Immuo-suppressive role of tumor-derived GDF-15 on myeloid cells

Christine Schubert-Wagner1, Irina Giese1, Matthias Kist1, Neha Vashist1, Sabrina Genßler1, Beatrice Haack1, Julia Weigand1, Marlene Auer1, Jorg Wischhusen2, Kathrin Klar1, Eugen Leo1 and Markus Haake1

1CatalYm GmbH, Planegg-Martinsried, Germany; 2Würzburg University Hospital, Department of Obstetrics & Gynecology, Section for Experimental Immunology, University of Würzburg, Würzburg, Germany

BACKGROUND

GDF-15, a divergent member of the TGF-β superfamily, is an important factor of fetomaternal tolerance. Beyond that, GDF-15 is induced in stressed and damaged tissues limiting local inflammation. GDF-15 correlates with poor survival in different cancers and predicts the responsiveness to anti-PD-1 therapy. We have previously shown how GDF-15 interferes with transendothelial migration of immune, especially T cells, from the blood vessels into the tumor microenvironment. Our new data on macrophage polarization suggest that GDF-15 inhibits macrophage effector mechanisms and thus creates an immuno-compromised environment, which can be reversed by antibody mediated neutralization.

METHODS

M0 macrophages were generated from human PBMC derived CD14+ monocytes or THP-1 cells. M0 macrophages were treated with IFNγ (20 ng/ml), GDF-15 (100 ng/ml), TP-1 (40 nM) or CTL-002 (40 μg/ml). Supernatants were collected for CBA analysis, while cells were stained for flow cytometric analysis or used for RNA isolation followed by cDNA synthesis and RT-qPCR. In vivo, 2x10⁶ SK-MEL-6•, mock-transfected or genome-edited SK-MEL-5GDF-15ko cells (clones #15, #21, #25) were injected s.c. into T and NK cell-deficient NOG mice. Tumor growth was monitored. Study was terminated on day 38 or day 35 respectively. Tumor flow cytometry analysis was performed the same day.

RESULTS

Figure 1: GDF-15 interferes with IFNγ induced M1-polarization of M0 macrophages.

A Schematic of macrophage generation and treatment of CD14+ monocytes. B Analysis of cell surface markers by flow cytometry of primary macrophages generated from different donors (N=6). Reduced expression of all four surface was observed in samples treated with IFNγ and GDF-15. C Schematic of macrophage generation and treatment of THP-1 cells. D IFNγ induced M1 polarization of THP-1 derived macrophages which was counteracted by GDF-15 leading to a decrease of analyzed polarization markers. Data normalized to M0 condition and represented as mean ± SD. The schematics were generated with BioRender.com.

Figure 2: GDF-15 inhibits the expression and release of pro-inflammatory cytokines/chemokines and reduces surface expression of Fcγ – receptors.

A Cytometric bead array analysis of cell culture supernatants of primary macrophages after indicated treatments. N=3 of individual donors. Panels show individual donors from left to right for IL-6, IP-10 and I-TAC. B RT-qPCR analysis of primary macrophages after the indicated treatments. Individual donors were shown. Panels show individual donors from left to right for IL-6, CXCL10 and CXCL11. C Surface staining of Fcγ receptors on primary macrophages after the indicated treatments. Data normalized to M0 condition and represented as mean ± SD.

Figure 3: GDF-15 mediated effects can be neutralized by addition of CTL-002 (anti-GDF-15 clinical candidate) or interference with tyrosine phosphatase SHP-1.

A Surface marker analysis in primary macrophages (N=6). Co-stimulation with TP-1, a SHP-1 inhibitor, interferes with IFNγ induced surface marker down-regulation. CTL-002 neutralizes GDF-15 thereby preventing repressive effects on surface markers. Data for CCL2 from Fig.2C and for HLA-DRA Fig.1B. B HLA-DR staining of treated THP-1 WT and THP-1 SHP-1 KO derived macrophages (N=2). Loss of SHP-1 results in loss of GDF-15 effects. C CTL-002 neutralizes GDF-15 in THP-1 polarization assay. Data in A+C are normalized to M0 condition and presented as mean ± SD. D Cytokine secretion of primary macrophages with the indicated stimul with N=3 different donors. Analysis was performed by CBA. Panels from left to right: IL-6, IP-10 (CXCL10) and I-TAC (CXCL11) with individual donors plotted.

Figure 4: GDF-15 prevents adhesion of monocytes in vitro and infiltration of macrophages in the TME.

A Cell-free flow adhesion assay with THP-1: Comparison of adhered monocytes. B No attachment of monocytes to CCL2-coated surface. C and D Cytometric analysis of primary monocytes and THP-1 cells. Adherence of CCL2 and CXCL8 stimulated cells to an ICAM-1 coated surface. E and F Intratumoral immune cell analysis by flow cytometry. Macrophages had higher abundance in SK-MEL-5/GDF15KO tumors and showed a higher degree of activation.

CONCLUSIONS

• GDF-15 interferes with IFNγ stimulated M1 macrophage polarization
• GDF-15 counteracts several IFNγ mediated effector functions of myeloid cells:
  • Fcγ receptor expression (ADCC/ADCP)
  • HLA-DR expression (Ag presentation)
  • Secretion of critical cytokines and chemokines (Inflammation)
• Phosphatase SHP-1 is a central mediator of the anti-inflammatory effects by GDF-15
• GDF-15 modulates not only the quantity, but also the quality of immune responses within the tumor microenvironment
• Neutralization of GDF-15, currently in clinical development, might be a valid approach to reverse an unfavorable tumor microenvironment and to restore responsiveness to natural and therapeutically enhanced immune responses against solid tumors

Conflict of interest statement and contact

Christine Schubert-Wagner is a full-time employee of CatalYm and holds shares in MSD, CatalYm GmbH and RNHale GmbH. christine.schubert-wagner@catalym.com