Increased chemo-sensitivity via targeting testicular nuclear receptor 4 (TR4)-Oct4-interleukin 1 receptor antagonist (IL1Ra) axis in prostate cancer CD133+ stem/progenitor cells to battle prostate cancer

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Running Title: TR4 promotes chemo-resistance in PCa stem cells
Background: PCa stem/progenitor cells develop higher chemo-resistance.  
Result: High TR4 levels in PCa stem/progenitor cells were shown to be critical in conferring chemo-resistance to these cells.  
Conclusion: TR4-Oct4-IL1Ra signaling is important in conferring chemo-resistance to PCa stem/progenitor cells.  
Significance: This finding suggests targeting TR4 and its downstream molecules may be a better therapeutic approach to battle PCa stem/progenitor cells-originated chemo-resistance.  
Abstract  
Prostate cancer (PCa) stem/progenitor cells are known to have higher chemo-resistance than non-stem/progenitor cells, but the underlying molecular mechanism remains unclear. We found the expression of testicular nuclear receptor 4 (TR4) is significantly higher in PCa CD133+ stem/progenitor cells compared to CD133- non-stem/progenitor cells. Knocking down of TR4 levels in the established PCa stem/progenitor cells (PCSCs) and the CD133+ population of the C4-2 PCa cell line with lentiviral TR4-siRNA led to increased drug sensitivity to the two commonly used chemotherapeutic drugs, docetaxel and etoposide, judging from significantly reduced IC₅₀ values and increased apoptosis in the TR4 knocked down cells. Mechanism dissection studies found that suppression of TR4 in these stem/progenitor cells led to down-regulation of Oct4 expression, which in turn, down-regulated the IL-1 receptor antagonist (IL1Ra) expression. Neutralization experiments via adding these molecules into the TR4 knocked-down PCa stem/progenitor cells reversed the chemo-resistance, suggesting that the TR4-Oct4-IL1Ra axis may play a critical role in the development of chemo-resistance in the PCa stem/progenitor cells. Together, these studies suggest that targeting TR4 may alter chemo-resistance of PCa stem/progenitor cells and this finding provides the possibility of targeting TR4 as a new and better approach to overcome the chemo-resistance problem in PCa therapeutics.  
Introduction  
Prostate Cancer (PCa) is the most commonly diagnosed malignancy and the second leading cause of cancer-related death in men in the Western World. In the USA, it is estimated as the most prevalent cancer among males (43%) (1). Patients with advanced and metastatic PCa initially respond well to androgen deprivation therapy (ADT). But in most cases patients with PCa inevitably suffer a relapse and their tumors develop into castration resistant prostate cancer (CRPC), and further advance into metastatic CRPC (mCRPC) (2).  
The results of chemotherapy using docetaxel (a member of the taxane family) are successful in some patients with PCa
(3), but others develop chemo-resistance problems and most of these patients receive a second-line chemotherapy. However, many clinical trials of second-line chemotherapy have been disappointing as Colloca et al (4) summarizes in their review of taxane based or non-taxane based chemotherapy for the docetaxel-resistant CRPC patients. The taxane based chemotherapy includes carboplatin plus docetaxel or estramustine plus docetaxel (5-8), and non-taxane based chemotherapy includes calcitriol plus docetaxel (9), or replacing docetaxel with mitoxantrone (10,11) and prednisone (12). However, none of them showed satisfactory results. For example, combined use of abiraterone acetate with docetaxel in Phase III trial extended the survival of mCRPC patients (13), but recent clinical studies suggested that the activity of docetaxel post-abiraterone appeared lower than anticipated and no responses to docetaxel were observed in abiraterone-refractory patients (14) and some side effects were also reported (15). Even in the patients who responded to docetaxel without toxicity developed chemo-resistance when re-challenged with docetaxel as a second-line treatment (16). So, development of better chemotherapy strategies are urgently needed.

