Journal of Clinical Neuroscience 22 (2015) 1071–1076

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

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

Review

Transferrin receptors and glioblastoma multiforme: Current findings and potential for treatment

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ARTICLE INFO

Article history:
Received 4 February 2015
Accepted 8 February 2015

Keywords:
Glioblastoma multiforme
Immunotherapy
Transferrin

ABSTRACT

The current standard treatment for glioblastoma multiforme (GBM) is surgery followed by chemotherapy and external radiation. Even with the standard treatment, the 2 year survival rate for GBM is less than 20%, making research for alternative treatments necessary. Transferrin receptor 1 (TfR1) controls the rate of cellular iron uptake by tuning the amount of iron delivered to the cells to meet metabolic needs. Kawabata et al. (J Biol Chem 1999;274:20826–32) cloned a second TfR molecule known as transferrin receptor 2 (TfR2) in 1999. Multiple experimental studies have documented increased expression of TfR1 on both proliferating cells and cells that have undergone malignant transformation. Calzolari et al. concluded that TfR2 is frequently expressed in human cell lines in 2007 (Blood Cells Mol Dis 2007;39:82–91) and in GBM in particular in 2010 (Transl Oncol 2010;3:123–34). In GBM, a highly significant correlation ($p < 0.0001$) was found between the expression level of TfR2 and overall survival, showing that higher levels of TfR2 expression were associated with an overall longer survival. The data on which of the two transferrin receptors is the better target is also unclear and should be studied. The transferrin pathway may be a promising target, but more research should be completed on the antigenicity to discern the viability of it as an immunotherapy target.

1. Introduction

The current standard treatment for glioblastoma multiforme (GBM) is surgery followed by chemotherapy and external radiation. However, in patients with newly diagnosed GBM, concurrent radiation and temozolomide therapy followed by adjuvant temozolomide for at least 6 months only offered modest benefit with a median survival of 14.6 months compared to an average of survival of 12 months with radiotherapy alone [1,2]. Additionally, aggressive treatment can often damage surrounding normal brain tissue [3,4]. One of the main challenges of treating GBM is its highly invasive and diffuse spread throughout the brain parenchyma, which renders most local therapies ineffective, and systemic therapies are limited due to the blood brain barrier [5].

Even with standard treatment, the 2 year survival rate for GBM is less than 20%, making research for alternative treatments necessary. One such alternative treatment is immunotherapy, which encompasses active, passive and cytokine immunotherapy. Most research in transferrin-based treatment has been performed for passive immunotherapy, which includes immunotoxins. Immunotoxins are tumor-specific monoclonal antibodies (mAb) or other ligands covalently linked to toxic proteins that combine cell type selectivity with high potency [6–15]. One antigen that has been suggested as a possible cellular target for such immunotherapy is transferrin receptor (TfR), as GBM cells that do express TfR have been shown to be sensitive to immunotoxins (IT) created from conjugating anti-TfR mAbs with intracellular toxins [14,16,17]. Here we summarize the current advances in knowledge of the role of TfR1 and TfR2 in GBM and the potential they present for future treatments.

2. Transferrin biology

Iron-containing proteins catalyze a wide variety of vital metabolic processes, including hemoglobin synthesis in erythroid cells, DNA synthesis, electron transport and oxygen transport. The best characterized mechanism for iron uptake is the binding of the serum iron carrier protein transferrin-to-transferrin receptor 1 (TfR1) [18]. Increased transferrin expression could represent a strategy adopted by tumor cells to obtain optimal import of iron, an element crucial for cell proliferation, therefore potentially giving tumor cells a growth advantage over normal cells [19].

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http://dx.doi.org/10.1016/j.jocn.2015.02.002
0967-5868/

Transferrin consists of a polypeptide chain of 679 amino acids in humans [20]. The transferrin protein is formed of alpha helices and beta sheets that comprise two major domains (N-terminus and C-terminus). In between each N and C-terminal sequences' globular lobe is an iron binding site [20–22]. Two tyrosines, one histidine and one aspartic acid bind the iron ion to the transferrin in both lobes. An anion is also required, preferably carbonate (CO_3^{2-}), in order for the iron ion to bind [21].

A disulfide linked homodimer acts as a transferrin iron-bound receptor. In humans, each monomer consists of 760 amino acids and three main domains: the protease domain, the helical domain and the apical domain, which can bind one or two molecules of iron. With its three distinct domains, the shape of the transferrin receptor is a butterfly-like complex [20–25].

Although other organs, such as the brain, produce transferrin, the liver is the main site of synthesis [26]. Notably, transferrin plays a key role where erythropoiesis and active cell division occur. The main function of transferrin is to deliver iron from absorption centers in the duodenum and red blood cell recycling macrophages to all tissues [21,27–29]. In order for iron ions to be introduced into the cell, the TfR is required [20,21]. The TfR helps maintain iron homeostasis in cells by controlling iron concentrations [21]. Iron, toxic as free Fe^{2+} and insoluble as free Fe^{3+}, is transported throughout the body as Fe^{3+} bound to transferrin and introduced into cells via endocytosis of its complex with TfR [21,30,31].

