Targeting YAP-Dependent MDSC Infiltration Impairs Tumor Progression

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ABSTRACT
The signaling mechanisms between prostate cancer cells and infiltrating immune cells may illuminate novel therapeutic approaches. Here, utilizing a prostate adenocarcinoma model driven by loss of Pten and Smad4, we identify polymorphonuclear myeloid-derived suppressor cells (MDSC) as the major infiltrating immune cell type, and depletion of MDSCs blocks progression. Employing a novel dual reporter prostate cancer model, epithelial and stromal transcriptomic profiling identified CXCL5 as a cancer-secreted chemokine to attract CXCR2-expressing MDSCs, and, correspondingly, pharmacologic inhibition of CXCR2 impeded tumor progression. Integrated analyses identified hyperactivated Hippo–YAP signaling in driving CXCL5 upregulation in cancer cells through the YAP–TEAD complex and promoting MDSC recruitment. Clinicopathologic studies reveal upregulation and activation of YAP1 in a subset of human prostate tumors, and the YAP1 signature is enriched in primary prostate tumor samples with stronger expression of MDSC-relevant genes. Together, YAP-driven MDSC recruitment via heterotypic CXCL5–CXCR2 signaling reveals an effective therapeutic strategy for advanced prostate cancer.

SIGNIFICANCE: We demonstrate a critical role of MDSCs in prostate tumor progression and discover a cancer cell nonautonomous function of the Hippo–YAP pathway in regulation of CXCL5, a ligand for CXCR2-expressing MDSCs. Pharmacologic elimination of MDSCs or blocking the heterotypic CXCL5–CXCR2 signaling circuit elicits robust antitumor responses and prolongs survival. Cancer Discov; 6(1): 1–16. ©2015 AACR.

INTRODUCTION
The tumor microenvironment (TME) is comprised of a complex mixture of tumor-associated fibroblasts, infiltrating immune cells, endothelial cells, extracellular matrix proteins, and signaling molecules, such as cytokines (1–3). Homotypic and heterotypic interactions between these cellular constituents play essential roles in cancer development and response to therapeutics (3, 4). Among the infiltrating immune cells, myeloid-derived suppressor cells (MDSC) represent a phenotypically heterogeneous population of immature myeloid cells that play a tumor-promoting role by maintaining a state of immunologic anergy and tolerance (5). In particular, activated MDSCs provide a source of secreted chemokines, cytokines, and enzymes, which suppress local T-cell activation and viability (5). In addition, MDSCs can suppress T-cell activity through deprivation of nutrients, such as l-arginine and l-cysteine, and interference with T-cell receptor functions via reactive oxygen species (ROS) and reactive nitrogen species.

Prostate cancer is the most common noncutaneous malignancy in men in the United States. Similar to many other solid tumor types, prostate cancer is characterized by a rich tumor–stroma interaction network that forms the TME (1–3). In prostate cancer, various signaling pathways have been implicated in the cross-talk between tumor and stroma, such as androgen receptor signaling, FGF, SRC, TGFβ, IGF, integrin, and Hedgehog pathways (1). Interestingly, MDSC abundance in the blood correlates with circulating PSA levels in patients with prostate cancer (6–8). MDSCs have been identified recently as a TME constituent in an indolent prostate cancer mouse model with conditional Pten deletion (9) and demonstrated to antagonize senescence during early tumorigenesis (10). However, the molecular mechanisms underlying the recruitment of MDSCs are not well understood, and the extent to which MDSCs facilitate prostate cancer progression has not been determined.

Previously, we have shown that deletion of Pten in the mouse prostate causes upregulation of SMAD4, which constrains cell proliferation and invasion, and, accordingly, dual deletion of Pten and Smad4 results in rapid prostate cancer progression, including metastasis (11). Comparative transcriptomic and cell profile analyses of PTEN- versus PTEN/SMAD4-deficient prostate cancer revealed a prominent immune signature and resident MDSCs as a major TME population in PTEN/SMAD4-deficient tumors. Biologic, molecular, and pharmacologic analyses established that a YAP1-mediated CXCL5–CXCR2 signaling axis recruits MDSCs into the TME and that...
MDSCs play critical roles in facilitating tumor progression. Our comprehensive analyses using a prostate cancer model coupled with clinical validation using patient samples support the view that targeting either MDSC recruitment or infiltrated MDSCs may represent a valid therapeutic opportunity in treating advanced prostate cancer.

RESULTS

Prominent Infiltration of Immune Cells in the Ptenpc−/−Smad4pc−/− Tumor Model

We previously reported that conditional deletion of Smad4 bypassed the senescence barrier instigated by Pten loss in the prostate epithelia, resulting in a highly proliferative and invasive prostate adenocarcinoma characterized by an exuberant stromal reaction and frequent metastasis to distant organs (11). Correspondingly, ingenuity pathway analysis (IPA) revealed prominent representation of cell movement, cell proliferation, and antigen presentation as the top three categories represented in the Ptenpc−/−Smad4pc−/− tumors (11). Further analysis revealed a prominent immune signature, including Granulocytes Adhesion and Diapedesis, Leukocytes Extravasation Signaling, and Agrandulocytes Adhesion (Fig. 1A). In addition, further grouped into various immune cell subpopulations (Fig. 2A and Supplementary Fig. S1A). Among the infiltrating immune cells, there was a striking age-dependent increase of CD11b+Gr1+ cells in tumors (Fig. 2B) and peripheral blood from Ptenpc−/−Smad4pc−/− mice (Fig. 2C); this trend was much less pronounced in the spleen or draining lymph nodes (Supplementary Fig. S1B; for gating strategy, see Supplementary Fig. S1C).

CD11b+Gr1+ Cells from Ptenpc−/−Smad4pc−/− Tumors Are Potently Immunosuppressive

To evaluate the potential immunosuppressive activity of intratumoral CD11b+Gr1+ cells from Ptenpc−/−Smad4pc−/− tumors, we examined T-cell proliferation using a standard cell coculture system. These CD11b+Gr1+ cells strongly suppressed CD3 and CD28 antibody-induced T-cell proliferation and activation (Fig. 3A and B; see Supplementary Fig. S2 for cell isolation strategy), establishing that CD11b+Gr1+ cells are indeed functional MDSCs.

