UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
PhD SCHOOL

PhD THESIS

THE STUDY OF THE EFFECT OF MOLECULAR TARGETED THERAPY IN HUMAN HIGH GRADE GLIOMA USING MULTIPLE *IN VITRO* METHODS

ABSTRACT

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Key words: glioblastoma, β-arrestina, eltd1, transfection, cytotoxicity, systematic analysis;

THE STAGE OF KNOWLEDGE

Despite the remarkable progress seen in the therapy of other cancers, treatment of malignant gliomas failed to show any significant improvements in the survival and quality of life of patients suffering from this cancer. The development of new therapies based on preclinical models and the association of these new therapies with older ones, which are already established as effective for the treatment of malignant glioma, is paramount for elaborating therapeutic protocols.

In our study, we focused on the following:

Determination of the cytotoxic effect of transfection using β-arrestin 1 and 2 plasmid in malignant glioma cell cultures;

Determining the cytotoxic effect of transfection using ELTD1 siRNA on in malignant glioma cell cultures;

-a systematic analysis based on the effect of dendritic cell-based immune therapies versus the well-established salvage therapy using the antineoplastic drug, Irinotecan alongside the monoclonal antibody bevacizumab, in patients suffering from recurrent malignant glioma.

PERSONAL CONTRIBUTIONS (EXPERIMENTAL STUDY)

Our study has achieved the following objectives:

OBJECTIVE NO.1. The comparison between the therapeutic effect of bevacizumab alongside irinotecan versus immune therapy using dendritic cells in patients diagnosed with malignant gliomas.

In our study, we performed a systematic review of literature studies for patients who received bevacizumab and irinotecan or dendritic cell vaccination. The results were compared in terms of mean survival, survival gain and weighted survival gain.

OBJECTIVE NO. 2 Evaluation of the effect of β-arrestin 1 transfection on the proliferation and treatment response to temozolomide in malignant glioma cell lines. In our study, we transfected two malignant glioma cell lines, U 343 MGa and CL 2: 6 with β-arrestin 1 plasmid to see how β-arrestin 1 overexpression influences both cellular proliferation and the way the malignant glioma cells respond when exposed to the alkylating agent temozolomide.

OBJECTIVE NO. 3 Evaluation of the effect of β-arrestin 2 transfection on the proliferation and treatment response to temozolomide in malignant glioma cell lines. In our study, we transfected two malignant glioma cell lines, U 343 MGa and CL 2: 6 with β-arrestin 2 plasmid to see how β-arrestin 2 overexpression influences both cellular proliferation and the way the malignant glioma cells respond when exposed to the alkylating agent temozolomide.

OBJECTIVE NO. 4 Evaluation of the effect of transfection with ELTD1 siRNA on proliferation in malignant glioma cell lines.
In our study we transfected two malignant glioma lines, GB5B and GB8B with the ELTD1 siRNA to see how inactivation of the ELTD1 gene influences the proliferation of tumor cells.

RESULTS AND DISCUSSIONS

In our study, we compared the effects of chemotherapy with irinotecan alongside molecular targeted therapy with bevacizumab versus dendritic cell-based therapy in patients with high grade malignant gliomas. A total of 381 patients from 14 different clinical trials were included in our study. 302 patients received irinotecan plus bevacizumab while only 79 of them were vaccinated with dendritic cells. According to the results obtained from processing data published in online literature, dendritic cell vaccination did not bring significant benefits for patients in the immune therapy group. While mean survival was significantly higher in patients receiving immunotherapy, this was not reflected in the survival gain calculated for all patients. Of the total of 7 clinical

trials that were based on dendritic cell therapy, only two of them have shown positive survival gain, proving that this option is similar to other palliative treatment variants for the treatment of high-grade malignant gliomas.

Regarding β-arrestin plasmid transfection, our experiment has demonstrated that β-arr1 gene overexpression enhances resistance to TMZ treatment in two malignant gliomas cell cultures (U 343 MGα and Cl 2: 6). It should be noted that the effect was more pronounced in the Cl 2: 6 cell line. A reasonable explanation would be the phenotypic differences accumulated by the two immortalized cell lines obtained during countless passages. In our experiment, we also analyzed the way β-arr 2 overexpression influences the response to TMZ treatment. In both cell lines, β-arr 2 overexpression influenced the treatment response exclusively for the maximum dose admitted in our experiment, 300 μM TMZ, increasing the sensitivity of the transfected cells to the alkylating agent . For the remaining doses in our experiment, transfection with β-arr 2 produced contradictory effects or did not affect the response to TMZ exposure, at all. Also, β-arr 2 transfection did not significantly affect the proliferation of untreated cells. These results show that β-arr 2 activity is very poorly understood, having both a suppressive and enhancing role for various cancers, underlining the existence of unkwown mechanisms governing the activity of this gene.

In our study, the GB5B and GB8B cell lines were transfected with anti-ELTD1 siRNA and demonstrated unusually high cytotoxicity. These results create an extraordinary number of possibilities in the treatment of malignant gliomas through association with older or newer therapies which, individually, did not show the desired efficiency. Thus, the association between anti-ELTD1 siRNA and other such treatments could potentially, prove beneficial for patients suffering from this incurable cancer, in the future

CONCLUSIONS

STUDY NR.1 Systematic analysis of the effect of treatment with bevacizumab with irinotecan versus immunotherapy based on dendritic cell vaccination. Our study demonstrated that the therapeutic approach based on dendritic cell vaccination was not superior to the therapeutic combination between irinotecan and bevacizumab in terms

of both median survival (P = 0.535) and in terms of the weighted survival gain (P = 0.620) for patients diagnosed with recurrent high grade malignant gliomas.

STUDY NR.2 The effect of β-arrestin 1 plasmid transfection in the U-343 MGa and CL 2: 6 cell lines on proliferation and treatment response. In our experiment, we observed that β-arrestin 1 plasmid transfection caused an increase in cell proliferation in the U-343 MGα and CL 2: 6 cell lines. It was also been observed that β-arrestin 1 transfection counteracts the cytotoxic effect of the alkylating agent temozolomide. The effect of β-arrestin 1 transfection was more pronounced in CL 2: 6 cell culture than in the U-343 MGα cell culture.

STUDY NR.3 The effect of β-arrestin 2 plasmid transfection in the U-343 MGa and CL 2: 6 cell cultures on proliferation and treatment response.

In our experiment, β-arrestin 2 plasmid transfection determined contradictory effects on proliferation and response to treatment. For untreated cells, transfection with β-arr 2 produced both a decrease and an increase in proliferation compared to untransfected cells in the U-343 MGα and CL 2: 6 cell lines. Regarding the response to TMZ treatment, a consistent increase in cytotoxicity was observed for the 300 μM dose alone, with the remaining doses presenting either an increase or a decrease in cytotoxicity, or no statistically significant effect, at all. In order to draw definitive conclusions on the role of β-arrestin 2 on tumor proliferation and response to treatment, in-depth studies are required in order to understand how β-arrestin 2 interacts with various intrinsic mechanisms of malignant gliomas.

STUDY NR.4 Effect of transfection with ELTD1 siRNA on cell cultures GB5B and GB8B.

Transfection with anti-ELTD1 siRNA produced a decrease in proliferation in both cell lines directly proportionate to the dose of siRNA used and the exposure time. Thus, after 72h, the 50 nM ELTD1 siRNA dose inhibited the GBM cell proliferation capacity by a half in the GB5B and GB8B cell lines. This pronounced cytotoxic effect holds great potential because it can be associated with other therapeutic approaches in the hope of producing a synergistic cumulative effect superior to other treatments in monotherapy.

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