Tetraspanin CD81 Promotes Tumor Growth and Metastasis by Modulating the Functions of T Regulatory and Myeloid-Derived Suppressor Cells

Felipe Vences-Catalán, Ranjani Rajapaksa, Minu K. Srivastava, Aurélien Marabelle, Chiung-Chi Kuo, Ronald Levy, and Shoshana Levy

Abstract

Tumor cells counteract innate and adaptive antitumor immune responses by recruiting regulatory T cells (Treg) and innate myeloid-derived suppressor cells (MDSC), which facilitate immune escape and metastatic dissemination. Here we report a role in these recruitment processes for CD81, a member of the tetraspanin family of proteins that have been implicated previously in cancer progression. We found that genetic deficiency in CD81 reduced tumor growth and metastasis in two genetic mouse backgrounds and multiple tumor models. Mechanistic investigations revealed that CD81 was not required for normal development of Treg and MDSC but was essential for immunosuppressive functions. Notably, adoptive transfer of wild-type Treg into CD81-deficient mice was sufficient to promote tumor growth and metastasis. Our findings suggested that CD81 modulates adaptive and innate immune responses, warranting further investigation of CD81 in immunomodulation in cancer and its progression.

Introduction

Understanding the factors influencing tumor progression should have a great impact in preventing and treating human cancers. Tetraspanins are a family of proteins that influence a wide range of cellular functions, including proliferation, adhesion, migration, differentiation, activation, and cell signaling (1, 2). Tetraspanins serve as membrane “docking” molecules that interact with cell surface receptors, such as integrins (3) and with intracellular signaling molecules (1, 2), and have been shown to play a role in cancer progression (4, 5). Tetraspanins cluster with partner proteins into so-called “tetraspanin-enriched microdomains” (TEM). Included in these TEMs are cell surface molecules important in the immune system, such as CD19 in B lymphocytes and CD4 in T lymphocytes (6, 7).

Historically, the first tetraspanin molecule was identified by a monoclonal antibody (mAb) that recognized a human “antigen associated with early stages of melanoma tumor progression,” now renamed CD63 (8, 9). Expression of a specific individual tetraspanin molecule in human cancer has been correlated with either good or bad prognosis. For example, KAI1/CD82 was originally identified as a metastasis suppressor gene in a rat prostate cancer model; subsequently, the human homolog was shown to suppress metastasis in this model (10). Moreover, CD82 mRNA expression in several cancers is associated with a good prognosis (11, 12). By contrast, CD151, previously identified by an antimeatstatic mAb (13) and TSPAN8, originally identified as a colon-associated antigen (14) are markers of poor prognosis. Overexpression of these tetraspanins correlates with tumor progression and metastasis (15). Corroborating the role of CD151 in tumor progression are studies in CD151-deficient mice, which develop fewer metastases than their wild-type (WT) counterparts in carcinogen-induced skin cancer, melanoma (B16F10), Lewis lung carcinoma (LLC), transgenic breast cancer (MMTV-PyVmT), and adenocarcinoma prostate cancer (TRAMP) models (16–19).

CD81 was originally discovered as a target of an antiproliferative antibody (TAPA-1; ref. 20). It was subsequently identified as a cell entry receptor for hepatitis C virus (HCV; ref. 21). It is also noteworthy that entry of sporozoites, the liver stage of the malaria parasite, requires the presence of CD81 (22). Mice lacking CD81 have additional impairments, including female infertility and nervous system malfunctions (23, 24). Although many studies have addressed the function of CD81 in infection (25) and in the immune system (26), few have studied the involvement of CD81 in tumorigenesis and metastasis. Recently, it was shown that expressing exogenous CD81 in a human melanoma cell line enhanced its migrating, invasive, and metastatic abilities in a xenograft model (27). This evidence suggests that CD81 contributes to melanoma cell motility. However, the effect of host CD81 on tumor progression has not been addressed previously.