Increasing evidences indicated that PCa stem/progenitor cells, which are characterized with high expression of CD133, CD44, and Oct4 (17,18), are resistant to chemotherapeutic drugs (19,20) and early reports suggested that chemo-resistance in cancer stem cells could be due to their high expression of drug resistant related genes including ABCB1/MDR1, ABCG2/BCRP, and ABCC1/MRP1 (21,22).

The testicular nuclear receptor 4 (TR4) belongs to the nuclear receptor superfamily and was first cloned from human prostate and testis cDNA libraries (23). It has been known to modulate many signaling pathways by interacting with the thyroid receptor, androgen receptor, retinoic acid receptor/retinoid X receptor, and estrogen receptor (24-26). The TR4 knockout mice (TR4KO) studies showed that TR4 knockout resulted in defects in development and abnormalities in spermatogenesis and reproductive systems in both genders, which indicates that TR4 might play important roles in stem/progenitor cell differentiation (24). On the other hand, TR4 was also shown to play protective roles against oxidative stress and ionizing radiation induced damages (27). These results prompted us to investigate whether TR4 has a role in the development of chemo-resistance in PCa stem/progenitor cells.

Materials and Methods
Reagents and Cell Culture
The C4-2 human PCa cells and prostate
cancer stem cells (PCSCs, Celprogen, San Pedro, CA) were cultured in the recommended media (Celprogen) and maintained at 37°C in a humidified incubator at 5% CO$_2$. The chemotherapeutic agents docetaxel and etoposide (LC Laboratories, Woburn, MA) were dissolved in 100% DMSO and stored at −20°C until use. pCDNA3.3-OCT4 was purchased from Addgene (Cambridge, MA), purified, and used in transfection experiments.

**Magnetic Bead Isolation of CD133+ Stem/Progenitor Cells**

Cells ($2 \times 10^7$) were detached with 5 mM EDTA, and incubated with streptavidin magnetic beads (Invitrogen, Carlsbad, CA) that have been conjugated with biotinylated CD133 antibody (Miltenyi Biotec, Cambridge, MA). The bead bound cells were separated by placing tubes in a magnetic field. The stem/progenitor marker expressions in the isolated CD133 positive (CD133+) stem/progenitor cells were confirmed by qPCR or immunofluorescence staining. The isolated CD133+ stem/progenitor cells were cultured in keratinocyte serum free medium (Invitrogen, Carlsbad, CA) with 2% FBS and 0.1% leukemia inhibitor factor (Sigma, St. Louis, MO) as described (17) and cells of less than two passages were used in the experiments.

**Plasmids and Cell Infection**

TR4-siRNA was cloned in pLKO plasmid. For incorporation of TR4-siRNA or scramble control plasmids into PCa cells, lentivirus carrying either control (pLKO-vector) or TR4-siRNA (pLKO-TR4-siRNA), was transfected into 293T cells with a mixture of pLKO-TR4-siRNA, psPAX2 (virus packaging plasmid), and pMD$_2$G (envelope plasmid) (4:3:2 ratio) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). After viral infection, media was replaced with normal culture media, subcultured, and stable clone cells were selected by adding 2 µg/ml puromycin (Sigma, St. Louis, MO) and then maintained in media containing 1.0 µg/ml puromycin.

**RNA extraction, cDNA synthesis, and qRT-PCR**

RNAs were extracted using Trizol reagent (Invitrogen, Carlsbad, CA) based on the manufacturer’s instructions. RNAs (1 µg) were then subjected to reverse transcription using iscript™ cDNA synthesis Kit (BIO-RAD, Hercules, CA) and the obtained cDNAs were used for qPCR analysis in a Bio-Rad CFX96 system. The primer sequences for TR4, stem cell markers (CD133, Oct4, Nanog, Notch, and Sox2), drug-resistance genes ($ABC1/MDR1$, $ABCG2/BCRP$, and $ABCC1/MRP1$), and Oct4 downstream genes are listed in supplementary Table 1. GAPDH was used as control and all reactions were run at least in triplicate.
Western Blot Analysis