MDSCs can be further classified as a Ly6G+Ly6C− subset with polymorphonuclear phenotype (PMN-MDSC) and a Ly6G+Ly6C− subset with monocytic phenotype (M-MDSC; ref. 13). PMN-MDSCs represented the major MDSC population in Ptenpc−/−Smad4pc−/− tumors (Fig. 3C and D), consistent with previously observed preferential expansion of PMN-MDSCs in tumor-bearing mice of various syngeneic models (5, 9, 13). The abundance of PMN-MDSCs was further confirmed by IHC for Ly6G, as shown by quantification of both intraepithelial and stromal Ly6G+ cells in tumors from Ptenpc−/−Smad4pc−/− mice and Pten−/− mice (Fig. 3E and F). It has been shown previously that ROS production by PMN-MDSCs is one of the mechanisms driving immune suppression (5, 14–16). Correspondingly, IPA revealed that pathways involved in ROS and nitric oxide (NO) production are among the top pathways activated in Ptenpc−/−Smad4pc−/− tumors (Fig. 1A, arrow). Consistent with the increased infiltration of PMN-MDSCs in the Ptenpc−/−Smad4pc−/− tumors, the expression of several subunits of NADPH oxidase (Nox2, p40phox, and p47phox), which are responsible for ROS production in PMN-MDSCs (5), was significantly upregulated in Ptenpc−/−Smad4pc−/− tumors relative to Pten−/− tumors (Fig. 3G). Moreover, Arg1, but not Nos2, was highly upregulated in the Ptenpc−/−Smad4pc−/− tumors (Fig. 3G). Together, MDSCs in autochthonous Ptenpc−/−Smad4pc−/− tumors display strong T-cell-suppressive activity and are predominantly the PMN-MDSC subtype.

Immunodepletion of MDSCs Impedes Tumor Progression in Ptenpc−/−Smad4pc−/− Mice

Enrichment of MDSCs in advanced Ptenpc−/−Smad4pc−/− tumors prompted us to explore the possible role of MDSCs in tumor progression. Using a well-characterized anti-Gr1 neutralizing monoclonal antibody (clone RB6-8C5; ref. 17), MDSCs were depleted in Ptenpc−/−Smad4pc−/− mice at 14 weeks of age, a point coincident with progression to the early invasive carcinoma stage (see Supplementary Fig. S3A for treatment scheme). The potent MDSC depletion activity of anti-Gr1 monoclonal antibody was evidenced by significantly decreased PMN-MDSCs and M-MDSCs in peripheral blood as early as day 2 after treatment (Supplementary Fig. S3B). In
Figure 1. Prominent infiltration of immune cells in the Pten<sup>pc−/−</sup>/Smad4<sup>pc−/−</sup> tumors as compared with Pten<sup>pc−/−</sup> tumors. 

A, the top 10 activated pathways in Pten<sup>pc−/−</sup>/Smad4<sup>pc−/−</sup> tumors (n = 5) as compared with Pten<sup>pc−/−</sup> tumors (n = 5) identified by IPA. RAR, retinoic acid receptor. 

B, a significant increase in the infiltration of immune cells as shown by IHC for CD45 in Pten<sup>pc−/−</sup>/Smad4<sup>pc−/−</sup> tumors as compared with Pten<sup>pc−/−</sup> tumors (n = 3). AP, anterior prostate; VP, ventral prostate; DLP, dorsolateral prostate. H&E, hematoxylin and eosin staining. Scale bars, 200 μm. 

C, quantification of tumor-infiltrating CD45<sup>+</sup> cells (AP, VP, and DLP combined) in Pten<sup>pc−/−</sup> tumors and Pten<sup>pc−/−</sup>/Smad4<sup>pc−/−</sup> from 16-week-old mice (n = 3), assessed by CyTOF. D, percentages of various immune cell populations within the CD45<sup>+</sup>-infiltrating immune cells in prostate tumors from 16-week-old Pten<sup>pc−/−</sup> and Pten<sup>pc−/−</sup>/Smad4<sup>pc−/−</sup> mice, assessed with CyTOF (9-marker) and analyzed with FlowJo. CD11b<sup>+</sup> myeloid cells are significantly greater in Pten<sup>pc−/−</sup>/Smad4<sup>pc−/−</sup> tumors as compared with Pten<sup>pc−/−</sup> tumors (n = 3; P < 0.05).
Figure 2. CD11b+Gr1+ cells are significantly increased in Pten−/−Smad4−/− tumors as compared with Pten−/− tumors. A, SPADE tree derived from CyTOF [17-marker] analysis of whole-tumor cell population from Pten−/−Smad4−/− mice at 5 weeks, 8 weeks, and 14 weeks of age (n = 3). Live single cells were used to construct the tree. Cell populations were identified as prostate cancer (PCa) cells (EpCAM+CD45−), nonimmune TME cells (EpCAM−CD45−), T cells (CD45+CD3+TCRβ+), B cells (CD45+B220+CD19+), natural killer (NK) cells (CD45+NK1.1+), dendritic cells (CD45+CD11c+), putative MDSCs (CD45+CD11b+Gr1+), and macrophages (CD45+CD11b−Gr1−). On the right plots, the tree is colored by the median intensity of individual markers shown on the top to highlight infiltrating immune cells (EpCAM−CD45+), epithelial prostate cancer cells (EpCAM+CD45+), total myeloid cells (CD45+CD11b+), and putative MDSCs (CD45+CD11b+Gr1+). B and C, CyTOF analysis of tumors (B) or peripheral blood (C) from 5-, 8-, and 14-week-old Pten−/−Smad4−/− mice revealed an age-dependent increase in the MDSC infiltration. Prostate from wild-type (WT) mice at 16 weeks old was used as control (n = 3 for each genotype). See also Supplementary Fig. S1.
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Figure 3. MDSCs from Ptenpc−/− Smad4pc−/− tumors display potent immunosuppressive activities and are dominated by PMN-MDSCs. A, CD11b+Gr1+ cells from Ptenpc−/− Smad4pc−/− tumors display potent immune-suppressive activity toward T-cell activation as demonstrated by CFSE dilution assay in triplicate. B, summarized result from A. C and D, flow cytometry analysis shows PMN-MDSCs as the major population in the infiltrated MDSCs in established Ptenpc−/− Smad4pc−/− tumors at AP, DLP, and VP (n = 5). SSC, side scatter. E and F, a significant increase in Ly6G+ cells in Ptenpc−/− Smad4pc−/− tumors as compared with the Ptenpc−/− tumors as shown by IHC for Ly6G and quantified by location of positively stained cells in the intraepithelial or stromal compartment of the tumor at AP, DLP, and VP (n = 3). G, quantification of the mRNA expression of subunits of NADPH oxidase (Nox2, p40phox, and p47phox), Arg1, and Nos2 in the Ptenpc−/− Smad4pc−/− tumors and the Ptenpc−/− tumors (n = 5). In B, D, F, and G, * P < 0.05; ** P < 0.01; *** P < 0.001; ns, nonsignificant. Also see Supplementary Fig. S2.
addition, a systemic reduction of MDSCs in spleen, bone marrow, and prostate tumors was documented following a 30-day treatment regimen of anti-Gr1 monoclonal antibody (Fig. 4A and Supplementary Fig. S3C). This MDSC depletion was accompanied by an increase of CD8+ T cells (so-called killer T cells; Fig. 4A), consistent with elimination of the T-cell suppression activity of MDSCs. Importantly, in line with the CD8+ T-cell expansion, we observed that the Gr1-treated prostate displayed remarkable weight reduction in ventral and dorsolateral prostates (VP and DLP; Fig. 4B). The lack of difference in the weight of the anterior prostate (AP) is likely due to the fact that the AP tends to develop cysts with fluid accumulated inside the gland (18, 19), which also prevents the accurate measure of the prostate weight (Supplementary Fig. S3D). Histopathology analysis revealed adenocarcinoma was the predominant pathology in mice treated with the control IgG, whereas mouse prostatic intraepithelial neoplasia (miPNI) was the predominant morphologic presentation in prostates from mice treated with anti-Gr1 monoclonal antibody (Fig. 4C and Supplementary Table S2). In addition, by immunohistochemical staining for CD45, Ki67, vimentin, smooth muscle actin (SMA), and Trichrome staining, we observed that tumor remnants in mice treated with anti-Gr1 monoclonal antibody displayed markedly reduced levels of cellular proliferation, stromal reaction, and inflammation as compared with those tumors treated with control IgG antibody (Supplementary Fig. S4A).

