Biochemical Pharmacology 130 (2017) 1–9

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Research update

The network of immunosuppressive pathways in glioblastoma

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ARTICLE INFO

Article history:
Received 10 October 2016
Accepted 13 December 2016
Available online 22 December 2016

Keywords:
Glioblastoma
Immunosuppression
Immunotherapy
Blood brain barrier
Molecular and cellular mechanisms
Immune checkpoints

ABSTRACT

Glioblastoma remains a fatal tumor despite increased knowledge regarding the complex signalling pathways that drive this devastating disease. Recently, immunotherapeutic approaches have shown remarkable and durable responses in various cancers including metastatic melanoma and advanced non-small cell lung cancer. So far, it remains unclear whether these immunotherapeutics may also work against glioblastoma and other tumors residing in the central nervous system. It is well known that patients with glioblastoma suffer from profound local immunosuppression that represents the major hurdle to overcome in the context of immunotherapy. Several studies have demonstrated that this immunosuppressive phenotype is orchestrated by glioma-derived membrane-bound and soluble factors as well as the particular microenvironment within the brain. Here, we discuss the molecular and cellular pathways involved in glioblastoma-mediated inhibition of the immune system and highlight possible treatment approaches aiming at reinvigorating anti-tumor immune responses.

Contents

Abbreviations: APC, antigen-presenting cells; BBB, blood brain barrier; CAR, chimeric antigen receptor; CSF-1R, colony stimulating factor-1 receptor; CTLA-4, cytotoxic T lymphocyte antigen 4; dcLN, deep cervical lymph node; DC, dendritic cells; FoxP3, forkhead box P3; GBM, glioblastoma; GDF-15, growth and differentiation factor-15; HIF-1α, hypoxia inducible factor; IFN, interferon; IDO, indoleamine-2,3-dioxygenase; iTreg, induced Treg; CD25, interleukin-2 receptor alpha chain; LLT-1, lectin-like transcript-1; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; MDSC, myeloid-derived suppressor cells; NK, natural killer; nTreg, natural Treg; NO, nitric oxide; PD-1, programmed cell death 1; PD-L1, PD ligand-1; RTF, regeneration and tolerance factor; Treg, regulatory T cells; TGF-β, transforming growth factor-β; TDO, tryptophan-2,3-dioxygenase; TAM, tumor-associated macrophages; VEGF, vascular endothelial growth factor.

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http://dx.doi.org/10.1016/j.bcp.2016.12.011
0006-2952/

1. Introduction

Glioblastoma is the most common and deadly primary malignant brain tumor in adults [1]. Despite maximal safe surgery, radio- and chemotherapy, the prognosis remains dismal with a median survival around 16 months within clinical trial populations [2]. Great promises held by targeted treatments directed against vascular endothelial growth factor (VEGF) or specific integrins were not fulfilled since these drugs failed to prolong overall survival in randomized clinical trials [3–5]. Hence, new therapeutic modalities are urgently needed. Immunotherapy has been regarded a promising treatment option for decades, however, without making significant progress. However, the therapeutic success achieved by "immune checkpoint inhibitors" in several tumor entities such as metastatic melanoma and non-small cell lung cancer with so far unseen response rates, paved the road for a renewed interest in exploring immunotherapeutic strategies also against glioblastoma [6–8]. Most therapeutic strategies employing the immune system are based on the consideration that T cells can recognize and respond against genetic and cellular alterations which occur during cancer development and progression [9]. Preclinical data from experiments using various methylcholanthrene-induced tumors demonstrated that knockout mice lacking either components of the interferon (IFN)-γ pathway or the perforin gene are more susceptible to tumor formation [10,11]. Subsequent studies proved the existence of tumor-specific T cells directed against mutated or overexpressed proteins confirming the presence of anti-tumor immune responses [12]. However, selective pressure on tumor cells by the immune system may lead to the emergence of immune-edited clones that escape recognition and ultimately grow undisturbed [13]. GBM is peculiar for its ability to escape from immune surveillance and two major challenges represent a major obstacle for the successful administration of immunotherapies: the tumor location in the brain which comprises an immunoprivileged microenvironment as well as the presence of several glioma-derived mechanisms of active immunosuppression. In this review article, we illustrate the pathways that are mainly involved in mediating glioblastoma-associated immunosuppression and therapeutic strategies which aim at reinvigorating immune responses against the tumor.

