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Review Article

Consequence of evolutionary loss of seasonal breeding by humans for prostate cancer chemoprevention

John T Isaacs

Department of Oncology, Sidney Kimmel Comprehensive Cancer Center (SKCCC), Johns Hopkins University, Baltimore, Maryland, USA

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Abstract: During mammalian evolution, circulating levels of gonadotropins [i.e., luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] acquired regulation by environmental (e.g., light, temperature, water, food, predators, etc.), and social (e.g., sound, sight, aggression, crowding, etc.) inputs that determine the level of testosterone production and secretion by the testis and systemic levels in the blood. This regulation became coordinated by interaction between the retinohypothalamic-pineal and the hypothalamic-pituitary neural axes, which resulted in androgen levels and its ligand-dependent transducing receptor being the master downstream determinant of male reproduction. A major factor in this selection of androgen levels relates to the unique danger of mammalian reproduction for survival of the individual. During mammalian evolution, breeding needed for survival of the species became episodically (i.e., seasonally) timed by androgen levels. Seasonal breeding has great reproductive advantage in restricting energy requirements for reproduction and limiting dangers associated with procreation (i.e., survival of the species) at the expense of suppression of the flight instinct (i.e., survival of the individual) to the minimal time frame of the breeding season. Human males evolved away from strict seasonal breeding by chronically maintaining androgen levels, enabling human males to reproduce year-round and worldwide, rather than "locking" them into specific indigenous breeding ranges, like other mammals. The price for the reproductive "freedom" that arises from the loss of seasonal breeding is an increased probability of developing prostate cancer as a result of chronically maintaining a hyperplastic state in the prostate. In human males, this results in the loss of episodic pruning of genetically-mutated prostate cancer precursors that normally occurs during seasonal breeding. Instead, the continuous androgen-dependent stimulation of the growth of such precursors occurs during prostate carcinogenesis. This review provides the rationale for the development of a therapeutic approach using PSA-activated prodrugs to selectively deplete prostate-specific AR protein for chemoprevention of prostate cancer.

Keywords: Androgen receptor, AR, seasonal breeding, evolution, prostatic carcinogenesis, prodrug

Evolution of the steroid receptor family

Our planet is 4.5 billion years old with life on Earth starting 3.5 billion years ago, **Figure 1**. Development of an oxygen rich atmosphere occurred 2.5 billion years ago with eukaryotic cells first evolving 800 million years later. After another billion years, the animal kingdom started. Over the next 500 million years, evolution continues eventually producing mammals characterized by the development of complex endocrine systems allowing the organism to coordinate the survival of the species vs. the individual via steroid receptor (SR) dependent transcription. SRs are ligand-activated transcription factors that belong to the diverse nuclear receptor (NR) superfamily of proteins. Bacteria, yeast, and plants do not contain NRs, which are restricted to animals. All members of the SRs descend from a single ancestral receptor (AncSR), which branched off from the rest of the NR superfamily early [700 million years ago (Mya)] in animal evolution [1]. Subsequent duplications of the AncSR gene 430 Mya produced three major SR subgroups: estrogen receptor (ER), corticosteroid receptor (CR), and the 3-ketogonadal steroid receptor (3-KGR). Over the next several hundred million years within the gnathostome lineage of bilaterally symmetric animals (i.e., jawed vertebrates), additional duplications within each of the 3 SR subgroups produced six steroid receptors: the ER to create estrogen receptors, ERα and Erβ; the CR to create a separate receptor each for

