CD38-Expressing Myeloid-Derived Suppressor Cells Promote Tumor Growth in a Murine Model of Esophageal Cancer

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Abstract

Myeloid-derived suppressor cells (MDSC) are an immunosuppressive population of immature myeloid cells found in advanced-stage cancer patients and mouse tumor models. Production of inducible nitric oxide synthase (iNOS) and arginase, as well as other suppressive mechanisms, allows MDSCs to suppress T-cell–mediated tumor clearance and foster tumor progression. Using an unbiased global gene expression approach in conditional p120-catenin knockout mice (L2-cre;p120ctn/f/f), a model of oral–esophageal cancer, we have identified CD38 as playing a vital role in MDSC biology, previously unknown. CD38 belongs to the ADP-ribosyl cyclase family and possesses both ectoenzyme and receptor functions. It has been described to function in lymphoid and early myeloid cell differentiation, cell activation, and neutrophil chemotaxis. We find that CD38 expression in MDSCs is evident in other mouse tumor models of esophageal carcinogenesis, and CD38high MDSCs are more immature than MDSCs lacking CD38 expression, suggesting a potential role for CD38 in the maturation halt found in MDSC populations. CD38high MDSCs also possess a greater capacity to suppress activated T cells, and promote tumor growth to a greater degree than CD38low MDSCs, likely as a result of increased iNOS production. In addition, we have identified novel tumor–derived factors, specifically IL6, IGFBP3, and CXCL16, which induce CD38 expression by MDSCs ex vivo. Finally, we have detected an expansion of CD38+ MDSCs in peripheral blood of advanced-stage cancer patients and validated targeting CD38 in vivo as a novel approach to cancer therapy. Cancer Res; 75(19): 4074–85. ©2015 AACR.

Introduction

The immune system (both innate and adaptive) plays an essential role in limiting tumor growth and, therefore, tumor progression requires escape from immune surveillance. One mechanism that allows for tumor escape is the activation and progression of immunosuppressive cell populations, including...
CD38 expression is a common characteristic to several immunosuppressive cell types. Foxp3+CD25+CD4+ Tregs expressing high CD38 levels possess a greater immunosuppressive activity than CD38low Tregs (18). CD38+CD8+ T cells suppress proliferation of CD4+ effector T cells, which requires IFNγ secretion and cell-to-cell contact (19). Similarly, CD19+CD24highCD38high B cells inhibit differentiation of Th1 cells in an IL10-dependent manner, and their dysfunction may play a role in autoimmune disorders such as systemic lupus erythematosus (20).

CD38 is a member of the ribosyl cyclase family and is expressed on the surface of diverse immune cells, including B cells, T cells, NK cells, and myeloid cells (21). CD38 possesses independent ectoenzyme and receptor functions. As an ectoenzyme, CD38 catalyzes synthesis and hydrolysis of cyclic ADP-ribose (CADPR), converting NAD+ to ADP-ribose (ADPR), as well as CADPR into ADPR (21, 22). Furthermore, at acidic pH, CD38 catalyzes synthesis and hydrolysis of nicotinic acid adenine dinucleotide phosphate (NAADP; refs. 21, 22). Both reactions are essential for calcium signaling, specifically for mobilization of intracellular Ca2+ (22). Receptor activity of CD38 has been documented in multiple immune cell types, where it is dependent on localization to the lipid rafts and association with professional signaling complexes (21). In both mouse and human myeloid cells, ligation of CD38 receptor leads to suppressed growth and survival, resulting in loss of the most differentiated immune populations (23).

