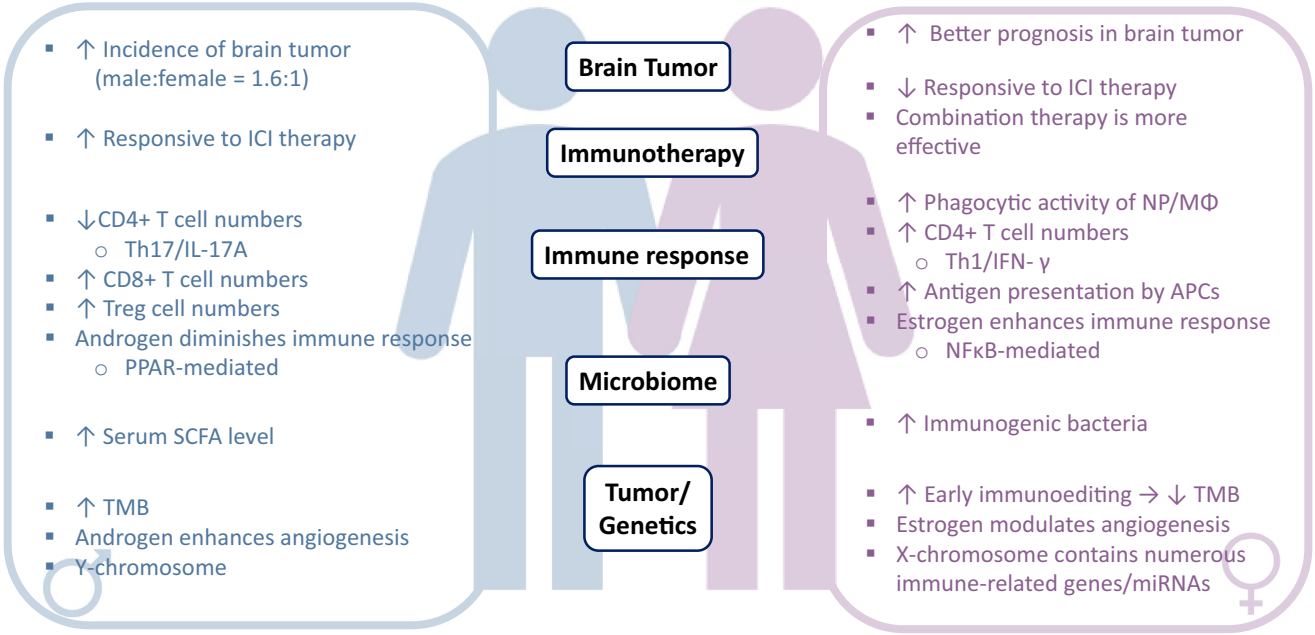

Fig. 1 Factors underlying sex differences in GBM. Schematic depicting differences in GBM incidence and outcome as well as distinct responsiveness to immune checkpoint inhibitor (ICI) therapy between male and female cancer patients, along with differences in immune response, microbiome, tumor genetic mutation, and sex chromosomes (*PPAR* peroxisome proliferator-activated receptor, *SCFA* short chain fatty acid, *TMB* tumor mutational burden, *NP* neutrophil, *MΦ* macrophage, *APC* antigen-presenting cell)

Sex Differences in Myeloid Cells

The importance of sexual dimorphism in neuro-inflammation becomes apparent when considering microglia, the innate immune cells of the brain. Sex hormones are a key determinant of brain development in rodents and are partially responsible for differences observed in microglia number and morphology in male and female mice (Wright-Jin & Gutmann, 2019). These sex hormone-induced differences in microglia also help regulate masculinization of the brain in rodents, a process that drives male copulatory behavior (Wright-Jin & Gutmann, 2019). New evidence suggests that these sex-specific differences in male and female microglia persist into adulthood. RNA sequencing has revealed distinct expression patterns of microglia isolated from the brains of adult male and female mice, while further examination of their activation states highlights an elevated inflammatory phenotype of male microglia compared to female (Villa et al., 2018). Notably, gene expression patterns of female microglia are retained when transplanted into male mice, suggesting that hormonal influence early in development may have lasting effects on these cells (Villa et al., 2018). These expression patterns are also matched by functional differences, as shown in experimental stroke models where female microglia are protective and can attenuate the severity of stroke in male mice (Villa et al., 2018). These microglial phenotypes become increasingly relevant in a disease context, as seen in children with neurofibromatosis type 1 (NF1). NF1 predisposes both male and female children to the development of optic pathway gliomas; however, girls develop vision loss at a much higher rate than boys (by 3- to 5-fold), an observation that has since been attributed to the effect of estrogen on microglia (Wright-Jin & Gutmann, 2019). While further work is needed to determine how sex-specific differences in microglia influence tumor maintenance and progression in GBM, the work highlighted above and recent observations revealed initial sex-specific molecular mechanisms for microglia activation (Turaga et al., 2020).