Here we used CD81-deficient hosts on both C57BL/6 and BALB/c mouse backgrounds in which we analyzed several tumor models to determine the contribution of CD81 to tumor progression and metastasis. Our results provide the first evidence that host CD81 facilitates tumor growth and metastasis. Furthermore, we demonstrate that lack of CD81 severely impairs the function of regulatory T (Treg) cells and myeloid-derived suppressor cells.
involve in tumor growth and metastasis.

Materials and Methods

Mouse tumor cell lines

C57BL/6 tumors were purchased in 2014. LLC from ATCC (then transfected with luciferase using pNL4.3 HIV Luc to generate LLC-luc cells) and the breast cancer E0771 from CH3 Biosystems. The BALB/c 4T1 mammary carcinoma and 4T1-luc cells were gifted in 2012 by Dr. S. Strober and Dr. C. Contag, respectively (both at Stanford University, California). 4T1, 4T1-luc, and E0771 were cultured in RPMI 1640 media (Corning Cellgro), LLC and LLC-luc in DMEM media (Corning Cellgro) both media contain 10% (v/v) heat-inactivated FCS (HyClone), 1% L-glutamine (Corning Cellgro), 100 U/mL penicillin (Gibco), 100 μg/mL streptomycin (Gibco), and 50 μg/mL 2ME (Gibco) at 37°C in a 5% CO₂ incubator. All cell lines were tested according to the manufacturer’s protocol and proved to be mycoplasma free (MycoAlert Mycoplasma Detection Kit; Lonza).

Mice

Mice were backcrossed to the BALB/c and C57Bl6 backgrounds more than 10 generations. Because of female infertility both colonies were maintained by breeding of C4b81+/− heterozygous (HT) mice. Six to twelve weeks old WT, HT, and C4b81+/− (CD81KO) female or male littermates mice were used in these studies. All animal experiments were approved by the Stanford Administrative Panel on Laboratory Animal Care and conducted according to the Stanford University Facility and NIH guidelines. Mice were bred and housed at the pathogen-free animal facility of the Stanford University Medical Center.

Genotyping

The following primers were used to genotype all mice: CD81FP 5’-AACCACGTCTCTGCATCCTC-3’, CDS71RB 5’-CAAGGTCGCC-CITCCTGCTACT-3’, and CDS81NEO 5’-ATTCCGACGGCATC-CITCCT-3’. PCR conditions were as follows: DNA was denatured at 94°C, followed by 35 cycles of amplification using Taq DNA Polymerase (New England Biolabs), 94°C for 1 minute, 55°C for 45 seconds, 72°C for 1 minute, and a final extension step at 72°C for 5 minutes. PCR products were separated in 1.5% agarose gel electrophoresis, expected size were 301 bp for WT and 565 bp for CD81KO.

Tumor growth assays

4T1 or E0771 tumor cells were injected either orthotopically or into the tail vein (intravenously) and LLC tumor cells were injected subcutaneously or intravenously as detailed in each study. Growth of primary tumors was monitored with a digital microscope.

Flow cytometry

Single-cell suspensions from spleens, tumors, and peripheral blood cells from naïve and tumor-bearing mice were filtered through a 70-μm cell strainer (BD Biosciences) and resuspended in 1% BSA in PBS, then stained with fluorochrome-conjugated antibodies (Supplementary Table S1) on ice for 30 minutes. Cells were washed twice in BSA/PBS and fixed in 2% paraformaldehyde, intracellular staining of anti-FoxP3 (clone FJK-16s) was performed according to the manufacturer’s protocol (eBioscience). Cells were acquired using the FACS Calibur or LSRII flow cytometers (BD Biosciences). Data analysis was performed using the FlowJo software (Treestar).

