Mashhad University of Medical Sciences (MUMS)

Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

Glioblastoma and the significance of MGMT gene methylation

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ARTICLE INFO

Article type
Review article

Article history
Received: 6 Apr 2014
Revised: 15 Apr 2014
Accepted: 21 Apr 2014

Keywords
Chemotherapy
Glioblastoma
MGMT gene
Radiotherapy
Temozolomide

ABSTRACT

In this research Glioblastoma has been studied as one of the most common brain tumors and a short review of the available therapeutic methods have presented including surgery, radiotherapy, chemotherapy and particularly adjuvant chemotherapy with temozolomide, as the most effective developed treatment. Moreover, MGMT gene promoter methylation has been introduced as an important predictive factor of treatment response to temozolamide. The different mechanisms of methylation and the available literature on its association with patient survival and disease recurrence have been summarized. Taken together, Glioblastoma is a tumor in which the MGMT gene expression can potentially deliver the highest amount of data in comparison to other tumors; as almost every related study has emphasized on the direct association between MGMT methylation and patient survival. Regarding this debate, the pseudoprogression pattern in Glioblastoma patients and the laboratory methods studying MGMT gene methylation have been examined. At the end of this review, the obstacles to its development have been briefly mentioned.

Please cite this paper as:
Izadpanahi P, Anvari K, Fazl Ersi M. Glioblastoma and the significance of MGMT gene methylation. Rev Clin Med.2014;1(3):135-140.

Introduction

Glioblastoma accounts for 75% of malignant glial tumors of the brain. This tumor is the most common primary brain tumor in adults, which has poor prognosis and high mortality rate despite different surgical, chemotherapy and radiotherapy modalities, which occurs in most patients in less than two years from diagnosis. Histologically these tumors are characterized by high cellular proliferation, formation of small blood vessels and local necrosis (1).

Today, the standard treatments applied for GB patients include the use of utmost surgical resection (2) chemoradiotherapy

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with temozolomide (TMZ) (3) and subsequently adjuvant chemotherapy with temozolamide (4).

Several studies have also shown that the addition of temozolomide to radiotherapy might result in the improved progression free survival and overall survival rate of patients. Regarding the high success rate of treatment with this drug, oncology guidelines have recommended the administration of temozolomide concurrent with radiotherapy in patients with good performance status since 2005 (5).

Surgical-based treatment

Surgery is the best opportunity to achieve an accurate and definite diagnosis of the disease. Several studies have shown that complete resection is associated with a more precise diagnosis besides the identification of the oligodendroglial component. Tumor resection resolves the pressure effects and improves the neurological symptoms caused by the tumor. In addition, response to chemotherapy is great following the surgery and leads to prolonged survival. In a study performed in Mashhad, Iran, patients' survival rate doubled after optimal surgical removal of the tumor compared to suboptimal surgery (6).

Many retrospective studies have also shown a higher survival rate following surgical resection of glial tumors of either high or low grade.

Radiotherapy-based treatment

Randomized studies have shown a certain benefit in adding radiotherapy to surgery. Regarding that tumor recurrence mainly occurs around the tumor bed, brain radiotherapy in GB is performed in a local field. Accordingly, the radiotherapy field is defined as 2-3 cm margin around the tumor, which is initially diagnosed by contrast MRI. The total radiotherapy dosage is 60 Gray, which is given through 30-33 sessions. Various studies have shown no additional benefit in using higher doses.

In patients with reduced function and a predicted low survival, radiotherapy with a lower total dose and by higher dose per fraction (30 Gy in two weeks) can be applied for alleviating clinical symptoms (7).

Chemotherapy-based treatment

The role of chemotherapy in the treatment of GB is still a major debate among experts. Adjuvant chemotherapy based on nitrousureas has been used with a moderate increase in patients' survival rate. In most studies so far, chemotherapy has been used based on nitrousureas or in combination with other medications.

In the recent years, the role of chemotherapy in the treatment of GBM has improved due to the introduction of temozolomide and regarding its desirable distribution to the brain and spinal cord. In a phase II study in 1990, temozolomide (TMZ) was found effective in limiting the recurrence of high grade glial tumors.

Thereafter, since 1999, TMZ was used as an alternative for nitrousureas. In recent randomized trials, increased survival was seen in newly diagnosed GB cases under concurrent treatment with radiotherapy and TMZ.

