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Anti-Tumour Treatment

Immunological challenges for peptide-based immunotherapy in glioblastoma

Malte Mohme [a,b,*,1], Marian C. Neidert [a,1], Luca Regli [a], Michael Weller [c], Roland Martin [c]

[a] Department of Neurosurgery, University Hospital Zurich, 8091 Zurich, Switzerland
[b] Center for Molecular Neurobiology Hamburg, University Medical Center Eppendorf, 20251 Hamburg, Germany
[c] Department of Neurology, University Hospital Zurich, 8091 Zurich, Switzerland

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ABSTRACT

Glioblastoma is the most aggressive primary tumor of the central nervous system with a medium overall survival of 7–15 months after diagnosis. Since tumor cells penetrate the surrounding brain tissue, complete surgical resection is impossible and tumor recurrence is almost a certainty. New treatment modalities are therefore needed, and these should be able to trace, identify, and kill dispersed tumor cells with great accuracy. Immunological approaches in principle meet these needs. Unfortunately, due to profound tumor-associated mechanisms of immunosuppression and -evasion, immunotherapeutic strategies like peptide vaccination have so far not been translated into clinical success. If future, peptide-based vaccination approaches shall be successful in glioblastoma therapy, multiple questions need to be solved including identification of suitable antigens, route and mode of vaccination, preparation of the tumor-bearing "host" and antagonizing, as much as this is possible, glioblastoma-associated mechanisms of immune evasion and poor vaccination response. In this review we will address the immunological challenges of glioblastoma and discuss key aspects that have rendered successful immunotherapy difficult in the past.

Introduction

Glioblastoma is the most common primary malignancy of the central nervous system and represents almost 50% of all primary intracranial neoplasias [1]. Because of the early infiltration of surrounding tissue, the high recurrence rate with fast progression, and the inability to completely eliminate the tumor glioblastoma evades successful treatment so far. Despite substantial advances and treatment refinements during the last decades, conventional therapies like neurosurgical resection and multimodal radio- and chemotherapy have limited effects on disease progression, recurrence rate or clinical outcome. Consequently, patients with newly diagnosed glioblastoma experience a median overall survival of only 7–15 months [2,3]. Despite extensive research, the prognosis for these patients has only improved by 3–6 months over the past decades [4].

Although the clinical outcome is influenced by individual factors like MGMT-promoter-methylation, [5] isocitrate dehydrogenase (IDH) mutations, age and Karnofsky performance score (KPS) at initial diagnosis [6], as well as the location, configuration and surgical accessibility of the tumor, the dominating pathological feature of glioblastoma remains its high recurrence rate. While metastases to other organs are rare, and less than 10% of malignant gliomas reoccur distant to the original site, local recurrence is almost certain [7]. A major factor responsible for the high recurrence rate is the ability of malignant cells to migrate and penetrate deeply into the surrounding parenchyma. Using white matter fiber tracts as well as feeding blood vessels as guiding pathways, tumor cells can spread and infiltrate anatomical structures adjacent to the primary tumor, so that dispersed tumor cells can be found centimeters away [8,9]. Since the brain does not allow expanded surgical en-bloc resection, neurosurgical treatment is restricted to reducing tumor burden. Therefore, while neurosurgical intervention remains one of the most important treatment approaches and the introduction of microscope-guided surgery improved the extent of surgical resection, complete surgical tumor removal remains impossible [10].

Other treatment modalities such as radio- or chemotherapy using alkylating agents like temozolomide have increased the overall life expectancy, but their effects are limited. One reason for the intractability of glioblastoma is the transforming nature and dynamic molecular phenotype of glioblastoma, which includes multiple mechanisms to resist drug- and radiation-induced antitumor activity [11,12]. In addition, malignant gliomas are characterized by their heterogeneity, which is promoted by tumor-initiating cells that drive a constantly mutating cancer cell population [13]. The transforming nature of a heterogeneous

* Corresponding author at: Department of Neurosurgery, University Hospital Zurich, Frauenklinikstrasse 10, 8091 Zurich, Switzerland, Tel.: +41 44 255 1111.
E-mail address: malte.mohme@zmnh.uni-hamburg.de (M. Mohme).
[1] These authors contributed equally to this work.

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tumor further facilitates the generation of defense mechanisms against radio- and chemotherapy. Although both treatment modalities prolong overall survival, they lack specificity and are accompanied by substantial side effects. New effective and more specific treatment modalities are therefore urgently needed.

Key players in immunotherapy

The immune system is not only engaged in defending the body from foreign pathogens, but it is also involved in eliminating cells that underwent malignant transformation in a process called "immune surveillance" [14]. Processes of malignant transformation are driven by genetic instability including changes in genes that are involved in cell cycle control, migration, angiogenesis, apoptosis, and also mutations of genes encoding for "normal" proteins not directly involved in tumor biology [15]. The immune system is able to recognize transformed cells and in analogy to vaccinations for infections, immune responses against tumor tissue can in principle be enhanced in an active or a passive fashion [16]. In contrast to prophylactic vaccinations against infections, cancer immunotherapy aims at eradicating established diseases, i.e. is analogous to therapeutic vaccination.

