GPR56 role on chemotherapy resistance on acute lymphoblastic leukemia (ALL)

Centro Infantil Boldrini

Silveira, A.B.[1] **(IC)**, Laranjeira, A.B.A.[1], Brandalise, S.R.[1], Yunes, J.A.[1]

[1] Molecular Biology Laboratory, Centro Infantil Boldrini, Campinas, SP, Brazil.

Interaction of acute lymphoblastic leukemia (ALL) cells with bone marrow (BM) stroma enhances their resistance to chemotherapy. We found that culture of ALL with BM stroma cells causes downregulation of G protein-coupled recetor_56 (GPR56), a gene reported to have lower expression levels on ALL cases more sensitive to L-asparaginase chemotherapy. We hypothesize that downregulation of GPR56 may somehow directly induce ALL resistance to L-asparaginase. To verify if the modulation of GPR56 by stromal contact is specific or shared by other adhesion G protein-coupled receptors, we analyzed the effect of culturing ALL cells on BM stromal layers on the expression of 4 genes of this family (GPR56, GPR124, LPHN1 and CD97). The expression levels of 17 ALL patients' cells were quantified by Real-Time PCR after 0h or 6h of culture. GPR56 was downregulated after 6h of culture and GPR124 was upregulated. LPHN1 and CD97 were not differentially expressed, which supports the hypothesis that downregulation of GPR56 and upregulation of GPR124 are responses specifically driven by ALL adhesion to BM stromal layers. We quantified GPR56 expression levels on a series of 80 children with ALL, looking for associations between GPR56 expression and patients' clinicobiologic features. Previous findings suggest that GPR56 is implicated in cell adhesion, therefore affecting cancer invasion and metastasis. We found that patients presenting with extramedullary disease showed higher GPR56 expression than others , suggesting a role of GPR56 in ALL invasiveness. Also, patients presenting with very low expression of GPR56 showed less probability of overall survival than others. To investigate whether the dismissed survival of that group is associated with higher chemotherapy resistance and to lower GPR56 expression levels, we are currently silencing GPR56 in ALL cell lines.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most frequent type of cancer in children and treatment failure has been associated with resistance to the chemotherapeutic regime. Microarray analysis of 173 patients' B leukemia cells showed a set of 124 genes differentially expressed in ALL cells resistant or sensitive to four chemotherapeutic drugs. Among them, GPR56, an orphan receptor of the adhesion G protein-coupled receptors family (adhesion GPCRs), was found to have lower expression levels on ALL cells more sensitive to L-asparaginase chemotherapy. GPR56 was also associated with tumor growth, invasiveness and metastasis in other types of cancer exclusively in vivo, raising the hypothesis that GPR56 depends on the presence of tumor microenvironment factor(s) to act.

We found that culture of ALL with bone marrow (BM) stroma cells causes downregulation of GPR56. Interaction of ALL cells with BM stroma enhances their resistance to chemotherapy. This study, aproved by CEP/Boldrini and CONEP, was conducted to investigate whether downregulation of GPR56 has any role in the stromal-induced leukemia resistance.

RESULTS

Co-culture assays

To verify if the modulation of GPR56 by stromal contact is specific or shared by other adhesion G protein-coupled receptors, we analyzed the effect of culturing ALL cells on BM stromal layers on the expression of 4 genes of this family (GPR56, GPR124, LPHN1 and CD97) (Figure 1).

