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Short communication

# Histone deacetylase 11: A novel epigenetic regulator of myeloid derived suppressor cell expansion and function☆

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ABSTRACT

Myeloid-derived suppressor cells (MDSCs), a heterogeneous population of cells capable of suppressing anti-tumor T cell function in the tumor microenvironment, represent an imposing obstacle in the development of cancer immunotherapeutics. Thus, identifying elements essential to the development and perpetuation of these cells will undoubtedly improve our ability to circumvent their suppressive impact. HDAC11 has emerged as a key regulator of IL-10 gene expression in myeloid cells, suggesting that this may represent an important targetable axis through which to dampen MDSC formation. Using a murine transgenic reporter model system where eGFP expression is controlled by the HDAC11 promoter (Tg-HDAC11-eGFP), we provide evidence that HDAC11 appears to function as a negative regulator of MDSC expansion/function *in vivo*. MDSCs isolated from EL4 tumor-bearing Tg-HDAC11-eGFP display high expression of eGFP, indicative of HDAC11 transcriptional activation at steady state. In striking contrast, immature myeloid cells in tumor-bearing mice display a diminished eGFP expression, implying that the transition of IMC to MDSC's require a decrease in the expression of HDAC11, where we postulate that it acts as a gate-keeper of myeloid differentiation. Indeed, tumor-bearing HDAC11-knockout mice (HDAC11-KO) demonstrate a more suppressive MDSC population as compared to wild-type (WT) tumor-bearing control. Notably, the HDAC11-KO tumor-bearing mice exhibit enhanced tumor growth kinetics when compare to the WT control mice. Thus, through a better understanding of this previously unknown role of HDAC11 in MDSC expansion and function, rational development of targeted epigenetic modifiers may allow us to thwart a powerful barrier to efficacious immunotherapies.



## 1. Introduction

In treatment of cancer, successful immunotherapy hinges on the effective function of antigen-presenting cells (APCs) and T cells. In fact, the concept of immunity is based on the capacity of T cells to mount an effective immune response against malignant cells and harmful pathogens. One of the major hurdles in cancer immunotherapy is the failure of T cells to attain an effective response to malignant cells. Such problems arise when T cells become unresponsive to tumor specific antigens due to physiological changes in the tumor microenvironment (Wells, 2003). In the past several years, mounting evidence has demonstrated that negative regulation of the host immune response is due to two groups of cells: 1-regulatory T cells ($T_{regs}$) (Wang, 2006) and 2-myeloid-derived suppressor cells (MDSCs) (Youn and Gabrilovich, 2010).

*Abbreviations:* MDSC, myeloid derived suppressor cells; IMC, immature myeloid cells; HDAC, histone deacetylase; eGFP, enhanced green fluorescent protein; APCs, antigen presenting cells; ROS, reactive oxygen species; NO, nitric oxide; GVHD, graft vs host disease; PBMC, peripheral blood mononuclear cells; BM, bone marrow; DAPI, diamidino-2-phenylindole; DCs, dendritic cells; EL4, murine lymphoma cell line; Panco, murine pancreatic adenocarcinoma cell line; LPS, lipopolysaccharide.

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MDSCs are a distinct population of cells with the ability to suppress various T cell functions. They are a heterogeneous population of cells generally composed of precursors to dendritic cells, granulocytes, macrophages, as well as myeloid cells at various stages of differentiation (Serafini et al., 2006). In cancer patients, MDSCs are defined as cells that express the common myeloid marker CD33 but lack expression of mature myeloid and lymphoid cells (Almand et al., 2001). In mice, these cells are recognized by co-expression of CD11b and GR-1 and have been more precisely identified by their immature myeloid origin, and most significantly their strong suppressive ability in various facets of immune response, most importantly in T-cell activation, proliferation and cytokine production (Marigo et al., 2008; Youn and Gabrilovich, 2010). In recent years, these cells have been further sub-categorized into two subsequent subsets based on their expression of two molecules Ly-6C and Ly-6G (Hestdal et al., 1991; Youn et al., 2008). $CD11b^{+}$ $Ly\text{-}6G^{-}$ $Ly\text{-}6C^{high}$ cells have been identified to have monocytic-like morphology and are subsequently termed monocytic-MDSCs (M-MDSCs) and $CD11b^{+}$ $Ly\text{-}6G^{+}$ $Ly\text{-}6C^{low}$ cells have been identified to have granulocytic-like morphology and are termed granulocytic MDSCs (G-MDSCs) (Condamine and Gabrilovich, 2011). MDSCs have a very fast proliferative capacity and rapidly accumulate in lymphoid organs of mice with infectious diseases (Gomez-Garcia et al., 2005), inflammation (Ezernitchi et al., 2006), sepsis (Delano et al., 2007), and more importantly in mice bearing tumors (Sawanobori et al., 2008). Since the identification of this suppressive subset, numerous studies have convincingly demonstrated possible molecules such as arginase, nitric oxide (NO), and reactive oxygen species (ROS) as major culprits responsible for the immunosuppressive ability of these cells (Bronte and Zanovello, 2005; Rodriguez and Ochoa, 2008; Gabrilovich and Nagaraj, 2009). It has been described that MDSCs have the potential to promote *de novo* development of Tregs (Foell et al., 2007; Rodriguez and Ochoa, 2008; Gabrilovich and Nagaraj, 2009).