The basic principle of cancer immunotherapeutic approaches is to evoke a tumor-specific cellular immune response resulting in the selective elimination of cancer cells. The central effector population for targeted cancer cell lysis is comprised of CD8+ T cells, also called cytotoxic T lymphocytes (CTL) [17]. CD8+ T cells can identify antigenic peptides, which are presented by human leukocyte antigen (HLA) class I molecules on the surface of cancer cells with their antigen-specific T cell receptor (TCR) [18]. The interaction of the TCR with the presented tumor antigen, together with costimulatory molecules like B7-1/2 will result in the targeted release of CTL effector molecules like perforin and granzyme, which induce apoptosis, as well as cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α/β (TNF-α/β). The potent CTL response is supported by a complex interaction of other immune cells, responsible for priming and amplification of the anti-tumor effect. An important part of the priming/activating immune cells are professional antigen-presenting cells (APCs), mainly represented by dendritic cells (DCs) which can take up tumor-associated proteins and/or peptides and, after intracellular processing, present them via HLA class I and -II molecules on their surface [19]. Although there is some cross-talk between these two processing and presentation pathways, presentation via HLA class I will mainly prime CD8+ CTL, while peptide binding to HLA class II will induce a CD4+ T_{helper} cell response [20]. Type 1 helper cells specific for tumor antigens are able to amplify CTL proliferation and enhance their anti-tumor effect. By creating a local proinflammatory environment, for example, by secreting cytokines like IL-2 or IFN-γ among others T_{helper} cells promote the local reactivation of CTL by APC. This means, in order to mount an efficient tumor-specific immune response, both CTL as well as T_{helper} cell activation against tumor antigens that are presented in the context of HLA-class I and -class respectively are required (Fig. 1).

As we would like to focus on immunological challenges that are relevant to improve immunotherapeutic protocols in a clinical setting, we will not introduce all molecules and cell populations, for example NK- and NK-T-cells, that are additionally involved in the complex interaction between immune- and cancer cells, but focus on the adaptive T cell-mediated and tumor specific immune responses.

Peptide-based immunotherapeutic approaches

Tumor vaccination protocols can either be based on vaccination with a peptide or protein, ideally one that is specific and relevant for the respective tumor. Different protocols using tumor-specific peptide antigens have been established. Crude peptide digests of the autologous tumor can be used for vaccination, but in most cases synthetically manufactured peptides are injected subcutaneously or intranodally to prime the host immune system and to expand existing tumor-specific CTL [21]. Theoretically, if the immune response is strong enough and sufficient numbers of tumor-specific CD8+ T cells expand, cell-mediated lysis of the tumor cells could lead to tumor regression [22], and a cure is at least a possibility. Another immunotherapeutic approach is the adoptive transfer of autologous or genetically engineered tumor-specific T cell populations. Although adoptive T cell transfer harbors great potential, we will focus here on the more clinically feasible peptide-based immunotherapeutic approaches.

Another approach of inducing a tumor-specific immune reaction is dendritic cell vaccination. Ralph Steinman's discovery of DCs and their potent antigen presenting function provided the rationale for DC vaccination protocols. Compared to peptide vaccination, vaccination with peptide-pulsed DCs is considerably more labor-intensive, requires a GMP laboratory, and therefore poses both technical and financial hurdles. It involves the isolation of large numbers of peripheral blood mononuclear cells (PBMCs) from the patient using leukapheresis. Out of the heterogeneous cell mixture of PBMCs, CD34+ or monocytic cells can be isolated, cultured and further differentiated into mature DCs using standardized protocols. After exposure to whole tumor cells, tumor cell lysates, tumor RNA or specifically selected peptide mixtures, the pulsed dendritic cells are readministered to the patient. Due to the excellent antigen presentation and density of costimulatory molecules, mature DCs in consequence are able to effectively stimulate the expansion of tumor-specific T cells [23]. As for peptide vaccination, the selection of the appropriate antigen for the loading of DCs is of major importance. Approaches that use unspecific tumor extracts carry the risk of severe autoimmune collateral damage [24].

Limits of current immunotherapeutic approaches

A substantial number of clinical vaccination trials for malignant gliomas have been conducted. While tumor immunotherapeutic approaches in animals have led to significant tumor reduction and produced long-term tumor immunity, anti-tumor efficacy in human trials has so far been disappointing throughout all cancer entities including glioblastoma. Despite many phase I- and an increasing number of phase II trials, only one peptide vaccine for hormone-refractory prostate cancer was approved by the FDA [25]. A metaanalysis by Rosenberg et al. summarized the poor results of peptide vaccination trials that were performed until 2006. Using objective criteria of tumor response, of 440 patients only 12 (2.6%) responded to vaccination treatment [26].

The *in vivo* response rate has been particularly low in high-grade gliomas and resistance towards an immune-mediated tumor regression is a hallmark of glioblastoma [27]. It appears that not only the initiation, but also the execution of tumor-directed effector functions poses considerable obstacles in the treatment of glioblastoma. To achieve clinical success, several factors like efficient T cell activation, selection of the best antigens and peptides, immunogenic presentation as well as ways to overcome glioblastoma-associated immune evasion mechanisms or immunosuppression, have to be considered. Considering the sobering results of clinical trials, but also the at least theoretically promising advances in tumor immunology we decided to review and critically discuss immunological aspects of vaccination strategies to treat glioblastoma.

Promising data from clinical studies

It has been shown for other malignancies that peptide-based immunotherapy is able to mount a safe and effective immune