

Androgen receptor in human endothelial cells

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Abstract

Androgen receptor (AR) is a ligand-inducible transcription factor, and a member of the steroid-thyroid-retinoid receptor superfamily, that mediates the biological effects of androgens in a wide range of physiological and pathological processes. AR expression was identified in vascular cells nearly 20 years ago, and recent research has shown that AR mediates a variety of actions of androgens in endothelial and vascular smooth muscle cells. In this mini-review, we review evidence indicating the importance of AR in human endothelial cell (HUVEC) homeostatic and pathogenic processes. Although a role for AR in the modulation of HUVEC biology is evident, the molecular mechanisms by which AR regulates HUVEC homeostasis and disease processes are not fully understood. Understanding these mechanisms could provide critical insights into the processes of pathogenesis of diseases ranging from cardiovascular disease to cancer that are major causes of human morbidity and mortality.

Key Words

- ▶ endothelium
- ▶ androgen receptor
- ▶ angiogenesis
- ▶ cancer

Journal of Endocrinology
(2015) **224**, R131–R137

Androgen receptor and vascular cells

Androgens are male sex hormones that are critical for the development and maintenance of the male reproductive system. Given the extensive role of androgens in normal physiology, abnormal androgen activity has been implicated in a wide variety of pathological conditions, including androgen-dependent prostate cancer (CaP; Matsumoto *et al.* 2013).

The incidence of cardiovascular and vascular disease is greater in men compared with age-matched premenopausal women. However, during menopause the incidence in women increases dramatically supporting a long-standing hypothesis that estrogens might provide vascular protection. However, the results of clinical trials raise an important controversy about the risks and benefits of hormone replacement therapy (Campelo *et al.* 2012).

Epidemiological and clinical data have indicated that the male hormones, androgens, are independent factors that contribute to the higher male susceptibility to atherosclerosis through adverse effects on lipids, blood pressure, and glucose metabolism (Liu *et al.* 2003, Nheu *et al.* 2011). There is evidence that androgen use has been associated with premature coronary disease in athletes and impaired vascular reactivity in female-to-male trans-sexuals (Death *et al.* 2004). On the other hand, it has been shown that a decrease in androgens, particularly testosterone, as a result of aging in men or bilateral ovariectomy in women, is associated with hypertension, diabetes, and atherosclerosis and that testosterone replacement therapy may benefit these people (Nheu *et al.* 2011). Restoration of physiological concentrations of testosterone may have a beneficial

influence on the hemostatic system through enhancement of anti-coagulant activity (Jin *et al.* 2007), and may exert anti-thrombotic effects (Jin *et al.* 2009, 2010). Furthermore, short-term administration of testosterone to men with coronary artery disease reduced myocardial ischemia and improved endothelial vasomotor function (Yu *et al.* 2010). However, despite the growing evidence of the protective effect of androgens on atherosclerosis, the picture is far from clear (Liao *et al.* 2012).

Vascular endothelial cells (EC) and vascular smooth muscle cells (VSMC) are key cellular components of blood vessels that play important roles in vascular health and disease. During the development and progression of atherosclerosis, changes occur both in the structure and function of these cells resulting in a wide range of abnormalities that affect growth, death, and physiological function. These cells contain functional androgen receptor (AR) and are targets for hormone action (Liu *et al.* 2003). Results have indicated that exposure to testosterone (mediated through AR) enhanced tumor necrosis factor α (TNF α)-induced apoptosis after serum deprivation in cultured human endothelial cells (HUVECs; Ling *et al.* 2002). In ECs dihydrotestosterone (DHT) induced VCAM-1 expression that resulted in increased monocyte binding to the endothelium. The pathway leading to VCAM-1 expression was dependent of the interaction of functional AR with the NF- κ B signaling pathway (Death *et al.* 2004, Nheu *et al.* 2011). In addition, testosterone rapidly induced nitric oxide (NO) production in human aortic endothelial cells (HAEC) that was associated with phosphorylation/activation of eNOS that was inhibited by incubation with nilutamide, or an AR siRNA (Yu *et al.* 2010, 2012). The biological action of testosterone in ECs is mediated predominantly by AR; however, some consequences may be mediated by ER after conversion of testosterone to estradiol (Mukherjee *et al.* 2002). Indeed, in ECs, estrogens alone rapidly activated eNOS and stimulated NO production in an ER-dependent manner (Yu *et al.* 2010). However, the nonaromatizable DHT and DHT analog, R1881, elicited significant eNOS phosphorylation and NO production (Goglia *et al.* 2010, Yu *et al.* 2010). Therefore, the critical effects of testosterone on eNOS phosphorylation and NO production appear to be AR-dependent (Koizumi *et al.* 2010, Yu *et al.* 2010, Campelo *et al.* 2012). DHT at physiological concentrations stimulated AR-mediated proliferation of HAEC, probably through upregulation of the expression of VEGF-A, cyclin A, and cyclin D1. The stimulation of EC proliferation in the cardiovascular system by activation of AR could contribute to the repair of EC injury/damage,

