Acute Leukemias

DECIPHERING THE CROSSTALK BETWEEN STROMA AND AML CELLS: AN *IN VITRO* MODEL TO UNCOVER THE MECHANISMS UNDERLYING EXTRAMEDULLARY PROGRESSION IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) may present with extramedullary localizations (EM), in which leukemic blasts infiltrate anatomical districts other than the bone marrow (BM) or peripheral blood (PB). Changes in leukemic cells and in the tumor microenvironment (TME) may support this phenomenon. We previously demonstrated that the “metastatic” dissemination linked to EM involvement is triggered by the activation of the epithelial-mesenchymal transition (EMT) program and it is supported by the capability of blasts to digest the extracellular matrix (ECM), thus promoting their motility. We now aim at evaluating the contribution of the tumor microenvironment, particularly mesenchymal stromal cells (MSCs), in enhancing the metastatic potential of leukemic cells *in vitro*.

We successfully established direct co-cultures between OCI-AML3 cells and primary MSCs freshly isolated from AML patients (n=3) and healthy donors (HDs, n=3). Leukemic cells ± 20 mM metformin, which we showed to strongly reduce their migration and invasion potential, were plated on the MSC feeder layer once they reached approximately 80% confluence. At the stopping point of the experiment (48h), Boyden Chamber assays were performed to assess the proportion of transmigrated cells. To study the functional interactions of MSCs and OCI-AML3, we used an 8-well chamber slide where we plated the cells previously labeled with DiD and DiI lipophilic tracers, and we subsequent observed them at confocal microscopy.

Co-cultures of MSCs from AMLs and HDs revealed that MSCs boost OCI-AML3s migration and, in the case of AML-MSCs, also invasion ($P<0.05$). Indeed, healthy MSCs, (HD-MSCs) significantly supported migration of OCI-AML3 cells ($P<0.05$), but we could not appreciate an increased number of leukemic cells capable of invading the ECM coating. Additionally, MSCs mitigated the efficacy of metformin, as compared with OCI-AML3 monoculture. We also hypothesize that the invasive ability of co-cultured leukemic cells relies on a close physical interaction with AML-MSCs involving direct cell contact (**Figure 1A, B**). To prove that, we counted OCI-AML3 cells bound to the surface area of MSCs (AML or HD, ± metformin) and we demonstrated that OCI-AML3 adhere more tightly to AML- than to HD-MSCs (**Figure 1C, D**). Moreover, phalloidin staining underlined the formation of stress fibers in co-cultures with MSCs, which are known to be involved in cell adhesion and migration.

Our results proved that AML-MSCs boost the migration and invasion potential of OCI-AML3s as compared to monoculture and may reduce the impact of treatment. We showed that these processes are linked to the formation of deep intercellular connections with the MSCs substrate, accountable for the aggressive phenotype and tumorigenicity associated with EM spreading.

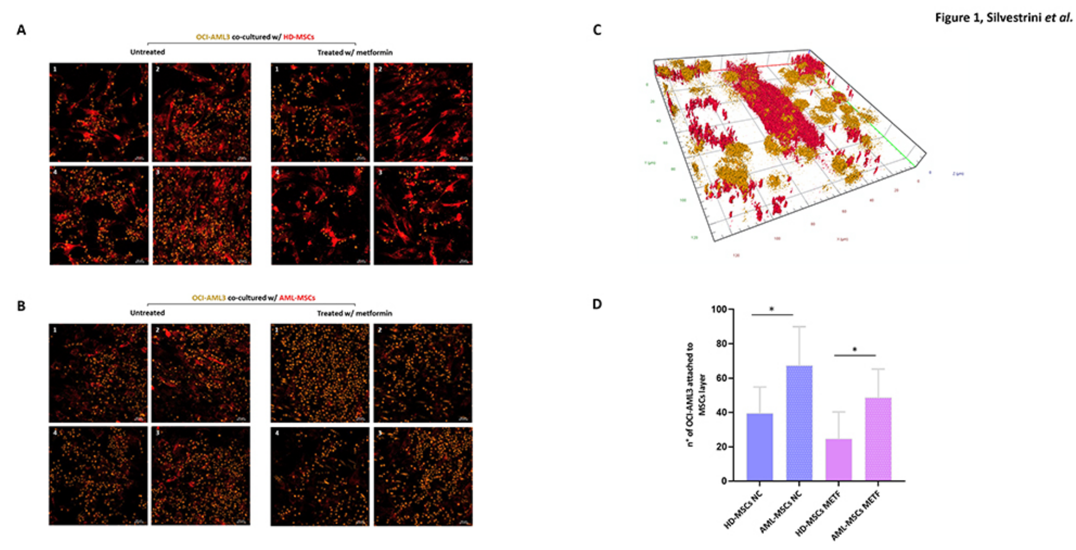

Figure 1. (A,B) Co-culture analysis using high-resolution confocal microscopy for the detection of cell-to-cell adhesion dynamics. Capture images of 4 different fields from each slide. Images refer to the merge of an average of 40 planes along the Z-axis. Images were acquired with a ×10 objective. **(C)** Snapshot 3D image of OCI-AML3 co-cultured with MSCs. OCI-AML3 cells were stained in yellow with DiI (565 nm), whereas MSCs were stained in red with DiD (665 nm). **(D)** Bar chart showing the number of OCI-AML3 cells attached to the substrate of MSCs. Data are presented as media with SD.