Targeting adhesion and migration by ezrin inhibitor in acute lymphoblastic leukemia cells

Jean Carlos Lipreri da Silva[1], Juan Luiz Coelho-Silva[2], Fabiola Traina[2], Mariana Lazarini[3], Leticia Veras Costa-Lotufo[1], Anne Ridley[4], João Agostinho Machado-Neto[1]

1 Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, Brazil.
2 Department of Medical Imaging, Hematology, and Oncology, Ribeirão Preto Medical School, University of Sao Paulo, Ribeirão Preto, Brazil
3 Institute of Environmental, Chemical and Pharmaceutical Sciences, Federal University of São Paulo, São Paulo, Brazil.
4 School of Cellular and Molecular Medicine, University of Bristol, Bristol, UK.

Background

Acute lymphoblastic leukemia (ALL) is a hematological neoplasm characterized by a block of differentiation of lymphoid precursors at an early stage and that may invade the bone marrow, peripheral blood, and extramedullary sites, being this migratory and invasive effect one of the main causes of relapse and death. Ezrin is an important cytoskeleton-associated protein that allows signal transduction between membrane proteins and actin filaments essential in cell migration and invasion. Given the above the aim of this work was verify the impact of pharmacological inhibition of ezrin on cell adhesion, and migration in ALL models.

Methods

Jurkat, NALM6, and REH cells were treated with ezrin inhibitor, NSC305787. Migration was accessed by transwell assay: the lower compartment was filled with 0.5% BSA RPMI containing CXCL12 and vehicle or NSC305787 (3.2 µM). Then, cells were added to the upper compartment in the presence of a vehicle or NSC305787 and allowed to migrate for 16h. For adhesion assay, 48-well plates were pre-coated with fibronectin (1 µg/well) overnight at 4°C. Non-specific binding sites were blocked with BSA. ALL cells were treated with NSC305787 and then added to fibronectin-coated plates, incubated for 30 min at 37°C. Non-adhered cells were removed by washing. The number of migrated and adhered cells was determined by counting and expressed as a percentage of vehicle-treated cells. Statistical analyzes were performed using ANOVA and Bonferroni post-test, p-value <0.05 was considered statistically significant.

Results

Ezrin signaling contributes migration and adhesion in leukemia cells

Acute lymphoblastic leukemia cells, has a marked tendency to adhere, migrate across the endothelium and disseminate through the central nervous system (CNS) and survive therapy. The capacity of ALL blasts for lodging behind the blood-brain barrier (BBB) would expose them to suboptimal levels of drugs, contributing to poor prognosis and high relapse rates. This phenomenon can be mediated by ezrin and downstream signaling like Rho GTPase proteins. (**Figure 1**)

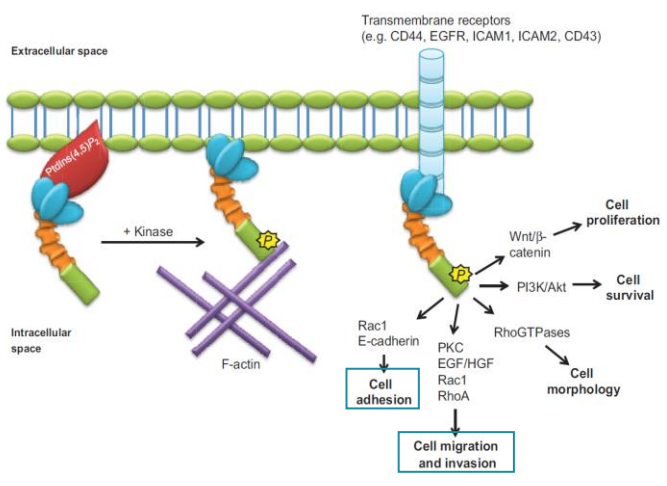

Figure 1. EZR activation and signaling pathways. ERM proteins bind to PtdInsP2 and are then recruited to specific areas of the cell membrane through their N-terminal (**A**) which exposes the conserved threonine residue of the F-actin domain (**B**). Specific kinases can now phosphorylate the conserved tyrosine residue. Once activated, ERM proteins can bind one of several transmembrane receptors and initiate a range of signaling transduction pathways, controling proliferation, survival, **migration, and adhesion** (**C**). Adapted from Clucas & Valderrama. ERM proteins in cancer progression. J Cell Sci 2014;127:267-275

