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Generation of a C57BL/6 MYC-Driven Mouse Model and Cell Line of Prostate Cancer

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Abstract

INTRODUCTION.—Transgenic mouse modeling is a favorable tool to reflect human prostate tumorigenesis and interactions between prostate cancer and the microenvironment. The use of GEMMs and derived cell lines represent powerful tools to study prostate cancer initiation and progression with an associated tumor microenvironment. Notably, such models provide the capacity for rapid preclinical therapy studies including immune therapies for prostate cancer treatment.

METHODS.—Backcrossing FVB Hi-MYC mice with C57BL/6N mice, we established a Hi-MYC transgenic mouse model on a C57BL/6 background (B6MYC). In addition, using a conditional reprogramming method, a novel C57BL/6 MYC driven prostate adenocarcinoma cell line was generated.

RESULTS.—Our results demonstrate that disease progression is significantly delayed in B6MYC when compared to their FVB counterparts. Current data also indicates infiltrating immune cells are present in pre-cancer lesions, prostate intraepithelial neoplasia (PIN). Further,

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AUTHORS' CONTRIBUTIONS

SK performed research, analyzed data, and wrote the manuscript; GA analyzed data; QL, DGT, JS, and BO provided vital reagents and performed experiments; CSN and SIA performed experiments and analyzed data; DWG analyzed data; RP designed experiments and wrote the manuscript; LE designed experiments, performed research, analyzed data, and wrote the manuscript.

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SUPPORTING INFORMATION

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immunophenotyping of this immune infiltrate demonstrates the predominant population as myeloid-derived suppressor cells (MDSC). Also, we successfully generated a B6MYC-CaP cell line, and determined that this new PCa cell line express markers of luminal epithelial lineage.

DISCUSSION.—This novel model of PCa provides a new platform to understand the cross talk between MYC driven prostate cancer and the microenvironment. Importantly, these models will be an ideal tool to support the clinical development of immunotherapy as well as other novel therapeutic strategies for prostate cancer treatment. *Prostate* 76:1192–1202, 2016.

Keywords

prostate cancer; MYC; mouse model; cell line

INTRODUCTION

Developing robust prostate cancer models including prostate derived cell lines, genetically engineered animals, and more recently prostate organoids [1,2], facilitates the field to investigate molecular mechanisms underlying disease initiation and progression. Further, such models facilitate the design and validation of new therapeutic strategies to test in human clinical trials. Numerous genetically engineering mouse models (GEMMs) have been generated to carry genetic alterations that commonly existed in human prostate cancer, including *MYC* amplification [3], *PTEN* loss [4], *p53/RB1* deficiency [5], and *TMPRSS2-ERG* fusion [6]. These GEMMs are capable of recapturing prostate cancer initiation and progression from prostatic intraepithelial neoplasia (PIN), to invasive adenocarcinoma, and rarely progress to metastatic disease. Increased expression of MYC has been frequently observed in human PIN and retained in human primary and metastatic prostate cancer samples, suggesting that MYC exhibits pleiotropic functions to drive prostate cancer initiation and progression [7–9]. To uncover the functions of MYC contributing to prostate carcinogenesis, Ellwood-Yen et al. generated the *Hi-MYC* GEMM of prostate cancer. This model developed rapid mouse PIN (mPIN) with progression to localized invasive adenocarcinoma of the prostate. Further, gene expression analysis revealed significant overlap with gene signatures from human prostate cancer [3].

Several mouse strains have been widely utilized to generate GEMMs. These strains carry diverse genetic backgrounds, which have influences on the presentation of modeling human disease [10,11]. For instance, it was previously demonstrated that specific deletion of PTEN in prostate luminal epithelium in C57BL/6 mice demonstrated delayed disease kinetics in the comparison to other mixed backgrounds of the same genotype [12]. Delayed kinetics of disease progression was associated with rapid recruitment of immunosuppressive Gr-1⁺CD11b⁺ myeloid derived suppressor cells (MDSCs). This GEMM highlights the role of inflammation, a known risk factor associated with human prostate cancer [13], during the early stages of prostate cancer progression. In support, overexpression of Vav3 in C57BL/6 mouse prostate luminal epithelium also displayed early chronic inflammation associated with development of prostate adenocarcinoma [12,14].

