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Inhibition of TAMs improves the response to docetaxel in castration-resistant prostate cancer

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Abstract

For men with castration resistant prostate cancer (CRPC), androgen-deprivation therapy (ADT) often becomes ineffective requiring the addition of docetaxel, a proven effective chemotherapy option. Tumor associated macrophages (TAMs) are known to provide protumorigenic influences that contribute to treatment failure. In this study, we examined the contribution of TAMs to docetaxel treatment. An increased infiltration of macrophages in CRPC tumors was observed after treatment with docetaxel. Prostate cancer cells treated with docetaxel released more macrophage colony stimulating factor (M-CSF-1, or CSF-1), IL-10 and other factors, that can recruit and modulate circulating monocytes to promote their protumorigenic functions. Inhibition of CSF-1

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Declaration of Interest

There's no conflict of interest to be disclosed

receptor kinase signaling with a small molecule antagonist (PLX3397) in CRPC models significantly reduces the infiltration of TAMs and their influences. As such the addition of PLX3397 to docetaxel treatment resulted in a more durable tumor growth suppression than docetaxel alone. This study reveals a rational strategy to abrogate the influences of TAMs and extend the treatment response to docetaxel in CRPC.

Keywords

CRPC; TAMs; docetaxel; CSF-1; CSF-1R

Introduction

Prostate cancer (PCa) is the second most common cancer in men after skin cancer, as 1 out of 7 men will be diagnosed with this disease in the United States (Siegel, et al. 2017). It is estimated that 161,000 newly diagnosed cases and 27,000 deaths will be attributed to this disease in 2017 (Siegel et al. 2017). A great majority of PCa patients, 70–80%, present with localized, organ-confined disease and their outcome is very favorable, having 10-year survival rate above 95%. However, 20–30% of patients will present with characteristics of high risk, advanced disease such as high Gleason grade or distant metastases. In these cases, the 5-year survival rate drops precipitously to about 30% (Siegel et al. 2017).

For PCa patients with advanced disease, androgen-deprivation therapy (ADT) is the first line of treatment, developed by Dr. Huggins more than 75 years ago to deplete androgen, a key growth factor for prostate cancer cells (Esch, et al. 2014). Over the years effective strategies of ADT include the depletion of the body's source of androgen by inhibiting androgen biosynthesis pathways and by blocking the activation of androgen receptor (AR) (Merseburger, et al. 2015). Abiraterone and enzalutamide are two newly approved potent ADT agents that inhibit CYP17A1 androgen synthetic enzyme and AR, respectively (de Bono, et al. 2011; Scher, et al. 2012). Both agents are effective in prolonging the survival of castration-resistant prostate cancer (CRPC) patients who had progressed on first line ADT (Ryan, et al. 2015). However, a significant proportion of CRPC patients either do not respond to either abiraterone or enzalutamide, or initially respond but subsequently progress on treatment (Silberstein, et al. 2016). Potential mechanisms of resistance include AR mutations, amplification and splice variant (Antonarakis, et al. 2014; Azad, et al. 2015; Romanel, et al. 2015).

Docetaxel has been established as the standard first line chemotherapy agent to treat CRPC since 2004. It was approved by FDA for this purpose as several large clinical trials showed docetaxel containing regimens provided survival benefits over other chemotherapies for CRPC patients (Petrylak, et al. 2004; Sweeney, et al. 2015; Tannock, et al. 2004). Belonging to the taxane family, docetaxel was initially postulated to suppress prostate cancer growth by interfering with microtubule function (Petrylak 2003). However, subsequent research supported that the therapeutic activity of taxanes in prostate cancer could arise from its interference with androgen signaling via the nuclear translocation process (Gan, et al. 2009).

Given taxane-based chemotherapy is one of a few effective treatments for CRPC, we investigate a rational combination regimen to improve its therapeutic efficacy. Recent findings from our group and others showed that tumor associated macrophages (TAMs) contribute significantly to treatment failure in PCa and other solid cancers via their wound healing and protumorigenic functions (Brown, et al. 2017; Escamilla, et al. 2015; Xu, et al. 2013). In this study, we employed a small molecule CSF1R kinase inhibitor (CSF-1Ri), PLX3397, to block TAMs in CRPC models. In combination with ADT and docetaxel, PLX3397 was able to significantly reduce the number of infiltrating TAMs and lower their protumorigenic influences. We showed that the addition of PLX3397 extended the therapeutic response to ADT and docetaxel in CRPC models.

Materials and Methods

Cell culture and drugs

The murine macrophage RAW264.7 (RAW) cells (ATCC), MyC-CaP cells (a kind gift from Dr. Charles Sawyers, Memorial Sloan Kettering New York) were cultured with DMEM (high glucose) while PC3 (ATCC), CWR22Rv2 (a kind gift from Dr. David Agus, Cedars-Sinai Medical Center), LNCap-C4-2 (C4-2) cells (ATCC) were cultured in RPMI-1640. Both media were supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin. PLX3397, 5-[(5-chloro-1H-pyrrolo[2,3-b]pyridin-3-yl)methyl]-N-[[6-(trifluoromethyl)-3-pyridyl]methyl]pyridin-2-amine was synthesized at Plexxikon Inc. The detailed synthetic procedure is shown by Tap et al. (Tap, et al. 2015).