In another therapeutic trial, we also utilized the recently developed MDSC-specific peptide–Fc fusion protein (i.e., peptibodies) that has been shown to effectively eliminate MDSCs in vivo through targeting the S100A9 surface protein (20). Employing a hydrodynamic injection approach for nucleic acid delivery (21), intravenous injection of either Pep-H6 peptibody expression vector or irrelevant control peptibody vector was initiated at 14 weeks every 4 days in Pten+/− Smad4−/− tumors as compared with those tumors treated with control IgG antibody (Supplementary Fig. S4B). Pep-H6 peptibody significantly reduced the MDSCs in peripheral blood, whereas such effect was not observed using the irrelevant control peptibody (Supplementary Fig. S4B). Pep-H6 peptibody treatment for 1 month led to a dramatic decrease in cancer cell content in the prostate tumors (Fig. 4D) and provided significant survival benefit for tumor-bearing mice (Fig. 4E). Together, our data strongly support the view that MDSC depletion blocks prostate tumor progression in the Pten+/− Smad4−/− model.

**CXCL5–CXCR2 Signaling Promotes MDSC Recruitment and CXCR2 Inhibition Delays Tumor Progression in Pten+/− Smad4−/− Mice**

To elucidate the cellular origins and signaling molecules governing MDSC recruitment to prostate tumors, we incorporated the mTmG dual fluorescence reporter allele into the Pten+/− Smad4−/− model where signaling events between tumor cells and stroma can be precisely delineated. The mTmG allele (22) allows Cre-dependent GFP expression in prostate epithelial cells and ubiquitous tdTomato expression in all other non–Cre-expressing cells (Fig. 5A). Transcriptomic and IPA analyses of FACS-sorted GFP+ tumor cells and Tomato+ stromal cells showed distinct expression patterns by hierarchical clustering (Fig. 5A) with tumor cells enriched for pathways involved in cell adhesion molecules and tight junctions (consistent with their epithelial nature) and stromal cells displaying activation of more diverse pathways involved in chronic inflammation, such as cytokine/cytokine receptor interaction, chemokine, JAK–STAT, T-cell receptor, and B-cell receptor signaling (P < 0.01, data not shown). This result is consistent with the immunopathologic and histopathologic analyses showing a massive infiltration of immune cells in the Pten+/− Smad4−/− tumors.

Employing this new model, we sought to identify genes that were upregulated in Pten+/− Smad4−/− cancer cells relative to Pten+/− cancer cells that might illuminate mechanisms involved in the recruitment of MDSCs by classifying the upregulated genes into either stroma- or tumor-enriched genes. To this end, we previously generated list of 242 genes with greater than 2-fold increased expression in Pten+/− Smad4−/− relative to Pten+/− tumors (11) was intersected with 486 genes preferentially expressed in Pten+/− Smad4−/− GFP+ cancer cells relative to Tomato+ stroma cells (fold change ≥4; Supplementary Tables S3 and S4), yielding 28 genes that are markedly enriched in Pten+/− Smad4−/− cancer cells (Supplementary Table S5). Among these 28 genes, Cxcl5, which encodes a key cytokine involved in MDSC recruitment (23, 24), is the most significantly upregulated cancer cell–specific cytokine in Pten+/− Smad4−/− tumors as compared with Pten+/− tumors (Fig. 5B and Supplementary Fig. S5A). Notably, CXCR2, the cognate receptor for Cxcl5, is also upregulated in Pten+/− Smad4−/− tumors as compared with Pten+/− tumors and is significantly enriched in Pten+/− Smad4−/− Tomato+ stroma cells (Fig. 5B). The upregulation of Cxcl5 expression in Pten+/− Smad4−/− prostate tumors was further confirmed by IHC (Fig. 5C). In addition, we performed FACS analysis of CD11b+Gr1+ cells and CD11b+Gr1− cells from bone marrow, spleen, peripheral blood, and tumors for CXCR2 expression. As shown in Supplementary Fig. S5B, CD11b+Gr1− cells (largely lymphocytes) are devoid of CXCR2 expression, whereas a large fraction of CD11b+Gr1+ cells express CXCR2. When CXCR2 expression was further separated into CXCR2hi and CXCR2lo, we observed an enrichment of the CXCR2hi subpopulation in the CD11b+Gr1− cells in prostate tumors compared with CD11b+Gr1+ cells from bone marrow, spleen, or blood (Supplementary Fig. S5B). This is consistent with the model of active recruitment of MDSCs by tumors through CXCR2-mediated chemotraction.

