Introduction

Early stages of prostate cancer are sensitive to androgen deprivation therapy, but upon progression, the disease develops to metastasis-resistant prostate cancer, where bone is the dominant site of metastasis. Despite of recent advances in drug development, this advanced stage is still incurable. Tumor microenvironment in metastatic locations differs from the primary site and may cause resistance to used therapies by changing tumor growth properties. Therefore it would be very important to use proper models in preclinical drug development to mimic these conditions in patients.

The aim of this study was to establish in vitro and in vivo prostate cancer models that can be used when targeting cancer cells or tumor microenvironment at different stages of preclinical drug development.

Materials and Methods

Androgen receptor (AR) positive human prostate cancer cell line LNCaP (ATCC) was used in all studies. In the in vitro assay, the effects of the antiandrogen enzalutamide (Selleckchem) on cell viability were determined using CellTiter Glo assay in the presence and absence of a synthetic androgen R1881 (Sigma Aldrich). In in vivo studies, male NMRI nude mice (Janvier) were used. In a subcutaneous (s.c.) model (n = 7 per group), part of the mice received dihydrotestosterone (DHT; release rate of 50 or 100 µg per day, MedRod™, PreclinApps) supplement prior to LNCaP cell inoculation (5 x 10⁶ cells in matrigel). In a bone metastasis model, the cells (2 x 10⁶ cells in PBS) were inoculated into tibia bone marrow. The mice were randomized to treatment groups (n = 10 per group) based on similar serum PSA levels (PSA screening) and cancer-induced changes in bone determined by X-ray imaging (Faxitron) at 6 weeks. The mice were treated with 300 kBq/kg, i.v., of Ra-223 dichloride (Oak Ridge National Laboratory) or vehicle as study weeks 6 and 10. Bone lesions were followed by X-ray imaging and tumor burden by measuring serum PSA levels during the study. The study was terminated at 12 weeks and the mice were processed for histological analysis (hematoxylin-eosin, orange staining, HE-orange) and AR immunohistochemistry (IHC) staining (antibody AR, clone SP107, Spring Biosciences).

In vitro assay

FIGURE 1. Effects of enzalutamide on LNCaP cell viability in the presence and absence of 0.1 mM (A) and 0.01 mM (B) R1881 at day 3. The results are shown as luminescence (CPS). The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. BL = Baseline (no added compounds). COMP = Study group used for statistical comparison. * p < 0.05, ** p < 0.01, NS = Not significantly different from the COMP group.

Subcutaneous LNCaP model

FIGURE 2. A) Prostate weight at sacrifice (ng, mean ± SEM) in LNCaP tumor growth curves (mm², mean ± SEM) up to 12 weeks. Tumor take rate of 80%, 100%, and 80% was gained in the control group and in the groups supplemented with 50 µg/d and 100 µg/d of DHT, respectively. The mice were sacrificed individually when the maximum tumor volumes (1500 mm³) were reached, earliest at study week 10.

Intratibial LNCaP model

FIGURE 3. A) IH staining in s.c. tumors from A) vehicle group, B) and C) groups supplemented with 50 µg/d and 100 µg/d of DHT, respectively. Strong AR expression was observed in all study groups.

Summary

- In the in vitro assay, 0.1 and 0.01 nM Ra-223 increased LNCaP cell viability.
- Enzalutamide reduced cell viability in the presence of R1881 compared to the group with only R1881.
- In the s.c. model, DHT supplement increased prostate weight dose-dependently. Maximum tumor volume was reached within 10-12 weeks. Tumor growth was observed in the absence of DHT, and 50 µg of DHT further supported tumor growth. However, no tumor growth was observed in the group supplemented with 100 µg of DHT in NMRI nude mice. No major changes were observed in tumor AR expression between the study groups.
- In the bone metastasis model, LNCaP tumors induced osteoblastic-mixed bone lesions, and IHC staining demonstrated strong AR expression in tumor cells. Corresponding AR expression was observed in tumors growing s.c. and in bone.
- Ra-223 dichloride decreased serum PSA levels and tumor area analyzed by histology, and reduced the progression of tumor-induced bone lesions analyzed from X-ray images.

Conclusions

This study showcases relevant preclinical prostate cancer models that can be used at different phases of drug development including evaluating efficacy of therapeutics as mono- or combination therapies. The preclinical model and key readouts should be carefully selected based on whether developing therapeutics for localized or bone metastatic prostate cancer.

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