Phd THESIS

ABSTRACT

NEW TREATMENT STRATEGIES FOR GLIOBLASTOMA MULTIFORME IN VITRO

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TABLE OF CONTENTS

1. BRAIN TUMORS. OVERVIEW .................................................................................. 1

2. GLIOBLASTOMA ..................................................................................................... 1
   2.1. Tumor heterogeneity and recurrence (classical, proneural, neural and mesenchymal subtypes) ............................................................................................................ 1
   2.2. The genetic and molecular factors in glioblastoma ........................................ 1
       2.2.1. Epithelial growth factor receptor (EGFR) ................................................. 1
       2.2.2. PDGFR ...................................................................................................... 2
       2.2.3. PTEN ......................................................................................................... 2
       2.2.4. MGMT ...................................................................................................... 2
       2.2.5. IDH1/2 ...................................................................................................... 2
       2.2.6. β-arrestin .................................................................................................. 2
   2.3. GBM therapy ................................................................................................... 2

3. PERSONAL CONTRIBUTION .................................................................................... 3
   3.1. WORK HYPOTHESIS AND GENERAL OBJECTIVES ............................................. 3
   3.2. RESULTS AND DISCUSSION .............................................................................. 4
       3.2.1. Meta-analytic investigation of dendritic cell vaccination and viral therapy in patients with malign glioma. .......................................................... 4
       3.2.2. The effect of EGFR inactivation on malign glioma cells viability .......... 4
   3.3. STUDY OF Β-ARRESTINE 1 TRANSFECTION INFLUENCE ON MALIGN GLIOMA CELL PROLIFERATION AND ON THE RESPONSE OF E TREATMENT ............... 5

4. CONCLUSIONS ........................................................................................................ 9

BIBLIOGRAPHY ……………………………………………………………………………………………………..9
1. BRAIN TUMORS. OVERVIEW

Brain tumors are the most severe form of tumors, considering the fact they are malign cell masses that affect vital systems in neurological balance regulation (1). They are considered to be primary, classified according to the type of cell that generated them and secondary – cerebral metastases (2).

Gliomas are the most frequent type of central nervous system tumors, representing almost 80% of primary brain tumors. These types of tumors can develop at any age, with a peak in the 5th-6th decade. Glial tumors are characterized by an important intratumoral heterogeneity, proliferative potential and tumor recurrence (1). Taking into consideration these properties, the overall survival is less that 15 months, despite the latest therapeutic approaches (3).

The study of histological characteristics of gliomas is crucial in identifying the molecular mechanisms underlying the actual limitations of therapy and their resistance to chemotherapy and radiotherapy.

2. GLIOBLASTOMA

The overall survival in glioblastoma, the most aggressive, invasive and undifferentiated brain tumor, is 14.6 months (4), despite modern multimodal therapeutic modalities.

2.1. Tumor heterogeneity and recurrence (classical, proneural, neural and mesenchymal subtypes)

Glioblastoma is known for its’ heterogeneity regarding signaling pathways, tumor site, phenotype, genetics, epigenetics and molecular properties.

2.2. The genetic and molecular factors in glioblastoma

2.2.1. Epithelial growth factor receptor (EGFR)

Specialized studies from the last decade have shown that over 50% of GBM present EGFR mutations. The most common is EGFR amplification (approximately 40% of EGFR amplification are EGF mutant) (3).
2.2.2. PDGFR

PDGFR overexpression is present in ~23% cases of GBM, having unfavorable repercussions on patients prognosis and survival with IDH1 mutation.

2.2.3. PTEN

40% of GBM cases have shown an early diminution of PTEN expression (5-7), and in 50-70% of recurrent GB, respectively in 50-90% of primary GB was found a loss of chromosome 10 heterozygosis (8-9).

2.2.4. MGMT

MGMT methylation determine a growth in tumor cell sensitivity to the action of alkylant agents like TMZ. In low grade gliomas, the role and clinical impact of MGMT methylation status is still in scientific debate.

2.2.5. IDH1/2

Recent studies have shown that the most frequent mutation in gliomas is IDH1 R132H (10). Also, there have been observed IDH mutations in 80-90% of grade II and III gliomas (10-11).

2.2.6. β-arrestin

A recent in vitro study conducted on GBM tumor cells has shown a growth of their sensitivity to TMZ through β-arrestine1 gene activation (12). The exact role of β-arrestine in gliomagenesis, proliferation and therapeutic response are still insufficient.

2.3. GBM therapy

GBM is still a therapeutic challenge despite multimodal and interdisciplinary approaches (13). The classic therapeutic methods are surgery, chemotherapy and radiotherapy. The new generation of therapeutic modalities consists in molecular target therapy, immunotherapy, viral therapy, adoptive therapy, dendritic cell vaccination and genetic therapy.
3. PERSONAL CONTRIBUTION

3.1. WORK HYPOTHESIS AND GENERAL OBJECTIVES

OBJECTIVE NO. 1
Meta-analytic investigation of viral therapy effect comparing to immune therapy with dendritic cells in malign gliomas.

OBJECTIVE NO. 2
In this study we analyzed the potential of EGFR-targeting on malign glioma cells viability.

OBJECTIVE NO. 3
Study of β-arrestine 1 transfection influence on cell proliferation and on the response of malign gliomas to the treatment.