Cells were harvested and washed with cold PBS and lysed in RIPA buffer supplemented with protease inhibitor cocktail tablets. The protein concentration was estimated using the Bio-Rad protein assay (Bio-Rad, Hercules, CA). Samples (20-40 μg protein) were separated on 10-12% SDS-PAGE gel, transferred to PVDF membranes (Millipore, Billerica, MA), and nonspecific binding was blocked using 5% milk in TBST. Membranes were incubated with primary antibodies overnight at 4°C, washed in TBST solution, incubated with HRP conjugated second antibody, and the protein bands were visualized with an enhanced chemiluminescence detection system (Bio-Rad, Hercules, CA).

Cytotoxicity test using docetaxel and etoposide

Cytotoxicity of docetaxel and etoposide was tested using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, St. Louis, MO) assay at 5 mg/ml. Target cells were seeded on 24-well plates (2×10^4 cells/well) and treated with various concentrations of docetaxel and etoposide. At the end of 48 hrs incubation, MTT tests were performed and absorbance at 570 nm was measured. Cell viability was calculated using the formula, OD sample/OD blank control × 100. Triplicate experiments were performed and average values with mean ± SEM were presented. IC_{50} value was calculated using Graphpad Prism 5.0 software.

TUNEL assay

PCSC-sc and PCSC-siTR4 cells were treated with docetaxel (2 nM) or etoposide (2 μg/ml) and and TUNEL assay was performed according to the manufacturer's protocol (Roche, Branchburg, NJ).

Statistical Analysis

Graphpad prism 5.0 was used for data analysis. Values are expressed as mean ± SEM and statistical analysis was performed using one-way ANOVA and student’s t test. The values were considered as statistically significant if the p-value was less than 0.05.

Results

TR4 and drug resistance associated genes were more highly expressed in PCa CD133+ stem/progenitor cells than CD133- non-stem/progenitor cells

Early reports suggested that PCa stem/progenitor cells showed higher chemo-resistance compared to non-stem/progenitor cells (19,21,28,29). We isolated CD133+ stem/progenitor and CD133 negative (CD133-) non-stem/progenitor cells of the PCa C4-2 cell line by magnetic sorting method using CD133 antibody and found that the C4-2 CD133+ cells showed high stem cell
marker expression (Fig. 1a). We then investigated TR4 level in those two populations of cells and found that TR4 was significantly more highly expressed in C4-2/CD133+ stem/progenitor cells compared to the non-stem/progenitor cells (Fig. 1b, left panel, mRNA level; right panel, protein level). We also found that TR4 was highly expressed in PCSCs, the established PCa stem cell line (Fig. 1b, right panel Western blot data). The PCSC cell line was originally obtained from a human PCa patient and immortalized by Celprogen (San Pedro, CA) (30-32). These cells were shown homogenous and positive for stem cell markers such as Oct4 and Nanog (data not shown).

Since some drug resistance associated genes, such as ABCB1/MDR1, ABCG2/BCRP, and ABCC1/MRP1, are known to contribute to the taxel related chemo-resistance in cancer stem cells (21,22), and in liver cancer cells (33), we investigated their expressions in C4-2/CD133+ stem/progenitor and CD133- non-stem/progenitor cells. We found that these genes were more highly expressed in the C4-2/CD133+ stem/progenitor cells compared to the CD133- non-stem/progenitor cells (Fig. 1c). These results imply that high levels of TR4 in PCa stem/progenitor cells might be important in conferring chemo-resistance properties to these cells.