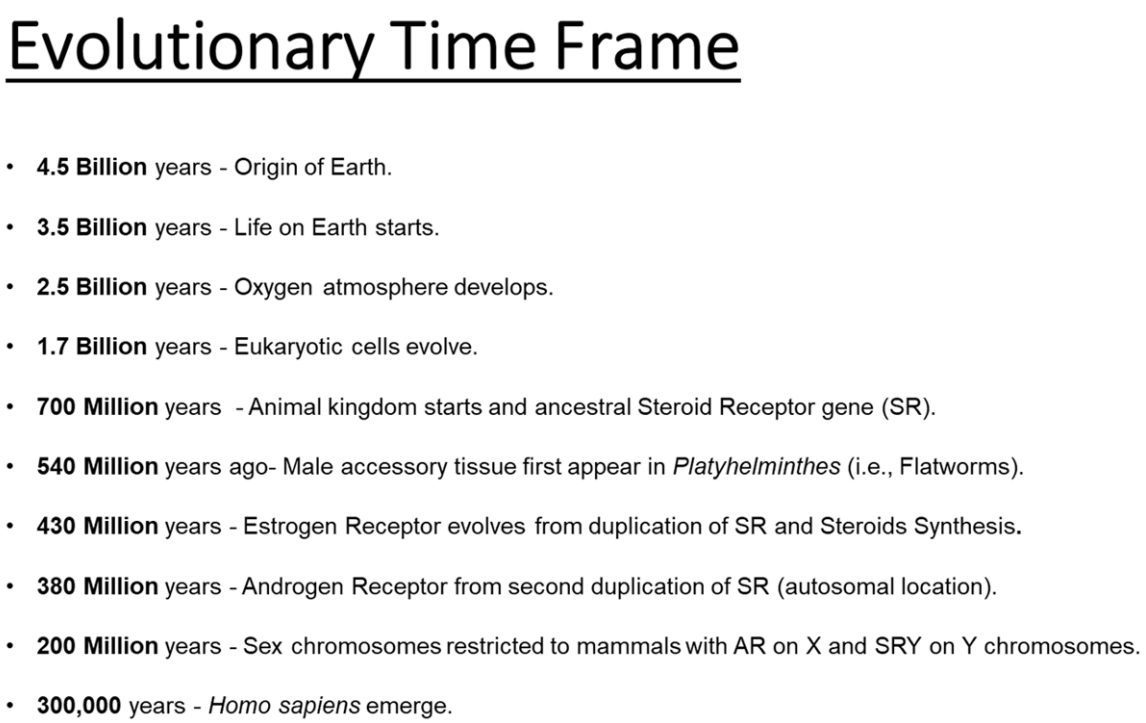

Figure 1. Summary of key events during mammalian evolution.

mineralocorticoids (MR) and glucocorticoids (GR); and the 3-KGR to create a separate receptor each for progestagens (PR) and androgens (AR). Significantly, the AR is the most recent evolutionary receptor in this family [1].

The elaboration of the SR family by gene duplication and ligand exploitation allowed increasingly specific hormonal control over physiological functions, particularly important for regulating the balance between survival of the individual vs. the species in mammals. ER regulation of reproductive maturation and function is the most ancient (i.e., 200 Mya) of all modes of steroid/receptor control. PR regulation over ovulation, oviposition, and other aspects of reproduction is also quite ancient, but does not precede estrogen signaling [2]. AR control over sexual dimorphism (i.e., male vs. female phenotype) and spermatogenesis in mammals is a more recent (>150 Mya) evolutionary novelty.

Evolution of the AR gene location on the X-chromosome in mammals

The mammalian X and Y sex chromosomes originated within the last 300 million years from an ancestral pair of autosomes in a reptile ancestor, which diverged over time as the Y chromosome progressively degraded [3-5]. Birds and reptiles do not share the mammal X and Y chromosomes. In mammals, 5 strata of decreasing evolutionary age are observed linearly along the X chromosome. The oldest (i.e., 200-150 Mya) being the centromere plus the majority of the q-arm which contains the AR at Xq12. The youngest (i.e., 60-50 Mya) being a portion of the proximal p-arm, suggesting that suppression of recombination occurred in a stepwise manner during evolution between the X and Y chromosomes [3-7]. During the evolution of mammals, before the split of protheria (i.e., monotrenes) from the metatheria (i.e., marsupials) and eutheria (i.e. placentals) between 200-150 Mya, only the AR among the 6 SRs became fixed on the long arm of the X chromosome (i.e., Xq12) in contrast to its location on autosomal chromosome 4 in chickens [8]. Thus, in mammalian males (i.e., XY), AR is a hemizygous (i.e., single copy) gene and is the non-redundant integrator of androgen-activated transcriptional signaling.