Chemotherapy with alkaloid drugs mainly temozolomide and nitrousureas results in DNA damage with displacement of the alkyl group in different DNA positions including O6-guanine. MGMT (O6- methylguanine-DNA methyltransferase) plays an important role in DNA repair by synthesizing DNA repair proteins. MGMT promoter methylation results in reduced expression of this gene and an increased response to alkylating agents (1).

Chemotherapy response predictor factors

In general, the addition of temozolomide to radiotherapy has proven survival benefits

in the treatment of high-grade glial tumors. One main approach is the determination of certain biological markers, which can outline the group of patients who respond to this type of treatment. For example, in patients with anaplastic oligodendrogliomas, the treatment-response predictor biomarker has a deletion in chromosome 1p19q. In patients suffering from GB, MGMT gene promoter methylation is a key factor that predicts response to treatment with TMZ (7).

The association between level of drug response and MGMT gene methylation

The MGMT gene located on the 10q26 chromosome produces the DNA repairing protein, which removes the alkyl group from the O6 guanine position, the main position for DNA methylation. The alkylation, which takes place in the O6 guanine position due to temozolomide and nitrousureas, causes the methylated guanine to be double with thymidine instead of cytidine in the following DNA replication. This will induce apoptosis and the cytotoxic effects of the drug. The cell can reconstruct guanine by the DNA repair enzyme. Therefore, it inhibits the effects of alkylating agents (Figure 1) (8).

The epigenetic silencing of the MGMT DNA-repair gene by promoter methylation, compromises DNA repair and increases the effect of alkylating agents, thus it is associated with longer survival in GB patients who receive drugs such as temozolamide (Figure 2) (9,10).

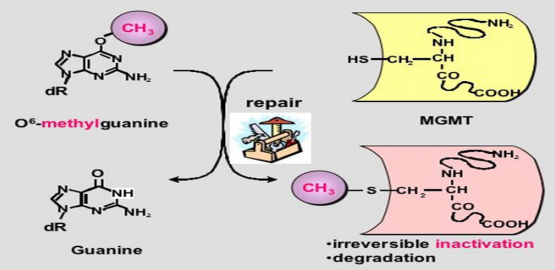

Figure 1. The mechanism of action of temozolomide and the DNA repairing enzyme (DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology. 9th ed)(1)

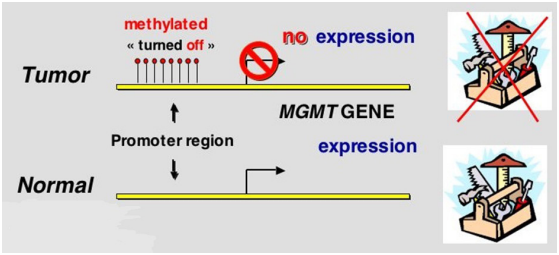

Figure 2. Epigenetic silencing of MGMT mechanism (DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology. 9th ed) (1)

GB Treatment and patient survival

Glioblastom is categorized as grade 4 glioma in the WHO grading system. Despite the aggressive treatment, most patients have a survival of 1 to 2 years (11). Only 3-5% of such patients survive over 3 years and long-term survival is rare (12). Although age, resection and performance status are the most reliable prognostic factors in the survival of such patients, studies have been performed on the factors predicting patient survival and allowing for more effective and beneficial treatment. The effect of surgical resection level has been studied in various studies and maximum tumor resection has been recognized to be associated with a better prognosis in such patients (6).

In addition, surgical resection reduces the need for steroid therapy in the treatment of such cases (13). On the other hand, the surgeon can rarely perform complete resection resulting in no recurrence due to the infiltrative nature of this tumor (14).

The standard treatment for GB is external radiotherapy, which has been associated with an increased survival rate in several randomized trials (15).

On the other hand, all efforts made to use novel methods such as 3D conformal radiotherapy and IMRT with escalating dose have not resulted in improved survival. In addition, studies have similarly shown that hyper- fractionation, accelerated fractionation and boost dose prescription

with brachytherapy has had no substantial benefit in increasing patients' survival; but such new techniques have resulted in fewer neurologic damages (16).

Concurrent chemoradiotherapy and subsequently adjuvant therapy with temozolomide has resulted in remarkable progress in the treatment of GB. As in the study by Stupp et al. (4), in 2005, it was introduced as an standard chemotherapy regimen. In the final analysis of the study published in 2009, the 5-year survival rate of patients under treatment with temozolomide was reported as 10% in contrast to 2% with radiotherapy alone (17).

