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Comparison of prostate cancer cell lines for androgen receptor-mediated reporter gene assays

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Abstract

In order to select a better prostate cancer cell model for androgen receptor (AR)-mediated reporter gene assays, we assessed the androgen response characteristics of three cell lines, LNCaP, PC3/AR$^+$ and 22Rv1, in this study. Both the mRNA and the proteins of AR and glucocorticoid receptor (GR) were expressed in all three cell lines. Among the three cell lines, only in LNCaP cells, DHT concentration-dependently stimulated proliferation. DHT induced the luciferase activity in three cell lines which were transiently transfected with pMMTV-Luc, in a concentration-dependent manner. The maximum induction was 24.0-fold and 13.4-fold in 22Rv1 and in the LNCaP respectively. PC3/AR$^+$ were more sensitive to respond to DHT at a minimal concentration of 10^{-12} M by 14.0-fold induction. The transcriptional activity induced with 10^{-8} M DHT was inhibited about 50–75% in the PC3/AR$^+$ and 22Rv1, and 98% in the LNCaP, by vinclozolin. Dexamethasone concentration-dependently induced the luciferase activity in PC3 and 22Rv1, but not in the LNCaP. However, the response to dexamethasone in 22Rv1 was very weak compared to DHT. The (anti)androgencity of seven pyrethroids was assessed via an AR-mediated luciferase reporter assay. None of them showed the androgenic action in all three cell lines. Permethrin inhibited the DHT induced luciferase activity about 22%, 35.8% and 75.5% in 22Rv1, PC3/AR$^+$ and LNCaP, respectively. Based on results from in this study and cell line character, 22Rv1 cells seemed to be an appropriate model for the screening of androgenic endocrine disruptors, although it needs further studies with other steroid receptor and thyroid receptor.

Keywords: 22Rv1; LNCaP; PC3; Dexamethasone; AR-mediated reporter gene assay

1. Introduction

Some environmental chemicals are known or suspected to interfere with reproductive development in wildlife and humans by either mimicking or inhibiting the action of the gonadal steroid hormones, estradiol and testosterone (Colborn et al., 1993; Danzo, 1998; Rajesh, 1999). Among these, environmental chemicals which are able to bind the androgen receptor (AR) may be involved in abnormalities of the developing male reproductive system (Kelce et al., 1998). The number of environmental chemicals identified to exhibit anti-androgenic properties is steadily growing. Therefore, the identification of androgen-active chemicals is quite relevant in several fields, including food manufacturing, toxicological monitoring, and risk assessment.

In the androgen target cells, the effects of androgens appear to be mediated by the AR, which is, in the absence of ligand, localized primarily in the cytoplasmic fraction. Upon ligand binding, the cytosolic AR translocates to the nucleus, where it binds to hormone response elements (HREs) within the regulatory DNA sequences of the androgen-responsive genes, subsequently stimulating their transcription (Roy and Chaterjee, 1995). The enhancer region of the mouse mammary tumor viral long terminal repeat (MMTV-LTR) promoter constitutes the most frequently used enhancer in the study of AR function (Cato et al., 1987; Darbre et al., 1986). HRE within the MMTV-LTR enhancer bound by AR is composed of the four inverted repeats of the 5′-TGTTCT-3′ core sequence, and can also be recognized by the glucocorticoid receptor

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(GR), progesterone receptor (PR), and mineralocorticoid receptor (MR) (Gowland and Beutti, 1989). Thus, the selectivity and sensitivity of the assay may rely on the levels of these receptors and transcription related proteins.

Most environmental anti-androgenic agents antagonize the actions of androgens within the target cell via competition with the AR, or via the reduction of the transcriptional activation of target genes at the crucial period (Andersen et al., 2002; Kang et al., 2004; Wong et al., 1995; Wolf et al., 2000). In order to characterize the androgenic properties of various chemicals, a number of in vitro methods have been developed, based on the AR binding assay and/or AR-dependent reporter gene assays, using mammalian cell lines or yeast cells (Lee et al., 2003). Numerous cell lines, including prostate carcinoma cells (LNCaP, PC3 and DU-145) or other cells (HepG2, CV-1, COS-1, COS-7 and CHO) have been employed in androgen reporter gene assays, and involved transient or permanent transfection with the MMTV-luciferase reporter gene (Stone et al., 1978; Hartig et al., 2002). The selection of an appropriate cell line, then, is crucial to the ultimate sensitivity and specificity of the assay.