Proliferation assays

Purified spleen T cells from naïve mice were negatively isolated using a pan T-cell Isolation Kit (MACS Miltenyi Biotec) and labeled with 2.5 to 5 μmol/L CFSE (Gibco, Life Technologies) for 10 minutes (according to the manufactures’ protocols). Reactions were terminated by 10 volumes of cold 10% FCS in RPMI. Labeled naïve T cells were stimulated with anti CD3/CD28 dynabeads (Gibco, Life Technologies) in a U-bottom 96-well plate and cocultured at the indicated ratios with Treg cells from naïve or tumor-bearing mice (also isolated using a MACS Miltenyi Biotec CD4+CD25+ Kit according to the manufactured protocol) or with blood MDSCs from tumor-bearing mice. Cocultures were incubated for 5 days at 37°C in a 5% CO₂ incubator, followed by staining with anti-CD3, CD4, and CD8 mAb. T-cell proliferation was analyzed by flow cytometry using FACS Calibur. Division index and percentages of proliferating cells was calculated using FlowJo software (Treestar).

Macrophage polarization assay

Naïve mice were injected with sterile 3% thioglycolate 4 to 5 days before macrophage isolation. Four to five days later, macrophages were collected from the peritoneum by washing several times with PBS using an 18-gauze needle. The cell pellet was centrifuged and resuspended in DMEM containing 10% FCS and incubated for 3 hours at 37°C in a 5% CO₂ incubator. Nonadherent cells were removed by washing several times with PBS. Adherent macrophages were then stimulated with 100 ng/mL of LPS and incubated with either WT or CD81KO blood MDSCs for 24 hours at 37°C. After 24 hours supernatants were collected and IL10, IL12p70, latent TGFβ3 and IFNγ secretion was measured by ELISA Kit (Legend Max; Biolegend) according to the manufacturer’s protocol.

Adaptive transfer of Tregs

4T1 cells were injected into either WT or CD81KO donor mice and after 10 and 17 days of tumor injection spleens were collected for Treg isolation. Tregs were purified using an MACS Miltenyi Biotec CD4+CD25+ Kit according to the manufactured protocol.
Puriﬁed CD3⁺CD4⁺CD25⁺FoxP3⁺ from WT or CD81KO donor mice were coinjected with 1 × 10⁴ 4T1 cells orthotopically into CD81KO recipient mice, a second dose of puriﬁed Tregs was injected 1 week later after tumor injection. Tumor growth was monitor by caliper and metastasis was assessed after 30 days.

IL10 and latent TGFβ determination
Puriﬁed WT or CD81KO Tregs from tumor-bearing mice were isolated as described above and culture in RPMI media supplemented with 10% FCS for 48 hours. Supernatants were collected and IL10 and latent TGFβ cytokines were quantiﬁed by ELISA Kit (Legend Max; Biolegend) according to the manufacturer’s protocol.

Statistical analysis
Results are presented as the mean of triplicates ± SD of at least three independent experiments. Data were analyzed using Prism 6.0 (GraphPad Software) by either unpaired t test or one-way ANOVA when more than two groups were compared. Differences are indicated in the figures. A P value of less than 0.05 was considered statistically signiﬁcant.

Results
Lack of CD81 expression in the host affects tumor growth
To investigate the role of CD81 in tumor growth, we generated a Lewis lung carcinoma (LLC) cell line expressing luciferase (LLC-luc) to monitor tumor growth in vivo. LLC-luc cells were injected subcutaneously into WT, and CD81 knockout (KO) C57BL/6 mice, followed by analysis of tumor volume by caliper measurements and bioluminescence imaging. We found that locally injected tumor growth was signiﬁcantly reduced in CD81KO by comparison to WT and HT mice (Fig. 1A and B). We then injected the tumor intravenously and found that lung metastases were signiﬁcantly reduced in CD81KO mice compared with WT mice (Fig. 1C and D). These studies suggested that the lack of CD81 in the host affects tumor growth and metastases.

To ascertain the role of CD81 in the host versus the tumor, we analyzed the growth of breast cancer cells (EO771), which in contrast to LLC tumor do not express CD81 (Supplementary Fig. S1). EO771 tumor volumes were equal in WT and HT mice, as monitored by caliper measurements (Fig. 2A and B). By contrast, tumor volume was considerably smaller in CD81KO mice throughout the monitoring period (Fig. 2A and B). Moreover, by monitoring individual tumor growth (Fig. 2A, right), we observed tumor shrinkage in more than half of the CD81KO mice. Furthermore, we found that tumors regressed in 10 of 25 of the CD81KO mice on day 25 after injection (Fig. 2C). This result establishes that lack of CD81 in the host plays an important role in susceptibility to tumor growth and because a subset of the mice actually rejected the tumor, suggests that the immune system might play a role.