In this study, we have identified CD38 as a novel marker for MDSCs that possess greater immunosuppressive capacity, thereby promoting tumor growth in vivo. We have identified a mechanistic role for CD38 in promoting expansion of the monocyte MDSC population, as well as in regulating expression of the effector molecule iNOS by these cells. In addition, we have established for the first time that several cytokines, specifically IFNγ, TNFα, IFGBP3, CXCL16, and IL6, are capable of inducing CD38 expression in MDSCs. Finally, we have demonstrated that administration of an anti-CD38 monoclonal antibody slows disease progression in tumor-bearing mice. As we have detected an expansion of CD38-positive MDSC-like population in peripheral blood of advanced-stage cancer patients, this study introduces the concept of anti-CD38 monoclonal antibody therapy for potential treatment of certain solid tumors.

Materials and Methods

Cell lines
AKR and HNM007 mouse esophageal squamous cell carcinoma (ESCC) tumor lines have been described previously (25, 26). Cells were maintained in DMEM + 10% FBS and passaged or harvested at approximately 80% confluency. We have propagated cells from frozen stocks of the original vials that were authenticated by short tandem repeat analysis for highly polymorphic microsatellites FES/FPS, vWA31, D22S417, D105526, and DSS592 so as to validate the identity of cells by comparing cells from the earliest stocks and those grown more than 8 to 12 passages. All cell lines have been tested for mycoplasma contamination on a regular basis.

Generation of MDSCs
All animal studies were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania (Philadelphia, PA). The L2-Cre;p120ctn+/+ mouse model of oral-esophageal cancer developed by us was described previously (24). The mouse ESCC tumor lines have been described previously (25, 26). A total of 2.5 × 106 AKR or HNM007 cells/animal were injected subcutaneously into C57BL/6J or CD38+/− mice (gift from Dr. Eduardo Chini, Department of Anesthesia, Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN). Subcutaneous tumor-bearing mice were aged until tumors reached a volume of 0.8 cm3. Spleens and bone marrow were harvested upon euthanasia for MDSC isolation.

FACS
Single-cell suspensions were prepared from mouse bone marrow or spleen by mechanical disruption. For NFκB and iNOS staining, cells were fixed (BD Cytofix) and permeabilized with methanol. Peripheral blood from previously untreated, advanced-stage head and neck cancer (HNC) patients was obtained with informed consent under University of Pennsylvania Institutional Review Board protocol #417200 or Philadelphia VA Medical Center protocol #01090. Patients’ peripheral blood mononuclear cells (PBMC) were separated using gradient centrifugation.

T-cell suppression
CD11b+Gr-1−, CD11b+Gr-1+CD38low, and CD11b+Gr-1−CD38high cell populations were sorted by FACS. Antigen-specific CD8+ T-cell suppression was tested as described previously (24). OVA-peptide was used to stimulate proliferation of OT-1 T cells.

RNA microarray and qPCR
RNA was isolated from FACS-sorted MDSCs to perform microarray (GeneChip Mouse Exon 1.0 ST Array, Affymetrix). Ingenuity Pathway Analysis software was used for data analysis. cDNA was generated using oligo-dT primers and Superscript II Reverse Transcriptase. qPCR was performed using validated SYBR Green primers and ABI7000 (Applied Biosystems).

Ex vivo MDSC differentiation
Generation of MDSCs from bone marrow has been described previously (27). Cytokine concentrations used: 0.1 ng/mL (GM-CSF and IL4), 10 ng/mL (TNFα and IFNγ), and 100 ng/mL (IL6, CXCL16, and IFGBP3). HNM007 or AKR-conditioned media (CM) were used at 50% v/v. Anti-CD38 or IgG2a isotype control antibodies were used at 10 μg/mL.

Colony formation and cell recovery assays
Isolation of MDSCs from tumor-bearing L2-cre;p120+/− mice by magnetic cell sorting was described previously (24). A total of 200,000 cells were cultured in MethoCult medium (Stem Cell Technologies). Anti-CD38 or IgG2a isotype control antibodies were used at 10 μg/mL. Colonies were counted after 7 days. For recovery assays, 5 × 103 MDSCs were seeded in complete RPMI1640 medium supplemented with antibiotics; cells were quantified by Trypan Blue exclusion using a Countess automated cell counter (Invitrogen).

