Molecular and Cellular Endocrinology 457 (2017) 43–48

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Natural extranuclear androgen receptor ligands as endocrine disruptors of cancer cell growth

Marilena Kampa*, George Notas, Elias Castanas

Department of Experimental Endocrinology, University of Crete, School of Medicine, Heraklion, Greece

ARTICLE INFO

Article history:
Received 16 September 2016
Received in revised form
13 February 2017
Accepted 13 February 2017
Available online 16 February 2017

Keywords:
Androgen disruption
Androgen receptor
OXER1
Extranuclear actions
Cancer

ABSTRACT

Even though the term endocrine disruption primarily designates environmental chemicals that can interfere with the action of hormones, in recent years it has been extended to include also plant derived compounds that can reach the human body, naturally, or have been identified and studied as alternative pharmaceutical agents. In fact, for a large number of them, their antihormonal action was appreciated by different traditional herbal medicines. In the present review we report the majority of the plant derived compounds that exhibit an antiandrogenic effect and the known mechanisms of action. These include a disruption at testosterone production level and at the classical androgen receptor triggered pathways, including membrane initiated ones. Finally, for the first time we describe the possible involvement of alternative cell membrane androgen receptor systems and the lipid signaling disruption by natural androgen, providing hints about a novel class of therapeutic involvement of androgens.

1. Introduction

The classical term of *endocrine disruption* includes any agent that interferes with the action of hormones within the human body; these agents are therefore named *endocrine disruptors*. Among these agents, that are mainly environmental chemicals (pollutants), known to alter male reproduction and increase reproductive tract cancers (in the case of androgens), there is a significant number of plant derived compounds that can also interfere with their action. They have been characterized as antiandrogens, due to their ability to block or suppress testosterone action through several mechanisms, including the competition with androgen for their receptor binding sites. In this review we will present an up to date summary knowledge on how natural compounds can interfere with the classical androgen receptor mediated actions, along with their interaction with extranuclear and membrane initiated androgen effects. Additionally, we extent the notion of hormone disruption by presenting novel, surprising evidence that androgens as well as natural androgen-interacting ligands can interfere with other cell membrane receptor systems providing an alternative mode of extranuclear androgen action.

2. Natural agents and the androgen receptor

In contrast to estrogen actions (with breast cancer being the prototype disease), through the estrogen receptor (ER) cluster, integrating ERα and β, ER variants and GPR30/GPER1, for which several molecules has been developed and introduced in clinical practice, androgen receptor competition and disruption has not received a similar attention. This might be due to the fact that prostate cancer, an androgen receptor (AR)-related disease, was found to express almost ubiquitely AR, and therefore this molecule is not routinely assayed in prostate cancer, neither as a diagnostic biomarker or companion therapeutic assay (Pelekanou and Castanas, 2016). Nevertheless, in recent years, an increased interest in AR detection and disruption of its action was expressed, as AR have been detected in a number of additional pathologies (including breast cancer), while a deeper insight of AR action has been found, together with alternative modes of action (Pelekanou et al., 2013, Pelekanou et al., 2007).

The classical mode of action of androgens is mediated by their binding to a specific androgen receptor, that belongs to the nuclear receptor superfamily. AR is a 919 aminoacid protein that contains four distinct domains: the N-terminal domain, the DNA binding domain, a hinge region and the ligand binding domain (Claessens et al., 2008). All domains contain one signal for the nuclear transport of AR, while the N-terminal domain and the ligand binding domain have transcription activation functions that allows AR

* Corresponding author. University of Crete, School of Medicine, Laboratory of Experimental Endocrinology, P.O. Box 2208, Heraklion, 71110, Greece.
E-mail address: kampam@uoc.gr (M. Kampa).

http://dx.doi.org/10.1016/j.mce.2017.02.021
0303-7207/

interaction with co-regulators (Dubbink et al., 2004, Kaku et al., 2008). AR is mainly found in the cytoplasm as a dimer and is complexed to heat shock proteins. When androgen binds to AR, the AR dimer is released, becomes phosphorylated and interacts with several co-regulators that promote its translocation to the nucleus, and its binding to DNA at specific sites, the so-called androgen response elements (AREs). As a consequence, the transcription of a number of genes is affected and a specific cellular androgenic response is initiated (Heemers and Tindall, 2007).

Most plant-derived compounds exert an anti-androgenic action by lowering testosterone levels, either by preventing its conversion to the more potent DHT, by promoting its conversion to estrogens, or by reducing prolactin release and subsequent FSH and testosterone levels, these hormones being directly related to testosterone production-secretion by Sertoli cells. For example epigallocatechins from green tea (*Camellia sinensis*), a red reishi mushroom (*Ganoderma lucidum*) extract (mainly its triterpenoids containing fraction) inhibit the 5-alpha-reductase conversion of testosterone into DHT (Fujita et al., 2005, Liao and Hiipakka, 1995), while paeoniflorin, a compound found in white peony (*Paeonia lactiflora*), was found to inhibit the production of testosterone and increase aromatase activity promoting its conversion to estrogens (Takeuchi et al., 1991). Additionally, a chaste tree extract (*Vitex agnus-cactus*) reduces testosterone via dopaminergic effects that result in reduced prolactin from the anterior pituitary (Nasri et al., 2007, Webster et al., 2011) although this effect on testosterone is contradicted by others (Jarry et al., 1994). A decrease in testosterone levels has been also described by a Licorice extract (*Glycyrrhiza glabra*) that decreases total testosterone levels (Armanini et al., 1999) and for spearmint (*Mentha spicata*) that specifically reduces free testosterone (Akdogan et al., 2007), without any effect on total testosterone concentration.

