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Interaction of hematopoietic cells with the extracellular matrix

Cell interactions with the extracellular matrix (ECM) regulates cell behavior, development, and reaction to external stimuli. In particular, they affect cell proliferation, differentiation of stem and progenitor cells to more mature forms, resistance to apoptosis induction, or cell migration. Leukemic transformation often includes changes in adhesion interactions of immature hematopoietic cells with the bone marrow microenvironment, as well as of more mature cell forms with specific tissues. Leukemia cell engraftment to the bone marrow provides protection against chemotherapy and contributes to residual disease maintenance.

While many regulatory mechanisms implicated in cell adhesion have already been described in adherent cell types, the number of studies focusing on hematopoietic cells is still limited. To analyze leukemia cell adhesion to ECM proteins, we use both the standard end-point method (determination of the cell fraction attached to a coated surface at a given time point) and an alternative approach enabling real-time monitoring of cell interaction with the selected coated surface by means of microimpedance measurement. Cell morphology and adhesion structures are studied using immunofluorescence staining and confocal microscopy (Fig. 1). Interference reflection measurement (IRM) is used to visualize those cell parts, which are in a close contact (less than 100 nm) with the coated surface (Fig. 1, right). Changes in expression or posttranslantional modifications of selected proteins are analyzed by western blotting or by flow cytometry.

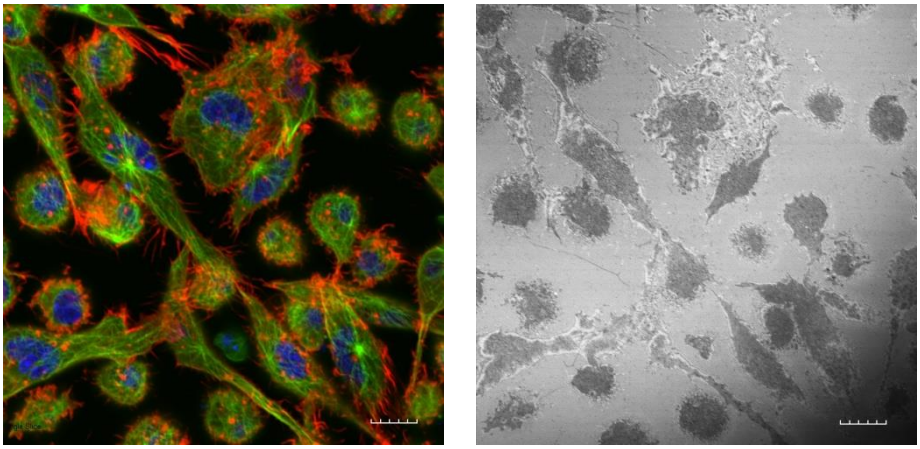

Fig. 1: MOLM-7 cells interacting with a fibronectin-coated surface. Left: immunofluorescence staining, red - actin polymers (stained with phalloidin), green - tubulin, blue - nuclei (DAPI). Right: the same optical field in interference reflection settings (IRM). Scale: 10 µm.

Characteristic proteins can be used to visualize known adhesion structures of adherent cells (focal adhesions, invadopodia) or of some mature forms of hematopoietic cells (podosomes). However, any of the known protein markers we tested (paxillin, vinculin, Lyn, parvin alpha and gamma, HS-1) cannot reliably identify adhesion structures of leukemia cells, which are more dynamic and resemble immature focal adhesions. The only antibody displaying relatively specific localization to adhesion points binds to a phosphorylated residue of the Src family of kinases (predominantly Lyn in leukemia cells) [1]. The kinases of the Src family (SFK) are major regulators of cell adhesion and migration in adherent cells, and the founding member of the family, c-Src, belongs to well-known oncogenes. Increased c-Src activity is associated with higher cell migration, tumor invasivity, and worse prognosis in many types of solid tumors. In chronic myeloid leukemia, SFK activation contributes to development of resistance to therapy by imatinib, and a dual inhibitor of BCR-ABL and SFK (dasatinib) is used to treat patients resistant to imatinib. According to our results, however, SFK have no important role in regulation of leukemia cell adhesion, maybe because the adhesion structures in these cells are not connected to the cytoskeleton [2].

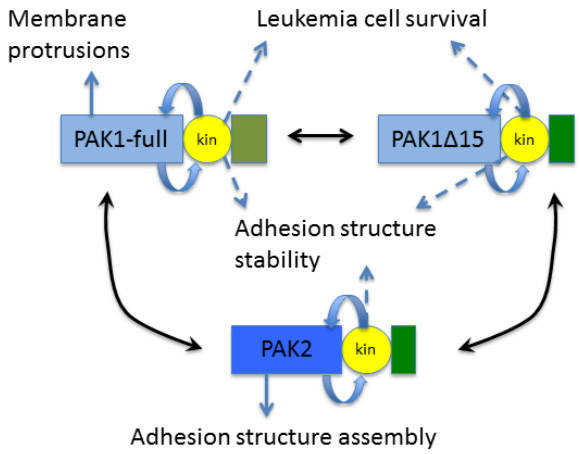

Fig. 2: Scheme illustrating mutual regulation of different PAK isoforms and of kinase and non-kinase PAK functions.

The family of p21-activated kinases (PAK) are other known important regulators of cell interaction with ECM, which were studied mainly in adherent cell types. Nevertheless, it was reported that PAK inhibition using interacting peptide lowered the ability of transplanted stem and progenitor hematopoietic cells to regenerate the hematopoiesis in irradiated mouse recipients. In addition, pharmacological inhibition of PAK induces apoptosis in leukemia cells, indicating PAK involvement in signaling pathways, which are required for cell survival. Possible PAK targeting in cancer therapy is hampered by unsufficient characterization of individual proteins of the PAK family, which have at least partially not overlapping functions. In the frame of a grant project supported by Czech Science Foundation (16-16169S), we described differences between PAK1 and PAK2. We found that a shorter

transcript variant of PAK1 is predominant, and its C-terminal sequence is similar to that of PAK2 [3]. We showed that all PAK isoforms form homo- and heterodimers and thus likely mutually affect their functions (Fig. 2). We have also found that both PAK1 and PAK2 are involved in cell metabolism regulation: PAK2 stimulates the aerobic glycolysis, whereas PAK1 activity is rather associated with a preference for oxidative phosphorylation (4). We studied in detail PAK functions in leukemia cells (5). In collaboration with colleagues from Lausanne and Geneva, we described functional consequences of PAK2 mutation causing Knobloch syndrome. The point mutation E435K results in a marked reduction of PAK2 kinase activity manifested by decreased phosphorylation at the autophosphorylation site Ser141 (6).

Integrins belong to the most important adhesion receptors mediating cell binding to extracellular matrix proteins. Increased expression of some integrin types was associated with worse prognosis of patients with acute myeloid leukemia (AML). According to our results, the stem/progenitor primary leukemia cells, characterized by CD34 expression, have higher surface amounts of the integrin αVβ3, specifically in patients with mutated nucleophosmin (7). Signaling through this adhesion receptor can thus contribute to residual disease persistance in this AML subgroup.

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