Cytokine array
Media from ex vivo differentiation cultures were collected and snap-frozen after 1 or 5 days of culture. Mouse cytokine array C3 kit (Raybiotech) was used according to the manufacturer’s
protocol. Results were quantified using the ImageJ protein array analyzer and normalized to positive controls.

ESCC/MDSC cotransplantation and anti-CD38 therapeutic study

C57BL/6j recipient mice from Jackson Labs were injected subcutaneously with a mixture of 2.5 × 10⁵ syngeneic HNM007 tumor cells with either 2.5 × 10⁵ CD38low or CD38hi MDSCs obtained from HNM007 tumor-bearing C57BL/6j mice. Recipient mice injected with 2.5 × 10⁵ syngeneic HNM007 tumor cells alone served as controls. For antibody treatment experiments, anti-CD38 monoclonal antibody or IgG2a isotype control antibody were administered intraperitoneally every 48 hours starting on day 5 after injection. Measurements were taken every 2 to 3 days once tumors became palpable.

Histology

Subcutaneous tumors were fixed in buffered formalin solution, paraffin-embedded, and stained with hematoxylin and eosin (H&E). Antigen-specific staining was performed as described previously (24).

Statistical analysis

The Student t test was used to determine whether there is significant difference between two experimental groups (P ≤ 0.05 was considered statistically significant).

Additional details (qPCR primers, antibodies, and detailed ex vivo differentiation protocol) can be found in Supplementary Materials and Methods.

Results

MDSCs from tumor-bearing L2-Cre;p120⁰⁰⁰ mice exhibit elevated CD38 expression

We have previously demonstrated that MDSCs play a fundamental role in tumor initiation and progression in a spontaneous genetic mouse model of ESCC (L2-Cre;p120⁰⁰⁰, referred to hereafter as p120⁰⁰⁰, ref. 24). Here we sought to identify genes associated with an immature myeloid phenotype that contribute to the tumor-promoting activities of MDSCs, thereby providing a platform to elucidate underlying molecular mechanisms. To that end, we performed microarray analysis of splenic MDSCs from 6- to 8-month-old tumor-bearing p120⁻⁻ mice and age-matched littermate controls (Supplementary Fig. S1; GEO accession number GSE71706). Among the 964 genes showing differential expression between the two groups (Fig. 1A), we identified Cd38 (ranked fifth highest among all genes tested; Supplementary Table S1) as a candidate gene of interest, as it has roles in both innate and adaptive immunity in mice and humans, including, but not limited to, chemotaxis of murine and human neutrophils (28, 29), early myeloid differentiation (23), and lymphoid cell activation (30). We validated Cd38 mRNA and protein expression in MDSCs from tumor-bearing mice, compared with those isolated from control mice (Fig. 1B–D). We also observed increased CD38 in splenic MDSCs isolated from L2-IL1β mice, a model of Barrett esophagus and esophageal adenocarcinoma (Supplementary Fig. S2; ref. 31).

CD38 expression correlates with ESCC progression and expansion of monocytic MDSC population

To determine the kinetics of CD38 expression in MDSCs, we analyzed splenic CD11bGr-1 mice from tumor-bearing p120⁺⁺ mice. A heatmap illustrating the results of microarray analysis performed using CD11bGr-1 cells from the spleens of six tumor-bearing p120⁻⁻ mice and three pooled samples from healthy littermate controls (n = 9). Increased expression of the Cd38 gene and protein in CD11bGr-1 cells from tumor-bearing mice was confirmed by qPCR (B; *P = 0.007) and FACS (C; n = 3; *P = 0.009). D, frequencies of CD38⁺ cells (F, *P = 0.003).

Figure 1.

