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Human macrophage programming by GDF-15

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GDF-15 is a multifunctional cytokine involved in immune tolerance that is elevated in stress conditions, correlating with disease severity and survival. GDF-15 is expressed by macrophages and can be endocytosed by the scavenger receptor stabilin-1. The role of GDF-15 in macrophages and its effects on the macrophage transcriptional program have been studied to only a limited extent. The aims of this study were: 1) to develop a model system to study the effect of recombinant human GDF-15 (rGDF-15) on macrophages; 2) to identify the transcriptional program induced by GDF-15 in macrophages by RNA-Seq and validate differentially expressed genes using RT-PCR; 3) to bioinformatically analyze the role of GDF-15, stabilin-1, and GDF-15-mediated pathways in most relevant cancers, particularly in kidney renal clear cell carcinoma (KIRC). Human monocytes were isolated from buffy coats by CD14+ positive selection and differentiated into M0 (non-stimulated), M1 (IFN-γ stimulated) and M2 (IL-4 stimulated) macrophages for 6 days. The selected protocol was as follows: on the day 6 of macrophage differentiation, a pretreatment with 50 ng/mL rGDF15 was performed for 1 hour, followed by a 6 hour challenge with 100 ng/mL LPS. RNA was isolated and total RNA Seq was performed in M0 and M2, revealing that rGDF15 altered the expression of 210 genes in M0 and of 372 in M2. rGDF15 and LPS in M0 and M2 exhibited changes in 230 and 295 genes, respectively. Top upregulated Gene Ontology (GO) terms, in both M0 and M2 treated with rGDF15 were associated with blood vessel morphogenesis and angiogenesis. In M0 and M2 under rGDF-15 and LPS, top upregulated GO terms belonged to TGF-β receptor and cytokine signaling pathways. Thirteen genes were selected for validation by RT-PCR. M1 phenotype was also included for validation. RT-PCR showed that rGDF-15 increased expression of CLEC12A in M0+LPS and M1+LPS, ID3 in M2 and M2+LPS, SMAD6 in M0, M2 ± LPS, and of IL17RB and PMEPA1 in all groups. rGDF-15 suppressed expression of CCL15 in M0+LPS and M2+LPS, GAS7 in M0+LPS and PTP4A3 in M0 and M2 + LPS. This pattern of gene expression suggests an anti-inflammatory effect of rGDF-15 in macrophages. Based on the TCGA database and using TIMER2.0 and the Xena platform, GDF-15 expression in cancer tissues was investigated. Contrary to the pattern seen in other cancers, GDF-15 expression was found to be lower in KIRC compared to normal tissue. Genes induced by GDF-15 (IL17RB and SMAD6) exhibited low expression levels in KIRC compared to normal tissue. Conversely, PTP4A3, downregulated by GDF-15, showed elevated levels in KIRC. High levels of IL17RB and SMAD6, along with the low levels of PTP4A3, correlated with better patient outcomes in KIRC. Stabilin-1 is the only known scavenger receptor for GDF-15. Functional endocytosis assay and confocal microscopy revealed that titanium nanoparticles, previously shown to enhance GDF-15 production by macrophages, can suppress the scavenging function of stabilin-1 encoded by the STAB1 gene. STAB1 expression was elevated in KIRC tissue and correlated with worse survival outcomes. Impairment of the stabilin-1 scavenging function may additionally increase the bioavailability of GDF-15 in KIRC, influencing patient outcomes. In summary, GDF-15 can program human macrophages into a tolerogenic phenotype, support angiogenesis and modify TGF-β signaling. Increased GDF-15 transcription, together with impaired stabilin-1-mediated clearance, can be suggested as a mechanism by which macrophages retain their ability to limit tumor growth.