To validate the CXCL5–CXCR2 axis in the recruitment of MDSCs to the TME of Pten+/− Smad4−/− tumors, we assessed the impact of pharmacologic inhibition of CXCL5 and CXCR2 in MDSCs using a transwell migration assay (23). First, anti–CXCL5–neutralizing antibody pretreatment of conditioned medium (CM) derived from Pten+/− Smad4−/− prostate cancer cell line resulted in decreased migration of MDSCs (Fig. 5D). Second, CXCR2 inhibitor SB255022 or anti-CXCR2 neutralizing antibody pretreatment also impeded migration of MDSCs (Fig. 5D). Third, in vivo blockade of the CXCL5–CXCR2 axis using SB255022 in 14-week-old Pten+/− Smad4−/− mice over a 14-day daily dosing schedule revealed a dramatic reduction in infiltration of MDSCs in the prostate tumors (Supplementary Fig. SSC and SSD). Notably, similar to mice treated with anti-Gr1 neutralizing antibody, these SB255022-treated Pten+/− Smad4−/− mice also showed significant reduction in tumor burden (VP and DLP) as compared with the vehicle-treated controls (Fig. 5E and Supplementary Fig. SSE). Strikingly,
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Figure 4. Targeting MDSCs with anti-Gr1 neutralizing antibody or MDSC-specific peptibody significantly delayed tumor progression in Pten<sup>pc−/−</sup>Smad4<sup>pc−/−</sup> mice. A, administration of Gr1-neutralizing antibody in vivo significantly reduced CD45<sup>+</sup>-infiltrating immune cells, reduced MDSCs, and increased CD8<sup>+</sup> T cells among total T cells in Pten<sup>pc−/−</sup>Smad4<sup>pc−/−</sup> tumors (n = 4), measured by flow cytometry. B, Gr1 antibody treatment of 14-week-old mice significantly reduced the weight of VP and DLP in Pten<sup>pc−/−</sup>Smad4<sup>pc−/−</sup> mice. C, Gr1 antibody remarkably altered the tumor histopathology in Pten<sup>pc−/−</sup>Smad4<sup>pc−/−</sup> adenocarcinoma, analyzed by hematoxylin and eosin staining of AP, VP, and DLP. D, one month of Pep-H6 peptibody treatment led to significant appearance and histology changes of the Pten<sup>pc−/−</sup>Smad4<sup>pc−/−</sup> adenocarcinoma. Irre-Pep, irrelevant control peptibody. E, Kaplan-Meier survival curve showing the significant delay of mortality caused by Pep-H6 peptibody treatment of Pten<sup>pc−/−</sup>Smad4<sup>pc−/−</sup> mice. In A and B, *, P < 0.05 and ***, P < 0.001; Also see Supplementary Figs. S3 and S4.
Figure 5. CXCL5–CXCR2 axis plays an indispensable role in recruitment of MDSCs and promotion of tumor progression. A, establishment of Pten<sup>pc<–/–</sup>Smad4<sup>pc<–/–</sup>mTmG<sup>F/+</sup> model allows fluorescent visualization of the GFP<sup>+</sup> tumor cells intermixed with Tomato<sup>+</sup> stroma (left; FACS isolation of GFP<sup>+</sup> tumor cells and Tomato<sup>+</sup> stromal cells from the prostate adenocarcinoma (middle); microarray analysis to identify differentially expressed genes (right). In the fluorescence image, Bl denotes bladder (completely Tomato<sup>+</sup>; n = 2). B, quantification of mRNA expression shows that Cxcl5 and Cxcr2 were both expressed at higher levels in Pten<sup>pc<–/–</sup>Smad4<sup>pc<–/–</sup> tumors than in Pten<sup>pc<–/–</sup> tumors, and Cxcl5 expression was enriched in GFP<sup>+</sup> tumor cells, whereas Cxcr2 expression was enriched in Tomato<sup>+</sup> stromal cells (n = 5). C, IHC for CXCL5 showed significantly higher expression levels of CXCL5 in Pten<sup>pc<–/–</sup>Smad4<sup>pc<–/–</sup> tumors than Pten<sup>pc<–/–</sup> tumors (n = 3). D, blocking the CXCL5–CXCR2 axis by CXCL5-neutralizing antibody, CXCR2 inhibitor SB225002, or CXCR2-neutralizing antibody significantly decreased migration of MDSCs toward conditioned medium from Pten<sup>pc<–/–</sup>Smad4<sup>pc<–/–</sup> tumor cells, evaluated with an in vitro transwell migration assay in triplicate. E and F, CXCR2 inhibitor SB225002 treatment of Pten<sup>pc<–/–</sup>Smad4<sup>pc<–/–</sup> mice for 14 days (n = 4) resulted in significantly reduced tumor weight of VP and DLP and significantly delayed progression for AP prostate cancer shown by hematoxylin and eosin staining. G, CXCR2 inhibitor SB225002 treatment of Pten<sup>pc<–/–</sup>Smad4<sup>pc<–/–</sup> mice significantly prolonged their overall survival. In B, D, and E, *, P < 0.05; **, P < 0.01; and ***, P < 0.001. Also see Supplementary Fig. S5.
all SB225002-treated tumors presented with prostatic intraepithelial neoplasia (PIN) pathology, whereas the control group uniformly possessed advanced adenocarcinoma (Fig. 5F and Supplementary Table S2). Furthermore, SB225002 treatment significantly prolonged the overall survival of the Ptenpc−/−/Smad4pc−/− mice as compared with the vehicle control (Fig. 5G). Thus, we conclude that the CXCL5-CXCR2 axis plays a prominent role in the recruitment of MDSCs to the Ptenpc−/−/Smad4pc−/− prostate TME and that inhibition of this axis profoundly impairs tumor progression.