To better model MYC-driven prostate cancer progression, we backcrossed FVB *Hi-MYC* mice to a C57BL/6 background (B6MYC). Our results phenocopy those described

previously, in that a switching of mouse background results in decreased kinetics of disease progression. Further, progression of prostate cancer in the B6MYC GEMM was associated with a spontaneous infiltration of myeloid-derived suppressor cells. In addition, we successfully utilized a conditional reprogramming method to establish a cell line (B6MYC-CaP) that displayed tumorigenic ability in vivo. B6MYC-CaP cells expressed full-length androgen receptor (AR) without evidence of de novo AR splice variant expression. Further, B6MYC-CaP maintained androgen dependency and responded to AR antagonists, bicalutamide, and enzalutamide in vitro and surgical castration in vivo. We further indicate that B6MYC-CaP cells express molecular features of prostate luminal and not basal or neuroendocrine cell lineage. Collectively, we believe that this is the first disclosed C57BL/6 MYC-driven prostate cancer GEMM and a syngeneic cell line representing prostate adenocarcinoma. With the cognate characteristics, B6MYC and B6MYC-CaP represent powerful tools to study prostate cancer initiation and progression with an associated tumor microenvironment. Notably, it will be applicable to examine the capacity of immune therapies for prostate cancer treatment.

MATERIALS AND METHODS

B6MYC Mouse Generation and Genotyping

A FVB *Hi-MYC* (ARR2/Pbsn-MYC) mouse strain was purchased from NCI, and backcrossed to C57BL/6N for more than seven generations to obtain *Hi-MYC* transgenic mouse model in pure C57BL/6 background, designated B6MYC. Genomic DNA was collected from tail snips following manufacturer's protocol (Qiagen, #69504). A pair of primers was used to examine ARR2/Pbsn-MYC transgene as following: 5'-aaacatgatgactaccaagcttggc3' and 5'-atgatagcatcttgttctt agtctttttcttaataagg3'. Then homozygosity of *Hi-MYC* was determined by crossing B6MYC with wild-type mice to produce all pups carrying ARR2/Pbsn-MYC.

B6MYC-CaP Cell Line Establishment

A conditional reprogramming method was used to establish a cell line from B6MYC transgenic mouse model [15]. Briefly, tumor samples from the prostate of an 18 months old B6MYC mouse was chopped into 2-mm fragments followed by digestion in a mixture of Collagenase/Hyaluronidase (Stem Cell Technologies, #7912) at 37°C for 3 hr. Cell clumps were then incubated with 0.25% trypsin for 1 hr on ice followed by Dispase (Stem Cell Technologies, #7913) and DNaseI (Sigma, #DN25) for 1 min. Cell suspensions were filtered through a 40-µm cell strainer, and then co-cultured with irradiated Swiss-3T3 feeder layer in F medium containing 10 µM Y-27632 (Enzo Life Sciences, #ALX-270-333) for 2 months. Epithelial colonies surrounded by irradiated fibroblast were harvested and trypsinized into single cells, plated in a 96-well plate. The B6MYC-CaP cell line was established from the homogeneous population in one single well and then continuously maintained in F medium with the supplement of 10 µM Y-27632 and 1 nM R1881. Preparation of F medium was described previously [15].

Cell Culture And Reagents

Myc-CaP cells was a kind gift from Dr. Charles Sawyers (Memorial SloanP Kettering Cancer Center). TRAMP-C2 cells was generously provided by Dr. Barbara Foster (Roswell Park Cancer Institute). PrEC-AR and LNCaP95 cells were provided by Dr. John Isaacs (Johns Hopkins). Myc-CaP and TRAMP-C2 cell lines were cultured using DMEM containing 10% fetal bovine serum. LNCaP and VCaP cells were purchased from ATCC. LNCaP, LNCaP95, and VCaP cells were cultured in RPMI1640 with 10% fetal bovine serum. PrEC-AR cells were cultured in keratinocyte serum-free media with added growth factors.

Flow Cytometry

Eighteen-month-old tumors or wild-type prostate tissue was disaggregated using collagenase/hyaluronidase (Stemcell Technologies, Cat #07912). Cells were stained as previously described [16]. Directly conjugated antibodies included those reactive with murine CD45.2 (eBioscience, clone 104), CD11b (eBio-science, clone M1/70), and Gr-1 (BD Biosciences, clone RB6-*C5). Cells were analyzed on an LSR II flow cytometer and data files were analyzed using FCS Express software.

