Fibronectin-adherent Peripheral Blood Derived Mononuclear Cells as Paclitaxel Carriers for Glioblastoma Treatment: An In Vitro Study

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Introduction

Glioblastoma (GBM) represents the most aggressive malignant brain tumor in adults, with a risible median life expectancy despite gold standard treatment. Novel drug-delivery methods have been explored. Here we evaluated the possibility to use mononuclear cells (MCs) belonging to the monocytic-dendritic lineage as drug-carrier.

Methods

MCs were obtained from ten patients harboring a GBM, and from healthy volunteers, considered as controls. GBM tissue was also obtained by patients. MCs were cultured and the adherent population on Fibronectin (FN-MCs), after immunocytochemistry and flow-cytometry characterization, was loaded with Paclitaxel (FN-MCs-PTX). Anti-proliferative and migration activity of FN-MCs-PTX was evaluated in two-dimensional and three-dimensional co-culture assays with red fluorescent U87-Malignant Glioma cells and primary GBM cells. Anti-angiogenic properties of FN-MCs-PTX were tested

Results

Phenotypical characterization showed a high expression of monocytic-dendritic markers in GBM cells and FN-MCs. FN-MCs demonstrated to effectively uptake PTX and to strongly inhibit GBM growth in vitro (**Figure 1**; $p<0,01$). Moreover, tumor-induced migration of MCs, although partially affected by the PTX cargo, remained statistically significant when compared to un-primed cells and this was confirmed in a 3D Matrigel model ($p<0,01$) and in a Trans-well assay (**Figure 2**; $p<0,01$). FN-MCs-PTX also disclosed considerable anti-angiogenic properties.

Figure 1

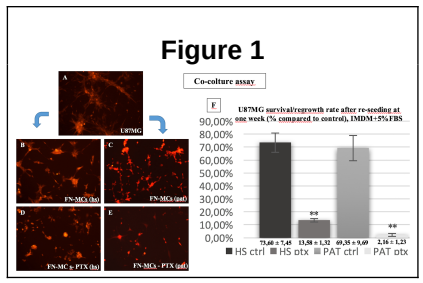
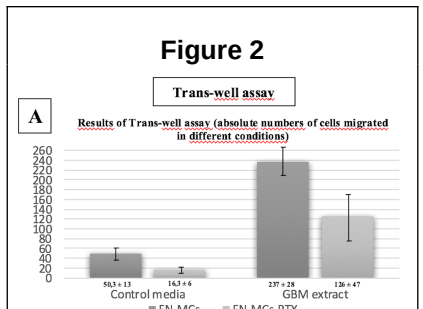


***Figure 1**. **A-E**. Microphotographs with red filter activated, 5th day of 1:1 FN-MCs and FN-MCs-PTX/U87MG co-culture (magnification 10X). A. U87MG control. B, C. Co-culture between U87MG and healthy subject (B) and patient (C) FN-MCs. D, E. Co-culture between U87MG and healthy subject (D) and patient (E) FN-MCs-PTX. Patient's cells demonstrated a higher anti-proliferative activity against GBM cells growth. **F**. Percentage of survived/regrowth U87MG cells after re-seeding at seven days at 1:1 co-culture proportions with tumor cells. The greatest activity was seen using FN-MCs-PTX from patients, where only 2,16 ± 1,23% of U87MG cells were still alive at culture day 7 ($p<0,01$) compared to control, but statistical significance was also found using FN-MCs-PTX from healthy subjects (13,58 ± 1,32% of U87MG cells still present at seven days, $p<0,01$). Un-primed FN-MCs from healthy subject and patient showed, instead, a not statistically significant efficacy in inhibiting tumor proliferation.*

Figure 2



B	FN-MCs vs FN-MCs-PTX (absolute numbers of cells/10X picture)	statistical significance
Control media	50,3 ± 13 vs 16,3 ± 6	p<0,01
GBM extract media	237 ± 28 vs 126 ± 47	p<0,01
	Control media vs GBM extract media (absolute numbers of cells/10X picture)	
FN-MCs	50,3 ± 13 vs 237 ± 28	p<0,01
FN-MCs-PTX	*16,3 ± 6 vs 126 ± 47*	*p<0,01*

Conclusions

Our results suggest that the Fibronectin-adherent population of MCs isolated from peripheral blood can be an effective tool to inhibit GBM growth. Given the relatively facility to obtain such cells, the short time needed for their culture and drug loading this approach may have potential as an adjuvant therapy for GBM.

***Figure 2**. **A, B**. Analytical results of Trans-well assay, comparing FN-MCs/FN-MCs-PTX migration in control media and GBM extract conditions. Results are reported as absolute numbers of cells per 20X-magnification microphotograph. As shown, PTX significantly affected FN-MCs motility, but migration capacity of FN-MCs-PTX, stimulated by GBM conditioned media, remained largely increased, if compared to FN MCs-PTX growth in control media (16,3 ± 6 126 ± 47 cells, $p<0,01$).*

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the importance of Fibronectin-adherent population of MCs isolated from peripheral blood in cell therapy for glioblastoma 2) Discuss, in small groups, of the future treatments in neuroncology, 3) Identify an effective treatment for Glioblastoma in vitro.

References

"Fibronectin-adherent peripheral blood derived mononuclear cells as Paclitaxel carriers for glioblastoma treatment: an in vitro study".
Marco Schiariti, Francesco Restelli, Paolo Ferroli, Anna Benetti, Angiola Berenzi, Anna Ferri, Valentina Ceserani, Emilio Ciusani, Moris Cadei, Gaetano Finocchiaro, Augusto Pessina, Eugenio Parati, Roberto Pallini and Giulio Alessandri.
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