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Inhibition effect of cypermethrin mediated by co-regulators SRC-1 and SMRT in interleukin-6-induced androgen receptor activation

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HIGHLIGHTS

- Cypermethrin can inhibit IL-6-mediated AR activation in androgen-independent manner.
- Cypermethrin represses IL-6-induced cell growth using the RTCA iCELLigence system.
- Cypermethrin suppresses the interaction between AR NTD and co-activator SRC-1 induced by IL-6.
- Cypermethrin suppresses the interaction between AR NTD and co-repressor SMRT induced by IL-6.

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ABSTRACT

It is hypothesized that the pesticide cypermethrin may induce androgen receptor (AR) antagonism *via* ligand-independent mechanisms. The Real-Time Cell Analysis (RTCA) iCELLigence system was used to investigate the inhibitory effect of cypermethrin on interleukin-6 (IL-6)–induced ligand-independent LNCaP cell growth. Then, the mammalian two-hybrid assays were applied to clarify whether the mechanism of IL-6–induced AR antagonism of cypermethrin was associated with the interactions of the AR and co-activator steroid receptor co-activator-1 (SRC-1) and co-repressor silencing mediator for retinoid and thyroid hormone receptors (SMRT). Cypermethrin inhibited the LNCaP cell growth induced by IL-6. The interactions of AR–SRC-1 and AR–SMRT mediated by IL-6 were suppressed by cypermethrin. The results indicate that the IL-6–mediated AR antagonism induced by cypermethrin is related to repress the recruitment of co-regulators SRC-1 and SMRT to the AR in a ligand-independent manner. Inhibition of the interactions of AR–SRC-1 and AR–SMRT mediated by IL-6 contributes to the AR antagonism induced by cypermethrin.

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1. Introduction

Endocrine disrupter chemicals (EDCs) are exogenous chemical substances that can interfere with endocrine system and are associated with homeostasis, reproduction, and process of development (Diamanti-Kandarakis et al., 2009). Because there are a growing number of studies about EDCs and health hazards, the scientific researchers and the general public have become increasingly concerned regarding the potential risk of EDCs on human health. EDCs have the potential to disrupt the endocrine system, triggering morphological and functional abnormalities through several mechanisms (Diamanti-Kandarakis et al., 2010). One of major mechanisms is that EDCs including some pesticides act as anti-androgen by binding to the AR resulting in preventing transcription of androgen-dependent genes. The reproductive malformations in human or experimental animals may be associated with the AR antagonism of EDCs (Gray et al., 2001).

Cypermethrin, a widely used type II synthetic pyrethroid pesticide, identified as an anti-androgen, is associated with certain male reproductive damages such as reduction of testicular sperm counts, daily sperm production and serum levels of testosterone, testicular lesions, sperm motility and morphologic changes (Elbetieha et al., 2001; Prakash et al., 2010; Singh and Singh, 2008). We have focused on the anti-androgenic activity of cypermethrin

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for years. It has been demonstrated that cypermethrin inhibits AR transcriptional activity by binding to AR as well as altering subsequent gene expression (Xu et al. 2006, 2008). Thus, it is inferred that the mechanism of cypermethrin has intimate connection with AR signaling pathway.

The AR belongs to the super-family of nuclear receptors (NRs) that regulate gene transcriptional process *via* AR nuclear translocation, binding to androgen response elements (AREs) on target genes and combining with multiple transcription factors (Culig et al., 2002; Lonergan and Tindall, 2011). The AR consists of an N-terminal domain (NTD) containing activation function-1 (AF-1), a DNA-binding domain (DBD), a hinge region, and a ligand-binding domain (LBD) containing activation function-2 (AF-2) (Bain et al., 2007). Evidence has indicated that the classical mechanism of AR to regulate gene expression is in a ligand-dependent manner (Brinkmann et al., 1999). However, ligand-independent AR activation, another crucial mechanism of AR gene expression, mediated by growth factors and cytokines, is becoming an issue of increasing concern (Dehm and Tindall, 2006; Jenster, 2000; Mellado et al., 2009). Interleukin-6 (IL-6), a multifunctional cytokine, plays a critical role in cytokine signaling network and can activate AR without androgens (Malinowska et al., 2009). The levels of IL-6 in serum are significantly increased in many patients with advanced, hormone-refractory prostate cancer and IL-6 could induce AR activity through up-regulation of AR gene expression in prostate cancer cells (Drachenberg et al., 1999; Lin et al., 2001). IL-6–induced AR transactivation is mediated by three potential signaling transducer pathway including Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT), Mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase/Akt kinase (PI3-K/AKT) pathways (Azevedo et al., 2011).