In GBM, breakdown of the blood–brain barrier results in infiltration of monocytes, macrophages, and other immune cells into the tumor microenvironment. Single-cell RNA sequencing studies of sorted microglia, monocyte, and macrophage populations from male and female tumor-bearing rodent brains has revealed an upregulation in MHC class II-associated genes in male microglia, consistent with previous data suggesting that these cells are more reactive in males compared to females (Ochocka et al., 2021). Analysis of human data from The Cancer Genome Atlas (TCGA) and single-cell sequencing data from patients with diffuse glioma (WHO grade II) corroborated these findings, demonstrating sex-dependent differences in MHC class II gene expression (Ochocka et al., 2021). A strong sexual dimorphic phenotype

has also been described in the two myeloid-derived suppressor cell (MDSC) subsets, monocytic (m-) and granulocytic (g-) MDSCs. Male mice with GBM tumors were found to have mMDSCs localized to the tumor microenvironment, whereas female tumor-bearing mice had enrichment of gMDSCs in the blood (Bayik et al., 2020). Importantly, these differences were shown to regulate a differential therapeutic response, which has important clinical implications (Bayik et al., 2020). These studies provide evidence for the importance of sex differences in the myeloid compartment in GBM and highlight the need for additional insight into how these differences can be leveraged for the treatment of GBM, especially in immunotherapy response.

Broader Sex Differences in the Immune System

Sex differences in immune response have been implicated/studied in autoimmune and infectious diseases for more than a decade. Typically, females have stronger and more robust innate and adaptive immune response compared to males (Fig. 2). In healthy females, neutrophils exhibit higher expression of genes associated with type I interferon signaling, whereas male neutrophils have significantly increased mitochondrial metabolism, an indicator of immature phenotype (Gupta et al., 2020). Females have a greater number of CD4+ T cells and a higher CD4:CD8 ratio, and males have higher CD8+ and regulatory T cell (Treg) counts (Klein & Flanagan, 2016). Upon activation, female CD4+ T cells tend to exhibit a Th1 phenotype, producing high levels of IFN-γ, whereas male CD4+ T cells become Th17 cells that mainly produce IL-17A (Zhang et al., 2012). Data suggest that this Th1/Th17-biased sexual dimorphism is driven by sex hormones. In vivo treatment of male mice with estrogen resulted in a marked increase in IFN-γ production in splenic T cells via upregulation of T-bet expression, mimicking the female phenotype (Karpuzoglu et al., 2007). In contrast, androgen negatively regulates NF-κB- and c-Jun-mediated IFN-γ production in T cells via the upregulation of peroxisome proliferator-activated receptor (PPAR)-α (Dunn et al. 2007). Androgen response elements were found in the promoter regions of PPAR-α (Zhang et al., 2012) as well as protein tyrosine phosphatase (PTPN)-1, which inhibits IL-12 signaling and thereby antagonizes Th1 cell differentiation (Kissick et al., 2014). A recent study using a murine model of bladder cancer showed that male CD8+ T cells adopt an exhausted phenotype over the course of tumor progression, while their female counterparts maintain their effector function (Kwon et al., 2020), suggesting divergent reprogramming of T cells in a sex-dependent manner. In addition to the sex hormone effect, genetic factors also contribute to sex differences in immune response. Given that many immune-related genes and micro-RNAs are encoded by the X chromosome and sex-specific immune responses are observed pre-puberty and are thus sex hormone independent (Klein & Flanagan, 2016), identifying sex hormone-versus sex chromosome-derived factors will be essential. The four