Histopathology

Mouse prostates were weighted and collected at appropriate time points. Dorsal, lateral, and ventral lobes were separated under a dissection microscopy (Zeiss, Oberkochen, Germany). Tissues were fixed in 10% neutral buffered formalin solution (Sigma, #HT5001128) for overnight and then transferred to 70% ethanol for an additional 24 hr. Four micron thick sections were cut from paraffin-embedded blocks and subjected to H&E and immunohistochemistry analysis. For immunohistochemistry, slides were first deparaffinized in xylene solutions and then rehydrated in graded ethanol. Further, slides were boiled in 10 mM sodium citrate solution (pH 6) in a microwave for 10 min in favor of antigen retrieval. The slides were then blocked in 2.5% normal horse serum (Vector Laboratories, #S-2012) for 30 min at room temperature and subsequently incubated with primary antibodies diluted in Tris-buffered saline containing 1.25% horse serum in a humidified chamber for overnight. Primary antibodies used for immunohistochemistry are anti-AR (Millipore, #PG-21, 1:250), anti-c-Myc (Abcam, #ab32072, 1:500), and Rabbit IgG (Cell Signaling, #3900). The slides were then washed by Tris-buffered saline containing 0.1% Tween-20 (Sigma, #P9416) following the incubation with secondary antibody conjugated with peroxidase (Vector Laboratories, #MP-7401). Slides were developed by DAB (Dako, #K3468) and subsequently counterstained with hematoxylin.

Cell Growth Assay

Five thousand cells were seeded in 6-well plates in phenol red-free DMEM medium with 10% charcoal-stripped FBS for overnight. Media containing 1 nM R1881, 10 μ M bicalutamide, and 10 μ M enzalutamide (MedChem Express, #HY-70002) were added, and replenished every the other day. Cells were stained with 0.4% trypan blue and counted by hemocytometer at indicated time points.

Real-Time PCR

RNA was extracted using TRIzol reagents (Thermo-Fisher Scientific, #15596-018) according to manufacturer's protocol. One microgram of RNA was applied to perform cDNA synthesis (Bio-Rad, Hercules, CA, #170-8891) followed by real-time PCR (Bio-Rad, #170-8882) using CFX Connect system (Bio-Rad). Primers for mouse FKBP5 are 5'gccgactgtgtgtgtaatgc3' and 5'cacaatacgcacttgggaga3'; for mouse TMPRSS2 are 5'gctgtcttgccttggaggtt3' and 5'ccgtcacaccagagagaga3'; for mouse SLC45A3 are 5'gacactatgatgaaggcattcgaa3' and 5'tctgtaccagcctgtccatgac3'; for mouse B2M are 5'atactcagccaccaccgg3' and 5'acggtcttgggctcggcc3'.

In Vivo Animal Studies

Mice were anesthetized in a chamber filled with Isoflurane. Five million cells mixed with matrigel (BD, #356234) were injected bilaterally into flank of wild-type C57BL/6 mice by a 26 g syringe. For tumor implantation, ~4mm² B6MYC-CaP derived tumors were engrafted subcutaneously, and continuously passaged as well as maintained in wild-type C57BL/6 mice. Tumor growth was determined by serial caliper measurement.

Immunofluorescence

Cells (5×10^4) were grown on sterile glass coverslips in a 24-well plate overnight followed by fixed in 4% (w/v) paraformaldehyde and permeabilized in 0.1% Triton X-100 phosphate buffered saline (Bio-Rad, #161-0780). Cells were then incubated with primary antibodies to visualize AR (Millipore, #PG-21, 1:250), c-MYC (abcam, #ab32072, 1:500), CK5 (BioLegend, #905501, 1:250), CK18 (Santa Cruz, #sc-28264, 1:200), and Chromogranin A (abcam, #ab45138). Secondary antibodies conjugated with either FITC (BD Biosciences, #554020, 1:250) or Alexa594 (Life technologies, #A-11012, 1:250) were subjected on the top of coverslips for 1 hr at room temperature. The coverslips were then mounted on microscopic slides using HardSet Antifade Mounting Medium containing DAPI (Vector Laboratories, #H-1500).