Evolutionary selection of androgen and the AR as the master regulator of mammalian male reproduction

During mammalian evolution, testicular production and secretion of testosterone locally

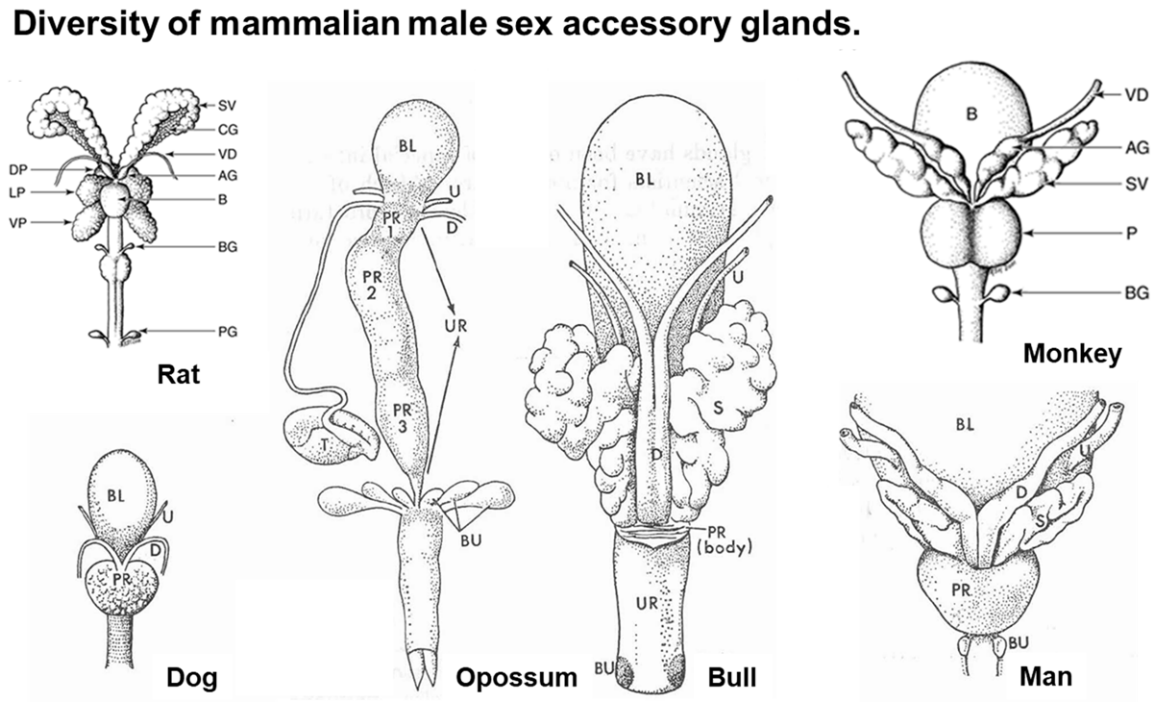

Figure 2. Comparative anatomy of male accessory sex tissue in a series of mammals (rat, opossum, bull, dog, monkey and man). BL or B = bladder; U = urethra; PR = prostate; S = Seminal vesicle; BU = Bulbourethral gland; D or AG = ampulla of ductus (vas) differens; T = testis; CG = Coagulating gland (aka anterior prostate lobe); VP = ventral prostate lobe; LP = later prostate lobe; DL = dorsal prostate lobe; and VD = vas deferens.