**YAP1 Is Activated in Ptenpc−/−/Smad4pc−/− Tumors and Directly Regulates Cxcl5 Transcription**

Having identified cancer cell-derived CXCL5 as a key signaling molecule governing recruitment of MDSCs into the TME, we sought to define the molecular mechanisms underlying the strong induction of CXCL5 expression in the Ptenpc−/−/Smad4pc−/− cancer cells. As CXCL5 expression is not significantly upregulated in the Pten−/− tumors (Fig. 5C), we performed unbiased Gene Set Enrichment Analysis (GSEA) to identify pathways that were activated in the Ptenpc−/−/Smad4pc−/− tumors as compared with Pten−/− tumors, aiming to identify potential regulators for Cxcl5 in Ptenpc−/−/Smad4pc−/− tumors. The YAP oncoprogenic signature emerged as the second most hyperactivated pathway (Fig. 6A and Supplementary Fig. S6A). Although it is known that the Hippo-YAP pathway plays an important role in development and cancer in organs such as the liver, skin, intestine, and pancreas (25–27), the role for the Hippo-YAP pathway in prostate cancer biology is emerging. Specifically, Hippo pathway components LATS1/2 have been implicated in anoikis and metastasis in prostate cancer (28), and ERG-induced YAP1 activation can promote age-related prostate tumor development (29). However, beyond the cancer cell–specific functions, the Hippo–YAP1 pathway has not been linked to signaling communication between cancer cells and immune cells in the TME. Consistent with the *in silico* analysis, IHC analysis documented a dramatic increase in the nuclear localization of YAP1 in Ptenpc−/−/Smad4pc−/− cancer cells as compared with Pten−/− cancer cells (Fig. 6B). As YAP1, a transcriptional coactivator and the downstream mediator of Hippo signaling, is regulated posttranscriptionally by either kinase-mediated degradation or cytoplasmic sequestration (25), our findings of increased nuclear localization of YAP1 are consistent with the hypothesis that the Hippo–YAP pathway is activated in the Ptenpc−/−/Smad4pc−/− tumors. In addition, unbiased oPOSSUM analysis (30) indicated that TEAD1, a member of the TEAD transcription factor family that is required for YAP1 function, ranked second among the top 10 transcription factors with overrepresented binding sites in the 70 cancer-specific genes that were upregulated in the Ptenpc−/−/Smad4pc−/− tumors as compared with the Pten−/− tumors (≥1.5 fold, Z-Score = 13.362; Supplementary Fig. S6B and S6C), an observation reinforcing the relevance of the Hippo–YAP pathway. Furthermore, we identified six YAP/TEAD binding motifs in the promoter of Cxcl5 gene (Supplementary Fig. S6D), suggesting YAP1 could be directly involved in the recruitment of MDSCs through regulating Cxcl5 expression. This hypothesis was supported by chromatin immunoprecipitation (ChIP) assay showing that YAP1 binds to Cxcl5 promoter (Fig. 6C) and that shRNA-mediated knockdown of Yap1 in Ptenpc−/−/Smad4pc−/− cancer cells drastically reduced the expression of Cxcl5 mRNA (Fig. 6D). In addition, overexpression of a constitutively active YAP1S127A mutant dramatically increased Cxcl5 mRNA expression in the Ptenpc−/−/Smad4pc−/− cell line (Fig. 6E), whereas overexpression of a TEAD binding defective YAP1 mutant S127A/S94A compromised its ability to activate Cxcl5 transcription (Fig. 6F). To examine the effect of YAP1-dependent cytokine signaling in the regulation of MDSCs recruitment, we first prepared CM from the Ptenpc−/−/Smad4pc−/− cell line either infected with shRNA against Yap1 or pretreated with verteporfin (25), a small-molecule inhibitor that disrupts YAP1–TEAD interaction. We then tested the effect of various CM on the migration of MDSCs in *vitro*. As shown in Fig. 6G and H, we observed significantly decreased MDSC migration in *vitro* when CM was from cells with either YAP1 knockdown or verteporfin treatment.

Finally, to test if targeting YAP1 in *vivo* can impair the infiltration of MDSCs and inhibit tumor growth, we used our recently isolated syngeneic murine prostate cancer line PPS, which is derived from the backcrossed Ptenpc−/−/Smad4pc−/−/Trp53pc−/− model (31) and can form subcutaneous or orthotopic tumors robustly in C57BL/6 hosts. Doxycycline-dependent shRNA knockdown of Yap1 (two independent shRNA designs #1 and #3) was established in PPS (Fig. 6I) and injected subcutaneously in C57BL/6 mice. YAP1 knockdown induced by switching to doxycycline-containing drinking water resulted in a reduction of MDSCs in the intratumoral CD45+ population (Fig. 6J and K) and impaired tumor progression (Fig. 6L). Although the observation supports the hypothesis that targeting YAP1-dependent MDSC infiltration impairs tumor growth, we acknowledge that the tumor growth impediment by YAP1 silencing is likely due to a combined effect of both cell-nonautonomous and cell-autonomous mechanisms. Together, these findings reveal a novel function for YAP1 in the recruitment of MDSCs through direct upregulation of Cxcl5 transcription in prostate tumor cells.

**YAP1 Is Activated in Human Prostate Cancer and Tracks with an MDSC Signature**

To determine whether YAP1 is overexpressed and activated in human prostate cancer, we performed IHC staining of a human prostate cancer tissue microarray (TMA) for YAP1. Interestingly, YAP1 is expressed in basal cells, but not in the luminal cells of the normal human prostate (Fig. 7A). In addition, we observed that YAP1 is overexpressed in a subset of human prostate cancers (Fig. 7A and B and Supplementary Table S6), consistent with a recent report (29). Given the lack of validated antibodies for human MDSCs for TMA analysis, we generated a list of 39 MDSC-related genes curated from literature analysis (Supplementary Table S7) to generate evidence of a link between YAP1 activation and MDSC prominence in human prostate. Using the prostate RNA-sequencing data from The Cancer Genome Atlas (TCGA), unsupervised clustering with the 39-gene MDSC signature categorized 498 TCGA primary prostate tumors into three subtypes: MDSC-high (*n* = 139), MDSC-medium (*n* = 158), and MDSC-low (*n* = 201; Fig. 7C), suggesting that a subset of human prostate tumors may have prominent infiltration of MDSCs. In addition, using GSEA, we found that several YAP1 signature genes are significantly overexpressed in MDSC-high samples as compared with MDSC-low samples (Fig. 7D; *P* value < 0.005), reinforcing the link between MDSC-high prostate tumors and YAP1 transcriptional activities. Furthermore,
**Figure 6.** Hyperactivation of YAP1 in Pten−/−;Smad4−/− tumors upregulates Cxcl5. **A**, GSEA analysis identified the YAP1 oncogenic signature as the top activated pathway in the Pten−/−;Smad4−/− tumors compared with Pten−/− tumors (n = 5). **B**, a significant increase in nuclear staining of YAP1 in the Pten−/−;Smad4−/− tumors compared with Pten−/− tumors (n = 3). **C**, ChIP shows that YAP1 can directly bind to Cxcl5 promoter using quantitative PCR in triplicates. **D**, shRNA knockdown of Yap1 in Pten−/−;Smad4−/− tumor cells resulted in a dramatic reduction in Cxcl5 mRNA expression using quantitative PCR in triplicate. **E**, overexpression of a constitutively active YAP1S127A mutant resulted in upregulation of Cxcl5 mRNA using quantitative PCR in triplicate. **F**, TEAD-binding defective YAP1S127A/S94A mutant significantly decreased Cxcl5 mRNA expression as compared with the YAP1S127A mutant using quantitative PCR in triplicate. **G** and **H**, conditioned medium prepared from Pten−/−;Smad4−/− cells infected with Yap1 shRNA (G) or treated with verteporfin (H), a small molecule that disrupts YAP1–TEAD interaction, induced less MDSC migration in vitro as compared with the control conditioned medium. Transwell migration was done in triplicate for each condition. **I**, Western blot analysis showed that two independent inducible shRNAs for Yap1 efficiently knock down Yap1 expression in the Pten−/−;Smad4−/− cells. **J–L**, inducible Yap1 knockdown strongly suppressed the intratumoral MDSC infiltration (J and K) and tumor growth (L) of the C57BL/6-syngeneic cell line isolated from prostate tumor of Pten−/−;Smad4−/−/Trp53−/−/ mice (n = 5). In **C, D, E** and **F,G,H,L**, *P < 0.05; **P < 0.01; ***P < 0.001. See also Supplementary Fig. 56.
Figure 7. YAP1 is activated in human prostate cancer and correlated with MDSC signature and CXCL6 overexpression. A, IHC analysis of YAP1 expression in basal cells of normal prostate tissue and human prostate cancers. Numbers in parentheses indicate YAP1 IHC intensity scores. B, YAP1 IHC intensity score representation in low-grade (n = 10) and high-grade (n = 60) prostate cancer. C, clustering of human TCGA prostate samples into MDSC-high, MDSC-low, and MDSC-medium groups using a 39-gene MDSC signature. D, YAP1 signatures are identified in MDSC-high prostate TCGA samples. E, CXCL6 expression is significantly higher in the MDSC-high group. See also Supplementary Fig. S7.
CXCL6, the human homolog of murine Cxcl5, is expressed at higher levels in the MDSC-high samples as compared with MDSC-low samples (Fig. 7E; P = 9.40E−29). Similar analysis was performed in a published dataset focused on tumor immunobiological differences in prostate cancer between African-American and European-American men (32). The 39-gene MDSC signature can cluster the 69 primary prostate tumors into MDSC-high (n = 40) and MDSC-low groups (n = 29), and YAP1 signatures were prominent in the MDSC-high groups (Supplementary Fig. S7A and S7B). Together, these human prostate tumor findings, which parallel our murine observations, suggest that activated YAP1 is integral to MDSC infiltration in both mouse and human prostate cancer, thus enhancing the translational value of the study.