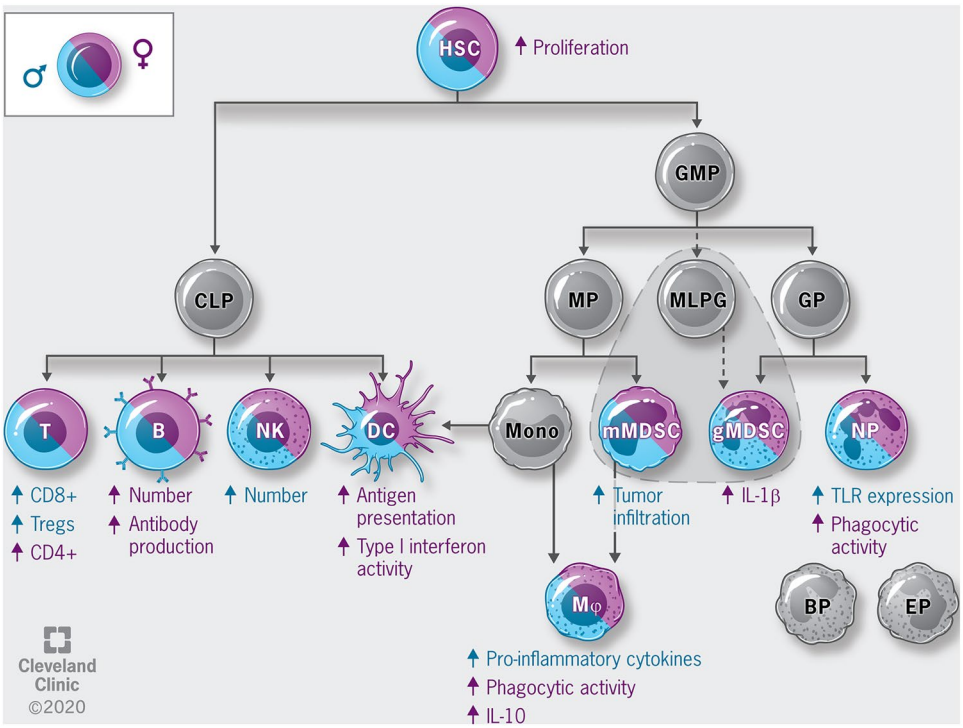

Fig. 2 Sex differences in the hematopoietic lineage. Schematic depicting sex differences in hematopoietic differentiation, with the dotted line indicating a cell differentiation process and sex differences specifically found in cancer (*HSC* hematopoietic stem cell, *CLP* common lymphoid progenitor, *NK* natural killer cell, *DC* dendritic cell, *GMP* granulocyte-monocyte progenitor, *MP* monocyte progenitor, *MLPG* monocyte-like precursors of granulocytes, *GP* granulocyte progenitor, *NP* neutrophil, *BP* basophil, *EP* eosinophil)

core genotypes model can be a useful tool to explore the mechanisms underlying sex differences. This model generates XX gonadal males or females as well as XY gonadal males or females, which enables to distinguish whether the sex differences arise from hormonal effects or sex chromosome complement.

Sex Differences in Immunotherapy

Despite clear sex-biased incidence and outcome of cancers including brain cancers, little is known about sex differences in cancer immunology. As ICI therapies have emerged, the impact of sex differences on anti-cancer immune functions has begun to be elucidated. In cancers which are responsive to ICI, the ICI treatment is predominantly more effective in male compared to female patients. Particularly, anti-CTLA-4 treatment led to prolonged overall survival in male patients, with the exception of melanoma patients, while no sex difference was observed with anti-PD-1/PD-L1 treatment (Grassadonia et al., 2018). Moreover, a more comprehensive meta-analysis study further showed that the level of PD-L1 expression by tumor cells has a critical impact on sexual dimorphism in anti-PD1/PDL-1 ICI efficacy (Li et al., 2020). Enhanced overall survival of both sexes was observed in patients with high PD-L1 expression (> 1%) whereas ICI was effective exclusively in female patients with low PD-L1 expression (< 1%). A previous murine study showed that PD-L1 expression on female Treg was critical for maintaining its suppressive function against estrogen-mediated functional reduction (Lin et al., 2010), suggesting that PD-L1-dependent regulation of Treg function may underlie the female-specific efficacy of PD-1/PD-L1 targeted ICI treatment. Including GBM, female tumors tend to have lower tumor mutational burden (TMB) due to strong MHC class II-based immune selection during tumor development (Castro et al., 2020). This weaker antigenicity of female tumors may lead to less effective anti-tumor immune response upon ICI treatment in female patients.