**TR4 knockdown led to enhanced chemo-sensitivity of PCa stem/progenitor cells**

To investigate the linkage between the high level of TR4 and chemo-resistance genes in PCa stem/progenitor cells, we performed in vitro manipulations of TR4 expression in the two sources of stem/progenitor cells, PCSCs and C4-2 CD133+. Successful knockdown of TR4 in PCSCs was shown in Fig. 2a (upper panel, mRNA level and lower panel, protein level). We then treated these cells with docetaxel and used MTT assay to analyze the cytotoxicity of these cells to docetaxel.

We found that PCSCs were more sensitive to docetaxel treatment in the TR4 knocked-down (PCSC-siTR4) cells compared to the scramble control (PCSC-sc) cells with IC$_{50}$ of 3.5 nM vs IC$_{50}$ 7.6 nM, respectively (Fig. 2b). We used another commonly used clinical drug, etoposide, and compared the cytotoxicity of PCSC-siTR4 and PCSC-sc cells to this drug. We obtained IC$_{50}$ value of 4.6 μg/ml for PCSC-sc cells vs 1.7 μg/ml for PCSC-siTR4 cells (Fig. 2c), also implying that TR4 knockdown increased drug sensitivity. We also performed TUNEL assay to investigate apoptotic death differences in the PCSC-siTR4 and PCSC-sc cells and obtained similar results showing higher apoptotic death in PCSC-siTR4 cells upon docetaxel and etoposide treatments (Fig. 2d).
We performed similar experiments using C4-2/CD133+ stem/progenitor cells, but lower concentrations of the two drugs were applied since the parental C4-2 cells showed higher sensitivity to these two drugs compared to the PCSCs (data not shown). We infected C4-2/CD133+ stem/progenitor cells with lentivirus carrying siTR4 or scramble control plasmid and Fig. 2e showed successful knockdown of TR4 in C4-2/CD133+ cells (upper panel, mRNA level; lower panel, protein level). We tested cytotoxicity of these cells to docetaxel and found that the TR4 knocked down C4-2/CD133+ stem/progenitor (C4-2siTR4-CD133+) cells showed increased drug sensitivity to docetaxel compared to scramble control (C4-2sc-CD133+) cells (IC_{50} of 1.37 nM in C4-2sc-CD133+ cells vs 0.90 nM in C4-2siTR4-CD133+ cells, Fig. 2f). Similarly, IC_{50} of 1.48 μg/ml in C4-2sc-CD133+ cells vs 0.78 μg/ml in C4-2siTR4-CD133+ cells were obtained in cytotoxicity tests against etoposide (Fig. 2g).

Together, results from Fig. 2 suggest that TR4 plays a critical role in conferring chemo-resistance to PCa stem/progenitor cells.

**TR4 conferred chemo-resistance to PCa stem/progenitor cells through up-regulation of Oct4 and IL1Ra**

Several previous reports have indicated that Oct4 contributed to drug resistance. Linn et al. (18) reported that drug resistant PCa cells express high levels of Oct4 and knockdown of this molecule attenuated growth of the drug resistant cells. Significant up-regulation of Oct4 was also observed in cisplatin-resistant patients with oral squamous cell carcinomas (34). In addition, knockdown of Oct4 in drug-resistant colorectal cancer cells showed increased cell apoptosis, decreased stem cell markers expressions, and weakened tumorigenicity. (35). Furthermore, TR4 regulation of Oct4 in embryonic stem cells at the transcriptional level was also demonstrated (36).

Therefore, we investigated the potential linkage between TR4 and Oct4 in altering chemo-resistance in PCa stem/progenitor cells and found that expression of Oct4 was significantly higher in C4-2/CD133+ stem/progenitor cells compared to CD133-non-stem/progenitor cells (Fig. 3a, mRNA level, and Fig. 3b, protein level). We used TR4 knocked down PCa stem/progenitor and scramble control cells and examined mRNA expressions of Oct4 and several Oct4 downstream genes associated with drug resistance and stem cells (18,33,37), including GATA6, GDF6, KLF5, and IL1RN, and found IL1RN gene expression was most significantly reduced in the TR4 knocked down PCSCs (Fig. 3c, left panel, mRNA level; right panel, protein level) and C4-2/CD133+ stem/progenitor cells (Fig. 3d, left panel, mRNA level; right panel, protein level).
We further investigated which drug resistance associated downstream genes are modulated by the TR4-Oct4-IL1Ra axis and found that some genes, such as RSP27, MyB, and MRP1, expressions were down-regulated, but expression of other genes, including BCRP1, MDR1, and MID1, were not modulated significantly (Fig. 3d left panel).

