UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

Phd THESIS

ABSTRACT

INVESTIGATING THE ROLE OF GROWTH FACTORS AND MESENCHYMAL STEM CELLS IN THE TREATMENT OF BRAIN CANCER

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INTRODUCTION

Glioblastoma, a WHO grade IV malignant brain tumor, is the most common and deadly primary brain tumor, accounting for a therapeutic challenge of the XXI century. Although there is a complex multimodal oncological treatment, the survival rate remains low. The data published in the literature show that the mesenchymal stem cells present a particular tropism for glioma and interact with tumor cells through direct contact or via secreted factors.

Within the tumor, there are disturbances in cell growth and proliferation, to which were added changes in expression of growth factors, resulting in aberrant activation of these receptors. The tumor response to conventional therapy is altered as a consequence to these phenomena.

Key words: glioblastoma, mesenchymal stem cell, growth factor

KNOWLEDGE

CHAPTER I

Glioblastoma is one of the most severe forms of malignant glioma with a poor prognosis and a median survival of 14.6 months despite complex treatment based on surgery, radiotherapy and chemotherapy with temozolomide (TMZ). (Frosina G et al., 2015)

Because glioblastoma treatment options are limited, it is necessary to identify other viable method of treatment. However, despite the overwhelming scientific information about molecular and genomic phenomena in glioblastoma, transforming this data into effective therapies remains mostly without clinical validation.

CHAPTER II

IMPORTANCE OF GROWTH FACTORS IN BRAIN TUMORS

Growth factors act on the tumor microenvironment by controlling angiogenesis and vascular remodeling, inflammation, stromal fibroblasts expansion and recruitment of stem cells, thus modulating tumor growth, invasion and metastasis.

(Hanahan D et al., 2011) For these reasons, investigating the therapeutic potential of inhibitors of growth factors is the correct approach in the glioblastoma treatment.

CHAPTER III

MESENCHYMAL STEM CELLS

The mesenchymal stem cells (MSC) based therapy was lately used both in vitro and in vivo, inducing tumor cells apoptosis and inhibiting liver and prostate cancer metastasis. (Alessandro R et al., 2014) Moreover, MSC therapy proved it's efficacy in a model of Kaposi's sarcoma, an inflammatory angiogenic tumor. (Khakoo Y et al., 2006) MSC also play a role in the development of malignant disease through secreted factors that activates various signaling pathways. The mechanism by which MSC act on tumor growth and metastasis is not yet fully elucidated. The main impediment in interpreting accurately the impact of MSC in malignant diseases is the use of various tumor cell lines and variations in experimental techniques and models. (Anja T et al., 2013)

PERSONAL CONTRIBUTIONS

CHAPTER IV

MATERIAL AND METHODS

The tumor cell lines were established from fresh tissue samples provided by 'Bagdasar – Arseni' Emergency Hospital, Bucharest, Romania from undergoing surgery glioblastoma patients.

HUC-1 and HUC-2 cell lines were established from umbilical cord tissue collected after natural birth in 'Emergency County Hospital', Craiova, Romania. Patients with cromosome anomalies, conenital malformations or other type of pathologies were excluded from this study.

SU1498 (VEGFR2 selective inhibitor) was acquired from Santa Cruz Biotechnologies.

AG1433 (PDGFR-β inhibitor, weak VEGFR2 and angiogenesis inhibitor) was obtained from Sigma Aldrich, Germany.

Tumor cells were treated with various concentrations of small molecule tyrosine kinase inhibitors: SU1498 (0,1 µM, 1 µM, 5 µM, 10 µM, 20 µM, 40 µM, 60

µM, 80 µM), AG1433 (0,1 µM, 1 µM, 5 µM, 10 µM, 20 µM, 40 µM, 60 µM, 80 µM). The MTT test was used in order to determine the antiproliferative effect of SU1498 and AG1433 on tumor cells.

Glioblastoma cells were seeded in 96-well culture plates at a concentration of $2x10^3$cells/well. Afterwards, they were treated with conditioned media from umbilical cord MSC and bone marrow MSC. The conditioned media treatment lasted for 24, 48, 72,and 96 hours. At the end of the tratment, cell proliferation was measured by MTT assay.

