1049 SHP-1 IS A CENTRAL MEDIATOR OF GDF-15 MEDIATED ADHESION INHIBITION IN T-CELLS

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Background GDF-15 (Growth and differentiation factor 15) is a distant member of the TGF-beta protein family and has a critical function during pregnancy as a component of the feto-maternal tolerance. It was shown that tumors overexpress GDF-15 to inhibit LFA-1 dependent immune cell infiltration and make use of its immunosuppressive function within the tumor microenvironment.[1 2] Chemokine sensing on the surface of endothelia activates LFA-1 via an inside-out signaling cascade promoting transendothelial migration.[3] While GDF-15 signaling via GFRAL in the brain-stem is well defined, the signaling pathways in T-cells downstream of GDF-15 impairing T-cell adhesion remain elusive. Recent work indicated an involvement of protein tyrosine phosphatase SHP-1 (SH-2 containing phosphatase-1) in GDF-15 mediated inhibition of myeloid cells.[4 5] In this study we investigated the signaling events downstream of GDF-15 inhibiting LFA-1 activation and the involvement of SHP-1 in T-cells.

Methods In flow-adhesion assays, primary T-cells or Jurkat cells pre-treated +/- GDF-15 were perfused over an activated layer of endothelial cells or recombinant adhesion molecules. Adhesion and transmigration were monitored by live imaging microscopy. Intracellular signaling was investigated in high-density-culture restore assays with human PBMCs. Inhibiting bioactive compounds or genetic disruption were used to interfere with pathways of interest. Effects were investigated and validated by intracellular flow cytometry, immunoblot and reporter assays.

Results Treatment with GDF-15 resulted in reduced adhesion of CXCL12 or CXCL9/10 stimulated primary and immortalized T-cells on activated endothelial cells. GDF-15 also reduced the amount of high affinity LFA-1 conformation induced by chemokine CXCL12 and CD3/CD28 co-stimulation. Intracellularly, GDF-15 prevented TALIN phosphorylation in a bead-binding assay and led to a reduction of phosphorylated ZAP-70 in a high-density culture. In flow-adhesion assays, inhibition or deletion of SHP-1 abrogated the GDF-15 mediated adhesion inhibition in T-cells.

Conclusions GDF-15 is an important immune modulator and regulator of immune cell infiltration. In this study we identified SHP-1 as a central mediator of GDF-15-related inhibition of T-cell adhesion. In addition, we confirmed that GDF-15 via SHP-1 inhibits phosphorylation of ZAP-70 that is involved in LFA-1 inside-out activation and T cell receptor signaling. These data connect immunosuppressive GDF-15 to SHP-1, a negative regulator of antigen-dependent activation and proliferation of T-cells.[4] This supports neutralization of GDF-15 as a promising new approach to enhance intratumoral T-cell infiltration and to increase antitumoral immune responses.[1] A clinical Ph1/2a study with our GDF-15 neutralizing antibody visugromab in combination with nivolumab is currently ongoing [GDFather-1/2 trial; NCT04725474, abstract 2122].

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