INTRODUCTION

High grade brain tumors, glioblastoma multiforme (GBM), anaplastic astrocytoma and medulloblastoma, are characterized by high progression, low effectiveness of traditional methods of chemo and radiotherapy, as well as high probability of tumor progression after tumor resection within healthy tissue. Due to these reasons average survival of patients with recurrent high grade tumors is about one year after diagnosis [1–3].

Insufficient effectiveness of surgical treatment of glioblastoma is a consequence of its rapid invasive growth, when tumor cells infiltrate intact healthy tissue and therefore they cannot be removed together with the main glioma tumor [4]. This also explains low effectiveness of radiotherapy of glioblastomas. Lack of clear borders of glioblastoma during neurovisualisation (Roentgen computed tomography, magnetic resonance and single photon emission computed tomography) cannot accurately determine value of surgical intervention. Application of cytostatic antibiotics for glioblastoma chemotherapy is essentially limited due to expression of multidrug resistance transport proteins in endotheliocytes of tumor microvessels [5]. Although the blood tumor barrier is rather structure-functionally imperfect these proteins prevent accumulation of xenobiotics in the tumor tissue. In addition, cytostatics effective against rapidly dividing cells do not act on mobilized glioma cells, which become a source of further glioma growth after chemotherapy [6].

Idea of employment of specific antitumor antibodies for tumor diagnostics and therapy was originally proposed by Pressman and Keighley in 1948 [7], however, practical employment of immunochemical detection of tumor localization in man became possible after discovery of tumor associated proteins: carcinoembryonic antigen (CEA) [8], alpha-fetoprotein [9, 10], and other tumor markers.

Anti-CEA polyclonal antibodies purified from antiserum and labeled with $^{131}$I were originally used for in vivo visualization of gastrointestinal tract tumors and ovarian tumors by Goldenberg et al. [11]. Subsequently this approach was also used for radioimmune localization of kidney tumors [12], reproductive tract tumors, germinogenic tumors and chorioncarcinoma [13]. The first attempt of radioimmune localization of glioma was undertaken by Day, Mahaley et al. (1965); they used antibodies to tumor antigens isolated from rabbit antiserum obtained after immunization with glioma preparations [14].

The development of hybridome technology opened a new stage in the radio-immunolocalization of tumors [15] and it became possible to obtain standard homogeneous antibodies specific to certain epitopes of tumor markers. Using the hybridome technology a wide screening for glial tumor antigens was performed and monoclonal antibodies recognizing particular antigens were found. These included antibodies recognizing antigens associated with thyroid carcinoma [16], melanoma [17], gastrointestinal tract carcinomas [18], and also antibodies recognizing such glioma markers as epidermal growth factors receptor [19–21], receptors of nerve growth factor [22] and insulin-like growth factor-1 [23, 24], fibronectin [25, 26], tenacin [27–32], nucleoprotein complexes [33, 34], and some other unidentified proteins of animal experimental gliomas and human glioblastoma.

REVIEWS

Monoclonal Antibodies in Diagnostics of High-Grade Gliomas

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Received January 11, 2008

Abstract—The review considers the modern methods of radioimmune diagnostics of high-grade gliomas using monoclonal antibodies and also other approaches of multi-component radioimmunolocalization with pretargeted bispecific antibodies. High-grade tumor-related proteins have been analyzed as potential targets for radiolabeled antibodies. Recent experimental and clinical data on immunolocalization of brain tumors and the most promising immunochemical approaches for diagnostic and targeted therapy of gliomas with tumor-specific antibodies are discussed.

Key words: glioblastoma multiforme, C6 glioma, monoclonal antibodies, radioimmunolocalisation, targeted delivery.

DOI: 10.1134/S1990750809020012
1. METHODOLOGY OF RADIOIMMUNOLOCALIZATION

1.1. Radioisotope Incorporation into Antigen Molecule

Pilot experiments on tumor radioimmunolocalization were performed with antibodies labeled with \( ^{\gamma}\)-radioisotopes. Now various types of isotopes with \( ^{\gamma}\), \( ^{\beta}\), and \( ^{\alpha}\)-emission are used. The most widely used \( ^{\gamma}\)-radioisotopes include iodine isotopes (\( ^{131}\text{I}, ^{132}\text{I}, ^{123}\text{I}, ^{125}\text{I} \)). In clinical studies the \( ^{131}\text{I} \) isotope is used more frequently [3, 11] because it is characterized by high energy \( ^{\gamma}\)-emission \( (E_{\text{max}} = 0.81 \text{ MeV}) \) and relatively short half-life time (seven days). Gamma-emission of \( ^{123}\text{I} \) is of lower energy and its half-life time is 13 h and so it is more preferable for radiodiagnostics than for therapy. The \( ^{125}\text{I} \) isotope is more convenient for experimental goals because it combines mild \( ^{\gamma}\)-emission \( (E_{\text{max}} = 0.35 \text{ MeV}) \) with rather high half-life time (60.1 days).