CHAPTER V

OBJECTIVES

1. Analysis of the effect of harvested conditioned media from cultured human MSCs (HUC-1-CM and HUC-2-CM) on the GB cells viability (GB1B, GB2B, GB8B).

2. Analysis of the effect of conditioned media collected from bone marrow cultured MSCs (CSM-CM) on the GB cells viability (GB1B, GB2B, GB8B).

3. Analysis of the effect of VEGFR receptor inhibition in glioblastoma (GB10B cell line).

4. Analysis of the effect of PDGFR receptor inhibition in glioblastoma (GB10B cell line).

The study intends to provide a new approach for the high grade primary brain tumor therapy and the required experimental bases.

RESULTS AND DISCUSSION

5.1. HUC-CM effect on GB1B cell line

Our results show that HUC-1-CM and HUC-2-CM inhibit the proliferation of all three cell lines GB compared to untreated control cells.

HUC-1-CM and HUC-2-CM produced a decrease in cell viability in GB1B line by about 30% at 24 hours after treatment. Also, the cytotoxic effect of HUC-1-CM and HUC-2-CM was irreversible on GB1B cell line, persisting until the end of the experiment.

Both HUC-1-CM treatment as well as the HUC-2-CM treatment had cytotoxic effect on GB1B cell line; extending the treatment to 96 hours, did not produce, however, a stronger cytotoxic effect, the percentage of dead cells remaining approximately at the same level, with a slight increase of the viable cells after 72 h of treatment (about 20%).

5.2. HUC-CM effect on GB2B cell line

Regarding the effects of HUC-1-CM treatment GB2B cell line, the highest inhibition was recorded at 48 h (about 37%). The stronger cytotoxic effect of HUC-2-CM (about 32%) was observed after 72 hours of treatment. These values weren't maintained until the end of treatment, GB2B cell viability showing a slight increase.
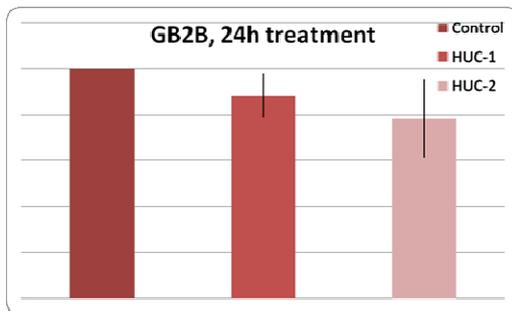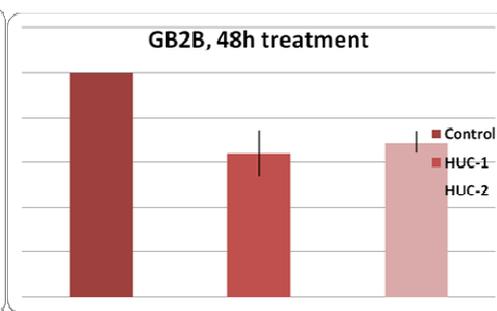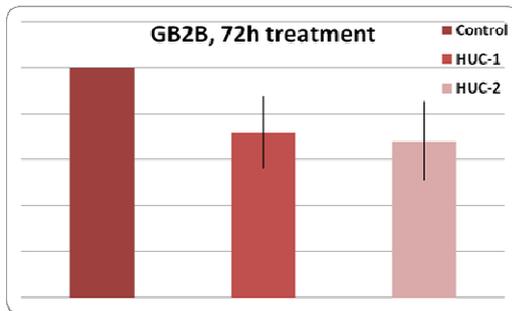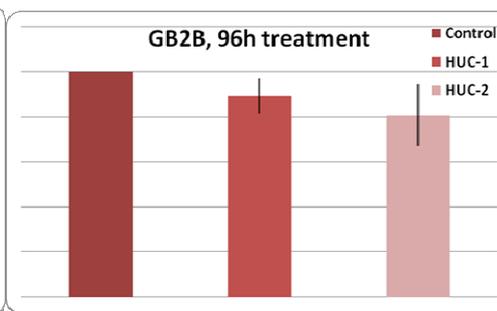

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Figures 1, 2, 3, 4 – HUC-1-CM and HUC-2-CM effect on GB2B cell line at 24, 48, 72 and 96 hours of treatment. Results are expressed as a percentage of control, and the values are the standard deviation of three different experiments.