**DISCUSSION**

Although a large number of studies have demonstrated a direct relationship between MDSC frequency and tumor burden (5), our understanding of the role of MDSCs in tumor progression, particularly prostate cancer, remains largely speculative. Here, using a highly invasive Pten/Smad4-deficient prostate cancer model, we established the signaling circuits involved in the recruitment of MDSCs to the TME and demonstrated a critical role of these cells in facilitating tumor progression.

Homozygous deletion of Pten in murine prostate elicited a strong senescence response that restricts tumor progression (33); thus, Pten-deficient prostate tumors are largely indolent and progress slowly to invasive prostate adenocarcinoma without metastasis to distant organs (11, 33). Recently, it was shown that infiltrating Gr1+ myeloid cells suppress Pten loss–induced cellular senescence through a paracrine signaling mediated by myeloid-secreted IL1RA (10). We have previously reported that deletion of Smad4 leads to bypass of Pten loss–induced senescence in prostate cancer progression, resulting in aggressive cancer cell proliferation and invasion/metastasis (11). Using the state-of-the-art CyTOF technology, we revealed that progression in the Pten+/−/Smad4−/− model is associated with abundant immune cell infiltration characterized by prominent representation of CD11b+Gr1+ MDSCs, which display potent immunosuppressive activities as shown by their strong antagonistic effect on T-cell proliferation (Fig. 3A and B).

The basis for the increased frequency of MDSCs in the TME and, specifically in the Pten+/−/Smad4−/− model, was not known and presumably could derive from either active chemoatractant or passive nonspecific responses to tissue stress associated with expanding tumor burden. Taking an unbiased approach to identify pathways that may recruit MDSCs, we deconvoluted cancer versus stromal cell transcriptions by exploiting a Cre-dependent dual fluorescence lineage tracing system in the Pten+/−/Smad4+/− model. This approach identified unique immune regulatory molecules that are activated prominently in Pten+/−/Smad4+/− cancer cells, most prominently CXCL6. We established that the CXCL5 chemokine plays a key role in the efficient recruitment of MDSCs which enables tumor progression, as blocking CXCL5–CXCR2 signaling with a CXCR2 inhibitor led to reduced MDSC infiltration with associated antitumor effects. It should be noted that the human homolog for murine CXCL5 is CXCL6, and CXCL6 has been shown to be upregulated in prostate cancer as compared with normal prostate and significantly associated with high Gleason scores 8 to 9 (34). Interestingly, it was shown that CXCL5 promotes recruitment of MDSCs to primary melanoma, resulting in epithelial–mesenchymal transition (EMT) and cancer cell dissemination (35). Thus, the possible role of CXCL5/CXCL6 in prostate cancer metastasis merits further study.

Our finding that CXCL5 is the main chemoattractant in the Pten+/−/Smad4−/− model also provided a framework to determine the cancer cell signaling pathways driving CXCL5 upregulation. By integration of bioinformatic analysis and experimental validation, we identified that YAP1 is activated in Pten+/−/Smad4−/− prostate tumors and that YAP1 directly regulates Cxcl5 transcription and MDSC recruitment. In addition, we showed that YAP1 is overexpressed in a subset of human prostate cancers, which is consistent with a recent publication showing a correlation of ERG and YAP1 coexpression in a subset of human prostate cancers (29). Importantly, a 39-gene MDSC signature clusters the prostate TCGA samples into three subtypes. By comparing the samples with high and low abundance of MDSC-related gene expression, YAP1 signatures and higher expression of CXCL6 are identified in the MDSC-high samples, which is consistent with our findings in the mouse model. Furthermore, the 39-gene MDSC signature can cluster primary prostate tumor samples from a published dataset (32) into two subtypes using MDSC-high and MDSC-low, with YAP1 signatures identified in the MDSC-high subtype. The Hippo–YAP signaling pathway is widely deregulated in human solid neoplasia and often associated with enhanced cancer cell proliferation and cancer stem cell phenotypes (25), and is implicated in the regulation of anoikis and metastasis in prostate cancer (28) and the development of age-related prostate cancers driven by ERG overexpression (29), yet how the Hippo–YAP pathway regulates the TME in prostate cancer has hitherto not yet been elucidated. Our finding of a novel non–cell autonomous function for Hippo–YAP signaling in MDSC recruitment in TME complements well the recently elucidated roles of YAP1 in promoting cell-autonomous functionality of cancer cells, including enhanced tumor survival, EMT, and bypass mechanism for oncogene addiction (26, 27).