Western Blot

Total cell lysates were extracted using RIPA buffer (Sigma, #R0278) containing protease and phosphatase inhibitors (Roche, Indianapolis, IN). Fifty micrograms of lysates were subjected to SDS-PAGE, and then transferred to nitrocellulose membrane. The membrane was blocked in 5% non-fat milk for 1 hr at room temperature, subsequently incubated with primary antibodies against AR (Millipore, #PG-21, 1:1000) and GAPDH (Cell Signaling, #5174, 1:2500) for overnight. Signals were detected using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Grand Island, NY).

Statistical Analysis

Data are displayed as the mean $\pm$ SE. Differences were determined using one or two-way ANOVA and two-tailed paired *t* tests where indicated, using GraphPad Prism software. *P* values less than 0.05 were assigned statistically significant.

RESULTS

Delayed Disease Progression in C57BL/6 ARR2/Pbsn-MYC Mice

Genetic background variation and immune system disparity within murine strains are vital determinants in precisely modeling disease development [10,17]. The C57BL/6 mouse strain displays a predominant increase of helper T cell-1 (Th1) cytokines, including tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ) compared to other strains [18]. A recent study also indicated that men with a Th1 cytokine profile were associated with a lower risk of prostate cancer [19]. To strengthen the merits of MYC-driven prostate cancer mouse model, we backcrossed the original FVB *Hi-MYC* (ARR2/Pbsn-MYC) transgenic mouse to a C57BL/6 background for at least seven generations to establish a C57BL/6 ARR2/Pbsn-MYC driven prostate cancer mouse model (B6MYC).

In the comparison to age matched wild-type C57BL/6 mice, B6MYC mice develop rapid prostate intraepithelial neoplasia (mPIN) lesions in dorsal (DP), lateral (LP), and ventral (VP) prostate lobes at 2 months of age (Fig. 1AaP_d). Formation of mPIN as early as 2 months was in line with the previously reported FVB *Hi-MYC* transgenic mouse model. Documented alterations indicative of mPIN were noted in B6MYC mice including multilayer epithelium with tufted and/or cribriform growth patterns associated with cytoplasmic hyperchromasia and multiple nucleoli [20]. In contrast to findings from Ellwood-Yen et al. where FVB *Hi-MYC* mice display progression to invasive prostate adenocarcinoma by 6 months [3], B6MYC mice maintained focal areas of mPIN in all prostate lobes till 12 months (Fig. 1AbP_o). At 15 months of age, all B6MYC mice exhibited microinvasive lesions indicative of adenocarcinoma (Fig. 1AdP_p, Supplement Fig. S1A and B, Table 1). Immunohistochemistry staining displayed AR nuclear expression throughout wild type and B6MYC DP lobes (Supplement Fig. S2aP_g). Similar AR positive nuclear staining was also observed in VP and LP lobes of wild type and B6MYC mice (data not shown). In addition, nuclear MYC protein expression was low or negative throughout all lobes within wild-type mice, whereas marked nuclear MYC protein expression was evident in lobes and at all ages (Supplement Fig. S2BiP_p).

As expected, increased prostate tissue growth resulted in increased overall genitourinary tract weight and significant increase in overall prostate tissue weight, when compared to aged matched wild-type control mice (Fig. 1B and C).

Myeloid Derived Suppressor Cells Are Recruited to Prostates of C57BL/6 ARR2/Pbsn-MYC Mice

Examination of prostate H&E sections over the course of disease progression in B6MYC mice revealed an increasing number on infiltrating lymphomononuclear cells, indicative of an inflammatory response (Fig. 2A). To further characterize this response, we dissected DP, LP, and VP lobes of 12-month-old mice and performed fluorescence-activated cell sorting of infiltrating lymphomononuclear cells. We stained for several categories of immune cells, including granulocytes, monocytes, macrophages, B cells, and T cells. Our results identified that the dominant subset of infiltrating cells to the prostate was CD45⁺Gr-1⁺CD11b⁺ cells, also known as myeloid-derived suppressor cells (MDSCs), are significantly enriched in

prostate lobes of B6MYC transgenic mice (Fig. 2B and E). The identification of infiltrating MDSCs has also been described in another C57BL/6 mouse model of prostate cancer deficient for *PTEN* [12]. Further, analysis of infiltrating CD45⁺Gr-1⁺CD11b⁺ cells in FVB wild type and MYC transgenic mice demonstrated no significant difference between either genotype (Supplement Fig. S3). Taken together, our results indicate that disease progression in B6MYC mice may phenocopy the inflammatory response seen in clinical prostate cancer cases.