Radioactive iodine isotopes can be readily included into immunoglobulin molecule. Antibody iodination is performed in neutral medium by means of various oxidizing agents (chloramines-T, lactoperoxidase, iodogen, etc.) and iodine is incorporated at ortho- position (versus –OH) of the phenolic group of tyrosine residue and more rarely into phenylalanine, tryptophan or histidine residues. Most researchers now use iodogen as the oxidizing agent. Although incorporation efficiency is rather low (about 25\%) employment of this method maximally preserves physicochemical properties and functional activity of antibodies and this is very important for subsequent manipulations with the radiolabeled antibody preparation.

Among other \( ^{\gamma}\)-isotopes technetium-99m (\( ^{99m}\text{Tc} \), \( T_{1/2} = 6 \text{ h} \)), indium-111 (\( ^{111}\text{In} \), \( T_{1/2} = 67 \text{ h} \)), and yttrium-90 (\( ^{90}\text{Y} \), \( T_{1/2} = 2.7 \text{ days} \)) are used for antibody labeling. Binding of metal radioisotope to antigens is achieved by heterofunctional chelating agents [35]. These isotopes are characterized by substantially higher energy of \( ^{\gamma}\)-emission (e.g. for \( ^{90}\text{Y} \) \( E_{\text{max}} = 2.3 \text{ MeV} \) than in the case of iodine. Some clinical trials of tumors of various etiology were performed used beta- (\( ^{188}\text{Re}, ^{186}\text{Re}, ^{177}\text{Lu}, ^{67}\text{Cu} \)) and alpha-emitters (\( ^{213}\text{Bi}, ^{211}\text{At} \)) [36].

Non-specific irradiation is a limiting moment during administration of any radiolabeled antibodies and until total uptake of circulating radiolabeled antibodies by target organs (or elimination from the body) all organs and systems are exposed to this irradiation; in the case of immunoglobulins this period may last for several days. Non-specific antibody sorption in liver and spleen may also increase organ background of the radioactivity. In addition effects of serum proteolytic enzymes may result in cleavage of radiolabeled peptides and amino acids from the antibody and their non-specific distribution in the body. These problems may be avoided by means of stepwise administration of antibody vectors and a radioactive hapten.

2. MULTICOMPONENT SYSTEMS FOR RADIOIMMUNOLOCALIZATION

Discovery of recombinant DNA technology [37] and subsequent biotechnological revolution resulted in the development of a new class of recombinant biospecific antibodies and their use for the development of multicomponent systems for radioimmunodiagnoses and therapy [38–41].

In 1988 Reardon et al. [42] and Goodwin et al. [43] described a methodology of multicomponent systems for tumor immunolocalization known as a “Pretargeting.” To avoid direct antibody binding to radioisotope they used chemically modified biospecific antibodies containing a recognition site for a radiolabeled hapten. Modern concept of the “Pretargeting” is based on employment of recombinant biospecific antibodies; one hypervariable site of such antibodies recognizes a target antigen whereas the other one interacts with the hapten with incorporated radioisotope. Biospecific antibodies (usually F(\(\text{ab}\))\(_2\) fragments with molecular mass of 100 kDa) are intravenously injected first and after one day (this time interval is required for accumulation of major proportion of antibodies in the target tumor) a radiolabeled peptide exhibiting affinity binding with the second antibody site is then injected [44]. This approach has evident advantages: on the one hand, it is safer for patient than administration of labeled antibodies because a small radioactive hapten is eliminated from circulation much faster than a large antibody and this decreases background irradiation of healthy tissues; on the other hand, employment of high-affinity low molecular weight ligands significantly increases sensitivity and specificity of radioimmunolocalization [45]. Most frequently, the avidin-biotin system is used in the case of non-recombinant antibodies [28, 46–48]. Avidin has four biotin binding sites of extremely high affinity: dissociation constant for this complex is \(10^{-15} \text{ M} \) (in most cases affinity of antigen-antibody interaction is characterized by dissociation constants not less than \(10^{-9} \text{ M} \)).

Employment of molecular biotechnology resulted in the development of modified biospecific antibodies exhibiting the same valency as full length immunoglobulins but having a (two times) lower molecular mass (75 kDa); these are known as small immunoproteins [49]. A smaller size of such immunoproteins promotes their better penetration through the blood tumor barrier and more rapid elimination from circulation [50] and the valency corresponding to that of full length antibodies preserves their immunochemical properties [51].

Now five types of two- and three-components systems have been constructed for tumor immunolocalization:

1. Recombinant biospecific antibodies containing one site for hapten binding and one or two sites for recognition of a target antigen [52].