Transwell coculture and migration assay

In coculture assay, 1.0×10^6 RAW macrophages were seeded in a transwell inserts with membrane pore size at 4µm (BD Falcon) in media supplemented with 2µM PLX3397, 1µM GW2580, or DMSO vehicle. The chamber was inserted in a 6-well plate with conditioned media from Myc-CaP, PC3, CWR and C4-2 cells treated with docetaxel (100nM for MyC-CaP, 5nM for CWR22Rv1, 30nM for PC3 and 2nM for C4-2 cells) or DMSO. Total RNA was extracted from tumor cells after 48 hours and analyzed by RT-PCR. The methods for RT-PCR is described in Supplementary Methods and primers are listed in Supplementary Table 1.

In migration assay, 1.0×10^5 RAW cells were seeded in transwell inserts with membrane pore size at 8µm assembled in 24-well plates. The number of migrated cells was evaluated after 6 hours of incubation at 37 °C, then treated with 3% paraformaldehyde (PFA) and stained with 0.1% (w/v) crystal violet solution. Random 10 fields/well at 4x magnification were sampled and quantified with ImageJ2.

ELISA assay

1.0×10^6 MyC-CaP, PC3, CWR and C4-2 cells were cocultured with or without RAW cells as mentioned above, with or without Docetaxel or PLX3397 at tumor cells' IC50 or IC10 concentrations.

Supernatant of all cell culture media were harvested after 48 hours. 96-well Nunc MaxiSorp Plates (Cat#44–2404-21, Thermo Scientific) were coated with the anti-M-CSF antibody (1:300, Cat#sc-365779, Santa Cruz Biotech) in coating buffer diluted from Coating Solution Concentrate Kit (KPL) at 4°C overnight. Then the plate was washed with 1x wash buffer (KPL) and blocked with 1% BSA Blocking Solution (KPL) for 1 hour at room temperature. Cell supernatant were added to the wells and incubate for 1 hour at room temperature in the shaker at 220rpm. After washing with 1x wash buffer (KPL), each well was incubated with the second anti-M-CSF antibody (1:300, Cat#sc-13103, Santa Cruz Biotech) overnight at 4°C. The wells were washed four times, 5 min for each and incubated with 100µl of HRP-conjugated goat-anti-rabbit IgG (1:5000, Cat# 111–035-045, Jackson Laboratory) for 1 hour at room temperature. The wells were washed four times, 5 min for each and incubated with 100µl of ABTS ELISA HRP Substrate (KPL). Absorbance at 410nm was measured by Synergy HT microplate reader (BioTek).

Flow Cytometry

MyC-CaP cells were cocultured with or without RAW cells, Docetaxel (IC10 or IC50) for 48 hours before cells were trypsinized. Single cell suspension was rinsed with PBS twice and incubated with APC conjugated anti-IL-10 antibody (Cat#17–7101-82, eBioscience) for 30min at 4°C at darkness. Cell acquisition was done on a BD LSR-II flow cytometer (Beckman Coulter) and data were analyzed by FlowJo software (TreeStar).

For tumor tissue analysis, single cells suspension were prepared by digestion from collagenase II at 0.1% for 1 hour. Then cells were counted and incubated with APC conjugated anti-CD11b antibody (Cat#17–0112-81, eBioscience) and PE conjugated anti-CSF1R antibody (Cat#12–1152-82, eBioscience) for 30min at 4°C at darkness.

MyC-Cap subcutaneous xenograft model

All animal experiments were approved by the Animal Research Committee of the University of California, Los Angeles. For MyC-CaP s.c. xenograft model, 16 FVB male mice at 6–8 weeks' old from Taconic Biosciences were adopted and kept at BSL2 animal facility. After trypsinization and rinsing with cooled PBS, 1.0×10^6 MyC-CaP cells were resuspended in 200µl PBS/Matrigel (1:2) (356230, Corning) and injected with insulin syringe into the subcutaneous space on the right back of FVB male mice (n=16). 1 week after the cell injection, mice were castrated and divided randomly into four groups, receiving DMSO vehicle + control chow, DMSO vehicle + chow containing PLX3397, docetaxel + control chow, or docetaxel + chow containing PLX3397. The PLX3397 dosage is 40mg/kg/day on average and docetaxel dosage is 40mg/kg/week. Tumor size was measured by digital calipers and calculated by the formula $V = 0.5 * a * b^2$, in which a is the larger and b is the smaller index of the two perpendicular indexes of the tumor.

CWR22Rv1 orthotopic xenograft model

After trypsinization and rinsing with precooled PBS, 1×10^5 CWR22Rv1 cells, stably expressing firefly luciferase, were resuspended in 10µl of PBS/Matrigel (1:2) (356230, Corning) and injected by insulin syringe into the left anterior lobe of prostate gland of 6–8 weeks old SCID-beige male mice (Jackson Laboratory). All mice were castrated on day 14

post injection and randomly divided into two groups, receive docetaxel + control chow or docetaxel + PLX3397 chow (40mg/kg/d). The docetaxel treatment started on day 19 at 10mg/kg/week. The in-vivo BLI were performed every week and the luminescence count were recorded as previously described²⁰. All mice were euthanized on day 42.

Statistical Analysis

Data are all presented as mean±SEM. Student t-test was used for comparison between two groups while two-way ANOVA was used for comparisons between multiple groups.