CD38 is significantly upregulated in CD11bGr-1 cells from tumor-bearing p120⁻⁻ mice. A, heatmap illustrating the results of microarray analysis performed using CD11bGr-1 cells from the spleens of six tumor-bearing p120⁻⁻ mice and three pooled samples from healthy littermate controls (n = 9). Increased expression of the Cd38 gene and protein in CD11bGr-1 cells from tumor-bearing mice was confirmed by qPCR (B; *P = 0.007) and FACS (C; n = 3; *P = 0.009). D, frequencies of CD38⁺ cells (F, *P = 0.003).

CD38 expression levels in tumor-bearing L2-Cre;p120⁰⁰⁰ mice were markedly lower than those in tumor-bearing CD11bGr-1 mice (Fig. 2A). CD38 expression in MDSCs from tumor-bearing p120⁻⁻ mice was significantly increased compared with control mice (Fig. 2B). CD38 expression was significantly increased in splenic MDSCs from tumor-bearing p120⁻⁻ mice (Fig. 2B), while a more mature subset of myeloid cells (CD11bGr-1) exhibited no change in CD38 levels (Fig. 2B).

We next tested two murine ESCC cell lines (AKR, ref. 25; and HNM007, ref. 26) for their ability to generate MDSCs in vivo using a syngeneic transplant model. We observed dramatically increased CD38 levels in all myeloid populations from spleens of HNM007 tumor-bearing mice, yet in AKR tumor-bearing mice CD38 levels were overall lower (Fig. 2C and Supplementary Fig. S3). Interestingly, while both cell lines induced
expansion of myeloid populations in spleens of tumor-bearing mice, it was significantly more pronounced ($P = 0.0009$) in HNM007 tumor-bearing mice (Fig. 2D). Furthermore, we observed differences in distribution of granulocytic and monocytic MDSCs (G-MDSC and M-MDSC, respectively), as well as mature monocytes (Fig. 2D). G-MDSCs ($CD11b^+Ly6G^+$) were...
less abundant ($P = 0.02$) in HNM007 tumor-bearing mice, compared with AKR. There also was a trend of M-MDSC (CD11b+Ly-6C+Ly-6G−) expansion, accompanied by a significant increase in mature monocytes (CD11b+Ly-6C−Ly-6G+) in HNM007, compared with AKR tumor-bearing and control mice ($P = 0.02$). These findings suggest that CD38 may be relevant to M-MDSC expansion in tumor-bearing mice.

**CD38**<sup>high</sup> MDSCs possess greater immunosuppressive and tumor-promoting capacity than CD38<sup>low</sup> MDSCs

As the CD38<sup>high</sup> MDSC population expands in tumor-bearing mice, we hypothesized that CD38<sup>high</sup> MDSCs possess greater immunosuppressive potential than CD38<sup>low</sup> MDSCs. To test this, we sorted CD38<sup>high</sup> and CD38<sup>low</sup> MDSCs from HNM007 tumor-bearing mice and assessed their capacity to suppress OT-1 T-cell growth following antigen stimulation. CD38<sup>high</sup> MDSCs demonstrated significantly greater T-cell suppressive capacity, compared with their CD38<sup>low</sup> counterparts (Fig. 3A), at 2:1 OT-1 to MDSC ratios (Fig. 3E).

Next, we investigated whether CD38<sup>high</sup> MDSCs may possess greater tumor-promoting capacity than CD38<sup>low</sup> MDSCs in vivo.

Next, we investigated whether CD38 is required for the immunosuppressive function of MDSCs by analyzing the capacity of MDSCs from Cd38<sup>−/−</sup> and Cd38<sup>+/−</sup> (wt) mice bearing HNM007 tumors to suppress OT-1 T-cell proliferation. Interestingly, Cd38<sup>−/−</sup> MDSCs exhibited significantly reduced immunosuppressive capacity at 1:1 and 4:1 OT-1 to MDSC ratios (Fig. 3E).