into the testicular microenvironment and into the circulation became highly regulated by environmental (e.g., light, temperature, water, food, predators, etc.), as well as social (e.g., sound, sight, aggression, crowding, etc.) inputs, coordinated via the interaction of the retinohypothalamic pineal and the hypothalamic-pituitary axes [9, 10]. This resulted in androgen and its ligand-dependent receptor (i.e., the AR) becoming the master downstream transducer of these neural outputs on embryonic development, postnatal growth, and maintenance of the male accessory sex tissues, as well as spermatogenesis, bone and muscle maturation, and libido. Accessory sex glands in males of various species are named either for their anatomical position in adult animals or for their assumed functions. Male accessory sex tissues first appear 540-480 Mya in the *Platyhelminthes* [i.e., flatworms] genera, *Planaria* and *Dendrocoelum* [11]. They are the most diverse organ system in the animal kingdom, varying widely even among mammals **Figure 2**. This diversity may have a selective advantage for maintenance of speciation during evolution, since this is in significance contrast to the remarkable similarities for most other organ systems (e.g., liver, lung, kidney, etc.) among mammals.

The acquisition of the dominant role of androgens and the elimination of estrogens as regulatory factors in male phenotypic development is a rather late (<150 Mya) event in mammalian evolution. It evolved in eutherian (i.e., placental) mammals due to the close connection between maternal and fetal circulation, which allows steroid hormones to pass the placental barrier. Estrogen and progesterone are required for the development and maintenance of the placenta, regardless of the sex of the developing mammalian fetus. Thus, the dichotomy of regulating sexual differentiation by male and female sex steroids, which occurs in earlier vertebrates, was lost. Instead, phenotypic male development (i.e., male accessory sex glands coupled with spermatogenesis) acquired an absolute dependence upon a critical level of testicular androgen production coupled with anti-Mullerian hormone production to induce regression of the Mullerian ducts without which female development is the constitutive pathway [12].

In both spermatogenesis and male accessory sex gland development and maintenance, this Androgen/AR dependence occurs via androgen-sensitive reciprocal paracrine interactions between mesenchymal stromal and epithelial/seminiferous tubule cell compartments [12-14]. Importantly, circulating testosterone is irreversibly converted to dihydrotestosterone (DHT) via the membrane-bound NADPH-dependent Δ4-3-ketosteroid 5α-oxidoreductase [i.e., SRD5A-1 & -2, (a.k.a. 5α-reductase type I & type II)] family of enzymes present in the stromal cells of the male accessory sex glands [15]. Both testosterone and DHT can bind to the AR, however, the metabolic pathways in the accessory sex glands are regulated so that DHT is present at a multifold molar excess compared to testosterone [16-18]. Additionally, DHT has a 10-fold higher AR binding affinity than testosterone (i.e., K_d of ~0.1 nM for DHT vs. ~1.0 nM for testosterone) [19]. Thus, as will be discussed below, 5α-reductase amplifies the levels of AR-dependent transcription within the male accessory sex glands as required for their rapid (i.e., within 1-2 weeks) hyperplastic growth induced by the increase in the circulating level of testosterone needed for seasonal breeding.

Once formed, DHT binds to AR in stromal cells, stimulating the transcription and subsequent secretion of paracrine peptide growth and survival factors [e.g., IGF-I, EGF, FGF, PDGF] [10, 19, 20]. Within the male accessory sex glands, there are a subset of epithelial cells expressing AR, which binds DHT to stimulate transcription and secretion of androgen-dependent tissue-specific differentiation proteins that differ between mammalian species [e.g., transglutaminase, prostate-specific antigen (PSA), human kallikrein 2 (hK2), prostatic acid phosphatase (PAP), microseminoprotein-beta (MSMB) in the prostate and semenogelins in the seminal vesicles in humans; PAP, MSMB, prostatein, transglutaminase, and probasin in the rat prostate; and PAP and arginine esterase in the dog prostate]. Such AR binding, however, does not stimulate their epithelial proliferation [19-22]. The AR-dependent locally produced stromal-derived paracrine growth and survival factors diffuse throughout their immediate microenvironment, entering their companion epithelial compartments in male accessory sex glands. There, these factors bind to cognate receptors inducing development and homeostatic proliferative maintenance of the epithelial compartment in the male sex accessory glands [19, 20]. Thus, a physiologically adequate level of circulating testosterone is chronically required for the maintenance of the male accessory sex glands in the adult male eutherian mammal. This chronic requirement for testosterone derives from the necessity for androgens for homeostatic maintenance of the total epithelial cell number. Androgen does this via a stromal cell-dependent paracrine stimulation of the rate of cell proliferation (i.e., agonistic ability of androgen) simultaneously coupled with inhibition of the rate of cell death (antagonistic ability of androgen) [23].