3. Transferrin in normal cells

TfR1 is a receptor that mediates cellular iron uptake by binding to iron-bound Tf [32]. TfR1 controls the rate of cellular iron uptake by tuning the amount of iron delivered to the cells to meet metabolic needs. Kawabata et al. cloned a second TfR molecule, known as transferrin receptor 2 (TfR2) in 1999 [32,33]. Unlike TfR1, TfR2 is not regulated by the same intracellular iron levels and instead seems to be regulated in accordance to the cell cycle [32,34,35]. TfR2 also has a unique, yet mostly unknown, role in iron homeostasis [32,36]. Calzolari et al. completed a study in 2006 showing that TfR2 is localized in lipid rafts which are cell membrane domains involved in the generation of receptor-mediated cell signaling [36]. The most distinguishable difference between TfR1 and TfR2 is their expression pattern, as TfR1 is expressed on all cells except mature erythrocytes and terminally differentiated cells, whereas TfR2 mRNA is highly expressed in the liver and in erythroid cells, spleen, lung, muscle, prostate and peripheral mononuclear cells to a lesser extent [32–34]. Both TfR1 and TfR2 are highly expressed in glioblastomas making them attractive for developing targeted therapies [32].

A comparison of TfR1 and TfR2 indicates many similarities between them. Both TfR1 and 2 encode a type II transmembrane glycoprotein that consists of a cytoplasmic, transmembrane and long extracellular domain [33–35]. The extracellular domain of TfR2 has a high degree of homology with TfR1 and also contains two cytokine residues, suggesting the TfR2 protein has a homodimer configuration similar to TfR1. TfR1 and TfR2 also support iron delivery to cells and increased expression in proliferating cells [33–35,37–43].

Differences are, however, also numerous between TfR1 and TfR2. TfR1 is essential for mammalian life whereas TfR2 is not. Also TfR1 is regulated by a number of factors, including cellular iron levels, proliferation and differentiation whereas TfR2 expression is not regulated by cellular iron levels. The tissue distribution and expression level of TfR2 mRNA is distinct from TfR1 mRNA. TfR1 binding affinity for transferrin is 25–30 times greater than that of TfR2 [37,41]. Finally, TfR2 is not able to compensate for TfR1 expression as TfR1 knockout mice die *in utero*, suggesting that the expression of TfR1 and TfR2 is tissue-specific and regulated by different control mechanisms [33–35,37–43].

4. Transferrin in cancer

Multiple experimental studies have documented increased expression of TfR1 on both proliferating cells and cells that have undergone malignant transformation [44–46]. Clinical studies have correlated tumor TfR1 expression with poorer outcomes in a number of neoplasms, including breast cancer and lymphoma [25,44,45,47–52]. Also, immunotherapies directed against TfR1 have been effective in modulating tumor growth in multiple tumor systems [24].

Transferrin is expressed in many different cancer types and has therefore been a target for alternative treatments. Human TfR2 mRNA is highly expressed in liver and normal erythroid precursor cells, erytholeukemic cell lines (especially K562, OCI-M1) and bone marrow samples from several patients with erythroleukemia and myeloid leukemia [33–35,38,40].

TfR1 is expressed in normal colonocytes and overexpressed in colon cancer [25,53]. A study by Prutki et al. showed that almost all (95%) of the normal colon mucosa samples showed weak staining of TfR1 on the cell membrane while 48% of tumor samples exhibited strong TfR1 staining ($p < 0.001$) [54]. Calzolari et al., in a study of 110 colon cancers of various histology, clinical progression, and grade found that TfR2 was expressed in 29 (26.3%) of the patients, providing evidence that TfR2 represents a membrane antigen frequently expressed in colon cancers while it does not seem to be expressed on normal colonic epithelium [53].

5. TfR1 and TfR2 in normal brain tissue and tumors

In normal brain tissue, the highest expression levels of TfR1 mRNA are in the medulla oblongata and the hippocampus, while expression is distinctly lower in the cortex, thalamus and cerebellum [55]. Brain tumors (meningioma, oligodendroglioma, oligoastrocytoma, medulloblastoma, astrocytoma) show no statistically significant expression of TfR1 mRNA when compared to normal human cortex. However, the astrocytoma cell lines have substantially higher TfR1 expression than the other brain tumor types [16,56–59].

The cerebellum is the only part of normal brain tissue that has been shown to express TfR2 mRNA. Some TfR2 mRNA expression has been shown to be present in a few brain tumor specimens, with possible differential expression seen in oligoastrocytomas. This study did not include GBM cell lines. TfR2 mRNA is not expressed in astrocytoma cell lines [55,58,59].

6. TfR1 expression in GBM

Recht et al. completed a study in 1990 detailing the extent to which TfR1 is expressed in GBM [16]. Out of the 10 GBM tissue samples stained with immunohistochemistry using TfR mAbs, more than 75% of the cells in nine (90%) of the samples stained positive and the other sample stained less than 25%. Among all of the tumor samples stained, the most intense staining was observed in GBM. The only sample of GBM that did have a high level of staining was obtained from a patient with a previously irradiated, recurrent dedifferentiated low grade tumor, and in this case, immunoreaction products were seen in less than 25% of cells and in a focal pattern [16].

Both the number of positively immunostained cells and the patterns of cellular staining (focal *versus* diffuse) are roughly correlated with the histopathological tumor type and, in most cases of glial tumors, the grade of the tumor [16]. The degree of staining