ELSEVIER

Toward an effective strategy in glioblastoma treatment. Part I: resistance mechanisms and strategies to overcome resistance of glioblastoma to temozolomide

Khaled Messaoudi[1,2], Anne Clavreul[1,2] and Frédéric Lagarce[1,2,3]

[1] LUNAM Université, Angers, France
[2] Inserm U1066, Micro et Nanomedecines Biomimétiques, IBS, Angers Cedex 9, France
[3] Service Pharmacie, CHU Angers, France

Glioblastoma multiforme (GBM) is a devastating disease and the most lethal of adult brain tumors. Treatment is based on surgery, radiotherapy and chemotherapy by oral temozolomide (TMZ), which is the most potent chemotherapy agent for the treatment of GBM. Despite TMZ efficiency, the prognosis of these tumors remains poor. This is because of inherent or acquired resistance of glioma tumor cells to TMZ. This resistance is caused by DNA repair enzyme activity, overexpression of epidermal growth factor receptor (EGFR), galectin-1, murine double minute 2 (Mdm2), p53 and phosphatase and tensin homolog (PTEN) mutations. Many strategies to overcome this resistance have been developed. In this review, we will describe the main mechanisms of GBM resistance to TMZ and different strategies developed to reverse the phenotype of these tumor cells. Finally, we will discuss the drawbacks and limitations of these strategies.

Introduction

Glioblastoma multiforme (GBM) is the most common and the most aggressive primary brain tumor in adults [1]. Median survival is 14.6 months [2] and the percentage of patients living for five years or more is less than 10% [3]. Despite the advances made during the past few years in brain tumor therapy, the prognosis of this disease has not improved as a result of tumor resistance. Current therapy includes surgical intervention, radiotherapy and chemotherapy with temozolomide (TMZ) [4]. TMZ is used at the beginning of GBM treatment at the daily dose of 75 mg/m^2 body surface area in association with radiotherapy for six weeks followed by six cycles of TMZ alone at the dose of 150–200 mg/m^2 for five days every 28 days. With this combination (TMZ plus radiotherapy), the median survival reached 14.6 months compared with 12 months for patients treated by radiotherapy alone [4]. The efficacy of TMZ for treating GBM is thus limited; this is partly because of the high levels of activity of O^6-methylguanine DNA methyltransferase (MGMT) DNA repair in tumor cells, which reduces the effect of this alkylating agent and leads to a resistant phenotype. Unfortunately, other mechanisms contribute significantly to the resistance to TMZ such as overexpression of epidermal growth factor receptor (EGFR), galectin-1, murine double minute 2 (Mdm2) and p53 gene mutation. To reduce acquired resistance, different therapeutic molecules have been developed, for example, O^6-benzyl-guanine which inhibits MGMT, tyrosine kinase inhibitors that act on EGFR, nutlin-3 which inhibits Mdm2, which contributes to the restoration of p53 activity. Despite their effectiveness, these molecules do not seem to be able to cure GBMs. However, the considerable development of RNA interference (RNAi)-based therapy seems to give hope to patients with GBMs. In this review, the main mechanisms of resistance of GBMs to TMZ and strategies that have been developed to overcome this resistance are described. Finally, we provide a critical evaluation of future prospects and challenges for treatment of GBMs.

Structure and mechanism of action of temozolomide

TMZ is an alkylating agent from the imidazotetrazine family which is stable only at acidic pH [5]. This prodrug undergoes rapid chemical conversion in the systemic circulation at physiological pH to the active compound 3-methyl-(triazen-1-yl)

Corresponding author: Lagarce, F. (frederic.lagarce@univ-angers.fr)

1359-6446/
http://dx.doi.org/10.1016/j.drudis.2015.02.011

Please cite this article in press as: Messaoudi, K. et al. Toward an effective strategy in glioblastoma treatment. Part I: resistance mechanisms and strategies to overcome resistance of glioblastoma to temozolomide, Drug Discov Today (2015), http://dx.doi.org/10.1016/j.drudis.2015.02.011

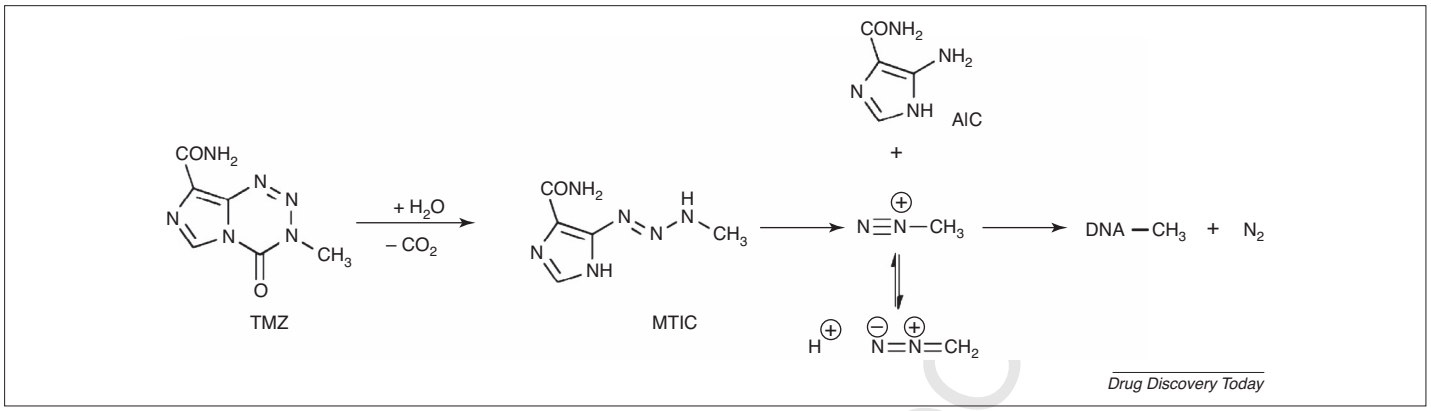

FIGURE 1

Structure and activation of temozolomide (TMZ) and production of active compound imidazole-4-carboxamide (MTIC) [5].

