HUMAN PLATELETS STRONGLY INTERACT WITH GLIOBLASTOMA (GBM) CELLS: DEVELOPMENT OF A RNAI-TRANSFECTED PLATELET STRATEGY FOR THE TREATMENT OF RELAPSING GLIOBLASTOMA

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INTRODUCTION

GBM is the most frequent malignant brain tumour in adults, with an annual incidence of about 3-4 cases per 100,000 inhabitants in Western Countries. Survival from diagnosis is usually less than 20 months, and after relapse less than 12 months. Current standard therapy consists in surgical resection + radiotherapy + temozolomide but virtually all patients experience tumor relapse [1]. Research is currently focused on new antiangiogenic drugs, immunotherapy, however so far these strategies have failed to produce consistent reduction of relapse and are limited by poor penetration in the central nervous system (CNS) and systemic and CNS toxic effects [2; 3].
Blood platelets have emerged as central players in tumor growth and metastasis [4]. Thrombocytosis is associated with poor survival in patients with GBM [5]. Moreover, GBM patients suffer increased incidence of thrombotic events compared with patients with malignancies outside the CNS and show increased markers of platelet activation, suggesting a strong interaction between GBM and platelets [6]. Platelet-targeted pharmacologic approaches are raising great interest for the prevention and treatment of cancer with promising results [7].

AIMS

- to assess the interaction between human platelets and GBM in vitro and ex vivo
- to test the delivery of RNAi from platelets to GBM cells in vitro and in vivo in mice
- to assess the blockade of GBM growth by therapeutically-designed RNAi delivered by platelets in vitro

METHODS

- Immunofluorescence (IF) of formalin-fixed-paraffin-embedded human brain biopsies from 10 GBM patients (Gliomas IV grade IDH wildtype) using an β1-tubulin antibody (platelet marker).
- Coincubation of U87 GBM cells in vitro with human washed platelets transfected with a fluorescent RNAi and assessment of RNAi localization by flow-cytometry.
- Infusion of human fluo-RNAi-transfected platelets in NSG mice intracranially-implanted with U87s and analysis of tumor mass explant by confocal microscopy for fluo-RNAi.
- Coincubation of U87 with platelets transfected with a RNAi (miRNA-135a mimics) targeting the EGFR pathway for 24h and assessment of cell proliferation and migration.

RESULTS

Human GBM brain biopsies show a strong infiltration of platelets while the adjacent normal brain tissue does not (10/10 biopsies analyzed).

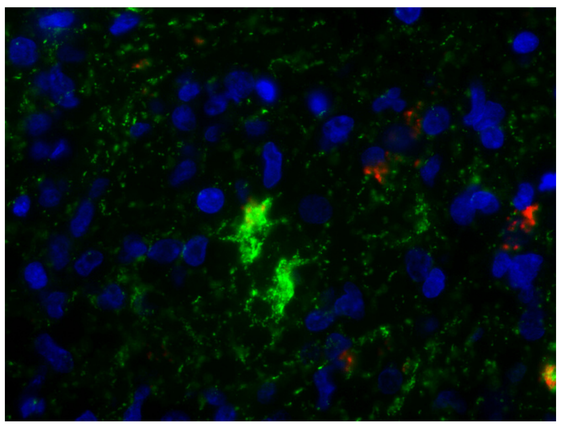
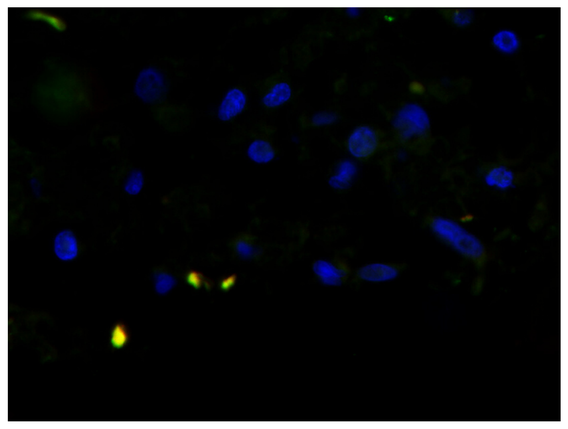


FIGURE 1
A Glioblastoma tissue.
B Healthy brain tissue.