Generation of a Novel Cell Line From the Hi-MYC C57BL/6 Transgenic Mouse

An isogenic prostate cancer cell line, which is compatible to immune competent mice, facilitates the field to closely investigate disease progression and therapeutic efficacy in the presence of a functional immune system. To date, only a few such PCa cell lines are available, including MYC-CaP [21] and TRAMP-C2 [22] in FVB and C57BL/6 mice, respectively. Previously, a C57BL/6 syngeneic prostate adenocarcinoma cell line, RM1 has been described. It was demonstrated that RM1 cells expressed the luminal lineage marker, CK18 and responded to androgen [23,24]. To achieve the development of our B6MYC-CaP cell line, we adopted a conditional reprogramming method to isolate cells from an 18-month-old mouse and seeded single cells in a 96-well plate to obtain a pure cell line. To further confirm that B6MYC-CaP cells originated from B6MYC mouse model, we performed PCR analysis to demonstrate positive expression of the ARR2/Pbsn-MYC transgene (Fig. 3A). The initial MYC-CaP cell line isolated from FVB ARR2/Pbsn-MYC transgenic mice displayed expression of both wild type and splice variant forms of AR [25]. To determine AR expression in B6MYC-CaP cell lines we performed western blot and PCR analysis. Both methods demonstrated that B6MYC-CaP cells were positive for full length AR and negative for variant AR isoforms (Fig. 3B and C). Also, AR full length mRNA was significantly increased in response to culturing of B6MYC-CaP cells in charcoal-stripped serum (Fig. 3C). Further validation of MYC and AR protein expression was performed by immunofluorescence microscopy (Fig. 3D and F). Because of positive AR expression, B6MYC-CaP cells were suggested to be of a luminal cell lineage. Additional immunofluorescence staining confirmed this, as B6MYC-CaP cells stained positive for the luminal marker cytokeratin-18, while negative for the basal cell marker cytokeratin-5 and the neuroendocrine marker chromogranin A (Supplement Fig. S4).

B6MYC-CaP Cells Retain In Vitro and In Vivo Dependency on Androgen Receptor Signaling

AR signaling pathway is a key driver in the development of murine and human prostate adenocarcinoma. In order to determine AR activity in our B6MYC-CaP cell line, we cultured B6MYC-CaP cells in androgen-depleted conditions over night before treating with the synthetic androgen R1881, with or without the AR antagonists, bicalutamide, or enzalutamide. Androgen supplementation stimulated B6MYC-CaP cell growth and AR transcriptional activity (Fig. 4A and B). Addition of AR antagonists reversed androgen/AR effects, as demonstrated by the ability of bicalutamide and enzalutamide to inhibit cell growth and AR transcriptional activity (Fig. 4A and B).

Additionally, we confirmed the tumorigenic potential of B6MYC-CaP cells by successful development of tumors in C57BL/6 mice (Fig. 4C). Following 2 weeks of tumor growth, a

subset of tumor bearing animals was surgical castrated to determine AR dependency *in vivo*. Our results illustrate that tumor growth rate was significantly decreased in castrated mice (Fig. 4C). Immunohistochemistry analysis displays that the expression level of MYC was reduced due to the fact that AR has been exported to cytoplasm (Fig. 4D). Collectively, this novel B6MYC-CaP cell line represents a powerful syngeneic *in vitro* and *in vivo* model for use in pre-clinical therapy trials and molecular studies involving prostate cancer progression.

DISCUSSION

Establishment of reliable model systems to mimic prostate tumorigenesis advances the field to delineate molecular mechanisms of prostate cancer progression and to investigate novel therapeutic strategies. Several genetically engineered mouse models have been generated to recapitulate prostate cancer initiation and progression. These models are driven by genetically relevant aberrations found in human prostate cancer [26]. Amplification of chromosome 8q resulting in increased the expression of the MYC oncogene is an early event observed in human PIN and prostate cancer [7,9]. Ellwood-Yen et al. reported a MYC-driven prostate cancer model (Hi-MYC) to demonstrate the ability of MYC to mediate prostate cancer initiation and progression. Further, similarities were also noted between gene expression signatures from this GEMM and human prostate cancer [3]. Due to the fact that genetic background and immune system variations within mouse strains may impact prostate tumorigenesis and the lack of syngeneic cell lines to model AR positive prostate adenocarcinoma in a C57BL/6 background, we backcrossed Hi-MYCFVB mice to a pure C57BL/6 back ground to generate a prostate cancer B6MYC GEMM.