and prevent EC dysfunction, minimizing a primary risk factor for vascular stiffness, hypertension, and atherosclerosis (Cai *et al.* 2011).

AR and angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing endothelium, is subject to a complex control system regulated by endogenous pro-angiogenic and anti-angiogenic factors. Physiologically, the formation of new vessels is crucial for wound healing, organ regeneration, enabling ovulation in the female reproductive system, implantation, and placenta formation. Pathological angiogenesis is an important factor in multiple disease processes, such as tumor growth, diabetic retinopathy, macular degeneration, and psoriasis (Hoeben *et al.* 2004). However, the role of angiogenesis in the repair of cardiovascular damage and the role of androgens in the causation versus the repair of cardiovascular disease (CVD) remain controversial (Sieveking *et al.* 2010a). Furthermore, anti-diabetic drugs increase serum testosterone levels in hypo-androgenemic obese men (Kapoor *et al.* 2008), whereas these drugs reduce androgen levels in hyper-androgenemic obese women (Sahin *et al.* 2007), indicating that men and women may respond to androgens differently or that insulin resistance has different effects on androgens in men and women. These results are indicative of a significant deficiency in our understanding of the differential role of androgens in men and women: is the expression of androgens and AR (and more importantly, androgen regulation) the same in males and females (Moulana *et al.* 2011)? Sieveking and colleagues showed that DHT, through activation of AR, increased EC migration, proliferation, tubulogenesis, and the production of vascular endothelial growth factor, a pivotal molecule in angiogenesis, in cells from male donors. However, in striking contrast, DHT treatment did not induce similar changes in ECs derived from female donors. These results indicate that androgens, through AR, might regulate vascular regeneration in a sex-dependent manner; these results could explain, in part, the observed sex differences in the outcomes of CVD (Sieveking *et al.* 2010b). In contrast, Yoshida *et al.* (2013), showed that AR is essential for robust re-vascularization in response to ischemia in both male and female mice. This work documented a sex-independent physiological role of AR in angiogenic potency and provided evidence of a novel cross-talk between androgen/AR signaling and the VEGF/kinase insert domain protein receptor signaling pathways. The differences between the studies of Yoshida and

colleagues and Sieveking and colleagues may reflect the differences in model systems: Sieveking and colleagues used cultured HUVECs, while Yoshida and colleagues utilized an *in vivo* model of ischemic rodent tissues, which included skeletal muscle, vasculature, bone, lymphatic tissue, and nerves. Results of a recent study (Annibalini *et al.* 2014) have indicated that under identical culture conditions, endothelial cells (HUVECs), regardless of sex, predominantly use pathways responsive to the action of androgens rather than those responsive to the action of estrogens. Also these results indicated that male and female HUVECs expressed high levels of AR and 5 α -reductase-1, but very low levels of estrogen receptors and aromatase.

