Obese microenvironment contributes to MDSC phenotype through epigenetic mechanisms

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Introduction

Patients with obesity are more susceptible to developing several different types of cancer. Myeloid derived suppressor cells (MDSC) promote tumor growth by blocking anti-tumor T cell responses. The expanded number and heightened function of MDSC have been found in mouse models of obesity. Our preliminary data show that patients with morbid obesity (body mass index [BMI] > 40 kg/m^2) also have increased numbers of MDSC in peripheral blood, which could contribute to the cancer risk. Here, we investigate whether the cholesterol, as a factor of obese microenvironment, regulates the immunosuppressive phenotype of MDSC via epigenetic mechanisms.

MDSC exert their immunosuppressive effects through the upregulation of several genes that codify for arginase-1 (*Arg1*), iNOS (*NOS2*), and PD-L1 (*CD274*). Transcriptome analysis by comparing purified MDSC from obese patients and normal weight controls (NWC) led us to hypothesize that expression of key genes such as arginase-1 is regulated by chromatin remodeling by the demethylase JMJD3. Additionally, ATAC-seq data obtained by comparing MDSC from mice fed with high-fat and low-fat diets under *in vitro* stimuli with cholesterol showed open chromatin regions uncovering motifs for EZH2 in regulatory elements of *NOS2* gene, suggesting that function of MDSC is also modulated by the methyltransferase EZH2. Together, the RNA-seq and ATAC-seq data led us to hypothesize that the inflammatory milieu or deregulated metabolic factors in obesity, such as cholesterol, have a key role in the regulation of immunosuppressive gene expression in MDSC through epigenetic mechanisms involving EZH2 and JMJD3.

Preliminary data

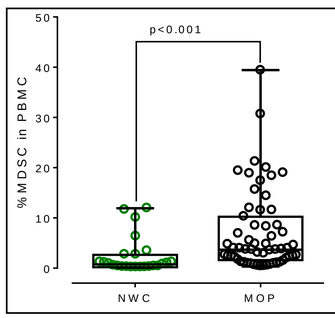

Figure 1: Morbidly obese patients (MOP) contain a higher percentage of MDSC in peripheral blood as compared to normal weight control patients (NWC)

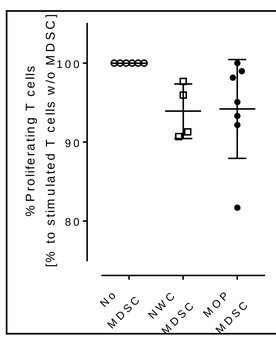

Figure 2: MDSC from morbidly obese patients (MOP) have similar immunosuppressive capacity as compared to MDSC from normal weight control patients (NWC)

MDSC in obese patients have altered gene expression

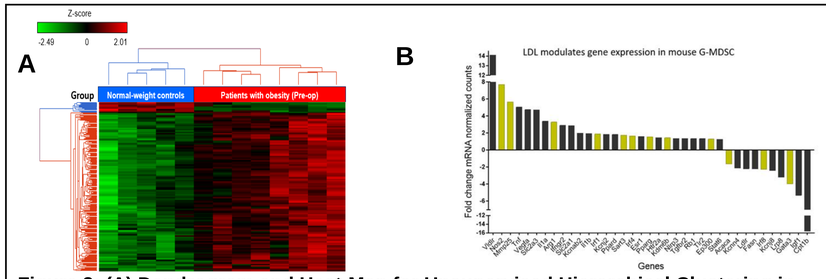

Figure 3: (A) Dendrogram and Heat-Map for Unsupervised Hierarchical Clustering in MDSC from 8 Patients with obesity and 5 Controls, based on the 238 genes differentially expressed. (B) Genes associated with MDSC function, such as *Arg1*, were upregulated in mouse bone-marrow derived MDSC pretreated with LDL. Upregulated genes displayed had significance with a p-value < .05

Results

LDL modulates the suppression capacity of MDSC *in vitro*

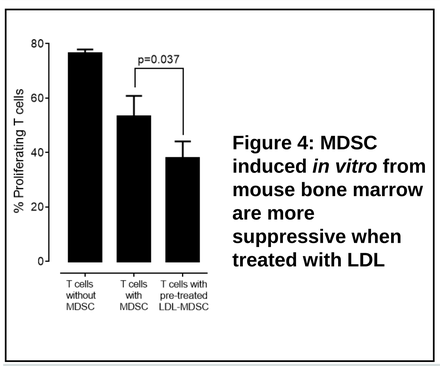

Figure 4: MDSC induced *in vitro* from mouse bone marrow are more suppressive when treated with LDL