5.3. HUC-CM effect on GB8B cell line

HUC-1-CM reduced the cell viability in the GB8B cell line by about 30% at 24

and 48 hours after treatment, then a decrease in the environment cytotoxicity of the cells belonging to the line GB8B was observed. However, at the end of the treatment there was a slight increase of the cell population. On the other hand, the effect of HUC-2-CM on GB8B cells was irreversible, cell viability decreased by approximately 30% after 24, 48, 72 and 96 hours of treatment.

5.4. CSM-CM effect on GB8B cell line

Regarding CSM-CM, there was a statistically significant enhancement in all glioblastoma cells within 24 hours, whereas prolonged treatment for 96 hours showed a minor decrease in GB1B and GB8B cell viability and a stimulated cell proliferation in GB2B cell line

5.5. AG1433 treatment effect on GB10B cell line

The GB10B treatment with the inhibitor of PDGFR, AG1433 determined a linear effect on cell viability at 48 hours, the maximum dosage of inhibitor causing a maximum decrease of cell population, while the minimum concentration of inhibitor determined the lowest decrease in cell viability.

5.6. SU1498 treatment effect on GB10B cell line

Treatment with minimum concentration of SU1498 inhibitor for 48 and 72 hours resulted in a minor cytotoxic effect on the GB10B cells. By progressively increasing concentrations of the inhibitor up to 80 µM was observed a continuous decrease of cell viability to the amount of 44.28% at 72 hours of treatment.

CONCLUSIONS

1. Factors secreted by mesenchymal stem cells isolated from umbilical cord caused a decrease in cell viability in all studied glioblastoma cell lines:

In GB1B cell line, after 24 hours of mesenchymal stem cells conditioned media treatment produced a decrease of cell viability with approximately 30%, prolonging treatment to 96 hours did not produce a stronger cytotoxic effect, but it was maintained a decrease in cell viability

In GB2B cell line, the highest decrease of cell viability (about 37%) was recorded at 48 hours of treatment with HUC-1-CM,while the strongest cytotoxic effect on tumor cells was recorded at 72 h from start of treatment with HUC-2-C

In GB8B cell line: HUC-1-CM reduced cell viability at 24 and 48 hours of treatment with 30%. At the end of the treatment was observed a slight increase in GB8B cell population. HUC-2-CM produced an irreversible cytotoxic effect up to 72 hours, and there was a slight recovery of cell viability at 96 hours of treatment, but the difference was not statistically significant ($p>0,05$).

2. In conclusion, the set of values with statistical significance is found for both lines of mesenchymal stem cells isolated from umbilical cord (HUC-CM-1 and HUC-2-CM) at 24-48 hours of treatment. The most significant inhibition was reached after 48 hours of treatment for HUC-1-CM cell line andafter 72 hours of treatmentfor HUC-2-CM cell line. On the last day of treatment with the conditioned media was observed an average percentage of tumor cell death of about 16% in all glioblastoma cell lines.

3. Factors secreted by mesenchymal stem cells isolated from bone marrow (CSM-CM) caused a statistically significant increase in all glioblastoma cell lines in the first 24 hours and a minor decrease of cell viability in GB8B and GB1B lines together with the proliferation of GB2B cell proliferation.

4. Inactivation of growth factor receptors by the use of PDGFR inhibitors - AG1433 induced a cytotoxic effect on glioblastoma tumor cell line.

5. Inactivation of growth factor receptors by the use of VEGFR inhibitors –SU1498 produced a linear decrease of glioblastoma cells viability.

6. Glioblastoma cell lines used in this study reacted differently to the analyzed treatment types in this study, suggesting the need to implement individual therapy.

7. The results from this study were consistent with those demonstrated in previous publications and shows that glioblastoma respond to therapy with both mesenchymal stem cells isolated from umbilical and to tyrphostins inhibitors monotherapy.

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