Cross-talk between Integrins and Colony-Stimulating Factor Receptor-1 in macrophages

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Adhesion to the extracellular matrix (ECM) is an important phenomenon for cells that is mediated by integrins. Integrins are among major cell surface receptors and play key roles in biological and cellular functions such as cell adhesion, migration, proliferation and differentiation. They can bind to ECM glycoproteins including collagens, laminins, fibronectins and are involved in the organization of the cytoskeleton and in the activation of several signaling pathways. One of these pathways involves the Focal Adhesion Kinase (FAK) which is important for the formation and remodeling of focal adhesions during adhesion and motility, but also for the survival in the absence of growth factors. The role of FAK in macrophages has been long debated. However, we showed that FAK is important for CSF-1R mediated cell spreading and adhesion in these cells. Data available in literature indicate that there is also a pathway that, starting from integrins, involves downstream RTK such as PDGF-R and EGF-R. In particular, adhesion to the ECM is able to promote, through tyrosine phosphorylation, a partial activation of these receptors in the absence of growth factors. The Colony-Stimulating Factor-1 Receptor (CSF-1R) is a RTK that supports the survival, proliferation, and motility of monocytes/macrophages, which are essential components of innate immunity and cancer development. Macrophages interaction with Fibronectin (FN) is recognized as an important aspect of host defense and wound repair.

The aim of the present study was to investigate on a possible cross-talk between integrins and CSF-1R in macrophages.

We used a murine macrophage cell line, BAC1.2F5 cells. Selected results were confirmed using human peripheral blood-derived primary macrophages or NIH/3T3 murine fibroblasts expressing ectopic human CSF-1R (NIH/3T3-Fms cells). For biochemical experiments, BAC1.2F5 cells and human macrophage cells were incubated for 18 h in the absence of CSF-1 before being stimulated or not with different concentrations of FN for different times. Then cells were lysed and protein lysates subjected to SDS-PAGE and immunoblotting. Cell migration induced by fibronectin was measured in 48-wells Boyden chamber.

We assessed first whether adhesion to FN is able to induce FAK phosphorylation/activation in these cells. Adhesion to FN induced phosphorylation of ERK1/2, FAK and paxillin, a FAK known substrate. FAK phosphorylation, depended on Src family kinases (SFK) and cytoskeleton organization since pretreatment with SFK inhibitors (10 μM PP2 or SU6656) or cytocalasin D, an inhibitor of actin polymerization, impaired FN-induced FAK phosphorylation. On the other hand, pretreatment with two different ERK1/2 inhibitors (10 μM UO126 or 30 μM PD98059) partially prevented FN-induced FAK phosphorylation pointing to an involvement of ERK1/2.

Moreover, adhesion to FN induced FAK-dependent CSF-1R phosphorylation. Indeed, CSF-1R phosphorylation was partially inhibited by genetic or pharmacologic FAK inhibition. The fact that FN induced FAK and CSF-1R phosphorylation was obtained also in NIH/3T3-Fms cells indicating that this effect is not restricted to macrophages.

FN dose-dependently induced macrophage migration in BAC1.2F5 cells and human macrophages. FN-induced macrophage migration was dependent on FAK, SFK, ERK1/2 and CSF-1R. Indeed, pharmacologic inhibition of these kinases (inhibitors used being: 1 μM PF-573228 for FAK; 10 μM PP2 or SU6656 for SFK; 1 μM CI-1040 or 10 μM UO126 for ERK1/2; 1 μM GW2580 for CSF-1R) blocked the effect of FN in inducing macrophage migration. Similar effects were obtained after genetic inhibition of FAK or CSF-1R using specific siRNA.

In conclusion, FAK is important for FN-induced macrophage migration and is an important connection between integrins and CSF-1R signaling. Our results indicated that CSF-1R is required for FN-induced migration.

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