Could Testosterone Have a Therapeutic Role in Prostate Cancer?

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Purpose: To discuss the role of membrane androgen receptors and to investigate the potential role of testosterone-albumin conjugate in the prostate cancer (PCa) treatment.

Materials and Methods: We identified studies published from 1990 onwards by searching the MEDLINE database of the National Library of Medicine. Initial search terms were "androgen receptors, cytoplasmic androgen receptor, and membrane androgen receptor" combined with "testosterone, testosterone-albumin conjugate, and prostate cancer treatment".

Results: The androgen receptor plays a critical role in both development and progression of PCa. The latter is associated with changes in the androgen receptor axis and more precisely, with its conversion from a paracrine dependent signaling pathway for proliferation and survival of prostatic cells to an independent autocrine process. This malignant conversion is due to functional changes in which the androgen receptor activates not only normal genomic, but also novel non-genomic signaling pathways, which are not present in normal prostatic epithelial cells. Thus, treatments for neoadjuvant, adjuvant, and recurrent disease, all center on the regulation and manipulation of the androgen pathway. Recent discoveries however offer strong evidence of a direct apoptotic action induced by activation of the membrane androgen receptor by testosterone-albumin conjugates.

Conclusion: Investigation of the molecular pathways of apoptosis through activation of the membrane androgen receptors in the androgen-independent PCa cell is important on the one hand because future manipulation of this mechanism can help with understanding and interpreting unknown to date characteristics of PCa and on the other hand, can contribute to the establishment of activators of membrane androgen receptors. In addition, study of the testosterone-albumin complex can constitute the basis for future treatments for PCa.

Keywords: androgen receptors, testosterone, prostatic neoplasms, therapeutics

INTRODUCTION

Steroid Hormone Receptors

The relationship between testosterone, prostate cancer (PCa) development, and its progression is controversial and the evidence is conflicting. In fact, while treatment for metastatic PCa is greatly affected by testosterone, serum testosterone levels of men treated with androgen deprivation therapy (ADT) are not associated with duration of ADT, ADT formulation, or disease status. Furthermore, there is evidence that even hormone-refractory PCa cells continue to be affected by androgen signaling despite ADT.[1] This fact indicates that androgen receptors are the key element in PCa development rather than androgens themselves.

According to current literature, steroid molecules, such as testosterone, enter the target cell, eg, of the prostate gland, by passive transport through the cell membrane by diffusion. After binding in the cytoplasm with intracellular androgen receptors (iAR), they undergo structural modifications resulting in the formation of a DNA binding area. This phenomenon results from the conversion of testosterone into its active form, dihydrotestosterone. The resulting complex (dihydrotestosterone and receptor) migrates to the nucleus and triggers off the transcription of genes into mRNA to produce protein molecules, which have strictly defined biological functions.[2] The time from initiation until the completion of the process, which results from the protein expression of this gene through the mechanism described, is at least 30 to 45 minutes while the time required for the production of significant amounts of protein is between 3 and 4 hours.[3,4]

However, the presence of steroid hormone receptors has also been demonstrated on the cell wall of the prostate cell, a fact which explains the high binding capacity of the testosterone-albumin complex.[5] It is also worthwhile noting that prostate cells have receptors for sex hormone-binding globulin (SHBG), a fact which allows the binding of the testosterone-SHBG complex with the cytoplasm.

It is believed that when the bound testosterone enters the target cell, passing through the cell membrane with the aid of transporting proteins, it acts on local cytoplasmic channels leading to the activation of cyclic AMP. This activity takes place in a matter of only a few minutes. It is a point of contention whether this rapid corticoid activity is really related to the cell membrane steroid hormone receptors or whether it represents an additional function of the classic cytoplasmic receptors. Indeed, the manifestation of rapid non-genomic phenomena resulting from steroid hormone signaling may be mediated by the classic receptors as is the case with oestradiol and progesterone. However, numerous functions of steroids, which are inconsistent with the existence of a sole classic receptor, point towards the existence of non-classic receptors for steroid hormones possibly located in the cell membrane, which are responsible for a non-genomic function. In any case, there are many cellular activities which occur without any prerequisite action on the genome, such as transcription and translation, which take place in a short span of time and are not influenced by inhibitors.