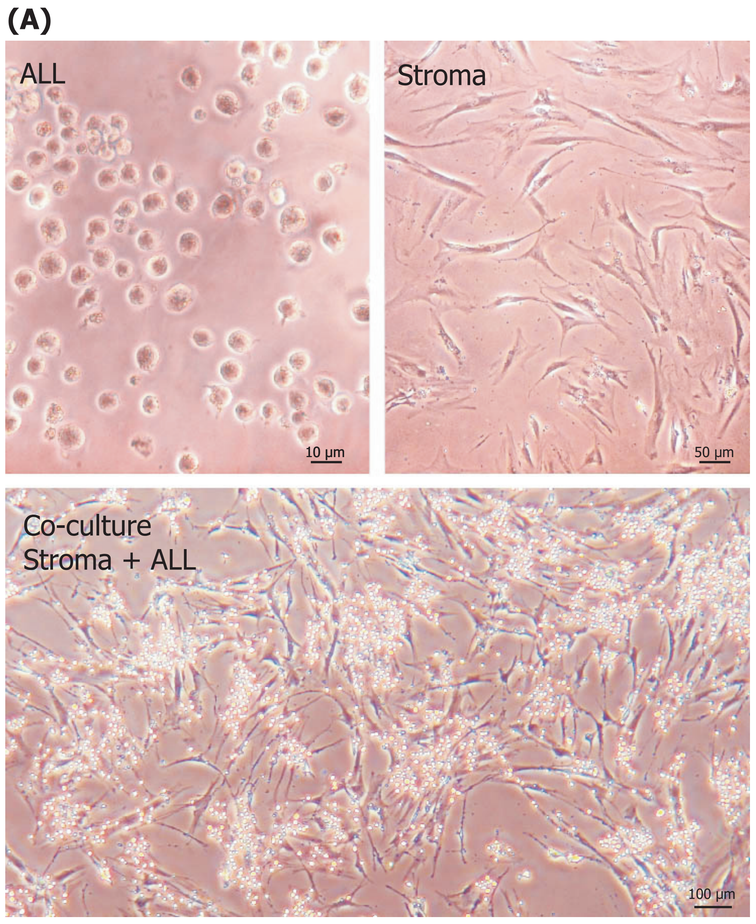

Figure 1. (A) Stromal adherent cells derived from normal donors BM samples were isolated and cultivated in vitro. On the co-culture assays, ALL-B cells from 17 patients were cultivated for 6h on plates covered by a monolayer of stromal cells. (B) GPCRs gene expression was quantified by Real-Time PCR on cryopreserved samples of ALL cells immediately after thawing (0h) or after 6h of co-culture with the BM stromal layers.

Prognostic relevance of GPR56

We quantified GPR56 expression levels by Real-Time PCR on a series of 80 children with ALL, looking for associations between GPR56 expression and the following patients' clinicobiologic features: at diagnosis, (1) age, (2) sex, (3) LLA subtype, (4) leukocyte counting, (5) risk, (6) extramedullary disease, (7) SNC situation, (8) DNA index, (9) presence of 12;21 chromosomal translocation and (10) presence of blasts in liquor; and during treatment, (1) leukocyte counting on day 7, (2) bone marrow situation on days 7 and 14, (3) achievement of remission, (4) occurrence of relapse, (5) overall survival and (6) event-free survival (Figure 2). Any association of GPR56 expression with dismissal prognostic factors would argue in favor of its role on ALL chemoresistance.