We then applied neutralization/interruption approaches to confirm if TR4 is required for the Oct4-IL1Ra signaling to modulate the chemo-resistance against docetaxel in these cells. Incorporating Oct4 into the PCSC-siRNA cells (Fig. 4a shows high Oct level in these cells) reversed the TR4 knockdown mediated drug sensitivity increase (Fig. 4b) and addition of the recombinant IL1Ra (rIL1Ra) to the PCSC-siTR4 and C4-2siTR4-CD133+ cells culture also reversed the TR4 knockdown effect in enhancing drug sensitivity (Fig. 4c and d), implying that IL1Ra is implicated in conferring drug resistance to PCa stem/progenitor cells.

Together, results from Fig. 3 and 4 suggest that TR4 may modulate chemo-resistant PCa stem/progenitor cells via up-regulation of the TR4-Oct4-IL1Ra signaling.

**Discussion**

ADT is the standard treatment strategy for locally advanced and metastatic PCa. Although there are some options for the treatment of CRPC, such as intermittent androgen blockade or second line androgen deprivation, these treatments can only partially postpone the progression to mCRPC.

Based on the TAX327 study results, in which docetaxel showed extended survival times when compared with mitoxantrone in treating mCRPC patients (38), the National Institutes for Health and Clinical Excellence (NICE) and American Urological Association (AUA) recommended docetaxel based chemotherapy as the first-line chemotherapeutic strategy for mCRPC. Subsequently, the effect of the combination therapy of luteinizing hormone-releasing hormone (LHRH)-agonist with docetaxel or abiraterone has been tested (STAMPEDE study) (39). Etoposide was also used in Phase II studies of the combination therapy targeting mCRPC (Culine group, 2011 Flechon) (40). In addition, docetaxel plus estramustine (anti-microtubule agent) chemotherapy represents an active and well-tolerated treatment in mCRPC patients in Japan (41). However, not all the mCRPC patients respond well to chemotherapy (40-43) at this stage, and the only expectancy is to allow mCRPC patients to live longer or have a better quality-of-life. Many attempts were made to overcome this drug resistance problem, for example, application of the new generation of taxel-derived medicines such as cabazitaxel, but whether this new
agent has a better effect remains inconclusive (43,44).

Earlier reports suggested that PCa stem/progenitor cells showed higher chemo-resistance compared to non-stem/progenitor cells (20,21,23). For example, the CD133+ stem/progenitor cells isolated from the highly invasive WPE1-NB26 PCa cell line were shown more resistant to docetaxel than the CD133- non-stem/progenitor cell (45). Similarly, the CD133+/CD117^{high}/ABCG2^{high}/nestin+ PCa cell subpopulation, isolated from the CWR22RV1 PCa cell line, was more resistant to the commonly used chemotherapeutic drugs, such as cisplatin, paclitaxel, and methotrexate than the CD133-/ABCG2^{low} cells (46). Early reports showed that the CD133+/CD44+ PCa cells isolated from a non-adherent suspension of PC-3 cells are more resistant to cisplatin (47,48). Recently, Zhang et al (47) suggested that tumorsphere forming PCa cells, which are characteristic of stem/progenitor cells, displayed higher chemo-resistance when compared with adherent cells. It was also shown that normal and malignant epithelial cells with stem-like properties have an extended G2 cell cycle phase that is associated with apoptotic resistance (49). All these results indicate that the CD133+ stem/progenitor cells have more chemo-resistance than CD133- cells although Yan et al (50) reported a contradictory result that the drug-tolerant PCa cells showed reduced tumor-initiating capacity due to loss of stem cell characteristics.