To establish that reduced tumor growth in CD81KO mice in these tumor models was not because of the host C57BL/6 genetic background, we moved to the 4T1 breast cancer model in BALB/c mice. This tumor expresses CD81 (Supplementary
Fig. S1). 4T1-luc cells were injected orthotopically into female mice and tumor growth was monitored by caliper and bioluminescence imaging. Once again, tumor volume in CD81KO mice was reduced by comparison to their WT littermates (Fig. 3A and B). We also injected 4T1 breast carcinoma cells into males, tumors grew but much slower than in females, yet, tumor volume was reduced in male CD81KO BALB/c, compared with their wild type littermates (data not shown). These results indicate that CD81 deficiency in the host affects the growth of tumor cells of different histologic types and the effect is independent of the genetic background of the host.

Diminished metastasis in CD81KO mice

Metastasis, which occurs during cancer progression, is the leading cause of death among all cancers. We therefore evaluated the role of CD81 in dissemination of tumor cells from the primary site to the lungs using a breast cancer model. 4T1 cells were injected orthotopically into the mammary fat pad of WT, HT, or CD81KO BALB/c mice and 28 to 30 days after injection lungs were perfused with India ink to visualize lung metastases, which appear as macroscopic white colonies on a black background (Fig. 3C, left). We found significantly fewer lung metastases in both female and male CD81KO BALB/c mice in comparison to their WT and HT counterparts (Fig. 3C, right). 4T1 cells are resistant to 6-thioguanine, which offers an alternative approach to the count of macroscopic colonies (28). Lungs from tumor-bearing mice were digested with collagenase IV and elastase and single-cell suspensions were then plated in the presence of 6-thioguanine. As expected, lungs from WT 4T1-bearing mice develop more tumor colonies in comparison to lungs from 4T1 bearing CD81KO mice (Fig. 3D).

To determine if the presence of CD81 in the host would affect colonization of tumor cells in the lung, 4T1 cells were injected intravenously—as with the orthotopic model we observed far fewer lung metastases in CD81KO mice by comparison to WT mice (Fig. 3E). Thus, lack of CD81 is associated with reduced colonization of tumor cells in both backgrounds, in LLC intravenously injected C57BL/6 (Fig. 1C) and in 4T1 intravenously injected BALB/c mice (Fig. 3E). Taken together, these results emphasize the importance of CD81 in the host in tumor progression and metastasis.

CD81 deficiency impairs Treg cells function in tumor-bearing mice

Expansion of Treg cells is a hallmark during cancer progression in both human and mouse (29). Treg accumulation contributes greatly to immune suppression in the tumor microenvironment promoting immune evasion, tumor growth, and dissemination.
Others have demonstrated that Treg depletion is effective in reducing tumor growth and metastasis of 4T1 tumor-bearing mice (30). Indeed, the percentage of Tregs in spleens of 4T1-bearing mice was increased by comparison to naïve mice (Fig. 4A, top). However, an equal accumulation of Tregs was observed in 4T1-bearing WT and CD81KO spleens (Fig. 4A, bottom). Interestingly, CD81 is upregulated in Tregs derived from tumor-bearing WT mice (Fig. 4B).

We proceeded to analyze the function of WT and CD81KO Tregs derived from tumor-bearing mice. Equal numbers of splenic CD4+CD25+ Tregs cells were isolated from tumor-bearing mice (Supplementary Fig. S2). Purified naïve T cells were labeled with tracking dye (CSFE) then stimulated to proliferate by beads coated with anti-CD3 and anti-CD28 mAbs (Fig. 4C, top). Proliferation was also assessed in the presence of the purified CD4+CD25+ Treg cells. This analysis revealed a considerable effect of CD81 deficiency on Treg function. CD81KO Tregs were severely impaired in their ability to suppress proliferation of both CD4 and CD8 T cells in comparison to WT Tregs (Fig. 4C–E). Moreover, analysis of the suppression activity of Tregs from LLC and E0771-bearing CD81KO C57BL/6 mice confirmed impairment in this alternative genetic background (Supplementary Fig. S3).

