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POSTER 13: Modeling the migration/adhesion of pediatric B ALL cells to study the implication of CD9 in the niche invasion

Océane Guého[1], Alizée Corbel[1,2], Anne-Gaëlle Rio[1], Virginie Gandemer[1,2], Frédéric Mazurier[1]

1. Gene Expression and Oncogenesis team, Institute of Genetics and Development of Rennes, CNRS UMR6290 - INSERM ERL U1305, University of Rennes, Rennes, France.
2. University Hospital, Department of pediatric oncohematology, Rennes, France.

Rational. The *ETV6-RUNX1* (ER) fusion is associated with a good prognosis of B-cell acute lymphoblastic leukemia (B-ALL), the most common pediatric cancer. But, relapse still occurs in 15 % of patients from bone marrow (BM) and extramedullary (gonads and central nervous system) sites, due to chemoresistance. We have found, a downregulation of tetraspanin 29 (CD9) expression in ER+ ALL, compared to other B-ALLs. CD9 KD slowed down the leukemia progression in NSG mice. Our data also showed that B-ALL cell migration rely on CD9 interaction with CXCR4 and activation of RAC1 pathway. In addition, CD9 has been shown to be upregulated by low level of oxygen (hypoxia), a common trait of hematopoietic and extramedullar niches.

Aim. We aimed to get a better view of the level of implication of CD9 in the interactions between B-ALL cells and their microenvironment. To do so, this study intended to set up *in vitro* and *in ovo* models to investigate the migration of B-ALL cells in a microenvironment, and then explore CD9 function in these models.

Methods. The mesenchymal stem cells (MSC) HS-27a cell line was used as a surrogate BM niche. Three B-ALL cell lines, ER+ (REH) and ER- (697, NALM-6) were used to evaluate the migration in Transwell® using HS-27a supernatant as chemo-attractants source for B-ALL cells, and *in ovo* by seeding cells on the chick chorioallantoic membrane (CAM) and quantifying human chimerism by human Alu sequences-based qPCR. B-ALL cells adhesion was examined by coculture with HS27a cells. Blocking antibodies or lentiviral inducible CRISPR/Cas9 vectors were used to inhibit CD9.

Results. MSC-supernatant triggered migration of REH and NALM-6 cells largely though SDF-1. Migration was abrogated by both CXCR4 inhibitor or anti-CD9 blocking antibody but not CD9 KO suggesting possible compensation effect by another tetraspanin such as CD81. In coculture, 697 and

NALM-6 were much prone to adhere to the stroma, but blocking CD9 did not affect the adherent ability of all cell lines. *In ovo*, B-ALL cells have formed a tumor on the upper CAM and have migrated into the lower CAM and in the embryonic tissues. However, there has been a variable capacity with 697-cell line migrating much more than others into the chick BM and brain.

Perspectives. CD9 could be involved in the migration rather than in adherence. We established B-ALL graft *in ovo*, and will test if blocking the CD9/CXCR4/RAC1 pathway unable the dissemination of B-ALL cells in the CAM and embryonic tissues.