imidazole-4-carboxamide (MTIC), which will react with water to produce 5-aminoimidazole-4-carboxamide (AIC) and the highly reactive methyldiazonium cation (half life = 0.4 s). This unstable cation transfers a methyl group to DNA causing the cytotoxic effect of TMZ [6] (Fig. 1). The methylation occurs at purine bases of DNA at the O^6 and N^7 positions of guanine and the N^3 position of adenine. The O^6 methylation accounts for 5% of the total lesions caused by TMZ in DNA but it is the main cause of the TMZ cytotoxicity [7]. The N^7 and N^3 methylations are very frequent and represent 80–85% and 8–18% of total alkylations, respectively [8].

Mechanisms of GBM resistance to TMZ

Through time, GBM cells become resistant to damages caused by TMZ. This resistance is related to the implementation of several mechanisms such as DNA repair mechanisms, the overexpression of EGFR, galectin-1 and Mdm2 and the mutation of p53 and phosphatase and tensin homolog (PTEN). In addition, certain microRNA (miRNA) expression profiles are altered in GBM with overexpression of some and a significant reduction in the production of others.

DNA repair mechanisms

MGMT

The main mechanism of GBM resistance involves MGMT DNA enzyme repair. MGMT is a 22 kDa protein, capable of removing not only the methyl groups attached to the O^6 position in guanines but also other alkyl groups such as ethyl, isopropyl and butyl [9]. However, MGMT removes methyl groups much faster than other alkyls [9]. It allows a direct repair of the lesion caused by TMZ by removing the methyl group in position O^6 of guanine [10] (Fig. 2). It acts as a suicide enzyme because the fixing of the methyl group on cysteine residue 145 located in its catalytic pocket causes its inactivation [11] (Fig. 2). MGMT is not recycled and undergoes degradation by the proteasome [12]. The levels of MGMT vary widely according to the type of tumor and also within the same type of tumor [13]. The MGMT gene is not commonly mutated or deleted [13]. MGMT expression is correlated to the methylation profile of the MGMT promoter. Some studies have shown that the level of MGMT is inversely related to the density of the cysteine-phosphate-guanine (CpG) methylation in CpG islands [14]. MGMT gene silencing is ensured by hypermethylation of promoter CpG islands [15]. Approximately, 45% of patients with newly diagnosed GBM present a methylation of the MGMT promoter and, thus, respond better to TMZ [16]. Therapeutic molecules that inhibit MGMT such as O^6-benzyl guanine (O^6-BG) and O^6-(4-bromothenyl) guanine have been used in clinical trials preceding treatment with TMZ [8,17,18] (Table 1). These pseudosubtrates gave promising results by enhancing TMZ activity *in vitro* and *in vivo* in tumor cells possessing high levels of MGMT. They react with MGMT by covalent transfer of the benzyl group or bromothenyl group to the active cysteine residue of MGMT and cause an irreversible inactivation of this enzyme. Despite the effectiveness of the association of these pseudosubstrates with TMZ, their high toxicity against normal cells especially bone marrow cells is an obstacle to the use of these molecules [19].

MMR alteration

DNA mismatch repair (MMR) is a system that corrects errors of nucleotide base mismatches generated during DNA synthesis [8]. In the absence of MGMT, the O^6-MG persists and it can pair with thymine. The resulting O^6-MG/T is recognized by MMR and only newly synthesized strands are excised, keeping O^6-MG intact. This repair cycle is repeated when another strand is generated. These futile cycles of insertion and excision of thymine occur and lead to cell cycle arrest and apoptosis [8,20]. An impaired MMR pathway caused by mutations in MMR protein complexes causes a failure to recognize and repair O^6-MG adducts produced by TMZ. This causes DNA replication and allows the cell cycle to continue and thereby makes TMZ less effective [21]. These mutations can be present naturally or acquired by TMZ regimens [22]. Because of its importance, strategies to restore the effect of the MMR system should be developed to improve the effect of TMZ.

BER

This system is involved in the repair of DNA damage caused by oxidizing agents, ionizing radiation or alkylating agents [23,24]. The base excision repair (BER) system comprises proteins and enzymes. Among them, poly(ADP-ribose) polymerase-1 (PARP-1) is an enzyme activated in response to DNA damage and plays an important part in the BER system. This enzyme binds to DNA and begins the synthesis of a poly(ADP-ribose) (PAR) from NAD+, which enables the recruitment of BER complex proteins (XRCC1, DNA polymerase, ligase, FEN-1) and the DNA repair [25].

Please cite this article in press as: Messaoudi, K. et al. Toward an effective strategy in glioblastoma treatment. Part I: resistance mechanisms and strategies to overcome resistance of glioblastoma to temozolomide, Drug Discov Today (2015), http://dx.doi.org/10.1016/j.drudis.2015.02.011