Results

Docetaxel-mediated tumor cell injury induces the expression of M2 cytokines

To study the impact of docetaxel in prostate cancer, we first examined the dose response of this chemotherapeutic agent on several prostate cancer cell lines (Figure 1A). We found that PCa cells exhibit a wide range of sensitivity to docetaxel, with C4-2 (IC50 = 2nM) and CWR22Rv1 (IC50 = 5nM) being the most sensitive, PC-3 (IC50 = 30nM) as an intermediate responder and MyC-CaP (IC50 = 100nM) being the most resistant. As we have shown in previous studies, conventional cytotoxic therapies such as radiation therapy and ADT all can induce PCa cells to express M2 cytokines (Escamilla et al. 2015; Xu et al. 2013). Here we further inquired whether docetaxel in addition to ADT would also induce the expression of M2 cytokines such as CSF-1 and IL-10. To mimic ADT, all prostate cancer cells were cultured in media supplemented with charcoal-treated fetal bovine serum (FBS) to remove the androgens. As shown in Figure 1B MyC-CaP or CWR22Rv1 cells treated with ADT plus docetaxel, dosed at each line's respective IC50, increased the expression of CSF-1 and IL-10. Likewise, the expression of these M2 cytokines were also induced when PCa cells, including MyC-CaP, CWR22Rv1, PC3 and C4-2, were treated in the presence of macrophages (Figure 1C). This ADT plus docetaxel treatment resulted in a significant reduction in cell proliferation, as indicated by the decrease in the proliferative marker Ki67. Interestingly, when the PCa cells were treated with a lower dose of docetaxel at the IC10 dose, the elevation of M2 cytokine expression was no longer observed (Figure 1D). Docetaxel treatment induced increase in CSF-1 and IL-10 in the tumor cells were further analyzed and verified at the protein level by CSF-1 ELISA (Figure 1E) and IL-10 flow cytometry (Figure 1F). This induction of M2 cytokines is likely not restricted to docetaxel alone. We observed very similar effects with paclitaxel treatment of all 4 PCa cell lines (Supplementary Figure 1). Collectively, these findings support that cell injury mediated by ADT plus docetaxel induces the heightened expression of M2 cytokines in PCa cells.

Docetaxel induces CSF-1 expression and increases the recruitment of macrophages *in vitro*

CSF-1 or M-CSF is a cytokine critical not only in the differentiation and proliferation of myeloid cells but also in the recruitment and polarization of protumorigenic M2 macrophages (Brown et al. 2017). Next, we examine the impact of macrophage recruitment in the setting of docetaxel treatment. As shown in Figure 2A and 2B, CWR22Rv1 and C4-2 PCa cells treated with docetaxel were able to recruit more macrophages in an *in vitro* transwell assay compared to chemo-naïve cells. The elevated CSF-1 produced by the

docetaxel-treated PCa cells likely contributed to the increased macrophage recruitment, as the addition of the CSF-1Ri PLX3397 attenuated the enhancement in macrophage recruitment *in vitro* (Figure 2A and B), as we and others have previously reported (Butowski, et al. 2016; Escamilla et al. 2015; Moughon, et al. 2015; Xu et al. 2013).

PLX3397 is known to also inhibit c-Kit (Tap et al. 2015). We employed a second highly selective CSF-1R kinase inhibitor GW2580 to substantiate that CSF1/CSF1R as the key signal axis for macrophage recruitment (Priceman, et al. 2010). As shown in Figure 2C and 2D, the enhancement of macrophage recruitment across a transwell mediated by docetaxel treated PCa cells were dampened significantly by the addition of GW2580.

Adding CSF-1R kinase inhibitor, PLX3397, to docetaxel regimen enhances therapeutic efficacy in CRPC

Next, we investigated the impact of docetaxel treatment on macrophage recruitment *in vivo* in CRPC tumors. We first evaluated TAMs in the MyC-CaP tumors engrafted subcutaneously in syngeneic FVB male mice. One week after tumor cell implantation tumor bearing mice were treated with surgical castration as ADT, and divided into 4 treatment groups receiving (i) diluent control, (ii) oral PLX3397, (iii) docetaxel or (iv) docetaxel plus PLX3397. The PLX3397 treatment was administered orally via rodent chow and docetaxel was administered IP at 40mg/kg/week. Comparing to diluent control treated tumors, PLX3397 only treatment significantly reduced the number of CD11b⁺ CSF1R⁺ TAMs, while docetaxel significantly increased TAMs (Figure 3A, B). Importantly, the addition of PLX3397 to docetaxel treated group was able to not only reverse the chemotherapy induced TAM influx but suppressed the TAM level in the tumor below that of the control treated group (Figure 3A, B). These results demonstrate the importance of CSF-1/CSF-1R axis in the recruitment of macrophages and the effectiveness of PLX3397 in blocking this CSF-1R mediated TAM recruitment *in vitro* and *in vivo*.

In our previous therapeutic studies, we consistently observed that CSF-1R blockade treatment alone can reduce the infiltration of TAMs but exert negligible impact on tumor growth *in vivo* (Butowski et al. 2016; Escamilla et al. 2015; Priceman et al. 2010; Xu et al. 2013). The same result was observed here in the MyC-CaP tumors: no significant reduction in tumor growth was observed after oral PLX3397 treatment alone despite clear reduction in the level of TAMs in the tumor (Figure 3C-E). As expected, docetaxel treatment significantly retarded the growth of MyC-CaP tumor compared to control (Figure 3C-E). More importantly, docetaxel plus PLX3397 achieved the most significant tumor growth suppression in the 4 treatment groups, more effective than docetaxel alone (Figure 3C-E).