Taken together these results suggest that CD81 mediates anti-tumor immune responses by affecting Treg cell function. Interestingly, Tregs derived from non-tumor-bearing CD81KO mice were as effective as WT Tregs in their ability to suppress T-cell proliferation (Supplementary Fig. S4). In addition, Tregs equally suppressed naïve WT and CD81KO T cells.

CD81 deficiency impairs MDSC function in tumor-bearing mice

MDSCs are a heterogeneous population that accumulates in response to proinflammatory mediators during infection or during cancer development (31). In addition to their immune suppression activity, MDSCs also promote tumor angiogenesis and metastasis (32). Moreover, MDSCs have been shown to suppress the adaptive immune response to tumors. Indeed, multiple previous studies have demonstrated sharp increases in the number of MDSCs in metastatic cancer models.
MDSCs circulating in 4T1-bearing BALB/c mice (33). Some of these studies have also revealed a suppressive effect of MDSC on T-cell proliferation (34). We therefore decided to analyze the impact of CD81 on MDSC function.

CD81 is expressed on the surface of MDSCs (Fig. 5A); however, its absence does not affect the maturation of MDSCs as naïve CD81KO, HT, and WT C57Bl6 and BALB/c mice have similar percentage (10–20%) of blood MDSCs (Fig. 5B). Even 4T1 tumor-bearing mice, which accumulate circulating MDSCs rapidly after tumor inoculation, show an equal increase over time in both WT and CD81KO mice (Fig. 5C). Similarly, MDSCs equally accumulate in the spleen, the primary tumor site and in the lungs (data not shown), independent of CD81 presence (Fig. 5D).

Next, we tested if CD81KO MDSCs suppress T-cell proliferation. As expected, MDSCs from WT 4T1-bearing mice suppressed the proliferation of naïve CD4⁺ T cells, whereas CD81KO MDSCs were severely impaired in their ability to suppress this proliferation (Fig. 5F and G). Similarly, CD8⁺ T-cell proliferation was suppressed by WT MDSCs, but to a considerably lesser extent by CD81KO MDSCs derived from tumor-bearing mice (Fig. 5H).

Several mechanisms by which MDSCs mediate immune suppression include arginine depletion through ARG-1-dependent consumption and l-cysteine deprivation via its consumption and sequestration (35, 36). However, Arg1 expression and other related genes did not differ between WT and CD81KO MDSC (Supplementary Fig. S5A and S5B).

Generation of oxidative stress, which is caused by the production of ROS and reactive nitrogen species, is another pathway utilized by MDSCs to mediate suppression (37), but we did not detect any difference in ROS production between WT and CD81KO MDSCs (Supplementary Fig. S6).

MDSCs have also been shown to modulate innate immune cells by polarizing M1 to M2 macrophages (38). As expected, WT MDSCs strongly polarize M1 macrophages into M2, as evident by secretion of high amounts of IL10 and by the expected inhibition in IFNγ and IL12 secretion; however, CD81KO MDSCs similarly polarize M1 into M2 macrophages (Fig. 5E).

CD81 in Tregs promotes tumor growth and metastasis

To determine if reduced tumor growth and metastasis was due to the impaired immune suppression in the absence of CD81, we adoptively transferred either WT or CD81KO Tregs from tumor-bearing animals together with 4T1 breast cancer cells into CD81KO recipient mice (Fig. 6A). Mice that received WT Tregs had increased tumor volumes in contrast to mice that received CD81KO Tregs (Fig. 6B). Furthermore, lung metastases were increased upon adoptive transfer of WT but not CD81KO Tregs (Fig. 6C). However, when MDSCs were adoptively transferred no differences were observed in tumor growth and metastasis (data not shown). Tregs mediate immune suppression by different mechanisms such as expressing inhibitory receptors that blocks activation of effectors cells. However, although CD81 expression