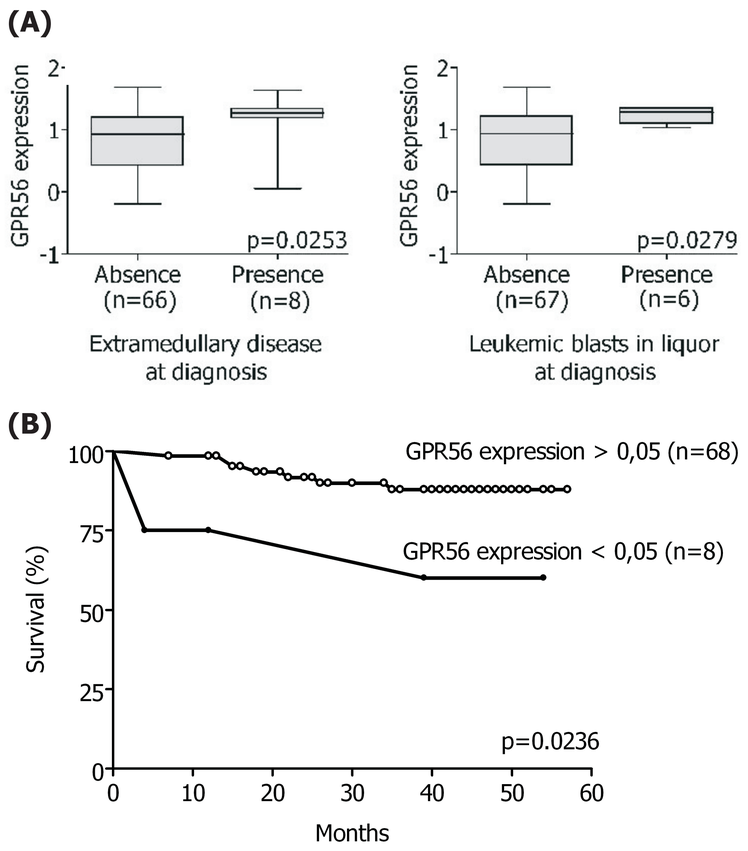

Figure 2. Expression data obtained by RQ-PCR by SYBR Green monitoring. ABL gene as endogenous control. (A) Clinocobiologic features significantly associated to GPR56 expression (Mann-Whitney). Continuous variables were considered using previously defined discrete categories according to the GBTLI LLA-99 protocol. (B) Overall survival of ALL patients categorized according to GPR56 expression levels. Survival curves were plotted according to the method of Kaplan and Meier, and comparison between the curves was performed using the log-rank test. The cut off point was arbitrarily defined.

DISCUSSION

As expected, GPR56 was downregulated after 6h of co-culture. Surprisingly, GPR124 was practically undetectable on cells deprived of contact with stroma (0h) and was upregulated after culture (Figure 1B). GPR124 codes a glycosaminoglicans receptor responsible for cell-cell and cell-matrix contact survival signaling on endothelial cells. We speculate that this protein acts likewise on ALL cells. LPHN1 and CD97 were not differentially expressed (Figure 1B), supporting the hypothesis that downregulation of GPR56 and upregulation of GPR124 are not simply due to a generalized effect of culture (and co-culture) conditions on ALL gene expression.

GPR56 expression at diagnosis was only associated to extramedullary disease and presence of leukemic blasts in liquor (Figure 2A). This may suggest a role for GPR56 on ALL's ability of invasion, which is consistent with its known function in detachment. We hypothesize that GPR56 upregulation may reduce anchorage stability and may enhance ALL potential of invasion.

Our data also indicates that very low expression levels of GPR56 (thus, in theory, an increased cell-cell adhesion) is associated to a dismissed survival (Figure 2B). This may suggest an important role for GPR56 on the induction of resistance patterns to the chemoterapeutic regime on ALL. This finding is consistent with previous reports that showed positive relation between cell-cell adhesion and chemotherapy resistance.

Adhesion GPCRs are known for their role both in signaling and cell adhesion, being able to elicit transduction of cell-cell and cell-matrix adhesion signals to the cell. GPR56 induces upregulation of PAI-1, a protein able to promote cell detachment by internalization of integrins-uPA-uPAR adhesion complexes (Figure 3). GPR56 also activates TCF, a transcription factor involved in regulation of adhesion, cell proliferation and death. Consequently, GPR56 may participate in a dynamic mechanism that controls attachment and detachment of ALL cells to bone marrow stroma and matrix.

PERSPECTIVES

To reinforce results obtained so far, we plan to quantify GPR56 expression in a series of 200 additional patients. To investigate whether the patients' dismissed survival is associated with higher chemotherapy resistance and to lower GPR56 expression levels, we are currently silencing GPR56 in ALL cell lines. These experiments may elucidate several aspects of GPR56 role on ALL.