Next we asked whether the benefit of PLX3397 in combination with docetaxel in the subcutaneous MyC-CaP model can also be observed in the orthotopic prostatic environment of the CWR22Rv1 model. SCID/Beige male mice received intraprostatic injection of firefly luciferase labeled CWR22Rv1 cells, such that tumor growth can be monitored in real time by bioluminescence imaging (BLI, Figure 3F, G). On day 14 after tumor cell implantation, mice received ADT via surgical castration. On day 19 tumor bearing mice received either docetaxel with control or docetaxel plus oral PLX3397 (Figure 3B). Treatment continued to day 42, at which point the animals were euthanized. Assessed either by BLI (Figure 3F, G)

or by terminal tumor volume (Figure 3H, I), the docetaxel plus PLX3397 group consistently showed significantly greater efficiency in suppressing tumor growth over docetaxel treatment alone. Again, corroborating our prior findings the added oral PLX3397 drastically reduced the level of CD11b+ CSF1R+ TAMs from 10.6% in the docetaxel only group to 0.1% in the docetaxel plus PLX3397 group, as analyzed by flow cytometry (Figure 3J). This finding was further verified by F4/80 immunohistochemistry stain to detect macrophages (Figure 3K). The functional consequences of TAM inhibition by PLX3397 included lowering angiogenic drive, tissue remodeling and immunosuppression as assessed by VEGF-A, MMP-9 and Arg-1 expression respectively (Figure 3F, G). Taken all together, we have shown that the use of a selective CSF-1Ri PLX3397 can block the infiltration of TAMs into prostate tumor and thus reduce the protumorigenic influences of M2 macrophages by lowering tumoral angiogenesis, tissue remodeling and immunosuppression leading to more effective treatment response to docetaxel.

Discussion

Docetaxel is a widely used chemotherapeutic agent in treating breast cancer (Palmeri, et al. 2008), head and neck cancer (Rapidis, et al. 2008), and non-small cell lung cancer (Fossella 2002). In the Chemohormonal Therapy versus Androgen Ablation Randomized Trial for Extensive Disease (CHAARTED) randomized phase III trial, men with hormone-naive metastatic PCa were randomly assigned to receive docetaxel plus ADT or ADT alone, with nearly 400 men in each arm. In particular, patients who had high volume disease benefited the most with docetaxel, achieving a very significant prolongation of their median survival by 17 months compared to ADT alone (Azad et al. 2015). Hence, docetaxel is an important therapeutic agent in the armamentarium against CRPC.

In this study, we investigated whether TAMs, an important component of the tumor microenvironment, could influence CRPC's response to docetaxel. We postulate that cellular damage sustained during docetaxel treatment induces PCa cells to produce cytokines and chemokines that recruit and polarize macrophages to the protumorigenic, alternatively activated M2 subtype (Brown et al. 2017). Congruent with this concept, we observed a significant increase in the expression of M2 cytokines, such as CSF-1 and IL-10 in all 4 prostate cancer cell lines, MyC-CaP, PC-3, CWR22Rv1 and C4-2, after docetaxel treatment. The elevated CSF-1 led to increased infiltration of macrophages *in vitro* and TAMs in MyC-CaP and CWR22Rv1 tumors after ADT and docetaxel treatment. We observed that treatment with another chemotherapeutic agent, paclitaxel, also elicited an increase in M2 cytokine expression in PCa, parallel the findings of a comprehensive chemotherapeutic study in preclinical breast cancer (DeNardo, et al. 2011). Importantly, these findings support the rational combination of CSF-1Ri with docetaxel to lower the recruitment and M2 polarization of TAMs, which in turn reduce the protumorigenic influences of TAMs and significantly increase the efficacy of tumor growth suppression of ADT and docetaxel treatment (Figure 4).

As the emergence of resistance to the current therapies is expected, what new and effective therapies will be incorporated to treat CRPC? A second line taxane, cabazitaxel, was developed to overcome this resistance problem. The effectiveness of docetaxel is limited by

its affinity for P-glycoprotein, an ATP-dependent drug efflux pump that decreases the intracellular concentrations of drugs (Bradshaw and Arceci 1998). Cabazitaxel exhibits low affinity for P-glycoprotein and has been shown to be effective in docetaxel-refractory PCa patients (de Bono, et al. 2010; Paller and Antonarakis 2011). Although the cancer vaccine Sipuleucel-T was approved for CRPC, current clinical experience suggests this therapy has limited efficacy for aggressive large volume disease (Mok, et al. 2014; Schellhammer, et al. 2013). New immunotherapeutic strategies for CRPC need further exploration. In this regard, TAMs could have multiple negative influences. For instance, M2 macrophages are well known to impair T-cell responses by depleting essential nutrients through arginase I or by inhibiting T-cell receptor CD3 ζ chain (Munder, et al. 2006; Rodriguez, et al. 2004). Interestingly, a recent study by Gordon et al (Gordon, et al. 2017) further implicated that PD-1 expressing TAMs are inhibiting tumor immunity, which might further empower the efficacy of the PD-1 or PD-L1 checkpoint blockade.