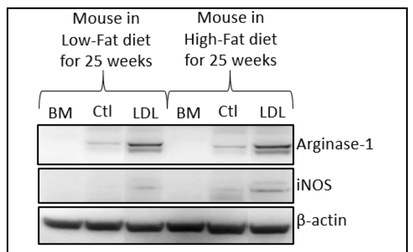

Figure 5: LDL-cholesterol induce higher expression of arginase-1 and iNOS. BM indicates bone marrow; Ctl, control; LDL, low-density lipoprotein

ATAC-seq data indicates that LDL could affect MDSC function through epigenetic mechanisms

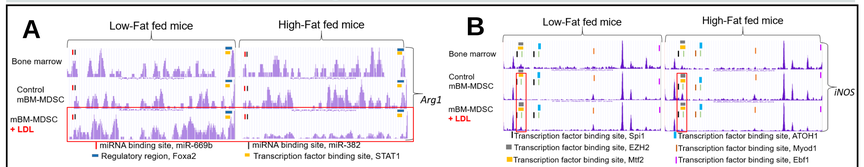

Figure 6: Differences in open chromatin pattern in *Arg1* gene and accessibility for histone 3 methyltransferase EZH2 in the regulatory region on *NOS2* gene in MDSC from obese and lean mice (high-fat vs low-fat fed mice) when treated with LDL *in vitro*. (A) Chromatin accessibility profile in *Arg1* and (B) *NOS2* genes from ATAC-seq analysis. mBM-MDSC indicates mouse bone marrow-derived MDSC

Inhibition of EZH2 and JMJD3 modulates MDSC function

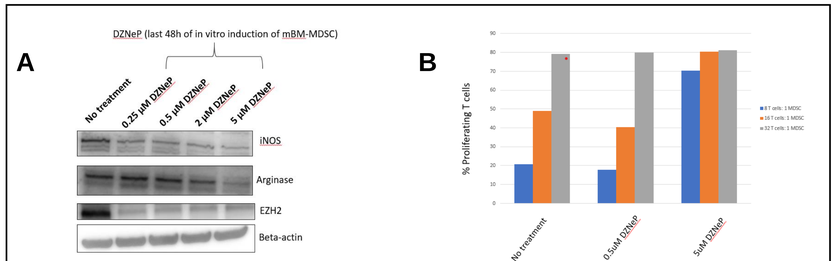

Figure 7: MDSC induced *in vitro* in presence of *EZH2* inhibitor, DZNeP, are less immunosuppressive. (A) Effect of DZNeP on protein expression levels of iNOS, Arginase-1 and EZH2 and (B) suppression capability of MDSC on T cell proliferation. DZNeP indicates 3-deazaneplanocin A; mBM-MDSC, mouse bone marrow-derived MDSC

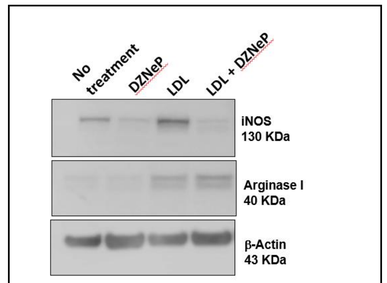

Figure 8: Inhibition of EZH2 blocks the induction of iNOS promoted by LDL

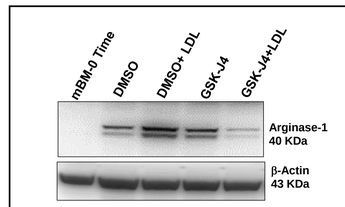

Figure 9: Inhibition of JMJD3, a H3K27m3 demethylase, with GSK-J4 reversed the effects that LDL has on Arginase-1 expression in MDSC in vitro. mBM-indicates mouse bone marrow; H3K27m3, trimethylated histone H3 lysine 27

Conclusions

- MDSC are increased in patients with morbid obesity
- Cholesterol-LDL increases expression levels of arginase-1 and iNOS and enhanced immunosuppressive capacity of MDSC induce *in vitro* from mouse bone marrow.
- Cholesterol-LDL differentially modify chromatin accessibility in key genes of MDSC from obese and lean mice
- Expression of iNOS and suppression function on T cells are significantly reduced by inhibition of EZH2 with DZNeP
- Inhibition of JMJD3 with GSK-J4 alters the expression of arginase-1 induced by LDL
- These findings suggest that epigenetic modulators such as EZH2 and JMJD3 could be a promising druggable targets to modify the function of MDSC in obesity and reduce their potential role in cancer development and progression.

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