3.2. RESULTS AND DISCUSSION

3.2.1. Meta-analytic investigation of dendritic cell vaccination and viral therapy in patients with malign glioma.

3.2.1.2. OS and PFS meta-analysis in dendritic cell vaccination
Our systematic analysis integrated 9 specialized studies. In these studies, were included 357 patients, from which 104 patients were in experimental groups and 253 in control groups. Of these, 207 patients were newly-diagnosed with HGG (70 patients in experimental group and 137 patients in control group) and 150 patients were diagnosed with recurrent HGG (34 patients in experimental group and 116 in control group). Regarding therapy combined with dendritic cell vaccination, a PFS improvement was noticed (HR 0.49), with CI 95% 0.21-1.16. Statistically, results of dendritic cell vaccination therapy of HGG patients was not significant (p=0.10).

3.2.1.3. OS and PFS meta-analysis of viral therapy
In the four studies integrated in the systematic analysis of viral therapy we identified 642 newly-diagnosed HGG patients, the experimental group included 237 patients, and control group included 405 patients. In total, the OS meta-analysis integrated 4 studies,
and PFS meta-analysis included 3 studies. In three studies we observed an OS progress, the most notable one being described by Wheeler et al (14) (HR 0,72, 95% CI: 0,54-0,97). On the other hand, Rainov (15) reported a benefit for the patients from the control group (HR 1.08, 95% CI: 0,81-1,46).

3.2.2. The effect of EGFR inactivation on malign glioma cells viability

3.2.2.1. The growth pattern of untreated HGG cells

In this study we analyzed the proliferation of 11 HGG cell line in 7 days.

3.2.2.2. The effect of EGFR inactivation on HGG cells

In this study we investigated the effect of AG556 on 11 HGG cells in order to conclude on the cytotoxicity of this agent. HGG cells were exposed to different concentrations of AG556: 10 µM, 20 µM and 30 µM. The cell proliferation rates were measured in day 3 and day 7. We observed that the most significant cytotoxic effect was reached at the concentration of 30 µM, with a diminish of survival malign cells with approximately 17% in day 3 (Fig 2). This value was constant and there were no significant changes the rest of the experiment, including day 7 of AG 556 treatment (Fig.3).
Fig. 2, 3. The effect of AG556 inhibitor on EGFR inactivation in 11 HGG cell line at 3, respectively 7 days of treatment.

3.3. STUDY OF β-ARRESTINE 1 TRANSFECTION INFLUENCE ON MALIGN GLIOMA CELL PROLIFERATION AND ON THE RESPONSE OF E TREATMENT

3.3.1. The TMZ treatment effect on malign glioma cells

Our experiment tested the cytotoxic effect of the alkylating agent TMZ on two different malign cell lines: 18 HGG and U-343MGa. We analyzed the decrease of malign cell proliferation at 24h, 48h and 72h after treating them with different concentrations of the alkylating agent (200 µM, 250 µM and 300 µM).
24 hours from the beginning of the treatment with 200 µM, we observed a decrease of the cell viability with 17.32%. 250 µM concentration determined a 17.88% decrease and the maximum concentration determined the highest cytotoxicity of 20.17% (Fig. 4). Regarding the prolonged treatment at 48h, we recorded a significant increase of the cytotoxic effect in U-343MGa cell line, as follows: at 300 µM we observed the most important cytotoxic effect (45.3%) (Fig 5). At 72 h from the TMZ treatment initiation, tumor cells cytotoxicity was reduced comparing with the one recorded at 48 hours (Fig 6).

Regarding the second cell line, line 18 HGG, treated with the same concentrations of alkylating agent for 24, 48 and 72 hours, we observed similar values with U-343MGa cell line (Fig 7, 8, 9).
3.3.2. Study of β-arrestine-1 transfection influence on the response of TMZ treatment in U-343MGa cell line

After 24 hours of treatment, β-arr1 transfection countered by ~4% the effect of 200 µM TMZ treatment, but this result cannot be considered statistically significant (p>0.05) (Fig. 10). The results showed that U-343MGa cell line proliferation recorded a significant
growth after 48 hours from β-arr1 transfection (26%), comparing with the control group. (p≤0.05) (Fig. 11). 72 hours from the beginning of TMZ treatment in high dose, the β-arr1 did not prevent cytotoxicity (p≥0.05) (Fig. 12).

3.3.3. Study of β-arrestine 1 transfection influence on the response of temozolomide treatment in 18 HGG cell line

β-arrestine 1 transfection caused the decrease of 18 HGG cell viability and presented a moderate influence on the cytotoxic effect of TMZ treatment.

Fig. 13, 14, 15. The influence of β-arr transfection on TMZ treatment at 24, 48 and 72 hours
4. CONCLUSIONS

Our analysis demonstrated that the therapeutic approach based on dendritic cell vaccination shows an improvement of patients with newly-diagnosed (HR=0.65) and recurrent (HR=0.63) high grade glioma overall survival and PFS (HR=0.49) comparing to viral therapy.

In our experiment, the EGFR inactivation with cytotoxic agent AG556 determined a proportional decrease of cell proliferation with the inhibitor dose. This effect was observed at 3 days of treatment, with no significant changes at the prolonged 7 days of treatment.

B-arr1 transfection determined a growth of cell proliferation in U-343MGa and countered the temozolomide cytotoxic effect.

B-arr1 transfection also determined the decrease of cell viability in 18 HGG cell line and presented a moderate influence on the temozolomide cytotoxic effect.

BIBLIOGRAPHY