In our collective experience of studying TAM's influences in cancer therapy, we observed that TAMs contribute to every stage of PCa progression and therapy. From the control of local disease by radiation therapy (Xu et al. 2013), to the implementation of ADT for more advanced disease (Escamilla et al. 2015), to the use of docetaxel in recurrent CRPC studied here, blocking TAMs with CSF-1Ri in conjunction with these conventional therapies consistently improved therapeutic outcome by prolonging the duration of tumor growth suppression. Of note, the use of CSF-1Ri alone has no therapeutic impact in numerous preclinical models we have studied, including PCa, melanoma and lung cancer (Priceman et al. 2010). A large volume of literature shows that macrophages are educated and polarized by the tumor microenvironment towards the protumorigenic M2 subtype (Brown et al. 2017). We deduced that in the face of cellular injuries induces by conventional therapies, tumor cells secrete a higher level of M2 cytokines and chemokines such as CSF-1, CCL2, and IL10 that accentuate the protumorigenic functions of TAMs. Thus, combining CSF-1Ri with conventional cytotoxic therapies is a rational approach to improve their effectiveness. As we have shown that CSR-1Ri can improve the efficacy of adoptive T-cell therapy (Mok et al. 2014), it will be prudent to consider the incorporation of TAM blockade in combination for future immunotherapy strategies developed for CRPC, be it checkpoint inhibition or CAR T-cell therapy or others (Bilusic, et al. 2017). Given the critical role of TAMs in therapeutic setting for PCa, we envision that the incorporation of TAM blockade could extend the efficacy of all phases of treatment. In doing so, we could extend the survival of PCa patients and achieve the goal of transforming PCa into a chronic and survivable malignancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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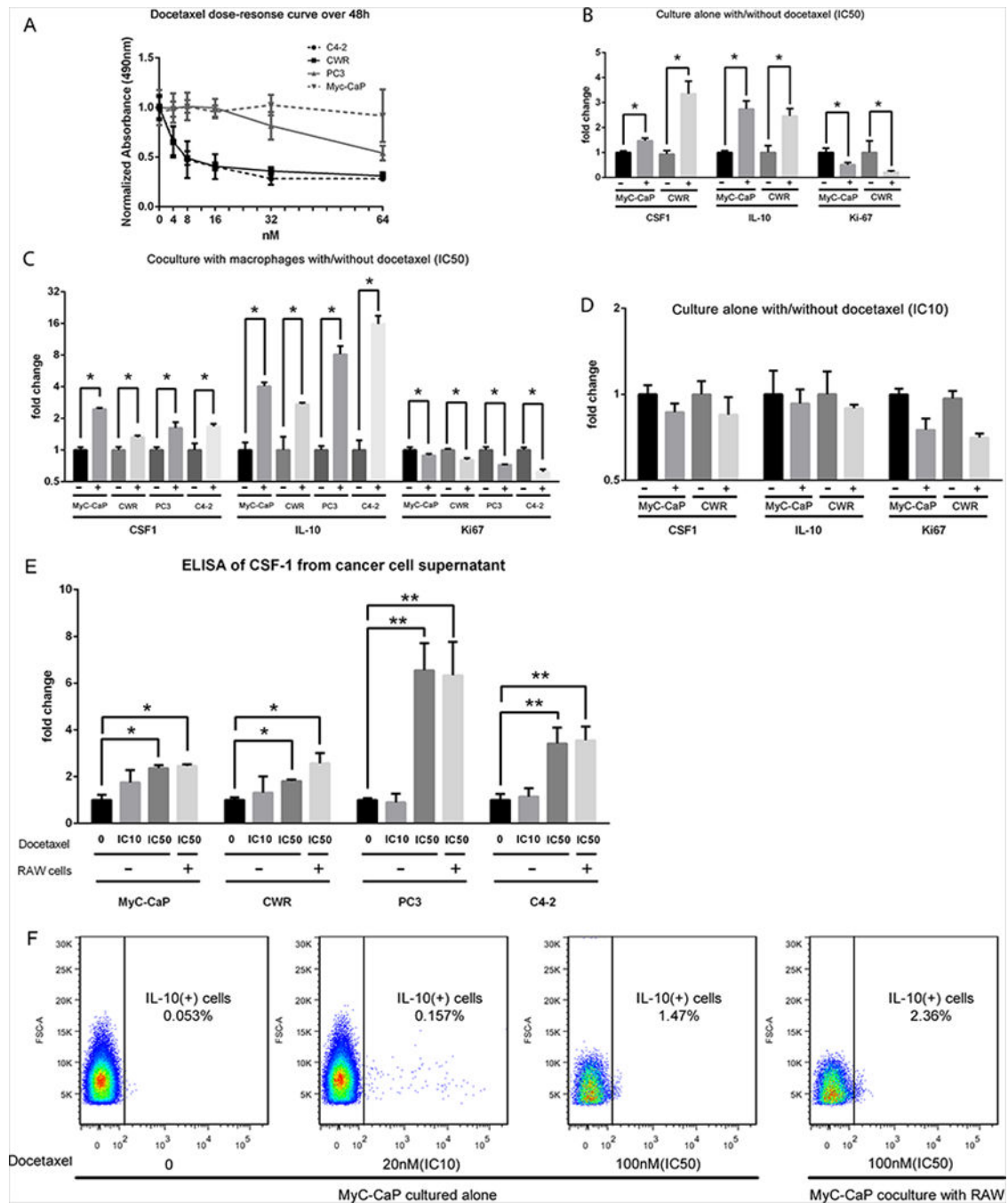


Figure 1. Cytokine expression upon docetaxel treatment in prostate cancer cells.