The prostate is one of the principal target tissues of androgens (Roy et al., 1999). Thus, we considered using a prostate cell line for our AR-mediated androgen reporter gene assay. PC3, derived from metastatic lesions to the bone, is one of the representative androgen-insensitive cell lines which tend to express AR at barely detectable levels (Kaighn et al., 1979). The proliferation of PC3 cells is not associated with any AR-mediated mechanisms. Although these cells are useful with regard to the evaluation of (anti)androgenic effects, it has proven fairly difficult to apply these cells without the transfection of the AR to androgenic endocrine disruptor screening (Tepper et al., 2002). One of the more widely used androgen-sensitive prostate cancer cell lines is LNCaP, which is derived from metastatic lesions of the supraclavicular lymph node. The mutant AR, T877A, in the LNCaP cells maintains androgen responsiveness at levels comparable to wild-type AR, but also exhibits broadened ligand specificity (Veldscholte et al., 1990). 22Rv1 is a recently developed human prostate carcinoma cell line. The 22Rv1 cell line was derived from the human prostatic carcinoma xenograft, CWR22R, and expresses both AR and prostate specific antigen (PSA) (Tepper et al., 2002; Hartel et al., 2003). Unlike any other commercially available prostate cancer cell lines, all of which express at least one additional steroid receptor, 22Rv1 cells express only the human androgen receptor (Hartel et al., 2003). Further validation experiments, however, will be required for confirmation that no other steroid receptors are expressed in the 22Rv1 cell line.

Synthetic pyrethroids are among the most common pesticides and insecticides currently in use worldwide. Recently, chemicals classified as synthetic pyrethroids are suspected as being endocrine disrupting chemicals. However, relatively few studies have reported on hormonal activities, particularly androgenic activities, and the results of these studies are in some respects contradictory (Garey and Wolff, 1998; Yamada et al., 2003; Kojima et al., 2004).

In order to select the most appropriate cell line for AR-mediated reporter gene assay, we conducted a comparison of the basal characteristics associated with androgen response, as well as the potential for the detection of (anti)androgenic action, using pyrethroids insecticides to three prostate cancer cell lines, i.e., LNCaP, PC3/AR$^+$, which had been transiently transfected with human AR, and 22Rv1 cells.

2. Materials and methods

2.1. Chemicals and reagents

The 5α-dihydrotestosterone (DHT) and testosterone were purchased from Wako Pure Chemical (Osaka, Japan). The AR (C19), PSA (A67-B/E3) and GR (H300) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase conjugated anti-rabbit IgG and anti-mouse IgG were purchased from Zymed (San Francisco, CA). Test pesticides and the dual luciferase assay kit were purchased from Supelco (Bellefonte, PA) and Promega Corp. (Madison, WI), respectively.

2.2. Cell culture

The human prostate cancer cell lines, PC3 (cat # CRL-1435), 22Rv1 (cat # CRL-2505) and LNCaP-FGC (cat # CRL-1740), were all obtained from the American Type Culture Collection (Rockville, MD). These cells were routinely maintained in RPMI 1640 medium (Gibco BRL, USA) containing 10% fetal bovine serum (FBS), penicillin (100 units/ml), 1% L-glutamine and streptomycin (100 μg/ml) in a 5% CO_2 atmosphere, at 37 °C. Steroids were removed from FBS essentially as described by Borras et al. (1994). Briefly, FBS (Gibco BRL, USA) was incubated with 0.5% activated charcoal and 0.05% dextran T-70 for 30 min. The charcoal particles were removed by centrifugation at 4 °C for 20 min at 4500*g*. This step was repeated and the stripped serum was sterile filtered and stored in aliquots at −20 °C.

2.3. Cell proliferation assay

Cells were seeded in 96-well plates at an initial concentration of 3×10^3 cells per well and changed to the experimental medium (phenol red-free RPMI 1640 with 10% dextran charcoal-stripped FBS) containing DHT and/or phenoxy compounds after 24 h. On the 4th day, the cells were fixed, and stained with sulforhodamine-B (SRB) as described in Villalobos et al. (1995). In brief, the cells were treated with 10% trichloroacetic acid, and incubated at 4 °C for 1 h, then washed with PBS. In order to stain the proteins, 50 μl of 0.4% SRB was added to each well for 1 h at room temperature. Cells were washed several times in 1% acetic acid, and air dried. The bound dye was solubilized