A B

The size of green spot that is positive to beta-1 tubulin indicates that the infiltration is realized by platelet-derived microparticles. In some biopsies it was detected the presence of entire platelets .

Beta-1 Tubulin
Nuclei



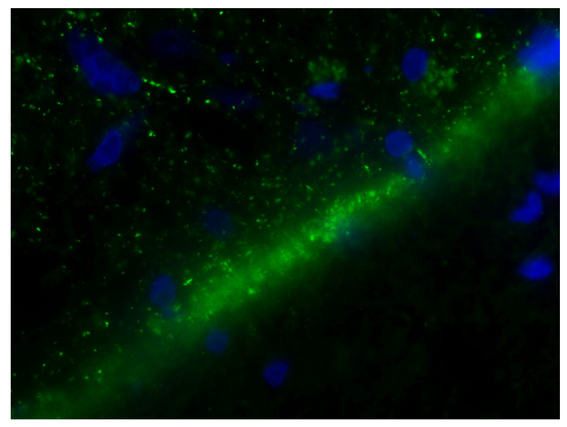


FIGURE 2
In the upper-left side a portion of Glioblastoma tissue.
In the lower right side of the figure the healthy brain tissue. The diffused green light represents the border and it is due to the cut line done with a mechanical pencil by the pathologist for discriminating between pathological and healthy tissue.

Beta-1 Tubulin
Nuclei

HUMAN PLATELETS INTERACT WITH GBM IN VITRO

The interaction between washed platelets and U87 cells causes the increase of P-sel expression (+10.94±1.67%) and PMP production (data not shown) of platelets and leads to the delivery of exogenous siRNA from platelets to GBM cells after 24h and 48h of coincubation (73.8% after 24h - 82.7% after 48h).

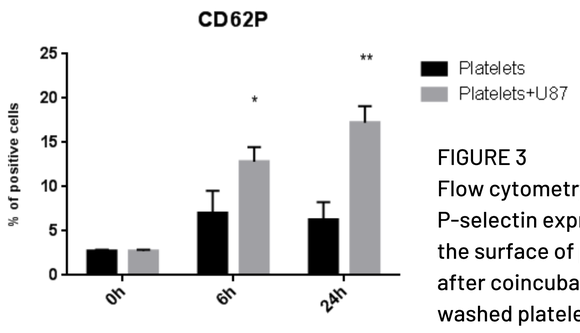


FIGURE 3
Flow cytometry analysis of P-selectin expression in the surface of platelets after coincubation of washed platelets with U87 cells.

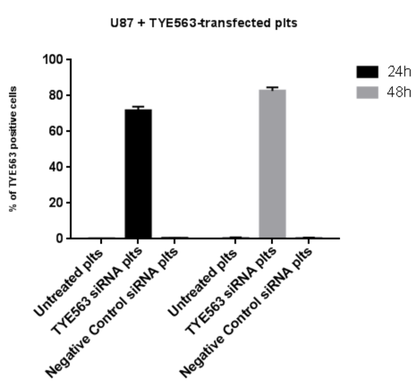
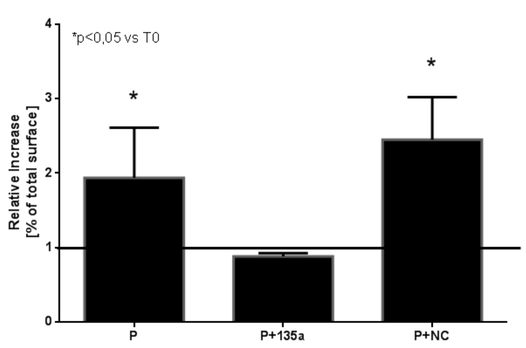


FIGURE 4
Flow cytometry analysis of TYE563-siRNA expression in U87 cells after coincubation with siRNA-transfected washed platelets.

THERAPEUTICALLY-DESIGNED RNAI IS DELIVERED FROM PLATELETS TO GBM

The delivery of miR135a mimics from platelets to GBM cells in vitro causes a significant reduction of proliferation (-54.41±3.14%) and migration (-54.55±7%) of GBM cells.