Experimental systems inferring the presence of steroid receptor molecules, which are not identical with the classic cytoplasmic receptors of steroid hormones, were the first indications of their existence.[6] Such steroid hormone receptors, which are additionally related to the production of rapid non-genomic phenomena, have already been described in various tissues in the past 25 years. It is noteworthy that such receptors were also identified in cells lacking in classic receptors (monocytes, T-lymphocytes).[7-9] Merely as of a decade, non-classic steroid receptors have been identified in the cell membrane of cells carrying classic androgen receptors, such as osteoblasts and cells of the prostatic glandular epithelium.[5,10] The exact mechanism that steroid molecules have through these receptors has not been fully elucidated. The established mechanisms up to date are kinase regulation, cyclic nucleotide modulation, and intracellular calcium changes.[11-17]

The aforementioned activities are very rapid: progesterone results in a significant rise in intracellular sperm cell calcium, which takes place within a matter of a few seconds,[18] while aldosterone induces the exchange of sodium and hydrogen ions in the small bowel cells in a time frame of less than a minute.[19,20] Testosterone increases intracellular calcium in the osteoblasts of male rats within only five seconds[21] and leads to the synthesis of the molecular messengers, such as inositol trisphosphate (IP3) and diacylglycerol (DAG), with-

in only ten seconds.[22] This activity is dose-related, it is not affected by external factors, and it takes place rapidly.

The influence of testosterone in normal concentrations (1 to 10 nM) on activated T-lymphocytes and in high concentrations (0.3 to 3 M) on isolated Sertoli cells has been shown to increase intracellular calcium by influx in a similarly short span of time.[8,23] The influence of 50 to 100 nM of testosterone on muscle cells of male rats and of androstenedione on human porcine ovarian cells resulted in a similar rise in intracellular calcium and trebling of the levels of IP3.[24,25] The rise in intracellular calcium seen in the above-mentioned studies was dose-related with maximum activity observed with 1 nM androstenedione in porcine cells and 10 nM in human cells. Rapid androgen action has also been described in the cardiac tissue with varying biological results,[26,27] as well as in the neuronal cells[7] and hepatocytes.[28]

The fact that the above-mentioned functions have been demonstrated in cells lacking a functional nucleus, such as erythrocytes, platelets, or sperm cells, as well as the fact that they are not inhibited by nuclear androgen receptor inhibitors (hydroxyflutamide and cyproterone) supports the view that these functions are affected by non-classic steroid receptors.[20] This view is further supported by similar findings in experiments with rat macrophages, which show a rapid response to androgen stimulation despite their lack of classic androgen receptors (IC-21).[29]

MATERIALS AND METHODS

We identified studies published from 1990 onwards by searching the MEDLINE database of the National Library of Medicine. Initial search terms were "androgen receptors, cytoplasmic androgen receptor, and membrane androgen receptor" combined with "testosterone, testosterone-albumin conjugate, and prostate cancer treatment". References in the selected publications were checked for relevant publications not included in the MEDLINE or PubMed search.

RESULTS

Androgen Receptors and Prostate Cancer

It is widely known that testosterone is possibly the most important factor affecting the proliferation and function of the normal prostate gland. Prostatic glandules and prostatic pores are covered by epithelium consisting of two cell layers. Basal cells do not carry classic androgen receptors, but are differentiated through a transitional form, which also does not carry classic androgen receptors, into secreting and neuroendocrine cells, which have androgen receptors. The epithelium is supported by a fibromuscular stroma, the cells of which (smooth muscle cells and fibroblasts) also carry androgen receptors.

Androgens play an important role in the development and maintenance of both normal and neoplastic prostate tissue. The dependence of PCa on androgens is known since the middle of the previous century since the discovery of androgen deprivation therapy for PCa by Huggins and Hodges. Androgen blockade in its complete form (elimination of testicular testosterone and inhibition of the classic androgen receptor) remains the golden standard management of metastatic PCa and is also used as adjuvant and neo-adjuvant therapy in advanced cancer.[30] Unfortunately, despite an initial positive response to androgen deprivation therapy, many cancers progress to androgen-independent or androgen-resistant forms and relapse ultimately. Because of this, androgen deprivation therapy is a helpful, but not definitive treatment irrespective of how complete the deprivation is.