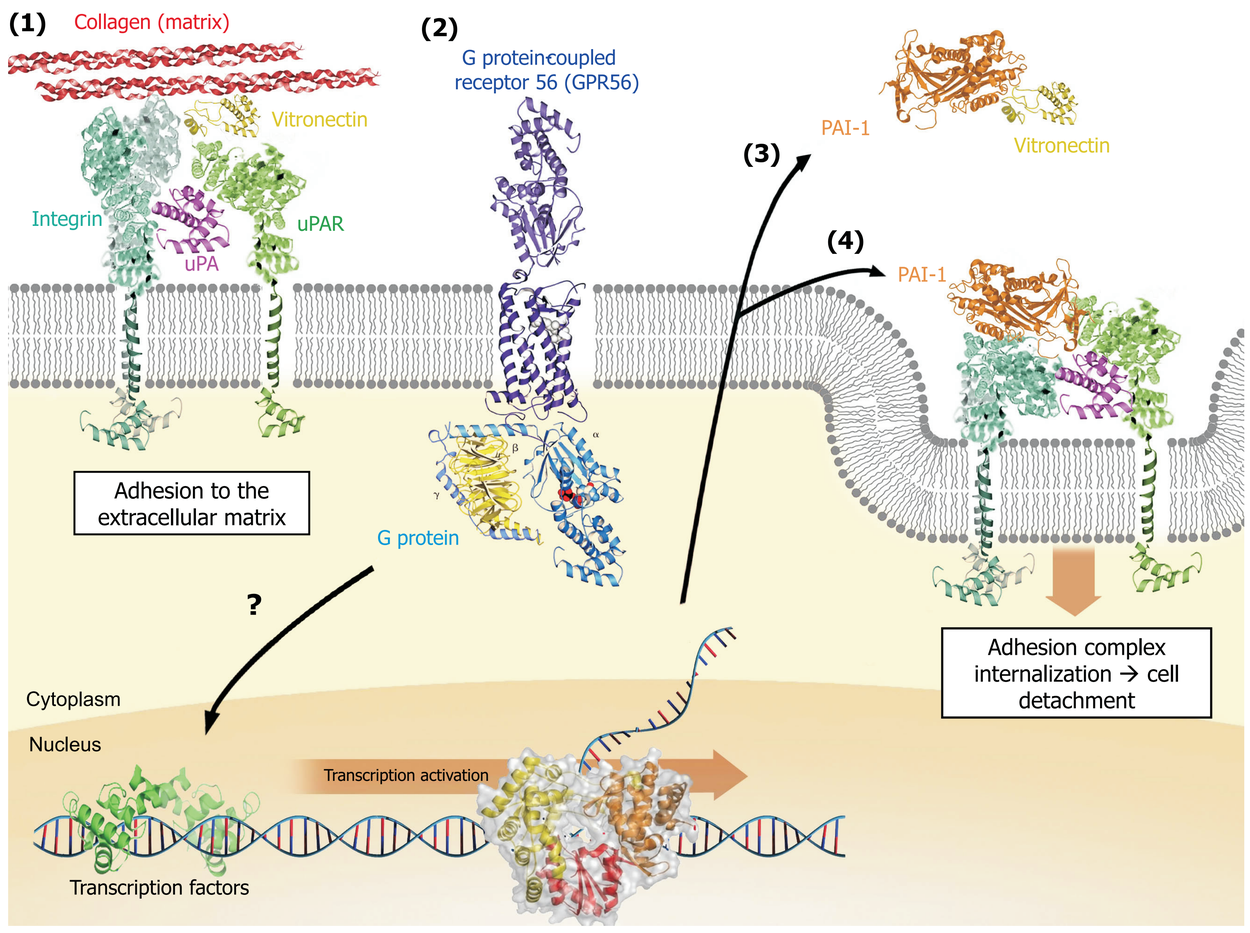

Figure 3. Model depicting proposed events in GPR56 induced cell detachment. (1) Cells attach to their preferred extracellular matrix proteins via integrins and adhesion complexes composed by integrins, uPA and uPAR. If the matrix contains vitronectins, the uPA–uPAR-integrin complexes will bind to it, strengthening cell adhesion. (2) GPR56 signaling induces PAI-1 gene transcription, activates TCF signaling pathway and NFκB by unknown means. (3) PAI-1 released from cells compete with uPAR for the interaction with vitronectins, causing cell detachment. (4) PAI-1 also binds to the uPA–uPAR–integrin complexes, leading to their deactivation and subsequent dissociation from the matrix and cell detachment. The presence of PAI-1 also mediates their LRP-mediated endocytosis. This pathway may represent a general mechanism, since PAI-1 also can detach cells from fibronectin and type-1 collagen by similar mechanisms.