In this study, we investigated the role of TR4 in PCa stem/progenitor cells in drug resistance using two sources of PCa stem/progenitor cells, the isolated CD133+ cell population of the C4-2 PCa cell line and the established PCSC cell line, and the two common clinically used drugs, docetaxel and etoposide, and showed a positive role of TR4 in affecting chemo-resistance. This positive role of TR4 is consistent with the previous reports showing a protective role of TR4 in oxidative stress and ionizing radiation induced damage (24-27).

There have been many attempts to investigate the underlying mechanism of chemo-resistance in CRPC. For example, it was reported that calcitonin induces apoptosis resistance in PCa cell lines against cytotoxic drugs via the Akt/survivin pathway (51). Methylseleninic acid therapy (52) and targeting p38/p53/p21 signaling (53) have also been suggested to battle chemo-resistance. In this study we investigated the mechanism conferring chemo-resistance to PCa stem/progenitor population cells and provided one critical target molecule, TR4.

Targeting TR4 directly may be a problem since there is no known specific inhibitor of TR4 on the market, although Metaformin, an activator of AMPK (54)
that could suppress TR4 signaling indirectly (55), could be used. Therefore, we attempted to reveal downstream signal molecules of TR4. We found that TR4 may exert its action through Oct4-IL1Ra signaling. The Oct4 role in drug resistant PCa cells has been reported (18) and TR4 regulation of Oct4 has also been suggested (36). Our results showed TR4 modulation of Oct4 in PCa stem/progenitor cells and revealed its downstream molecule, IL1Ra. It was suggested that an IL1Ra could be used for the treatment of cancer (56), which supports our findings. Therefore, we believe that the TR4-Oct4-IL1Ra signal axis may contribute to chemo-resistance in PCa stem/progenitor cells. However, in vivo studies should be done to confirm this.

In summary, in this study we clearly demonstrated a positive role of TR4 in rendering drug chemo-resistance in PCa stem/progenitor cells. Furthermore, we provide a possibility of using its downstream signaling axis, Oct4-IL1Ra, as a potential target to battle chemo-resistance originating from the PCa stem/progenitor cells.

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Figure Legends

**Figure 1.** Higher expressions of TR4 and chemo-resistance associated gene expressions in PCa CD133+ stem/progenitor cells compared to CD133- non-stem/progenitor cells. (a) Stem cell markers expressions in C4-2/CD133+ PCa stem/progenitor and CD133- non-stem/progenitor cells. (b) TR4 expression in C4-2/CD133+ PCa stem/progenitor and CD133- non-stem/progenitor cells. mRNA levels are shown in left panel and protein levels are shown in right panel. (c) Expressions of several taxel-related drug-resistance genes, including *ABCB1*(MDR1), *ABCG2*(BCRP), and *ABCC1*(MRP1) in C4-2/CD133+ PCa stem/progenitor and CD133- non-stem/progenitor cells.