The androgen receptors themselves are responsible for the progress of the cancer. Contrary to older views that they are gradually depleted following gradual depletion of available androgens, it is today believed that androgen receptors undergo mutations or genetic amplification. Although in the androgen-resistant state the levels of detectable prostate androgen receptors are reduced, there are strong evidences for the presence of androgen receptors in androgen-independent cancers, those under androgen deprivation, and cancers relapsing during androgen deprivation therapy.[31]

The fact that the androgen receptor protein is expressed in patients with androgen-independent PCa suggests the preservation or re-emergence of the androgen receptor.[31-34] Furthermore, the observation of an intermediate state of hormone independence between hormone sensitivity and hormone resistance (androgen deprivation syndrome) lends merit to the above view as it could be interpreted as a phenotypic manifestation of a mutation phase of the androgen receptor.[35] According to experimental data, cells positive for androgen

receptors produce a growth factor, which activates in a paracrine way the production of androgen receptor-negative cells.[36] Although, as already mentioned, in the hormone-resistant state the levels of detectable prostate androgen receptors are reduced and the androgen-resistant cells multiply irrespective of the presence of androgens, they still require the androgen receptors for their survival.[37] Indeed, if by intracellular injection, the expression of androgen receptors of cell lines coming from androgen-resistant cancers (LNCaP, LAPC-4, LAPC-9, MDA-PC-2B, V-Cap, DuCap, and LNCaP) is reduced beyond a certain point, these cells stop multiplying and die.[38-40] This property characterizes the non-classical androgen receptors expressed preferentially in human PCa cells.[41]

The Role of Androgens and Non-Classic Androgen Receptors

As opposed to the classic cytoplasmic androgen receptor, membrane androgen receptors have not been studied adequately. It seems however that they affect a variety of non-genomic functions, including kinase regulation, cyclic nucleotide modulation, and intracellular calcium changes.[11-17] The latter seems to be related to the apoptotic process and programmed cell death. Studies on both cultured human PCa cells (LNCaP) as well as on direct processed specimen of human PCa provided strong evidence of an immediate antineoplasmic activity after the activation of membrane androgen receptors by the testosterone-albumin complex. Specifically, what has been observed is inhibition of development of LNCaP cancer cells, induction of apoptosis, release of the pro-apoptotic protein FaS in LNCaP cells, and a significant reduction in migration, adhesion, and development of the androgen-resistant human PCa cells DU145.[41-44]

The fact that the above-mentioned antineoplastic functions remain even in the presence of the antiandrogen flutamide administration both in iAR-negative human cancer cells (DU145) as well as in processed (with iAR antisense oligonucleotide) positive for cytoplasmic androgen receptors cultured human cancer cells (LNCaP), shows that this function is expressed independently of cytoplasmic androgen receptors possibly at the level of membrane androgen receptors. It is merit noting that treatment with the testosterone-bovine serum albumin (BSA) complex at a dose 4.8 mg/kg body weight in rats immunized with LNCaP cells for a month led to a 60% reduction in the size of the tumor, while the result was not affected by the administration of antiandrogen as already mentioned.[41-44]

The function of the testosterone-albumin complex in the prostatic cell has not been fully clarified and it is not known if it is testosterone, albumin, or both that exhibits this proven anticancer activity. In fact, albumin inhibits the production of actin and therefore affects the formation of the cytoskeleton contributing thus to the apoptotic remission of human PCa cells.[44,45] On the other hand, testosterone and other androgens display also anticancer properties. For example, 3B-Adiol, a metabolite of dihydrotestosterone (5A-androstane-3B,17B-diol), is known to exert inhibitory activity on the migration of PCa cells by activation of estrogen receptor B (ERB) and the resultant signaling.[46,47] Another biochemical pathway possibly involved in the apoptotic process is activation of the receptor of tumor necrosis factor (TNF) RSF6 (FAS) by androgens, given that human PCa cells as well as cancer cell lines LNCaP and DU145 express the FasL gene and produce its end product.[48,49]

Contemporary studies demonstrated that androgens also exert anticancer properties in organs other than the prostate. Activation of membrane androgen receptors by the testosterone-albumin complex in the colon cancer cells results in both in-vitro and in-vivo signaling of Akt/bad and vinculin/actin pathways exerting thus inhibitory activity on the migration of cancer cells.[50]

Apoptotic Regression of Prostate Cancer Through Activation of Membrane Androgen Receptors

Among the possible mechanisms by which the activation of membrane androgen receptors by the testosterone-albumin complex affects the apoptotic process is its influence, through mobilization of intracellular calcium, on cytoskeletal actin. The latter is of vital importance for cell survival as it is a structural element of the cellular substrate and thus influences numerous cell activities, such as mobility, division (multiplication), and secretion. The mechanism by which the non-genomic influence of androgens is exerted on the cytoskeleton through membrane androgen receptors has not

been fully elucidated.