(A) The dose-response curve was plotted in different working concentrations of docetaxel for C4-2, CWR22Rv1, PC3, MyC-CaP cells to determine their respective IC₅₀ and IC₁₀ values. (B) MyC-CaP and CWR22Rv1 cells, cultured alone were treated with docetaxel at the IC₅₀ dose for each cell (Myc-CaP at 100 nM and CWR22Rv1 at 5nM). Expression of CSF-1 and IL-10 and Ki-67 in response to docetaxel treatment was shown. (C) The impacts of docetaxel treatment at IC₅₀ on PCa cells in the presence of macrophages (RAW cells) were shown for MyC-CaP, CWR22Rv1, PC3 (30nM) and C4-2 (2nM) cells were shown (D)

When treated at their respective IC10 doses of 20 and 1 nM, MyC-CaP and CWR22Rv1 cells showed no significant change in CSF-1, IL-10 or Ki67 expression. (E) The level of secreted CSF-1 in culture supernatant from MyC-CaP, CWR22Rv1, PC3 and C4-2 cells treated with docetaxel at IC10 or IC50, and with and without co-cultured with RAW macrophages, were analyzed by ELISA. (F) Intracellular IL-10 expression in MyC-CaP cells treated with docetaxel at IC10 or IC50, with and without co-cultured with RAW macrophages, were analyzed by flow cytometry. All cells were cultured in media supplemented with charcoal-stripped serum. (*: $p < 0.05$, **: $p < 0.01$)

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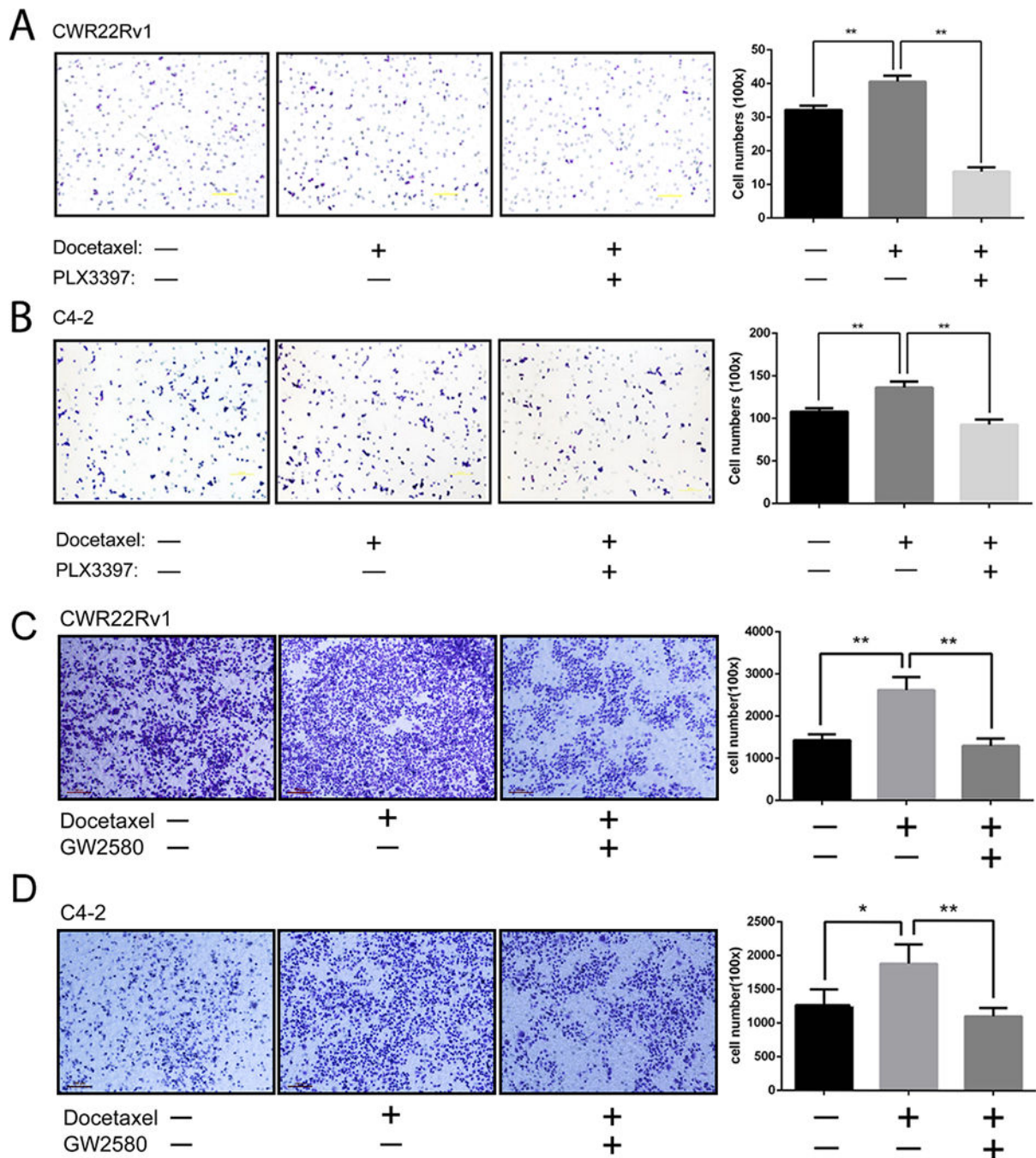


Figure 2. CSF-1R inhibitor PLX3397 abrogated the increased recruitment of macrophages induced by docetaxel treatment *in vitro*.