Figure 4.
Tregs are less suppressive in 4T1-bearing CD81KO mice. A, top, percentage of CD3⁺ CD4⁺ CD25⁺ FoxP3⁺ cells in naïve versus 4T1-bearing WT mice. Bottom, percentage of splenic Tregs (CD4⁺ CD25⁺ FoxP3⁺) in 4T1 tumor-bearing WT, HT, and CD81KO BALB/c mice. B, CD81 expression on splenic FoxP3⁺ cells in naïve and 4T1-bearing WT mice. C–E, purified WT and CD81KO splenic Tregs from tumor-bearing mice were cocultured at the indicated Treg:CD4⁺ ratios with CFSE-labeled naïve CD3/CD28-stimulated T cells. C, CD4⁺ T-cell proliferation was analyzed after 5 days and is shown as histograms. D and E, division index quantification of CD4⁺ (D) and CD8⁺ (E) T cells of five independent experiments are shown.
was increased in WT Tregs, both WT and CD81KO Treg expressed similar levels of CTLA-4, PD-1, OX-40, ICOS, CD137, and GITR (Supplementary Fig. S7). Finally, we also tested the ability of Tregs to secrete IL10 and TGFβ; cytokines known to mediate immune suppression. Although both WT and CD81KO Tregs secreted similar amounts of TGFβ (Fig. 6D), IL10 secretion was diminished in CD81KO Tregs (Fig. 6E).

Taken together, the absence of CD81 in Tregs (Figs. 4 and 6) and in MDSCs (Fig. 5) impairs their T-cell suppressive function.

Discussion

Over the past years several impairments have been described in CD81KO mice (23, 24, 39, 40). However, none of the studies have evaluated the contribution of CD81 in the host during cancer progression. Here we report for the first time that CD81 deficiency in the host has a profound effect on tumor growth and metastasis in two genetic backgrounds of CD81KO mice.

Tetraspanins are widely expressed in the body. Hence, tumor cells that arise from normal tissues also express these proteins. Indeed, some tetraspanin members have been shown to play a role in cancer progression. A definite role for CD151 was demonstrated in two independently derived CD151KO mice that were challenged with several tumor models (16, 19). In the same studies, diminished metastasis in CD151KO hosts was suggested to be due to impaired adhesion and trans-endothelial migration of CD151 expressing tumor cells (19).

Numerous studies have demonstrated that 4T1 tumors induce a strong suppressive microenvironment with an accumulation of MDSCs and Treg cells. Tregs, a subset of CD4+CD25+ T cells, infiltrate tumors and suppress antitumor activity of effector T cells. Previous studies have demonstrated that depletion of Tregs by anti-CD25 antibodies completely abrogates metastasis of 4T1
The fact that Tregs in 4T1-bearing mice upregulated CD81 expression suggested that CD81 could potentially mediate Treg function. As expected, Tregs accumulated in 4T1-bearing mice; however, an equal increase was seen in both WT and CD81KO mice. Remarkably, the suppression ability of these tumor-induced Tregs was severely impaired in CD81KO mice. Moreover, both CD4\(^+\) and CD8\(^+\) T cells proliferated in the presence of CD81KO Treg recipient mice. A second transfer of Tregs was given after 7 days after tumor injection. B, tumor growth was monitored by caliper. C, lung metastasis was assessed by India ink staining. D and E, latent TGF\(\beta\) (D) and IL10 (E) secretion was measured by ELISA of purified WT or CD81KO Tregs from 4T1-bearing mice after 2 days in culture. NS, nonsignificant.