MGMT methylation and GB patients' survival

Much attention has been paid to the manifestation of MGMT as a potential predictive marker of response to chemotherapy especially to alkylating agents such as temozolomide (18). The non-demonstration of the protein produced by the MGMT gene (DNA-repairing enzyme), which occurs by methylation of the gene promoter, results in reduced DNA repair and the accumulation of the alkyl groups allowing the alkaloid drugs to have a further effect (19). Although the MGMT protein manifests in many tumors including the colon, head and neck and lungs, GB is still one of those tumors that MGMT expression can potentially give a vast amount of information (8).

The mean methylation of the MGMT gene has been reported as 40-60% in different studies (18). In the largest study on GB patients with more than 3-year survival, the rate of MGMT methylation was reported as 74%, which showed a direct correlation between MGMT methylation and patient survival (20). Moreover, in two other case reports on two GBM patients with more than twenty years survival, additional mentioning of other factors responsible for their prolonged survival, MGMT methylation was also positive (21,22).

In 2013, a meta-analysis was published, which had surveyed the findings of 24 studies. In 22 studies, a significant relationship was found between MGMT methylation and overall patients' survival. In 12 studies, an association between MGMT methylation and disease-free survival was reported (23). Other studies have also confirmed this association (24).

In addition to the mentioned studies, other reports have been published investigating the quantitative association between the percentage of MGMT methylation and patient survival, mainly Dunn et al study. In this study, 109 patients diagnosed with GB underwent tumor surgical resection followed by chemoradiotherapy with temozolomide. Based on this study, all patients with MGMT methylation had a greater overall survival rate in comparison to the unmethylation group. Moreover, those with higher than 35% methylation had a better overall survival and disease progression-free survival compared to the group with lower methylation (25).

MGMT methylation study method

Different methods have been introduced for the detection of the level of MGMT methylation including DNA studies, RNA studies and studying the level of protein that each method have partial advantages and disadvantages. The Methylation Specific PCR (MSP) method is the most common and most sensitive method for studying methylation in the MGMT gene promoter, which is used in most clinical trials (26).

In the mRNA expression method, the methylation of MGMT gene promoter DNA can result in altered mRNA expression, which is only detectable in the fresh sample of the tumor (26). Eventually, in the MGMT expression by immunohistochemistry method (IHC), the MGMT protein can be evaluated by the IHC method with the anti-MGMT antibody. However, to date, all

studies even with large sample sizes, have failed to find an association between the results of this method and the MSP one (27).

MGMT methylation and GB recurrence

The prognostic and predictive value of MGMT gene promoter methylation in the recurrence of GBM has not been fully proven yet. Brands et al study of 33 patients with GB recurrence showed no significant difference between the median disease-free survival and the 6 month-disease-free survival between patients with methylated in comparison to unmethylated patients (28).

In another study, methylation was seen in 17 of the 36 cases with recurrence. However, the disease-free survival rate did not differ between the methylated and unmethylated groups (29).

MGMT methylation and pseudoprogression in GBM

Pseudoprogression has a specific neuroradiological pattern in which patients receiving chemotherapy with temozolomide, imitates the disease progression. It seems that this is a false positive sign, which is observed in the first trimester after completed chemoradiotherapy. It may also have a higher prevalence in patients with MGMT methylation. A study conducted in 2008 showed that among the 103 GB patients, pseudoprogression occurred in 38 cases from which a significantly greater number were methylated (30).

Conclusion

Finally, it can be concluded that many studies approved the association between MGMT gene methylation and the outcome in the GB. On the other hand, in order to be able to use MGMT gene methylation as a routine and successful lab technique for assessing the treatment response of GBM to temozolomide, progression in two parallel therapeutic fields seems essential. Firstly, parts of the MGMT promoter gene should be defined genetically, which can more precisely predict the response to the treatment. Moreover, other chemotherapic drugs should be introduced in addition to TMZ as an alternative in unmethylated patients so that the evaluation of methylation by itself does not rule out the contemporary most effective treatment for this group of patients. Taken together, reducing the cost of this lab test can surely result in its higher application.

Acknowledgement

We would like to thank Clinical Research Development Center of Ghaem Hospital for their assistant in this manuscript. This study was supported by a grant from the Vice Chancellor for Research of the Mashhad University of Medical Sciences for the research project as a medical student thesis.

Conflict of Interest

The authors declare no conflict of interest.

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