Histone deacetylases (HDACs) are enzymes that are frequently recruited by transcriptional factors or co-repressors to the gene promoters, where they regulate transcription through chromatin modification without directly binding response elements on DNA. It has also been suggested that some HDACs have a broad range of protein substrates, in addition to factors involved directly in transcription, and have the potential to deacetylase non-histone proteins (Glozak et al., 2005). Despite the rapidly increasing knowledge about the role of HDACs in cancer biology, as well as other pathological conditions such as autoimmunity, it is imperative to delineate specific mechanisms induced by these molecules which govern the physiological outcome of such diseases. Recently, it has been shown that HDAC inhibition enhances MDSC generation and expansion (Condamine and Gabrilovich, 2011). Also, important to mention are the new roles assign to specific HDACs which are particularly involved in controlling the immune response (Villagra et al., 2010). We recently unveiled the role of HDAC11 in the regulation of antigen presenting cells and T cell response (Villagra et al., 2009). This deacetylase is the newest member of the histone deacetylase family and has previously been identified as tissue-restricted and exclusively expressed in the brain, kidney and testis (Gao et al., 2002). Several studies have also highlighted the role of this HDAC in regulating the differentiation and development of neural cells (Liu et al., 2008, 2009). Beyond these studies, little was known regarding the role of HDAC11 in other cell types, until demonstrated by our group that HDAC11, by interacting at the chromatin level with the IL-10 promoter, down-regulates IL-10 transcription in murine and human APCs (Villagra et al., 2009). Unpublished data from our lab also suggests that HDAC11 is involved in hematopoietic lineage differentiation, as well as graft vs host disease (GVHD) (both manuscripts in preparation); however the mechanistic role of HDAC11 in myeloid differentiation and MDSC expansion/function still remains to be elucidated. Here we demonstrate that HDAC11 appears to be involved in the regulation of MDSCs *in vivo*. A better understanding of this previously unknown role of HDAC11 in MDSC biology may lead to the development of targeted epigenetic therapies, in order to modulate the suppressive ability of these cells and augment the efficacy of immunotherapy against autoimmunity, GVHD and malignancies.

## 2. Materials and Methods

### 2.1. Flow cytometry immunophenotyping

Peripheral blood mononuclear cells (PBMCs), bone marrow aspirates (BM), and splenocytes were harvested under sterile condition. Single-cell suspensions were prepared, and red blood cells were eliminated using ACK lysis buffer (Gibco). Flow cytometric analysis was performed using fluorochrome-labeled monoclonal antibodies (mAbs; anti-CD3, -CD11b, -Ly6C, -Ly6G, Becton Dickinson, San Jose, CA and eBiosciences, San Diego, CA) and the vitality dye 4′,6-diamidino-2-phenylindole (DAPI, Sigma). Data was acquired on an LSRII cytometer (Beckman Coulter), and analyzed with FlowJo software v9.52 (Tree Star, Ashland, OR). Flow cytometric sorting was performed using the same fluorochrome panel and utilizing the FacsARIA (Beckman Coulter) device.

### 2.2. IFN-gamma suppression/functional assay

Whole spleens and tumors were isolated from tumor burdened or naive mice, cells were isolated and sorted into MDSC population ($CD11b^{+}$/$GR\text{-}1^{+}$) and their subsets (M-MDSC & G-MDSC, $Ly6C^{high}$/$Ly6G^{-}$ & $Ly6C^{low}$/$Ly6G^{+}$ respectively) using FACSAria cell sorter (BD Bioscience) The purity of cell population was 99%. Anti-OVA $CD8^{+}$ T-cells (OT-I) in the presence or absence of cognate peptide (OVA $peptide_{323-339}$ for $CD4^{+}$ T-cells and OVA $peptide_{257-264}$ for $CD8^{+}$ T-cells) were incubated for 48 h with MDSCs. An MDSC:OT-I Splenocyte ratio of 1:3, ($5 \times 10^{4}$/well MDSC to $15 \times 10^{4}$/well OT-I splenocytes), was used and cells were stimulated with OVA peptide (10 μg/mL) and incubated at 37 C in a 96 well plate for 48 h in RPMI/10%FBS. Cytokine production by T-cells was determined using Enzyme-linked immunosorbent assay (ELISA) and Enzyme-linked immunosorbent spot (ELISPOT). For $CD8^{+}$ T-cells IFN-γ production was measured using an IFN-γ ELISA kit (DY485 R&D Systems) following manufacturer provided protocols.

### 2.3. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

Total RNA was prepared from centrifugally pelleted and presorted cells (RNeasy mini columns and RNAse free DNAse, Qiagen, Valencia, CA). cDNA was prepared using iScript cDNA Synthesis Kit (Bio-Rad) and qRT-PCR reactions were conducted using the SYBR green two-step qRT-PCR (Bio-Rad) with transcript-specific primers (Supplied upon request) and cDNA from MDSCs as templates. qRT-PCR amplification reactions were resolved on CFX iCycler (Bio-Rad) and fold changes were quantified ($2^{-\Delta\Delta Ct}$).

### 2.4. Mice and cell lines

OT-I mice were purchased from Jackson laboratories, Tg-HDAC11-eGFP (Gong et al., 2003) reporter mice were provided by Nathaniel Heintz through the Mutant Mouse Regional Centers, and HDAC11-KO kindly supplied by Merck and obtained from Dr. Seto's lab respectively. Mice were kept in pathogen-free condition and handled in accordance with the requirements of the Guideline for