Pharmacologic depletion of MDSCs using Gr1 antibody, Pep-H6 peptibody, or CXCR2 inhibitor arrested prostate progression at the high-grade PIN stage whereas controls exhibited full-fledged adenocarcinoma in the Pten+/−/Smad4−/− model. Given that treatment commences at 14 weeks of age (Supplementary Fig. S3A), when prostate tumors have uniformly advanced to the invasive adenocarcinoma stage (11) with significant MDSC infiltration (Fig. 2B), our findings support the view that anti-MDSC treatment provokes regression of advanced tumors. In addition, both Pep-H6 peptibody and CXCR2 inhibitor treatment significantly prolonged the overall survival of the Pten+/−/Smad4−/− tumor-bearing mice. Therefore, our preclinical data suggest that pharmacologic depletion of MDSCs may offer potential therapeutic benefits for patients with advanced prostate cancer, particularly those deficient for PTEN and SMAD4. In line with our findings, others have demonstrated that depletion of G-MDSCs promotes the intratumoral accumulation of activated CD8+ T cells and apoptosis of tumor epithelial cells in a Kras/Trp53 mouse pancreatic cancer model (36).
MDSCs are of myeloid cell lineage, and their coordinated regulation represents one of the most complex aspects of cancer-host interactions (37). The involvement of the myeloid compartment of the hematopoietic system in innate immunity, adaptive immunity, as well as in regulation of TME through nonimmune mechanisms highlights the need to understand more deeply how modulating different myeloid populations, including MDSCs, can positively or negatively affect tumor growth.

Pep-H6 peptibody, targeting S100A9 expressed on MDSCs, has been shown to have minimal toxicity in treated mice (20) and potent antitumor activity (Fig. 4D and E; ref. 18). Interestingly, tasquinimod, a small-molecular inhibitor for S100A9, has been shown to increase progression-free survival and overall survival for metastatic castration-resistant prostate cancer in a phase II clinical trial and has entered phase III clinical trials (38). Importantly, similar to the peptibody treatment in mice, tasquinimod or similar drugs targeting S100A9 could potentially be used as chemopreventive agents for patients with high-risk primary prostate cancer. The antiproliferative mechanism may explain why targeting CXCR2 in prostate cancer with abundant preexisting MDSC infiltration can lead to MDSC depletion, as MDSCs have been shown to undergo active proliferation inside the prostate tumor of the Pten−/− model (9). The effectiveness of targeting CXCR2 in our model suggests targeting mechanisms that specifically regulate MDSC recruitment as well as their proliferative and survival potential in human cancers would provide therapeutic benefit for patients with prostate cancer.

Targeting MDSCs as a cooperative approach for immunotherapy is clinically relevant, as increasing evidence indicates MDSCs represent a bona fide immunosuppressive cell population in patients with various solid tumors (39, 40). Immunosuppressive mechanisms by MDSCs in mice have been validated in humans, which include l-arginine depletion, NO and ROS production, TGFβ secretion, blocking T effector cells and inducing Treg cells, among others (39). Future studies are warranted to evaluate if combining MDSC depletion with immune checkpoint inhibitors, such as anti–CTLA-4, anti–PD-1, and anti–PD-L1 antibodies, may elicit synergistic efficacy in preclinical models of prostate cancer and eventually benefit patients with prostate cancer.

**METHODS**

**Mice Strains**

Pten−/− and Pten−/−; Smad4−/− models were developed previously (11) and were backcrossed to the C57BL/6 background for more than four generations. B6.129(Cg-Gt(RosA)26Sortm4(ACTB-tdTomato,-EGFP) Lnu)) (“mTmG”) strain was obtained from The Jackson Laboratory. Mice were maintained in pathogen-free conditions at the MD Anderson Cancer Center. All manipulations were approved under the MDA Institutional Animal Care and Use Committee.

**Cell Lines**

Pten−/−; Smad4−/− prostate cell lines, which have been described previously (11), were generated in 2010. PPS, a C57BL/6-syngeneic cell line isolated from prostate tumors of Pten−/−; Smad4−/−; Trp53−/− mice, was generated in 2013. All cell lines tested for Pten−/−; Smad4−/− inducible expression in vivo were negative within 6 months of performing the experiments. Cell line authentication was not performed.

**CytOKF and Flow Cytometry**

Prostate tumor single cells were isolated using the Mouse Tumor Dissection Kit (Miltenyi Biotec). Single cells were isolated from spleen, lymph node, and peripheral blood using standard protocol. All isolated cells were depleted of erythrocytes by hypotonic lysis. For CytOKF analysis, cells were blocked for FcR using CD16/CD32 antibody (clone 2.4G2, BD Biosciences) and incubated with CytOKF antibody (DVS Sciences, used at 0.5 test/1 million cells) for 30 minutes at room temperature. Cells were washed once and incubated with MAXPARNucleic Acid InterCalator-100Rh (DVS Sciences) for 20 minutes for viability staining. Cells were fixed with 1% formaldehyde for 1 hour and incubated with MAXPMWNucleic Acid InterCalator-Ir (DVS Sciences) at 4°C overnight to stain the nuclei. The samples were analyzed with CytOKF instrument (DVS Sciences) in the Flow Cytometry and Cellular Imaging Core Facility at the MD Anderson Cancer Center. Flow cytometry was performed using standard protocol on LSIsFortessa analyzer (Becton Dickinson) and analyzed with Flowjo software (Tree Star).

**T-cell Suppression and MDSC Migration Assay**

T-cell suppression assay was performed as described (9) using FACS-sorted MDSCs and CFSE (Invitrogen)-labeled MACS-sorted (Miltenyi Biotec) CD8+ or CD4+ T cells in anti–CD3- and anti–CD28-coated 96-well plates at an MDSC/T-cell ratio of 0.1, 1.1, 1.2, 1:4, with 3.0 × 10⁵ to 5.0 × 10⁵ MDSCs used in each ratio. Cells were analyzed after 72 hours by flow cytometry, and the suppression of T cells is calculated as described (41). The percentage of CFSE+ cells divided in the presence of MDSCs was compared with the percentage of CFSE+ divided cells in the absence of any added MDSCs. For the migration assay, an equal number of FACS-sorted MDSCs, untreated or pretreated with neutralizing antibody or inhibitor, were placed on the upper chamber of a transwell system (BD Falcon), and conditioned media from PTEN/SMAD4 deficient cells under various conditions were added to the bottom chamber. Cells were allowed to migrate to the bottom well for 6 hours at 37°C with 5% CO₂. Migrated cells were then analyzed by flow cytometry using BD Fortessa X20. Migrated FITC-positive cells were gated to count the absolute number of cells migrated through the transwell.

**MDSC Depletion In Vivo with Gr1 Antibody, Peptibody, and CXCRR2 Inhibitor SB225002**

Anti-Gr1 (clone RB6-8C5) and isotype control (clone LTF2) were purchased from BioXcell and dosed at 200 μg/mouse (i.p.) every other day. Endotoxin-free plasmin (15 μg) for irrelevant control peptibody (Irr-pep) and MDSC-specific Pep-H6 peptibody were injected into mice through tail vein using the established protocol (21) in TransIT-EE Delivery Solution (Mirus Bio LLC) every 4 days. SB225002 (Cayman Chemical) in DMSO was diluted in vehicle (0.9% NaCl, 0.3% Tween 80) for in vivo administration every other day (5 mg/kg).