2. Blood brain barrier and immunological routes to the brain

A first hurdle to overcome in the context of immunotherapies for brain tumors is represented by the tumor location within the CNS. The presence of the blood brain barrier (BBB) and the absence of classical lymphatic vessels are two major issues that may hamper the successful administration of any immunotherapy. The BBB is a cellular barrier formed by specialized brain endothelial cells, pericytes and astrocytes, which regulates the ionic composition of the brain thereby maintaining appropriate neuronal function by blocking the entrance of unwanted and possibly neurotoxic molecules. The latter property relies on the presence of several ATP-binding cassette (ABC) transporters, that actively export molecules and drugs out of the brain [14]. The BBB also regulates the entry of immune cells to the CNS. Under physiological conditions, only few immune cells are present in the brain parenchyma, whereas various pathological conditions result in disruption of the BBB which then becomes more permeable to immune cell entry [15–17]. Substantial work has shown that antigens released within the CNS are drained towards peripheral lymphoid tissues and can be presented by antigen-presenting cells (APC) to naïve T cells, which can subsequently be activated and upregulate the expression of α4 and β1 integrins [18,19]. Only when expressing these integrins, lymphocytes can interact with vascular cell adhesion molecule (VCAM)-1 expressed on the cerebral endothelium and pass across the BBB. Immune cell infiltration has been found in glioblastoma, however, to a variable extent [20–22]. Despite being partially disrupted during tumor progression, intact zones of the BBB may protect tumor cells invading the normal brain parenchyma from drug delivery, representing a possible explanation for the re-iterated failure of various systemic therapies in GBM and other brain tumors [23–25]. Therefore, it is unlikely that antibodies used as therapeutics for the targeting of immunomodulatory molecules, can reach all parts of the tumor in the brain and exert any effect unless they interact with target molecules in the periphery [26]. Of note, attempts to circumvent or block BBB drug-efflux activity to improve standard and targeted GBM treatments are currently being investigated [27]. Since it has long been believed that a classical lymphatic drainage system is absent in the CNS, it has been assumed that brain immune-surveillance occurs mainly in the meningeal compartment. Yet, recent evidence has questioned this long-held belief and shed new light on immune cell trafficking between the brain and extracranial sites. Indeed, two laboratories have independently found a dural lymphatic vascular system that drains fluids, macromolecules and immune cells from the cerebrospinal fluid, and is connected to the deep cervical lymph-nodes (dcLN) in rodent models [28,29]. Although further experiments are required to finally confirm the functional trafficking of T cells to and from the human brain, the existence of a cellular route is further supported by single reports suggesting dcLN metastasis in primary brain tumors [30]. The discovery of the dural lymphatic route, along with the recently described glymphatic system – a cellular pathway which facilitates CSF drainage and clearance of potentially toxic metabolites and unfolded proteins such as β-amyloid and tau protein into the brain parenchyma – suggests that the brain cannot be seen as an immune-privileged organ anymore but rather an immune-distinct site which is still accessible for immunotherapeutic approaches [31].

3. Mechanisms of immunosuppression in glioblastoma

3.1. Immunosuppressive immune cell subsets

3.1.1. Regulatory T cells

Regulatory T cells (Treg) account for 5–10% of all circulating $CD4^+$ T cells and are key modulators of the immune system maintaining tolerance to self and host antigens and inhibiting autoimmunity through resolution of tissue inflammation [32]. This T cell subset is characterized by the constitutive expression of the nuclear transcription factor forkhead box P3 (FoxP3), interleukin-2 receptor alpha chain (CD25), cytotoxic T lymphocyte antigen 4 (CTLA-4) and the glucocorticoid-induced tumor necrosis factor (TNF) receptor (GITR) [33]. Two main types of Treg exist: natural Treg (nTreg) which have developed in the thymus and induced Treg (iTreg) arising from FoxP3 induction in conventional $CD4^+$ T cells exposed to an immunosuppressive microenvironment. Despite marked lymphopenia, the Treg fraction in the peripheral blood and tumor specimens of glioma patients is increased and correlates with tumor grade and poor prognosis [34–36]. However, Treg presence in the blood or tumor as negative prognostic factor remains controversial since low percentage and no impact on survival was observed in several other studies [20,37,38]. Discrepancies may be due to the existence of different Treg subtypes that can only be dissected by high-dimensional analyses and not only based on FoxP3 staining. In experimental models, glioma-infiltrating Treg are mostly thymus-derived nTreg as tumor entrance is drastically impaired in previously thymectomized mice [39]. This finding supports the idea that the glioma microenvironment efficiently recruits nTreg from the periphery, mainly through