Pharmacological ezrin inhibition reduced adhesion in leukemic cell lines

To investigate the effect of EZR inhibitor treatment on cell adhesion in ALL cells, an adhesion assay was performed on fibronectin, a glycoprotein that has numerous reported functions, including cell-cell adhesion and cell basement membrane. We observed a 50% reduction in binding of Jurkat, NALM6 and REH cells to fibronectin after treatment with 3.2 µM for 6 hours with NSC305787. Using a transwell assay, we observed a strong reduction of CXCL12 (a crucial chemokine that induces cell migration)-induced migration in all NSC305787-treated ALL cell lines ($p<0.05$). This indicates that EZR inhibition may be acting to inhibit ALL cell adhesion. (**Figure 2**).

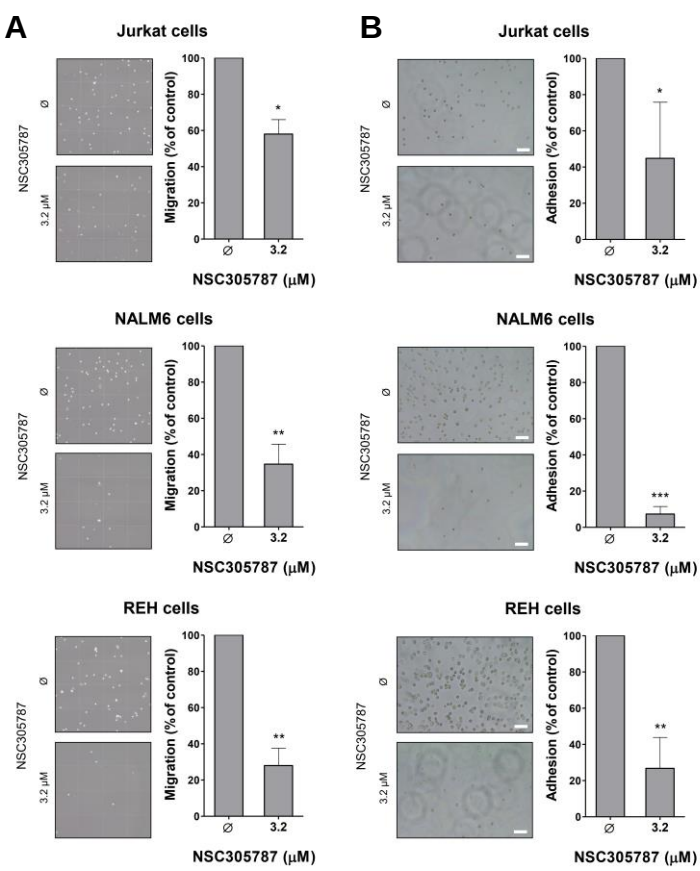

Figure 2. Ezrin inhibition reduces cell adhesion and migration in acute lymphoblastic leukemia cells. Jurkat, NALM6 and REH cells treated with 3.2 µM of NSC305787 and seeded in a Transwell plate and left migrate overnight. Cells were collected, counted and images illustrate one experiment and graphs show the mean ± SD of at least three independent experiments *p=0.05 **p=0.001 (**A**). Jurkat, NALM6 and REH cells treated for six hours with 3.2 µM of NSC305787 and seeded in a flat 48-well plate with a coat of 10 µg/mL of fibronectin. Cells were counted and images illustrate one experiment and graphs show the mean ± SD of at least three independent experiments. ***p<0.0001; (**B**). ANOVA test and post Bonferroni test

Conclusion

One of the major problems of acute lymphoblastic leukemia and one of the biggest causes of relapse and death is its invasive and migratory phenotype, leading to infiltration of various organs such as the liver, intestines and mainly the central nervous system.Our results indicate that the ezrin inhibitor, NSC305787, has anti-invasive effects on ALL by reducing adhesion and migration. Due to the high invasiveness of malignant lymphoblasts, pharmacological ezrin inhibitors may emerge as a novel drug class for the therapeutics of ALLs preventing organ invasion.

Supported by: FAPESP, CNPq and CAPES.

Contact

Jean Carlos Lipreri da Silva
PhD candidate. E-mail: jeanlipreri@usp.br
João Agostinho Machado-Neto
Supervisor. E-mail: jamachadoneto@usp.br