**Inducible Yap1 Knockdown**

Inducible Yap1 knockdown was constructed by cloning the two Yap1 shRNAs used previously (28) from the pLKO.1 into a doxycycline-inducible plasmid. Lentivirus was packaged in 293T and was used to infect PPS, a C57BL/6-syngeneic cell line isolated from prostate tumor of Pten−/−; Smad4−/−; Trp53−/− mice. Stable sublines were selected with puromycin (2 μg/mL) and injected subcutaneously to the flank of 5-week-old male C57BL/6 mice (Jackson Laboratory). Two weeks after injection, mice were fed with doxycycline water (2 g/L), a method used to execute doxycycline-inducible expression in vivo (42). Tumors were measured and extracted 6 days later to analyze for MDSC percentage in infiltrating immune cells.
Computational Analysis of Mouse Microarray Data and Human Prostate TCGA Data

RNA was isolated from FACs-sorted GFP+ and Tomato+ cells using \textit{Pem}^{+/−} \textit{Smad4}^{−/−} \textit{mTmG} prostate tumors, followed by microarray analysis at the MD Anderson Microarray Core facility using the Mouse Genome 430 2.0 Array (Affymetrix) to generate a \textit{Pem}^{+/−} \textit{Smad4}^{−/−} tumor/stroma dataset GSE71319. Dataset GSE25140 was downloaded from the NCBI Gene Expression Omnibus (GEO) database. Differentially expressed genes between two conditions (GFP+ vs. Tomato+ or PTEN/SMAD4 vs. PTEN) were subjected to IPA, GSEA, and oPOSSUM analysis. For analysis of human prostate data, we first generated a list of 39 human MDSC signature genes by literature mining (Supplementary Table S7). The gene expression data of 498 TCGA prostate samples were downloaded from the Broad GDAC Firehose (http://gdac.broadinstitute.org), which is the RSEM expression estimates normalized to set the upper quartile count at 1,000 for gene level and then with log2 transformation. The 498 TCGA prostate samples were downloaded from the Broad GDAC Firehose (http://gdac.broadinstitute.org), which is the RSEM expression estimates normalized to set the upper quartile count at 1,000 for gene level and then with log2 transformation. The 498 TCGA prostate samples were clustered using the 39 MDSC genes into MDSC-high, MDSC-low, and MDSC-medium (distance between pairs of samples was measured by Manhattan distance, and clustering was then performed using complete-linkage hierarchical clustering). Sixty-nine samples from Wallace and colleagues (32) were clustered into MDSC-low, and MDSC-medium (distance between pairs of samples was measured by Manhattan distance, and clustering was then performed using complete-linkage hierarchical clustering). Sixty-nine samples from Wallace and colleagues (32) were clustered into MDSC-low, and MDSC-medium (distance between pairs of samples was measured by Manhattan distance, and clustering was then performed using complete-linkage hierarchical clustering).

Immunohistochemistry and Western Blot Analysis

Tissues were fixed in 10% formalin overnight and embedded in paraffin. IHC was performed as described earlier (11). For Western blot analysis, cells were lysed on ice using RIPA buffer (Boston Bio-Products) supplemented with protease and phosphatase inhibitors (Roche). YAP1 antibody was obtained from Novus Bio and Cell Signaling Technology. CXCL5 antibodies were obtained from Bioss and R&D Biosystems. CCR2 antibody was obtained from Bioss and R&D Biosystems. CD45 and Ly6G antibodies were Biogened. Prostate tissue microarray was obtained from Folio Bioscience.

Chromatin Immunoprecipitation

ChiP was performed as described (26) using YAP1 antibody from Novus. Briefly, 5 μg of rabbit IgG (Santa Cruz) or YAP1 antibody was incubated with Protein A Dynabead magnetic beads (Invitrogen) for 4 hours, followed by extensive wash to remove unbound antibody. Antibody beads were then added to the chromatin and incubated over-night. The following primers were used for qPCR analysis: CXCL5_S: 5′-CTCCAGTTTCTCGCTGAAAG-3′ and CXCL5_as: 5′-GTGTTGGAGATTAGGGCTCA-3′.

Quantitative RT-PCR

RNA was isolated by the RNeasy Kit (Qiagen) and reverse transcribed using the SuperScript III cDNA Synthesis Kit (Life Technology). Quantitative PCR was performed using the SYBR-GreenER Kit (Roche). YAP1 antibody was obtained from Novus Bio and Cell Signaling Technology. CXCL5 antibodies were obtained from Bioss and R&D Biosystems. CD45 and Ly6G antibodies were Biogened. Prostate tissue microarray was obtained from Folio Bioscience.

Statistical Analysis

Data are presented as mean ± SD unless indicated otherwise. The Student \( t \) test assuming two-tailed distributions was used to calculate statistical significance between groups. Animal survival benefit was determined by the Kaplan–Meier analysis. \( p < 0.05 \) was considered statistically significant.

Disclosure of Potential Conflicts of Interest

A. Kapoor is a research investigator at Novartis. C.J. Logothetis has received commercial research grants from Astellas, BMS, J&J, Exelixis, Pfizer, Novartis, Bayer, AstraZeneca, and Helsinn HC. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors thank Drs. Larry W Kwak and Hong Qin for the pepti-body plasmands; Drs. Willem Overwijk and Yared Halenichemickel for advice in hydrodynamic injection; Samirkumar Amin and members of the DePinho laboratory for helpful suggestions and technical support; and the Flow Cytometry and Cellular Imaging Core Facility (Jared Burks, Duncan Mak, and Karen Dwyer) and the Sequencing & Non-coding RNA Core Services (Chang-Gong Liu) at the University of Texas MD Anderson Cancer Center (Cancer Center Support Grant CA16672).

Grant Support

The project was supported by U01CA141508 (to L. Chin and R.A. DePinho), Prostate Cancer Research Program (PCRP) W81XWH-13-1-0202 (to G. Wang) and W81XWH-14-1-0429 (to P. Dey); the Idea Development Award–New Investigator Option (W81XWH-14-1-0576; to X. Lu) from the Department of Defense; and the Clayton & Modesta Williams Cancer Research Fund. Additional support was provided by the Jane Coffin Childs Memorial Fund Postdoctoral Fellowship to X. Lu. E.-J. Jin is supported by Korean Governments (MISP) grant number 2011-0030130.

Received February 23, 2015; revised October 5, 2015; accepted October 16, 2015; published OnlineFirst December 23, 2015.
MDSCs Promote Prostate Cancer Progression

REFERENCES

Targeting YAP-Dependent MDSC Infiltration Impairs Tumor Progression

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Cancer Discov  Published OnlineFirst December 23, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/2159-8290.CD-15-0224

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