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Fibronectin-adherent peripheral blood derived mononuclear cells as Paclitaxel carriers for glioblastoma treatment: An *in vitro* study

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Abstract

Background. 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 10 patients harboring a GBM, and from healthy volunteers, considered as controls. GBM tissue was also obtained from 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). Antiproliferative and migration activity of FN-MCs-PTX was evaluated in two-dimensional (2D) and three-dimensional (3D) co-culture assays with red fluorescent U87 Malignant Glioma cells and primary GBM cells. Antiangiogenic properties of FN-MCs-PTX were tested on cultures with endothelial cells. *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* ($P < 0.01$). Moreover, tumor-induced migration of MCs, although partially affected by the PTX cargo, remained statistically significant when compared with unprimed cells and this was confirmed in a 3D Matrigel model ($P < 0.01$) and in a Trans-well assay ($P < 0.01$). FN-MCs-PTX also disclosed considerable antiangiogenic properties. *Discussion.* 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 relative facility to obtain such cells and the short time needed for their culture and drug loading this approach may have potential as an adjuvant therapy for GBM.

Key Words: *drug delivery, Glioblastoma, mononuclear cells, Paclitaxel*

Introduction

Glioblastoma (GBM) represents the most common and aggressive malignant brain tumor in adults.

Despite treatments with the current standard of care, composed of maximal safe surgical resection, followed by radiotherapy with concomitant and adjuvant Temozolomide, the prognosis remains poor [1]. Median life expectancy is limited to 16–19 months, with only 25%–30% of patients still alive at 2 years from the diagnosis [2,3]. To develop more effective therapies, recent years have seen many clinical trials based on kinase and growth factor inhibitors, anti-angiogenic agents, gene therapy and immunotherapy strategies with limited or no efficacy on progression-free survival and overall survival of patients [4–11].

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A major reason for the failure of many therapeutic approaches is the difficulty of delivering therapeutic agents specifically to the brain, since the blood-brain barrier (BBB) prevents the transport of most systemically delivered molecules into the central nervous system (CNS). In fact, although major disruptions occur mostly in the necrotic core of the lesion, the preservation of the BBB in tumor margins may prevent molecules from being successfully delivered. Therefore, it is often necessary to treat patients with high doses of chemotherapeutic agents, which may lead to multi-systemic toxicity [6,12,13]. In light of this, there is an urgent need for therapeutic strategies that can achieve higher local concentrations of therapeutic molecules, limiting collateral systemic effects. A rational approach to implement such a strategy may imply the delivery of therapeutic molecules to GBM. Multiple studies have explored different delivery methods, such as convention-enhanced systems, implantable reservoirs [14], nanoparticles [15], liposomes [16] as well as mesenchymal stromal cells and neural stem cells [9,17–19]. The role of mononuclear cells (MCs) belonging to monocyte and dendritic cell lineages as possible carriers of anti-cancer molecules is intriguing, given their massive presence in the glioma microenvironment [20–22], their ability to cross the BBB in case of inflammatory insults [23,24] and their specific tropism for intracerebral lesions if intravenously administered [25,26].

In this study we used a population of MCs that adheres to fibronectin (FN)-coated dishes because in this cell population are present the so-called endothelial progenitor cells (EPCs) that have been shown to participate in the angiogenesis processes and to specifically localize in hypoxic/ischemic environments such as those in tumors [27,28].

We therefore evaluated the possibility of using FN-adherent MCs (FN-MCs), isolated from the peripheral blood (PB) of patients affected by GBM and healthy donors, as carriers of Paclitaxel (PTX), a microtubule poison that proved to be effective against glioma [19,29], but limited in its clinical efficacy by its inability to cross BBB [30]. To assess if FN-MCs loaded with PTX (FN-MCs-PTX) could inhibit GBM proliferation, co-culture assays were carried out by using red fluorescent U87 Malignant Glioma cells (U87MG), a human GBM cell line [31] and a primary GBM (p-GBM) cell line, obtained from surgical specimens. Antiproliferative and migration capacities of these PTX-primed cells, along with their ability to prevent angiogenesis, were also studied using three-dimensional (3D) Matrigel assays and Trans-well migration tests. Here we demonstrate that the heterogeneous population that belongs to FN-MCs, when loaded with PTX, exerts a strong *in vitro* anti-tumor activity against glioma cells and thus it may represent a promising therapeutic tool for systemic or local administration.

Materials and methods

Patients, tumor specimens and blood samples

This study was approved by the Ethics Committee of the Fondazione IRCCS Istituto Neurologico C. Besta in Milan. Informed written consent was obtained from all the participants to the study. The total duration of the study was 12 months.

PB samples (12 mL) and 1 cc of tumor tissue were obtained during surgery from 10 patients (mean age 63.5 ± 9.9 years) affected by suspected first diagnosis GBM, then confirmed by intraoperative pathological analysis. PB samples (12 mL) were also obtained from 10 healthy donors (mean age 31.2 ± 11.8 years), considered as controls in this study.

All cell cultures were followed and photographed with a Nikon Eclipse TE300 microscope; cell counts were manually performed with trypan blue coloration technique, supported by Adobe Photoshop Premiere.

Immunocytochemical analysis of tumor tissue samples

Tumor tissue samples were collected during surgical removal of the lesion and processed within 1 h from the arrival in the laboratory. After mechanical disgregation with sterile micro-scissors in ˂1 mm pieces, cells were centrifugated at 1250 rpm for 10 min. Then, Collagenase (Sigma) was added to tumor cells to a final concentration of 1 mg/mL and enzymatic digestion proceeded overnight at 37°C. Cells were then centrifuged at 1250 rpm for 10 min and the pellet was re-suspended in Iscove's Modified Dulbecco's Medium (IMDM; Lonza) + 5% fetal bovine serum (FBS, Lonza) and then filtered using –40 and –10 μm filters (Corning), in this order. Immunocytochemical analysis through cytoinclusion technique was performed on –10 μm filtered and unfiltered cells. Briefly, cell pellets from the two different conditions were resuspended in 40 μL of Matrigel (Corning) and, after gelification for 1 h at 37°C, were placed in plastic boxes and fixed in 4% formaldehyde. Cells were analyzed for their expression of different astro-glial and hematopoietic markers: Glial Fibrillary Acid Protein (GFAP), S-100, Neurofilaments (NF), CD14, CD31, CD34, CD146, Factor VIII and VEGF-R2 (KDR) (BD Bioscience).

Isolation and cell cultures of FN-MCs isolated from PB

MCs were isolated from patient and healthy donor PB samples and processed within 1 h from the arrival in the laboratory. Then 12 mL of PB from each participant were collected in a sterile tube containing anticoagulant ethylenediaminetetraacetic acid. MCs were then isolated by density gradient centrifugation with Ficoll-Paque Plus (Amersham Biosciences), following the manufacturer's protocol, and resuspended