AR and endothelial progenitor cells

In the last decade, our knowledge of vascular homeostasis and repair has evolved significantly with the discovery of circulating endothelial progenitor cells (EPCs) in adult human blood. EPCs that originally reside in the bone marrow and other putative niches are mobilized to the peripheral circulation in response to many stimuli, and once in the bloodstream take an active part in EC replacement and formation of new blood vessels (Fadini *et al.* 2009). Because of their role in maintenance of functional endothelium, EPCs are currently considered to be an integrated component of the cardiovascular system. Subjects with risk factors for or with established CVD have a depletion of circulating EPCs. Interestingly, patients with lower levels of EPCs in the bloodstream have a higher risk of cardiovascular events. These results indicate that depletion of EPCs is a pathogenic event that contributes to an inability to maintain an intact endothelium and to promote angiogenesis, risk factors that can translate into the development and progression of CVD (Di Mambro *et al.* 2010).

Human EPCs express AR, and androgens influence EPC mobilization from bone marrow (Foresta *et al.* 2006, 2008). Furthermore, a direct effect of testosterone on EPC function was indicated by the evidence that hypo-gonadal men had lower numbers of circulating EPCs compared with normal men, and that the numbers of EPCs increased significantly after testosterone replacement therapy (Foresta *et al.* 2006). Moreover, subjects with Klinefelter syndrome (KS), a condition characterized by hypogonadism and associated with a significant morbidity related to vascular diseases, have a marked reduction in the number of EPCs in circulation (Di Mambro *et al.* 2010). These *in vivo* observations were supported by the results of *in vitro* studies that demonstrated that testosterone,

acting through AR-mediated pathways, increased EPC proliferation, colony formation, and migration (Foresta *et al.* 2008). Recently, it has been suggested that DHT modulated EPC proliferation and adhesion, and the PI3-K/AKT pathway played an important role in this process. The positive effects of androgen (DHT) on EPCs may explain the finding that low levels of circulating androgens are associated with increased male CVD morbidity and mortality (Liu *et al.* 2014).

AR and cancer-associated vasculature

Although the importance of tumor angiogenesis was initially met with skepticism, it is now accepted as a hallmark of cancer progression and has been explored as a therapeutic target in almost every type of neoplastic disease, including CaP (Galsky & Oh 2013). CaP is a common malignancy in humans, representing the second leading cause of cancer-related deaths in American men (Siegel *et al.* 2012). Increasing evidence has indicated that the tumor microenvironment has a role equally important as the cancer cells in the progression of a tumor (Dayyani *et al.* 2011). In the tumor microenvironment, one of the key components thought to have a critical role in tumor progression is the vasculature (Godoy *et al.* 2013).

Our group determined that human prostate endothelial cells (HPEC) isolated from fresh human clinical specimens of benign prostate and CaP expressed functional AR *in vivo* and *in vitro*, and that androgen modulated *in vitro* EC proliferation and gene expression in a cell-type-specific manner. Also dihydrotestosterone (DHT), through AR, directly increased proliferation of primary cultures of HPECs in a dose-dependent manner without affecting the formation of endothelial tube structures in the matrigel, indicating that the differentiation and migration processes involved in endothelial tube formation are independent of proliferation in prostate ECs. These studies provide evidence of a potential role for AR in regulation of human prostate vascular EC homeostasis (Godoy *et al.* 2008). Our group also demonstrated that androgen withdrawal induced acute involution of the human prostate vasculature in primary xenografts of human benign prostate or CaP tissues that had been transplanted into humanized SCID mice that had received implants of testosterone pellets to maintain a human level of circulating testosterone (Godoy *et al.* 2011). In this preclinical model, vascular involution was correlated temporally with the induction of apoptosis in the human prostate ECs, indicating that testicular androgen signaling had an important role in the maintenance of prostate EC homeostasis in intact men. This observation also indicated