Conditioned media of CWR22Rv1 (A) and C4-2 (B) prostate cancer cells treated with docetaxel was able to recruit more RAW macrophages migrating across a transwell porous membrane than media from untreated cells. The addition of 2 μ M CSF-1Ri PLX3397 to the docetaxel treated conditioned media abrogated the increased macrophage recruitment induced by both cell lines. In a second set of similar study, the increase in RAW

macrophages migration by CWR22Rv1 (C) and C4-2 (D) conditioned media was inhibited by 1 μ M GW2580, a selective CSF-1R kinase inhibitor. (*: p<0.05, **: p<0.01)

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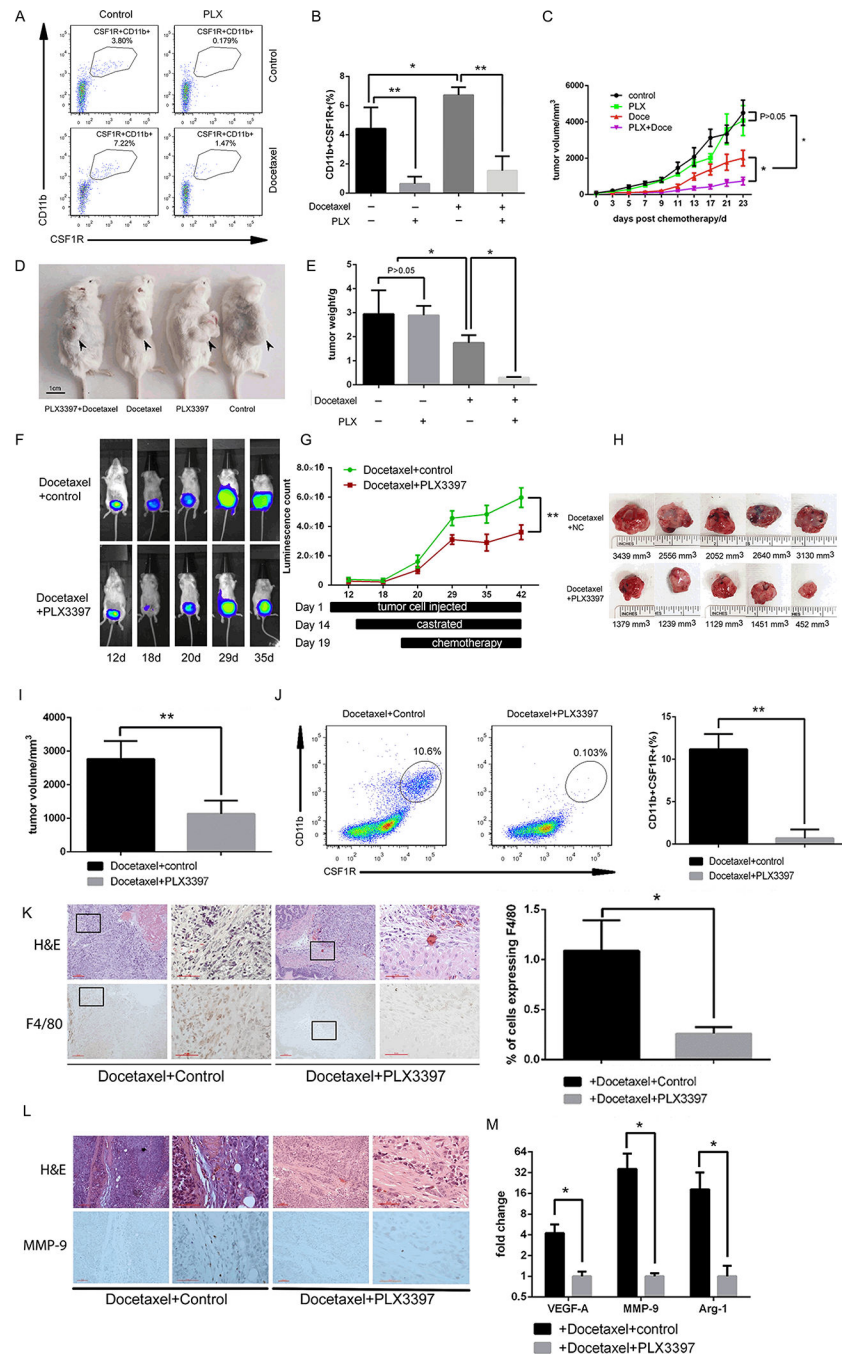


Figure 3. The addition of PLX3397 to docetaxel improves therapeutic efficacy in CRPC by reducing the protumorigenic influences of TAMs.

The therapeutic effects of combining CSF-1Ri PLX3397 with docetaxel were evaluated in subcutaneous MyC-CaP tumors established in FVB male mice. Seven days after tumor cell implantation, all mice received surgical castration and randomly assigned to 4 treatment groups: (i) control, (ii) PLX3397, (iii) docetaxel or (iv) docetaxel+PLX3397. Flow cytometric analyses of CD11b+CSF1R+ TAM population in the tumor were shown as individual representative flow plots (A) and for each treatment cohort (B). Longitudinal tumor volume (C) and final tumor size (D, E) were shown for the 4 treatment groups.