**Figure 2.** Higher expression of TR4 led to higher chemo-resistance in PCa stem/progenitor cells. (a) qPCR (upper panel) and Western blot (lower panel) analyses results showing successful TR4 knockdown in PCSCs. PCSCs were infected with lentivirus carrying either si-TR4 or scramble (sc) control sequence and TR4 mRNA and proteins levels were analyzed by qPCR and Western blot analysis, respectively. GAPDH served as controls in analyses. (b-c) Drug sensitivity test for (b) docetaxel, and (c) etoposide in PCSC-siTR4 and PCSC-sc cells. Cells were treated with various indicated concentrations of drugs for 48 hours and cell viability upon drug treatment was analyzed by MTT assay. IC₅₀ value was calculated using Graphpad Prism 5.0 software. Triplicate experiments were performed and mean ± SEM were presented. (d) TUNEL assay result. PCSCs cells were treated with indicated concentrations of docetaxel and etoposide and TUNEL assay was performed after 24 hrs incubation according to the manufacturer's instructions. Quantitation shown on right. (e) qPCR (upper panel) and Western blot (lower panel) analyses results showing successful TR4 knockdown in C4-2/CD133+ cells. Cells were infected with lentivirus carrying either si-TR4 or scr control sequence and TR4 levels were analyzed as in (a). (f-g) Drug sensitivity test for (f) docetaxel, and (g) etoposide in C4-2siTR4-CD133+ and C4-2sc-CD133+ cells. Cells were treated with various indicated concentrations of drugs for 48 hours and cell viability upon drug treatment was analyzed by MTT assay. IC₅₀ value was calculated using Graphpad Prism 5.0 software. Triplicate experiments were performed and mean ± SEM were presented.
viability upon drug treatment was analyzed by MTT assay as in (b) and (c). **p<0.01, ***p<0.001.

Figure 3. TR4 contributes to the chemo-resistance in PCa stem/progenitor cells through up-regulation of Oct4 and IL1Ra expressions. (a) qPCR (left panel) and (b) Western blot (right panel) analyses results showing high expression of Oct4 in C4-2/CD133+ stem/progenitor cells. (c) Expressions of TR4, Oct4, IL-1Ra, and several taxel-related chemo-resistance genes in PCSC-siTR4 and PCSC-sc cells. qPCR (left panel) and Western blot analysis (right panel) were shown. (d) qPCR (left panel) and Western blot (right panel) results showing expressions of Oct4, IL-1Ra, and several taxel-related drug resistance genes in TR4 knocked down C4-2/CD133+ PCa stem/progenitor cells. *p<0.05.

Figure 4. Rescue experiments showing chemo-sensitivity reversed by incorporating Oct4 and IL1Ra into PCSCs and C4-2/CD133+ PCa stem/progenitor cells. (a) Western blot analysis of TR4 and Oct4 protein levels in PCSCs-siTR4 cells, infected with either Oct4 or vector. (b) Effect of Oct4 incorporation in rescuing reduced PCSCs viability induced by TR4 knockdown. Cells were infected by lentivirus carrying Oct4 and then treated along with various concentrations of docetaxel for 48 hours, cell viability was analyzed by MTT assays. (c-d) Effect of recombinant IL1Ra (rIL1Ra) addition in rescuing reduced (c) PCSCs and (d) C4-2/CD133+ PCa stem/progenitor cells viability induced by TR4 knockdown. Cells were treated with 2 ng/ml rIL1Ra and then treated along with docetaxel for 48 hours and cell viability was analyzed by MTT assays. Triplicate experiments were performed and mean ± SEM presented. *p<0.05, **p<0.01.
Figure 1: Yang

(a) 

(b) 

(c) 

Relative mRNA expression

- CD133
- OCT4
- Nanog
- Notch
- Sox2

- C4-2/CD133-
- C4-2/CD133+

p = 0.0042

- TR4
- GAPDH

Relative mRNA expression

- CD133
- MDR1
- BCRP1
- MRP1

- C4-2/CD133-
- C4-2/CD133+

- PDX1
Figure 2_Yang
Figure 4_Yang

a

b

C4-2/CD133+

cell viability (%)

concentration (nM)

0 1 5 10

sc siTR4 siTR4+Oct4

TR4 Oct4 GAPDH

C4-2/CD133+

cell viability (%)

concentration (nM)

0 0.5 1.0 5.0

sc siTR4 siTR4+IL1Ra

PCSC

cell viability (%)

concentration (nM)

0 1 5 10

sc siTR4 siTR4+IL1Ra

P
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