that androgen ablation negatively affected EC viability in human prostate tissue independently of epithelial cell apoptotic death (Godoy *et al.* 2011). Supporting this hypothesis, withdrawal of androgenic signaling using AR antagonists (e.g., flutamide and bicalutamide) or inhibitors of steroid metabolism (e.g., finasteride and dutasteride) reduced hematuria during prostate surgery or in patients with benign prostatic hyperplasia (BPH) and in combination therapy with bicalutamide-goserelin (a gonadotropin-releasing hormone agonist) and dutasteride (an inhibitor of 5 α -reductase isoenzymes types 1 and 2) induced profound vascular collapse and reduced prostatic tissue vascularity in human CaP patients (Godoy *et al.* 2013). Results from other studies also indicated that prostate tumor cells regulated EC growth through a paracrine mechanism, which was mainly mediated by VEGF, and demonstrated that DHT was able to modulate EC growth via tumor cells (Wen *et al.* 2013) and that the induction of VEGF was mediated by binding of the transcription factor AR and SP1 to the core promoter region of VEGF (Eisermann *et al.* 2013).

Androgen deprivation therapy (ADT), the standard treatment for advanced CaP, is rarely curative and, in

virtually all cases, the initial response to ADT is followed by relapse of the disease as castration-resistant CaP, the lethal phenotype of the disease. The evidence of expression of functional AR in human prostate ECs in CaP tissue and of the acute effect of ADT on human prostate vascular integrity indicates that human prostate vasculature has a unique potential as a first-line target for ADT (Godoy *et al.* 2008, 2013). ADT-induced transient destabilization of the human prostate EC compartment may present a 'therapeutic window' for the delivery of chemotherapeutic agents. Therefore, the study of the regulatory role of androgens in the prostate microvasculature may provide the molecular basis for the development of new therapeutic modalities. This paradigm-shifting approach would change the monolithic paradigm of ADT as a first-line therapy, which is focused on induction of apoptotic death in CaP cells, to a dynamic paradigm where ADT is employed in a neo-adjuvant setting to improve therapeutic efficacy of conventional and new treatment modalities (Godoy *et al.* 2013). This new approach would capitalize on the 'therapeutic window' opened by the acute apoptotic death of androgen-responsive prostate ECs to allow access to the

Table 1 Summary of research studying the effects of androgens on endothelial cells

Model	Type of androgen	AR mediated ^a	Effect	Author
Human aortic endothelial cell (HAEC)	Testosterone	Not demonstrated	Increase in the production of nitric oxide	Goglia <i>et al.</i> (2010) and Yu <i>et al.</i> (2010, 2012)
	DHT	Yes	Increase in proliferation	Cai <i>et al.</i> (2011)
Human umbilical vein endothelial cell (HUVEC)	DHT	Yes	Increase in monocyte binding	Death <i>et al.</i> (2004) and Nheu <i>et al.</i> (2011)
	DHT	Yes	Increase in proliferation and tubulogenesis	Sieveking <i>et al.</i> (2010b)
	DHT	Not demonstrated	Induction of a pro-inflammatory state	Annibalini <i>et al.</i> (2014)
Human prostate endothelial cell (HPEC)	Testosterone	Yes	Increase in proliferation	Godoy <i>et al.</i> (2008)
	DHT	Yes	Increase in proliferation	Godoy <i>et al.</i> (2008)
Human endothelial progenitor cell (EPC)	Testosterone	Not demonstrated	Increase in proliferation, colony formation, and migration	Foresta <i>et al.</i> (2006, 2008)
	DHT	Not demonstrated	Increase in proliferation and adhesion	Liu <i>et al.</i> (2014)
Prostate vasculature (primary xenograft model)	Withdrawal	Not directly demonstrated	Increase in apoptosis ^b	Godoy <i>et al.</i> (2011)
Vascular endothelial cells (mouse hind limb ischemia model)	AR knockout	Not directly demonstrated	Reduced angiogenic capability	Yoshida <i>et al.</i> (2013)
Murine endothelial cell line (MEC)	Conditioned media from Prostate cancer cell line (DHT)	Not demonstrated	Increase in proliferation	Wen <i>et al.</i> (2013)

^aEffect mediated by the androgen receptor (AR).