**CD38**<sup>high</sup> MDSCs are phenotypically different from the CD38<sup>low</sup> MDSCs

Next, we analyzed CD38<sup>high</sup> and CD38<sup>low</sup> splenic MDSCs from tumor-bearing p120<sup>−/−</sup> mice via microarray (Supplementary Fig. S5; GEO accession number GSE71706) and detected differential expression of 498 genes (Fig. 4A; Supplementary Table S2). Among genes with the greatest increase in expression was inducible nitric oxide synthase (iNos). qPCR analysis further revealed that iNos expression was significantly elevated in CD38<sup>high</sup> MDSCs compared with CD38<sup>low</sup> MDSCs, while expression of arginase 1 (Arg1) and NADPH oxidase subunit (Nox2), two additional mediators of MDSC suppressive function, was comparable in these subpopulations (Fig. 4B). iNos protein expression was also validated in CD38<sup>high</sup> MDSCs (Fig 4C and Supplementary Fig. S5B). As iNos is a target of NFkB transactivation (32), we evaluated the levels of total and phosphorylated NFkB in CD38<sup>high</sup> and CD38<sup>low</sup> MDSCs and found increased phosphoNFKB-to-totalNFkB ratio in the CD38<sup>high</sup> population (Fig. 4C and Supplementary Fig. S5B). To test whether iNos contributes to the increased immunosuppressive capacity of CD38<sup>high</sup> MDSCs, we used an iNos inhibitor (L-NMMA), and found that it completely abrogated OT-1 T-cell suppression mediated by CD38<sup>high</sup> MDSCs (Fig. 4D). Finally, the CD38 inhibitor AraF-NAD (33) partially rescued OT-1 T-cell proliferation (Fig. 4E), suggesting that CD38 enzymatic activity is required for immunosuppressive capacity of CD38<sup>high</sup> MDSCs. Furthermore, iNos expression was decreased in MDSCs isolated from the spleens of HNM007 tumor-bearing Cd38<sup>−/−</sup> mice (Fig. 4F and Supplementary Fig. S5C).

Morphologic assessment of sorted CD38<sup>low</sup> and CD38<sup>high</sup> MDSCs revealed that the CD38<sup>high</sup> population consists of more immature cells, such as promyelocytes (~10%), myelocytes (5%-10%), metamyelocytes (5%-10%), and band cells (~70%), whereas the CD38<sup>low</sup> population consists of band cells (~10%), and mature neutrophils (~90% Fig. 4G), demonstrating that CD38<sup>high</sup> MDSCs are morphologically more immature than CD38<sup>low</sup> MDSCs.

**IFNγ, TNFα, CXCL16, IFGBP3, and Il6 induce CD38 expression**

Since we found that MDSCs from HNM007 tumor-bearing mice have increased CD38 expression, compared with AKR tumors (Fig. 2C), we sought to understand signaling pathways underlying this phenotype. We performed *ex vivo* bone marrow differentiation assays using GM-CSF, IL4 (both required for CD11b<sup>+</sup>Gr-1<sup>−</sup> cell generation from bone marrow progenitors; ref. 27) and conditioned media (CM) from either HNM007 or AKR cells. Only HNM007 CM induced CD38 expression (Fig. 5A). As IFNγ and TNFα are key components of the proinflammatory milieu and are known activators of CD38 transcription (21), we used these cytokines in *ex vivo* differentiation assays. Interestingly, both factors, individually or in combination, induced CD38 expression in CD11b<sup>+</sup>Gr-1<sup>−</sup> cells (Fig. 5A). A cytokine array using CM from *ex vivo* differentiation experiments revealed several factors, including CXCL16 and IFGBP3-3 that were present at higher levels in HNM007 cultures as compared with AKR cultures (Fig. 5B). In addition, the proinflammatory cytokine IL6, a predicted activator of CD38 transcription (21), was elevated in HNM007 cultures, albeit not as dramatically as CXCL16 or IFGBP3-3 (Fig. 5B). Next, we investigated the capacity of recombinant IL6, CXCL16, and IFGBP3 to increase CD38 expression *ex vivo*. Interestingly, addition of IL6, CXCL16, and IFGBP3 in combination induced a moderate, yet significant, increase in CD38 expression in AKR CM cultures (Fig. 5C).