Recent studies revealed that certain plant-derived compounds can also interfere with AR function. One way of such an interference is by modulating its expression levels. This is the case for the two flavonoids quercetin and luteolin that have been reported to repress the function of AR by inhibiting its protein expression in prostate cancer cells (Xing et al., 2001, Chiu and Lin, 2008). Similarly, guggulsterone, a constituent of the Indian Ayurvedic medicinal plant Commiphora mukul that has been shown to induce apoptosis in cancer cells, affects AR expression by inhibiting its promoter activity. Guggulsterone has been also reported to act as an AR antagonist (Singh et al., 2007). AR decreased expression has been also described as the mechanism for the anti-androgenic effect of resveratrol, a natural stilbene found in grapes and wine, on prostate cancer cells. However, in a recent work, Streicher and his colleagues (Streicher et al., 2014) have demonstrated that resveratrol also inhibits the dimerization of AR, explaining the previously observed disruption in AR-DNA-binding by resveratrol (Harada et al., 2011). On the other hand, emodin, a natural anthraquinone derivative isolated from the roots of *Rheum palmatum*a, targets AR and suppresses prostate cancer cell growth by inhibiting AR nuclear translocation, due to an increased association of AR with MDM2 and its subsequent enhanced proteasomal degradation (Cha et al., 2005).

Modulation of AR function by natural compounds has been also reported to be the result of their direct interaction with AR. This is the case of atraric acid, which binds to AR and blocks ligand induced AR translocation to the nucleus, additionally promoting cellular senescence of prostate cancer cells (Hessenkemper et al., 2014, Papaioannou et al., 2009). Equally, epigallocatechin-3-gallate (EGCG) was found to physically interact with the ligand-binding domain of AR (Siddiqui et al., 2011), inhibiting nuclear AR translocation and protein expression leading to inhibition of prostate cancer cell growth. Analogous action has been described for indole-3-carbinol (I3C), the major active compound in cruciferous vegetables along with its primary digestive derivative, 3,3_-diindolylmethane (DIM) (Le et al., 2003). DIM also exhibits a potent antiproliferative action in prostate cancer cells and was the first identified pure androgen receptor antagonist from plants. Finally, Jones and his colleagues have reported an additional type of AR activity inhibition by the natural compound harmol [the metabolite of harmine, a β-carboline compound naturally found in several medicinal plants including *Peganum harmala* (Zygophyllaceae) and *Banisteriopsis caapi* (Malpighiaceae)] (El Gendy et al., 2012, Herraiz et al., 2010). This type of AR inhibition does not involve modulation of ligand binding, but direct binding of the compound to AR in a non-competitive way that prevents normal conformational change of the receptor. The latter renders AR unable for DNA binding and gene expression modulation (Jones et al., 2009).

3. Natural agents and membrane initiated androgen actions

During the last decades, the mode of action of steroids has been extended: in addition to their classical nuclear transcriptional action, they can also trigger early cell signaling, initiated outside the nucleus and exert also rapid effects. Such actions have been described since 1967 by Szego and Davis, reporting an increase in uterine cAMP within 15 s after iv treatment with physiological doses of 17β-estradiol (Szego and Davis, 1967). This effect was not due to a nuclear transcriptional action, since it was not abrogated by transcription inhibitors. However, progress in this field has been slow until twenty years ago, when evidence accumulated supporting this mode of steroid action. Extra-nuclear steroid actions are characterized by an effect evident in seconds or minutes, an insensitivity to modulators of transcription or translation, evidenced at low, physiological, steroid concentrations and are triggered also by specific membrane-acting, impermeable, steroid analogs (Falkenstein et al., 2000). Androgens exert also extranuclear actions, detected in several cell types and involved in the development, growth, survival, and/or function of cells in different organ systems (osteoblasts, neurons, cardiomyocytes, endothelial, vascular smooth muscle, myometrial, Sertolli cells, spermatozoa, T lymphocytes, breast and prostate cancer). They include binding to specific membrane molecules, signaling cascades activation, rapid ion movements, cytoskeletal rearrangement and modulation of secretion (Kampa and Castanas, 2006, Kampa et al., 2008,Levin, 2008). Nevertheless, the mechanism by which such action of steroids occurs is not properly understood and contrasting reports on this topic have been made (Pelekanou et al., 2013).

A number of studies indicate the involvement of the classical AR or a splice variant that translocate to the membrane, via a palmitoylation mechanism, similar to ERα (Acconcia et al., 2005,Acconcia et al., 2004, Acconcia et al., 2003), since AR equally contains the required nine amino acid palmitoylation motif (Pedram et al., 2007, Yang et al., 2011). However, there are data that support the involvement of (a) different protein(s) at the membrane level. These include the inability of classical androgen receptor antagonists, such as flutamide or cyproterone acetate, to inhibit membrane initiated androgen actions (Hatzoglou et al., 2005, Kampa et al., 2002), the existence of such actions in cells lacking classical AR (Nifli et al., 2005) and their blockade with pertussis toxin that indicates a GPCR participation (Sun et al., 2006).

Our team being actively involved in the field of extra-nuclear steroid actions and especially that of androgens in prostate and breast cancer, have reported for the first time the presence of androgen membrane binding sites in prostate and breast cancer cell lines (Kampa et al., 2002, Kampa et al., 2005), patients' isolated neoplastic cells (Stathopoulos et al., 2003) and tissue specimens (Dambaki et al., 2005). Activation of these sites initially triggers