^bEffect observed between 1 and 4 days after androgen withdrawal.

interstitial tissue space to chemotherapeutic agents that are usually blocked by the intact endothelial barrier (Godoy *et al.* 2013). Furthermore, the potential generality of this therapeutic approach is indicated by the observation that ECs of all human hormonally responsive tissues, including breast, endometrium and ovary, as well as prostate, express AR.

Summary and conclusions

The role of androgen signaling and AR-mediated transregulation of the expression of genes associated with normal EC processes associated with viability, proliferation, and angiogenesis/repair, as well as with pathogenic processes, such as atherosclerosis and neoplasia, is poorly understood. To further cloud the issue, the literature is rife with reports espousing diametrically opposed conclusions (Table 1). AR is expressed in ECs in a largely sex- and species-independent manner in bone and bone marrow, skin, pancreas, brain, skeletal muscle, cornea, and endothelial progenitor cells (Liang *et al.* 1993, Abu *et al.* 1997, Mantalaris *et al.* 2001, Suzuki *et al.* 2001, Sinha-Hikim *et al.* 2004, Liu *et al.* 2005, Ohtsuki *et al.* 2005, Foresta *et al.* 2008, Morales *et al.* 2008, Fadini *et al.* 2009). Furthermore, AR is expressed in ECs in all steroid-responsive tissues in humans. However, a species difference was observed for prostate, in which ECs of both benign and malignant human prostate were demonstrated to express functional AR whereas ECs of rodent prostate and CaP were reported to lack expression of AR (Prins *et al.* 1991, Ralph *et al.* 2003, Europe & Tyni-Lenne 2004). Regarding sex differences, Sieveking and colleagues and Death and colleagues reported that, in both *in vitro* and *in vivo* models, androgens stimulated the proliferation and angiogenesis/vascular repair by ECs of male origin, or in male organisms, but not by ECs of female origin, even if the female ECs were supplemented with exogenous androgen or AR (Death *et al.* 2004, Sieveking *et al.* 2010b). However, Yoshida *et al.* (2013) have recently reported a novel sex-independent protective mechanism against ischemic injury mediated by AR.

The mechanisms by which AR mediates its biological effects in ECs are equally unclear. Demonstration that the nonaromatizable androgens, DHT and R1881, induced biological endpoints similar to those obtained with testosterone, and that the biological consequences were abrogated by flutamide, bicalutamide (casodex), nilutamide, or AR siRNA, clearly implicated AR in the modulation of EC function, proliferation, and gene expression via conventional nuclear-receptor-mediated transcriptional transactivation (Goglia *et al.* 2010, Yu *et al.* 2010, Cai *et al.* 2011, Nheu *et al.* 2011).

However, AR localized to the cell membrane in caveolae has been implicated in nongenomic regulation of EC function/gene expression via activation of the c-Src/PI3-K/AKT cascade that ultimately results in the activation of eNOS and NO production (Somjen *et al.* 2004, Goglia *et al.* 2010, Yu *et al.* 2010, 2012). The role of AR in the modulation of all responses of ECs to circulating androgens is complicated further by reports that the adrenal androgen DHEA(S), in addition to its role as the precursor of T/DHT and estrone/estradiol synthesis, also binds to a cognate receptor on the EC membrane and induces NO synthesis due to enhanced expression and stabilization of eNOS, and that this induction is not blocked by antagonists of ER, AR, PR, or GR (Simoncini *et al.* 2003, Williams *et al.* 2004, Zapata *et al.* 2005, Liu *et al.* 2008).

In summary, while it is clear that circulating androgens and their metabolites have significant effects on the normal biology and pathological processes that affect ECs, the processes through which AR-mediated mechanisms regulate EC homeostasis and angiogenic responses to injury or disease require significant clarification. Identification of these processes will be the key for the development of better models for investigating the biology of hormonally responsive tumors, tumor angiogenesis, and atherosclerosis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

A S G is supported by grants from the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) 1130051 and the Department of Defense (DOD) W81XWH-12-1-0341. G J S is supported by the National Cancer Institute (NCI) RO1-77739.

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Received in final form 24 December 2014

Accepted 6 January 2015

Accepted Preprint published online 6 January 2015