The mechanisms of AR transcriptional activation are involved in AR co-regulators known as co-activators and co-repressors. Co-activators expression activity may deeply alter the transcriptional activity of AR by associating with ligand-bound receptor and recruiting general transcription machinery (Tien et al., 2009). It has been confirmed that AR co-activator steroid receptor co-activator-1 (SRC-1), the first isolated nuclear receptor co-activator, can enhance AR transactivation (Hsu et al., 2005). AR co-repressor such as silencing mediator for retinoid and thyroid hormone receptors (SMRT) can repress the transactivation of AR by inhibiting AR amino- and carboxyl-terminal interaction and competing with co-activators in the presence of androgen (Liao et al., 2003). Co-regulators also have relation with IL-6–induced AR signaling pathway (Ueda et al., 2002). Co-activator SRC-1 plays an important role in IL-6–induced AR activation in castration-therapy resistant prostate cancer (Culig, 2016; Peng et al., 2011). Co-repressor SMRT also has influence on IL-6–mediated multiple myeloma cell growth and gene expression (Wang et al., 2004). Because the formation of a productive AR transcriptional complex depends on the structural and functional interaction of the AR with its co-regulators, the negative effects on the interactions of AR–SRC-1 and AR–SMRT will influence the expression of AR-dependent genes and finally cause male reproductive damages (Heemers and Tindall, 2007).

It is accepted that the anti-androgens suppress AR transcriptional activity. The mechanism of AR antagonism of some pesticides, including cypermethrin is associated with androgen-dependent AR signal pathway (Hu et al., 2012; Xu et al. 2006, 2008). However, few researchers have paid attention on AR antagonism of EDCs through ligand-independent manner. A newly developed pesticide pyrifluquinazon (PFQ) has been reported as a ligand-independent AR antagonist that could decrease cellular AR protein level (Yasunaga et al., 2013). In our previous studies, we have shown that cypermethrin may be characterized as ligand-independent anti-androgen, exhibiting inhibitory effects by suppressing IL-6–induced AR activation in the AR reporter gene assay (Pan et al., 2012; Wang et al., 2015).

In this study, we further proposed that the molecular mechanism of IL-6–mediated AR antagonism induced by cypermethrin may be related to the AR activation modulated by co-regulators SRC-1 and SMRT. The Real-Time Cell Analysis (RTCA) iCELLigence system was used to investigate the inhibition of cypermethrin on IL-6–induced LNCaP cell growth. Then the mammalian two-hybrid assays were developed to evaluate whether inhibition of AR–SRC-1 and AR–SMRT interaction in IL-6–induced AR signaling pathway was involved in the AR antagonism of cypermethrin.

2. Materials and methods

2.1. Reagents

Cypermethrin and 5α-dihydrotestosterone (DHT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Human IL-6 was from PeproTech Inc (Rocky Hill, USA). The chemicals were diluted to desired concentrations in phenol red-free RPMI1640 medium (Life Technologies, Carlsbad, California, USA) before use. The ESCORT V Transfection Reagent was from Sigma Chemical Co. (St. Louis, MO, USA) and the chloramphenicol acetyl transferase enzyme-linked immunosorbent assay (CAT-ELISA) kit was from Roche Molecular Bioch (Mannheim, Germany). The Bicinchoninic Acid (BCA) Protein Assay kit was obtained from Beyotime Institute of Biotechnology (Shanghai, China). The β-galactosidase (β-Gal) Enzyme Assay System with reporter lysis buffer was purchased from Promega (Madison, WI, USA).

2.2. Plasmids and plasmid construction

The Mammalian Matchmaker™ Two-Hybrid Assay Kit was purchased from Clontech Laboratories, Inc. (Palo Alto, CA, USA). The pG5CAT was used as the reporter vector. The plasmids include the fusion vector pVP16-ARNTD, pM-SRC-1 and pM-SMRT used in this study were constructed as previously described (Pan et al., 2012, 2013).

2.3. Cell culture

The LNCaP prostate cancer cell line and the African monkey kidney cell line CV-1 were purchased from Institute of Biochemistry and Cell Biology in Shanghai, Chinese Academy of Science (Shanghai, China). The cells were cultured in phenol red-free RPMI1640 medium supplemented with 10% charcoal-Dextran-stripped FBS (CDS-FBS) at 37 °C in an atmosphere of 5% CO_2/air.

2.4. Measurement of cell growth rate in the presence of IL-6/cypermethrin

The Real-Time Cell Analysis (RTCA) iCELLigence system (ACEA Biosciences Inc., San Diego, CA92121) based on electrical impedance measurement is an non-invasive and label-free approach for cell-based assays (Ke et al., 2011). The impedance of the device measured in each well depends on electrode geometry, ionic concentration in the well and whether there are cells attached to the electrodes. Cells attaching to the electrode sensor surfaces will alter the local ionic environment and lead to an increase of the impedance. The impedance data will be transferred to a computer, analyzed and processed by the integrated software (Atienza et al., 2005). The RTCA iCELLigence system was placed in a humidified incubator at 37 °C and 5% CO_2 condition. Initially, 150 μl RPMI1640 was added in 8-wells E-plates and background readings were recorded. Each well of the E-plate was added with 300 μl LNCaP

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