At an experimental level, it has been observed that subjecting PCa cells (LNCaP) to the testosterone-bovine albumin (testosterone-BSA) complex results in phosphorylation of a protein tyrosine kinase known as FAK (focal adhesion kinase), the ligation of which to the signaling molecule phosphatidylinositol-3 (PI-3) kinase leads to its activation as well as the activation of Cdc42/Rac1 GTPases. The above chain of events influences cytoskeletal actin and is followed by changes in its assembly.[51]

It is already known that various stimuli, including the integrins, growth factors, cytokines, neuropeptides, and steroid hormones, such as the androgens, modify the action of FAK, which in turn activates protein signals responsible for cytoskeletal reassembly.[17,52] However, a key factor in the control of cellular development, cell survival, malignant transformation, and invasiveness appears to be PI-3 kinase, which seems to be activated through different pathways by FAK (downstream) and the Cdc42/Rac1 (upstream), leading to changes in the assembly of the cytoskeleton with different outcomes depending on the direction.[53,54] Activation of PI-3 under the influence of the testosterone-albumin complex on membrane androgen receptors of human cancer LNCaP cells significantly increased the phosphorylation of FAK, the ligation of which to the p85 subunit of the PI-3 kinase resulted, by way of a rapid non-genomic action, in the reassembly of cytoskeletal actin and the formation of cell membrane evaginations (filopodia, lamellipodia).[42] Such cytoskeletal reassembly has been linked to malignant transformation, cellular migration, and cancer invasiveness.[55,56]

Confirming the above observations, FAK was found significantly activated in cancer tissue of patients with metastatic PCa.[57] It is worth noting that the attachment of phosphorylated FAK to the p85 subunit in this study was found to be significantly greater when the membrane androgen receptor was activated by the testosterone-albumin complex than when activated by dihydrotestosterone. In the same study, as already was mentioned, the effect of the testosterone-albumin complex results in a quantitative increase in Cdc42/Rac1 GTPases possibly through the upstream activation of PI-3 kinase.

Although small GTPases are considered factors that significantly affect the invasive potential of cancer cells (on the one hand, activation of Cdc42 results in the formation of filopodia, and on the other hand, activation of Rac1 results in the formation of wavelike cytoplasmic evaginations and lamellipodia), in fact their role in cellular response differs depending on cell type. In epithelial cells, such as in the prostate, activation of Cdc42 and Rac1 leads to retraction of cancer cells and inhibits migration and invasion.[58] It is therefore possible that the development or inhibition of development of the cancer cell depends on the direction of activation of PI-3. The parallel presence of elements of both the downstream and the upstream activation in the experimental model (human cancer LNCaP cells) and the different outcome depending on the case seem to support the above hypothesis.[44]

The phenomenon is dose-dependent and time-dependent. The antineoplastic activity of the complex persists for more than 48 hours even after the removal of the testosterone-albumin complex and membrane androgen receptors from the culture medium.[44] According to recent observations, chronic stimulation of membrane androgen receptors indeed leads to inhibition of cell development and to apoptosis through the activation of the actin disassembly mechanism whereas chronic androgen deprivation seems to increase the resistance of cancer cells to the induction of apoptosis through the same mechanism.[51] Despite the undoubtable observation that membrane receptors are the mediators of the above activity, many other mechanisms associating testosterone with membrane androgen receptors remain unclear.

CONCLUSION

In conclusion, investigation of the molecular pathways of apoptosis through activation of the membrane androgen receptors in the androgen-independent PCa cell is important on the one hand because future manipulation of this mechanism can help with understanding and interpreting unknown to date characteristics of PCa, and on the other hand, can contribute to the establishment of activators of membrane androgen receptors. The latter could constitute a novel aspect in the classic viewing of hormonal therapy if we only take into consideration that testosterone binding areas of the membrane constitute a constant feature of both positive for androgen receptors cancer cells as well as negative, regardless of the

expression of intracellular receptors. In addition, study of the testosterone-albumin complex can constitute the basis for future treatments for PCa. In such a case, the testosterone-BSA complex could either constitute a separate category of antineoplastic factors for PCa or could be combined with antiandrogens already in use.

CONFLICT OF INTEREST

None declared.

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