Intraprostatic CWR22Rv1 tumors were established with firefly luciferase marked cells, and longitudinal tumor growth were monitored by *in vivo* BLI (**F, G**). Tumor growth suppression was more effective in the docetaxel + PLX3397 group compared to the docetaxel only group (**H, I**) as assessed by terminal tumor volume. Likewise the PLX3397 containing treatment group was significantly reduced in the proportion of CD11b+ CSF1R+ TAM as analyzed by flow cytometry (**J**), and immunohistochemistry with F4/80 macrophages (**K**) and the tissue remodeling marker MMP-9 (**L**). Gene expression profiling by qRT-PCR revealed a reduction in VEGF-A, MMP-9 and Arg-1 with CSF-1Ri treatment (**M**). (*: P<0.05; **: p<0.01)

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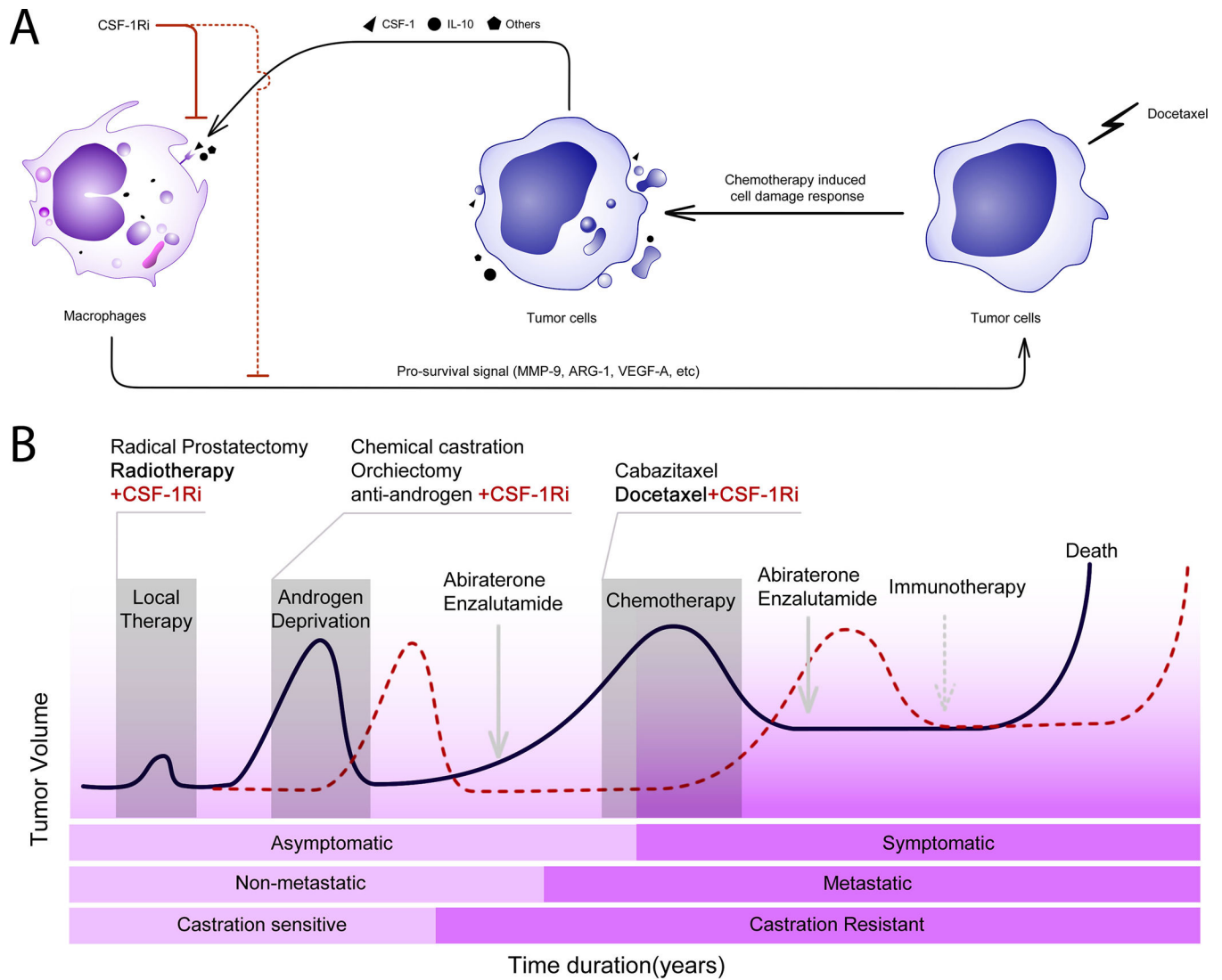


Figure 4. TAMs' contribution to docetaxel treatment failure in prostate cancer.

A schematic illustration of impact of docetaxel treatment in PCa. The cellular damage caused by docetaxel heightens expression of M2 cytokines such as CSF-1 and IL-10, which recruit and polarize more M2 TAMs to foster their protumorigenic influences in the tumor microenvironment. The use of CSF1-R inhibitors could disrupt this TAM mediated vicious cycle.