**Cross-linking of CD38 by an agonistic antibody impairs expansion and survival of CD11b<sup>+</sup>Gr-1<sup>−</sup> cells in vitro and suppresses tumor growth in vivo**

To test whether cross-linking of CD38 with a monoclonal antibody has an effect on MDSC function(s), MDSCs from spleens of tumor-bearing p120<sup>−/−</sup> mice were cultured in methyldcellose-based medium in the presence of an anti-CD38 monoclonal antibody (NIM-R5) or isotype control (IgG2a). Addition of anti-CD38 antibody inhibited growth of colonies from splenic MDSCs, and the effect of anti-CD38 antibody remained unchanged regardless of whether splenocytes were presorted (Fig. 6A and B), demonstrating that the anti-CD38 antibody inhibits MDSC proliferation and survival in vitro. In
suspension culture, sorted MDSCs survive only a few days, but their survival was further reduced in the presence of anti-CD38 antibody (Fig. 6C). We also tested whether CD38 cross-linking inhibits accumulation of CD11b$^+$Gr-1$^+$CD38$^{\text{high}}$ cells ex vivo in the presence of HNM007 CM. Using an additional anti-CD38 antibody (clone 90), we observed a dose-dependent decrease in CD38 expression within the CD11b$^+$Gr-1$^+$ population (Fig. 6D). Given that the proportion of CD11b$^+$Gr-1$^+$ cells...
within the culture remained consistent (25%–30%; data not shown), these data demonstrate that the CD11b^+ Gr-1^+ CD38^{high} population is likely depleted as a result of CD38 cross-linking. Finally, anti-CD38 antibody treatment resulted in decreased tumor growth rate in vivo in a subcutaneous HNM007 transplant ESCC model (Fig. 6E). In aggregate, these data demonstrate the importance of CD38 for MDSC-mediated ESCC progression and suggest targeting CD38 as an approach to ESCC therapy.

CD38 is expressed on human MDSC-like cell population that is expanded in peripheral blood of advanced-stage cancer patients

To determine whether our findings may be relevant to human cancers, we analyzed CD38 expression in the low-density CD15^{hi} CD33^{lo} population of PBMCs from advanced-stage head and neck cancer and non-small cell lung cancer patients and healthy donors. In contrast to our observations in mice, we found that CD38 expression levels were unchanged in CD15^{hi} CD33^{lo}
Figure 5. IFNγ, TNFα, IGFBP3, CXCL16, and IL6 induce CD38 expression and impair myeloid cell differentiation. A, CD38 expression in CD11b⁺Gr-1⁺ cells from ex vivo differentiation cultures was tested by FACS (MFI, mean fluorescence intensity; n = 3; *P = 0.0001; ** P = 2.5 × 10⁻⁴). B, cytokine array performed with media from ex vivo differentiation cultures (24 or 120 hours). Each cytokine was tested in duplicate. Difference in normalized expression between HNM007 and AKR groups is shown. C, ex vivo differentiation as in A with the addition of cytokines to the AKR-conditioned media (n = 3; *P = 0.05; ** P = 0.005). E-in mice, tumor progression leads to MDSC expansion. Tumor progression leads to amplified signals (such as cytokines) reaching MDSCs, which induces a differentiation halt and expansion of CD38⁺⁺ monocytyc MDSCs with enhanced immunosuppressive capacity [mediated by iNOS, which produces nitric oxide (NO)].
PBMCs from cancer patients, compared with healthy donors (Supplementary Fig. S8). However, this population was significantly expanded from 0.5% of total PBMCs in healthy donors to up to 17% in cancer patients (Fig. 7).