Figure 6.
CD81 in Tregs promotes tumor growth and metastasis. A, adoptive transfer of WT or CD81KO Tregs scheme: 4T1 were injected into WT or CD81KO donor mice and Tregs were purified on day 10 and 17 after tumor injection. Purified WT or CD81KO Tregs (CD3\(^+\)CD4\(^+\)CD25\(^+\)FoxP3\(^+\)) were then coinjected with 4T1 tumor cells into CD81KO recipient mice. A second transfer of Tregs was given after 7 days after tumor injection. B, tumor growth was monitored by caliper. C, lung metastasis was assessed by India ink staining. D and E, latent TGF\(\beta\) (D) and IL10 (E) secretion was measured by ELISA of purified WT or CD81KO Tregs from 4T1-bearing mice after 2 days in culture. NS, nonsignificant.
T-cell suppression (34, 50). However, molecules, such as Epstein-Barr virus induced gene 3 (EBi3, which encodes IL27b) and interleukin-12b (which encodes IL12a/p35), were shown to modulate Treg function (51). Eb3KO and Il12aKO Tregs had significantly reduced regulatory activity in vitro and also failed to cure inflammatory bowel disease in vivo, by comparison to WT Tregs.

Tregs and MDSCs modulate immune cells by plethora of mechanisms. Broadly, these mechanisms can be subdivided into cell–cell contact-dependent (Fasl–FAS, PD-1/PD-L1, CTLA-4/CD80–CD86) and those mediated mainly by secretion of immune-modulators molecules, such as IL10, TGF–β, IL37, and IL35 reviewed in ref. 32, or by sequestering factors required by effectors cells, such as decreasing the cysteine (36) and glutathione pools (52). Additional mechanisms observed in experimental mice models have shown that MDSCs, which are found at pre-metastatic distant organ sites, enable recruitment of colonizing tumor cells thereby promoting metastasis (reviewed in ref. 53).

Although we explored some of these inhibitory mechanism that MDSCs or Tregs use, we only found that CD81 KO Tregs derived from tumor-bearing mice have reduced IL10 secretion. Although the exact mechanism(s) by which CD81 modulates Treg and MDSC function still need(s) further investigation, it is clear that the presence of CD81 in the host has a major effect on tumor growth and that this effect is mediated partially by the immune system.

In regard to host–tumor interactions, it is well established that tumor cells secrete exosomes that modulate the microenvironment (54). CD81 and other tetraspanins are well-known markers of exosomes (55). Furthermore, a recent paper demonstrated that exosomes secreted by fibroblasts increased metastasis of MDA-MB-231 human breast cancer cells to the lungs of immunocompromised mice, importantly, the deletion of CD81 from these exosomes highly reduced metastasis of these tumor cells (56). However, uptake of exosomes was shown to require the presence of the integrin molecule CD29 and CD81, knocking down both molecules inhibited exosome uptake by mesenchymal stem cells (57). In view of these studies it is intriguing that although naive CD81KO and WT Tregs suppressed T-cell proliferation equally, when tumor was on board, the function of CD81KO Tregs was impaired. One possible scenario is that CD81KO Tregs have an intrinsic defect in the uptake of exosome from tumor cells, which is then followed by an inability to activate and suppress effector T cells.

In summary, we report that CD81 deficiency greatly contributes to tumor development as evident by reduced tumor growth and metastasis in three different tumor models in two different genetic backgrounds. We also demonstrate that the suppressive function of Tregs and MDSCs is impaired in CD81KO tumor-bearing mice. Ongoing studies are aimed at determining the contribution of CD81 on the host versus the tumor cells in growth and metastasis.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Grant Support**

This work was supported by the Translational Cancer Award from Stanford Cancer Institute and the Breast Cancer Research program from the Department of Defense grant W81XWH-14-1-0397 (both grants were awarded to S. Levy).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 15, 2015; revised July 22, 2015; accepted August 10, 2015; published OnlineFirst September 1, 2015.


Tetraspanin CD81 Promotes Tumor Growth and Metastasis by Modulating the Functions of T Regulatory and Myeloid-Derived Suppressor Cells


Cancer Res 2015;75:4517-4526. Published OnlineFirst September 1, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-15-1021

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2015/08/29/0008-5472.CAN-15-1021.DC1

Cited articles
This article cites 57 articles, 27 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/21/4517.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/75/21/4517.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/75/21/4517.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.