In the testis, AR is expressed in Sertoli cells, peritubular myoid cells, Leydig cells, and perivascular smooth muscle cells, but not in spermatogonia through mature sperm [24]. Here again, androgen binding to the AR-positive cells in the testis stimulates their transcription and subsequent secretion of paracrine factors (e.g., glioma-derived growth factor-I) which subsequently bind to their cognate receptors on spermatogonia to drive spermatogenesis [12]. Due to the presence of Leydig cells, testosterone concentration in the testes is >250-fold higher than in the peripheral blood in mammals, including humans [25]. Significantly, unlike the male accessory sex tissues where DHT is the major androgen, testosterone is the major androgen in the testes, exceeding the levels of DHT by 15- to 40-fold [26, 27]. The level of testosterone in the testes required for paracrine-dependent spermatogenesis (i.e., ~50 nM [28]) is more than 10-fold higher than that required for host anabolic effects and paracrine-dependent homeostatic maintenance of the male accessory sex tissues (i.e., 5 nM [29]). Thus, if exogenous testosterone is chronically provided to maintain a physiologic level of serum testosterone (i.e., 5 nM), which maintains libido and accessory sex organ function, endogenous testosterone production in the testes is lowered due to negative feedback hypothalamic-pituitary loop. This results in lowering testicular testosterone to the circulating blood level and spermatogenesis is prevented [30, 31].

Seasonal breeding optimized survival of the species vs. the individual

A major driving factor in the selection of androgen as the master regulator of male reproduc-

Table 1. Seasonal variation in serum testosterone among a representative series of feral mammals throughout the world

Feral Mammal	Serum Testosterone (nM)		Ratio (B/NB)	Reference
	Non-breeding Season (NB)	Breeding Season (B)		
Horse	5.5 +/- 1.0	10.4 +/- 2.1	1.9	[50]
Asian Black Bear	1.9 +/- 0.3	4.2 +/- 0.3	2.2	[51]
Muskrat	3.8 +/- 3.5	10.4 +/- 2.8	2.7	[52]
Desert Mouse	1.9 +/- 1.4	5.2 +/- 2.4	2.7	[53]
Arctic Ground Squirrel	3.1 +/- 0.3	11.8 +/- 0.3	3.8	[33]
White-tailed Deer	5.2 +/- 0.7	29.4 +/- 3.5	5.7	[54]
Coyote	1.4 +/- 2.4	11.4 +/- 3.5	8.1	[55]
Mole	4.5 +/- 0.7	36.9 +/- 11.8	8.2	[56]
Polar Bear	2.1 +/- 0.7	20.0 +/- 2.8	9.5	[57]
Racoon	0.7 +/- 0.3	9.3 +/- 3.5	13.2	[58]

tion during mammalian evolution relates to the unique danger of mammalian reproduction for the survival of the individual. During mammalian evolution, breeding needed for survival of the species became episodically (i.e., seasonally) timed for optimal survival of the individual parent and offspring. During the seasonal period when breeding is not optimal, levels of testicular and circulating testosterone are at a nadir in feral non-human mammals, and thus male accessory sex glands, as well as sexual libido and sperm maturation, are also at a nadir. When environmental conditions (i.e., food/temperature/light/social conditions, etc.) are appropriate, male mammals come into "breeding season", which is induced by an increase in testicular testosterone producing a >2-10-fold increase in circulating testosterone, depending on the species **Table 1**.