Aging of B6MYC mice showed that development of mPIN occurred rapidly at 2 months of age, which is in line with the original observation in Hi-MYCGEMM [3]. However, progression of disease was severely delayed, as development of invasive adenocarcinoma was not observed until 15 months of age in B6MYC mice, whereas the FVB Hi-MYC mouse was reported to progress to invasive adenocarcinoma by 6 months [3]. Our observed delay in disease kinetics in B6MYC mice have been observed by another study describing the generation of a C57BL/6 PTEN null model of prostate cancer [12]. Our study also demonstrates that MYC-dependent tumorigenesis is influenced by genetic background, which can also include variations in downstream gene expression signatures [10]. Whether, gene signatures are altered in our described B6MYC mice compared to the original FVB Hi-MYC GEMM was not investigated here, but should be considered in future studies.

Two previous prostate cancer mouse models generated on a pure C57BL/6 genetic background have reported disease progression is associated with an inflammatory phenotype, which was described to be a predominant lymphomononuclear cell infiltration [12,14]. Svensson et al. further identified their infiltrating lymphomononuclear population to show an enhanced recruitment of Gr-1⁺CD11b⁺ myeloid derived suppressor cells (MDSCs) [12]. These observations were in line with disease progression in the B6MYC mice, where a marked increased recruitment of Gr-1⁺CD11b⁺ MDSCs compared to aged matched wild-type control mice was observed. In this present study, we did not determine the contribution of MDSCs in MYC driven prostate cancer initiation and progression, however, it had been described from other mouse studies that recruitment of MDSCs had increased potential to

suppress T-cell proliferation [12]. Chronic inflammation is a known risk factor associated with human prostate cancer initiation [13]. This immune response results in the recruitment of pro-inflammatory immune cells into the prostate that produce reactive chemical compounds leading to DNA damage in epithelial cells. This is known as proliferative inflammatory atrophy (PIA) [27]. Previous literature have shown that the recruitment of MDSCs in prostate may create the inflammatory environment in favor to tumorigenesis and also demonstrate that the recruitment of immune cells in transgenic mouse models lead to occurrence of chronic inflammation in the prostate [14], suggesting that inflammation may serve as an initiation event during prostate cancer development. Recently it was indicated that PI3K-AKT and YAP signaling pathways play an important role in the recruitment of MDSCs [28]. Overall, B6MYC and other C57BL/6 prostate cancer GEMMS phenocopy the inflammatory immunophenotype observed in human prostate cancer. This greatly strengthens the capacity of such prostate cancer mouse models with C57BL/6 genetic background to carry extreme relevance for translation to human prostate cancer.