Discussion

Using spontaneous genetic and syngeneic transplant tumor models, as well as an ex vivo differentiation model, we have established for the first time that tumor-derived signals drive expansion of monocytic MDSCs by inducing CD38 expression. Expansion of the CD11b<sup>+</sup>Gr-1<sup>+</sup>CD38<sup>high</sup> cell population occurs after initial splenic MDSC accumulation is evident, which likely indicates a requirement of threshold levels of tumor-derived signals for induction of CD38 by MDSCs (Fig. 5D). Interestingly, two different ESCC cell lines exhibited differential capacities to signals for induction of CD38 by MDSCs (Fig. 5D). Interestingly, CD38<sup>high</sup> MDSCs, and iNOS is required for T-cell suppression by CD38<sup>high</sup> MDSCs. Interestingly, CD38 can induce iNOS upregulation in murine activated microglia (resident monocytes of the brain; ref. 34). Furthermore, CD38<sup>high</sup> MDSCs express elevated iNOS levels compared with CD38<sup>low</sup> MDSCs, and the tumor-derived signals do not promote the tumor cells are responsible for promoting CD38 expression on MDSCs. Based upon our ex vivo studies, the tumor-derived signals may act directly on immature myeloid cell populations present in hematopoietic tissues to promote CD38 expression. Furthermore, our data suggest that the tumor-derived signals do not promote enhanced proliferation of CD38<sup>high</sup> MDSCs (RB1 pathway was activated in CD38<sup>high</sup> MDSCs: Supplementary Fig. S6), but provide these cells with increased survival potential.

Herein, we demonstrate that CD38<sup>high</sup> MDSCs are halted at an earlier differentiation stage compared to CD38<sup>low</sup> MDSCs. CD38 ligation can contribute directly to the differentiation halt (23), which suggests that CD38 signaling may contribute to the maintenance of undifferentiated state observed in CD38<sup>high</sup> MDSCs. Although CD38 has been demonstrated to bind CD31 (21), we do not know whether this interaction contributes to the observed properties of CD38<sup>high</sup> MDSCs.

CD38<sup>high</sup> MDSCs express elevated iNOS levels compared with CD38<sup>low</sup> MDSCs, and iNOS is required for T-cell suppression by CD38<sup>high</sup> MDSCs. Interestingly, CD38 can induce iNOS upregulation in murine activated microglia (resident monocytes of the brain; ref. 34). Furthermore, CD38<sup>high</sup> MDSCs express elevated iNOS (Supplementary Fig. S8), and the tumor-derived signals do not promote tumor cells are responsible for promoting CD38 expression on MDSCs. Based upon our ex vivo studies, the tumor-derived signals may act directly on immature myeloid cell populations present in hematopoietic tissues to promote CD38 expression. Furthermore, our data suggest that the tumor-derived signals do not promote enhanced proliferation of CD38<sup>high</sup> MDSCs (RB1 pathway was activated in CD38<sup>high</sup> MDSCs: Supplementary Fig. S6), but provide these cells with increased survival potential.
induce bone marrow-derived CD11b+TNFα-density CD38+ cells. This immunosuppressive population in cancer treatment CD38 therapy may represent a novel approach to targeting MDSCs (Fig. 4B).

MDSCs contribute to the T-cell suppression repertoire found in cancer, which merits further investigation as a prospective therapeutic target (48). In this study, we have identified CD38 as being suitable for potential MDSC targeting and useful in identification of potentially immunosuppressive MDSC populations. Thus, anti-CD38 monoclonal antibody therapy (45) may hold potential for targeting CD38-expressing MDSCs (49) in patients with certain types of cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T.A. Karakasheva, T.J. Waldron, E. Eruslanov, P.D. Hicks, D. Basu, S. Singhal

Analysis and interpretation of data (e.g., statistical analysis, bioinformatics, computational analysis): T.A. Karakasheva, T.J. Waldron, E. Eruslanov, S.-B. Kim, J.-S. Lee, S. Singhal, A.K. Rustgi

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. O’Brien, A.K. Rustgi

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