Sex Differences in Genetic and Epigenetic Regulations

Genetic mutation also plays an important role in sexual dimorphism, as demonstrated by recent findings revealing the contribution of sex to brain tumor incidence and outcome. Independent of isocitrate dehydrogenase (IDH) mutation, the overall survival rate of female patients was higher than that of males (Ostrom et al., 2018). It was also suggested that the higher level and frequency of the O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation might correlate with increased survival in female patients (Ostrom et al., 2018). In addition, astrocytes in males with mesenchymal GBM display increased deactivation of the tumor suppressor retinoblastoma (RB), which leads to increased growth and induction of stem-like cell populations, indicating tumor-intrinsic sex differences (Sun et al., 2014). A recent study focused on tumor-intrinsic factors that drive sex differences expanded on this to perform intensive analysis of tumor transcriptome and outcome data from male and female GBM patients. Male and female tumors exhibited increased gene expression signatures associated with cell cycle and integrin signaling, respectively, and these sex-specific factors critically impact patient survival (Yang et al., 2019). Given the link between TMB and ICI response, determining how these genetic differences impact immunotherapy response remains an immediate priority.

Emerging Areas: Microbiome and Metabolic Alterations

With the growing availability of metagenomics sequencing, the gut microbiome is gaining additional attention, and sex differences in the gut microbiome have been confirmed over the past few years (Vemuri et al., 2019). The interaction of the gut microbiome with immune cells in the gastrointestinal tract can have both local and systemic effects, implicating a gut-microbiome-tumor interface that requires a thorough investigation. Studies have shown that gut dysbiosis caused by antibiotics decreases the efficacy of ICI therapy as a cancer treatment (Gopalakrishnan et al., 2018). Fecal microbiota transplants of "favorable" microbiota into mice with "unfavorable" gut microbiota restores the effectiveness of ICI treatment. As this treatment has had limited efficacy in GBM (Brahm et al., 2020), investigating the gut microbiome may provide a potential avenue to improve GBM patient response to immunotherapy.

Expanding from the conventionally assessed gut and skin microbiome areas, the human tumor intracellular microbiome was recently elucidated, illuminating distinct bacterial fingerprints found in different tumor types, including GBM (Nejman et al., 2020). Based on our understanding of sex differences in the gut microbiome and interactions between the gut microbiome and local immune cells, it is likely that sex differences in the tumor microbiome affect GBM and the local immune response. Gut bacteria populations in both mice and humans go through significant changes at sexual maturation, indicating sex hormones may drive selection of gut microbiota (Vemuri et al., 2019). The sexual dimorphism in the immune environment of GBM may add an extra layer of complexity to this interaction, as well. Other populations, such as the gut mycobiome and virome, are also in the early stages of investigation for cancer connections.

Sexual dimorphism may also play a role in GBM by affecting metabolic pathways. Glycolysis in grade 2 gliomas is not only different between males and females but is also predictive of prognosis when combined with gene expression and metabolite profiling data (Ippolito et al., 2017). Overall, nutrient utilization in GBM may differ between males and females, and this could contribute to the sex differences in survival and prognosis that have been observed. Metabolic phenotyping could provide further information on sex differences and their implications in GBM.

Conclusions

These examples demonstrate the breadth and depth of sex differences that likely underlie differences in GBM incidence, pathogenesis, and therapeutic response. Future studies should focus on fully elucidating these cellular and molecular mechanisms to drive the development of more effective therapies. Given these sex differences, preclinical therapeutic assessments would benefit from taking into account sex as a biological variable, as these studies may reveal differences in efficacy and sex-specific resistance mechanisms to inform clinical use. Finally, given the sex differences currently observed in GBM incidence and outcome, the structure of clinical interventions should be revisited, including the design of clinical trials that are also statistically powered to observe these sex differences. Taken together, sex differences may offer insight into multiple aspects of GBM, ranging from fundamental pathological processes to therapeutic response, and provide a new lens through which this complex disease can be viewed.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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