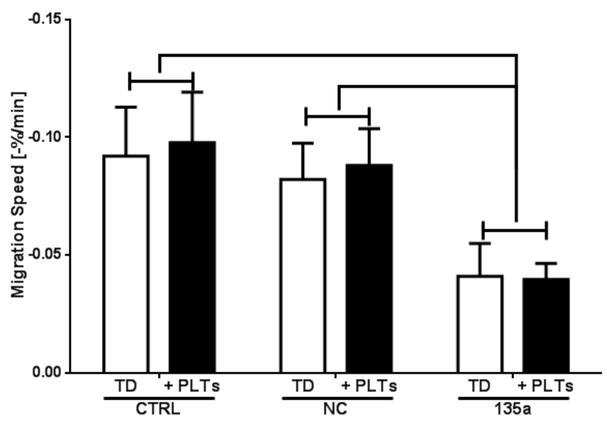
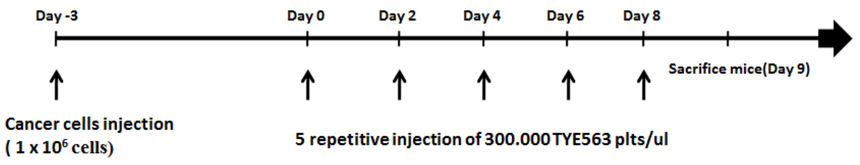


FIGURE 5
Proliferation of GBM cells after coincubation with platelets analyzed as covered surface.
P: Washed platelets.
P+135a: miRNA 135a-transfected platelets.
P+NC: Null control siRNA-transfected platelets.

FIGURE 6
Migration of GBM cells (scratch assay) after coincubation with platelets.
TD: Direct transfection of U87 cells.
PLTS: co-incubation of U87 cells with platelets.
CTRL: washed platelets or cells.
NC: Null control siRNA
135a: 135a miRNA.

RNAI IS DELIVERED FROM PLATELETS TO GBM CELLS IN VIVO IN MICE

In vivo delivery was assessed in NSG mouse model, where U87 cancer cells were injected intracranically (orthotopic xenofraft) and human platelets were I.V. injected 5 times.



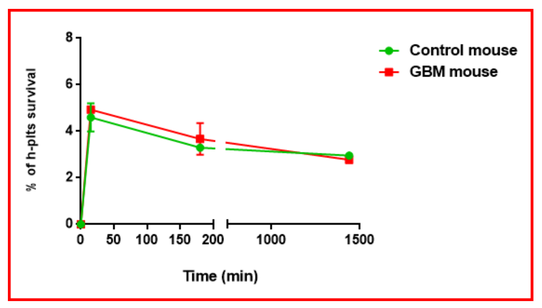


FIGURE 7
Blood was collected after 15 minutes, 3h and 24h post-infusion from tail vein.
Circulating human plts were stained with CD42b-FITC Ab and fluorescence was assessed by cytofluorimetric analysis.

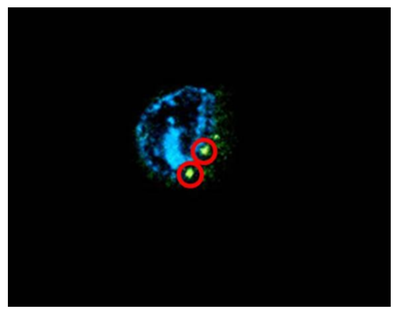


FIGURE 8
Cancer cells, disrupted from tumor area, were stained with hCD41-Alexa 488 antibody and analyzed by confocal microscopy.
Colocalization CD41 (AF488-T2) and siRNA's (Cy3-T1) emission frequencies was detected in cancer cell (highlighted with the red circle).

CONCLUSIONS

Platelets strongly interact with human GBM cancer cells in vitro and in vivo. The strong infiltration of platelets and PMPs in human GBM biopsies indicates a probable prominent role of platelets in GBM growth and/or relapse.
RNAi-transfected platelets (miR125a mimic) deliver their RNAi to GBM cells in vitro and in vivo and inhibit, in vitro, the growth and the migration of GBM cells.
Platelets in GBM pathogenesis, once well characterized, can be used as carrier for the treatment of GBM and the prevention of relapse.

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