In vitro cell culture systems can be a powerful tool to understand the biology of prostate cancer and test potential therapeutic applications. Several human prostate cancer cell lines have been utilized for decades [29,30]. However, most cell lines can only be applied to immunodeficient mice to perform in vivo studies, which carry limitations involving tumor cell and microenvironment cross talk. For this reason, the generation of syngeneic prostate cancer cell lines would advance the prostate cancer research. While few murine cell lines have the ability to generate syngeneic in vivo transplant models, to our knowledge, only one model exists that represent AR positive adenocarcinoma on a C57BL/6 genetic back ground [23,24]. Here, we adopted a reprogramming method [15] to establish a prostate cancer cell line from our B6MYC model, designated as B6MYC-CaP. Our results demonstrate that B6MYC-CaP express both nuclear AR and MYC, and this new cell line was derived from a luminal lineage, as indicated by positive expression of cytokeratin 18 and negative expression of the basal cell and neuroendocrine lineage marks cytokeratin 5 and chromogranin A, respectively. Previously, the original FVB MYC-CaP cell line was reported to express wild-type AR plus AR variants [25]. From our analysis B6MYC-CaP was restricted to only expressing full-length AR, without noticeable mRNA or protein expression of known AR variants. In addition, B6MYC-CaP cells retain dependency on AR signaling pathway as cell growth and AR transcriptional activity were induced by androgen and significantly repressed by AR antagonists, bicalutamide, and enzalutamide. Moreover, our in vivo studies display that B6MYC-CaP cells are able to grow in syngeneic C57BL/6 mice, and inhibited by surgical castration. Taken together, we report here that we successfully generated a new MYC-driven mouse model and a derived cell line on a C57BL/6 genetic background. These models will provide a useful background to conduct a superior platform to investigate molecular mechanisms to prostate cancer initiation and progression. To date, immune-check point inhibitors have failed to show significant clinical activity in patients with prostate cancer despite the success in other solid tumors. We believe that this model will provide a new tool to interrogate the cross talk between prostate cancer cells and immune cells, and to develop novel therapeutic strategies to enhance immuno-therapies for prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Histological disease progression in B6MYC mice. C57BL/6 wild type (wt) and B6MYC mice were analyzed for pathological changes to the prostate epithelium via histology (dorsal shown). **A:** H&E staining of DP in C57BL/6 wt male mice (**a-p**) and B6MYC transgenic mice (**i-p**) demonstrates the development of pathological changes associated with mPIN lesions and invasive adenocarcinoma. **B:** Whole genitourinary tract weights of C57BL/6 wt and B6MYC mice. **C:** Combined dorsal, lateral, and ventral prostate lobe weights of C57BL/6 wt and B6MYC mice. *** $P < 0.001$, **** $P < 0.0001$ determined by student t -test. Scale bar = 200 μ m.

Fig. 2. Microenvironment changes in B6MYC transgenic mice. **A:** H&E staining of C57BL/6 reveals increased mononuclear infiltrating inflammatory cells in prostate lobes of 12-month-old B6MYC transgenic mice compared to aged matched wt mice. **B-E:** Example scatter plots and absolute numbers of infiltrating $CD45^{+}Gr-1^{+}CD11b^{+}$ are increased in B6MYC transgenic mice. Scale bar = 100 μ m. Statistical significance for D was determined by a student's *t*-test.

Fig. 3.

Establishment of a B6MYC-CaP cell line. **A:** Genomic DNA was isolated from indicated cell lines and PCR was used to detect ARR2/Pbsn-MYC transgene with a 177 bp product size. B6MYC-CaP cell line displays positive expression for the ARR2/Pbsn-MYC transgene. **B:** Whole cell lysates were generated from indicated cell lines. B6MYC-CaP cells express full length AR without expression of AR variants. **C:** PCR analysis displays B6MYC-CaP cells express mRNA of full length AR when cultured in FBS (red) or CSS (blue). No mRNA expression of AR variants was detected. **D:** Statistical significance was determined by students *t*-test. Immunofluorescence microscopy validated PCR and western blot analysis displaying strong AR and MYC expression in B6MYC-CaP cells. Original MYC-CaP cells were used as positive control. Scale bar = 200 μ m.

Fig. 4.

In vitro and in vivo androgen dependency of B6MYC-CaP cells. **A:** B6MYC-CaP cells grown in androgen depleted culture conditions. R1881 (10 nM) stimulated cell growth is inhibited by the AR antagonists, bicalutamide (10 μ M), and enzalutamide (10 μ M). **B:** B6MYC-CaP cells grown in androgen depleted culture conditions. R1881 (10 nM) stimulated AR transcriptional activity is inhibited by AR antagonists. **C:** Intact C57BL/6 male mice harboring B6MYC-CaP tumor growth is significantly inhibited by surgical castration. * $P < 0.05$, ** $P < 0.01$ determined by two-way ANOVA. **D:** IHC staining for AR and MYC expression in B6MYC-CaP tumors. Intact mice display strong nuclear AR and MYC expression. Castrated tumors display decreased AR and MYC nuclear expression. Scale bar = 200 μ m.

TABLE 1.Pathological Changes to DP, LP, and VP Lobes of C57BL/6 Hi-*MYC* (B6MYC) Mice

Disease progression in B6MYC model			
Age (months)	Normal	PIN	Invasive carcinoma
2	0/3	3/3	0/3
6	0/3	3/3	0/3
12	0/9	9/9	0/9
15	0/11	2/11	9/11