This seasonal rise in testosterone induces the growth of the male accessory sex glands from their baseline to a "hyperplastic" condition needed for breeding within 1-2 weeks and stimulates spermiogenesis, which requires only 30-75 days depending on the species. Importantly, this elevation in testicular and circulating testosterone is only episodic, returning to a nadir usually within 1-2 months during which spermatogenesis stops and the "hyperplastic" male accessory sex tissues regress to their baseline state, thus defining a limited seasonal breeding period.

Such limited seasonal breeding has great reproductive advantage and was selected during evolution to restrict the energy requirements for maintaining male accessory sex glands, sperm maturation, and sexual libido. This limits the dangers associated with the maniacal focus upon procreation (i.e., survival of the species) at the expense of suppression of normal flight instinct in the presence of same species male competitors and/or different species predators (i.e., survival of the individual) to the minimal timeframe established by the limited breeding season [13]. Evolutionary pressure selected the development of a neuroendocrine (i.e., pineal gland-hypothalamic-pituitary) axis to restrict testosterone production in the testis to the high level needed for spermatogenesis and to elevate serum testosterone sufficiently to the level needed for male sex accessory gland growth to occur only the breeding season.

Arctic ground squirrels as a paradigm for androgen/AR control of seasonal breeding

The artic ground squirrels (i.e. *Spermophilus parryii*) **Figure 3**, is a paradigm for such seasonal breeding. They are the largest and most northern of the ground squirrels ranging from the Arctic Circle to northern British Columbia, and down to the southern border of the Northwest Territories, as well as Alaska and Siberia. Artic ground squirrels have unique physiological adaptations that allow them to survive during winter. Artic ground squirrels are obligate hibernators and adult males start hibernating as soon as they have enough body fat to survive the winter, often in late August when plenty of food is still available. During hibernation **Figure 3** (*left panel*), artic ground squirrels adopt the lowest body temperature ever measured in a mammal. The body temperature of hibernating squirrels drops below freezing, a condition referred to as supercooling. At intervals of two to three weeks, still in a state of sleep, hibernating squirrels shiver and shake for 12 to 15 hours to create heat that warms them back to a normal body temperature of ~98°F. When the shivering and shaking stops, body temperature drops back to the min-

Figure 3. Male arctic ground squirrel. *Left* - hibernating in winter; *Middle* - emerged from hibernation; and *Right* - post-mating season.

imal temperature. This type of hibernation is rare among mammals.

In early April, they stop hibernating and emerge from their burrow, **Figure 3** (*middle panel*), having lost up to 40% of their body weight (i.e., compare **Figure 3** *middle* vs. *right panel*) [32]. Despite this weight loss, their plasma testosterone level quickly (i.e., within days) increases nearly 4-fold, **Table 1**, and they become highly territorial with overt male-male aggression, and search for females [33]. Fights between males during this time are severe and lead to frequent wounding and even death. This aggression occurs even in the absence of females, which stop hibernating in late April. The peak of testosterone level in April is associated with spermatogenesis and coincides with the midpoint of when newly emerged females display estrus, as well as when mating takes place between mid-April to mid-May [32]. Importantly, this is followed by a rapid return to nadir in plasma testosterone and to aspermia beginning in June, which is a time when the surviving males now focus upon eating, not sex or fighting, to fatten up for their upcoming hibernation. Thus, the arctic ground squirrel is a paradigm for the ability of androgen and its receptor to override the survival instinct of the individual restrictively during the seasonal breeding period and instead reproduce for the survival of the species.

The problem with human males

In contrast to other mammals, human males evolved away from strict seasonal breeding by acquiring the ability to chronically maintain serum testosterone (i.e., <10% variation in both total and bioavailable serum testosterone during the year [34]) at a sufficiently high concentration (i.e., >15 nM) to maintain the male accessory sex glands, spermatogenesis, muscle mass/bone density, and libido in a fully stimulated adult state. Such a constant serum testosterone is a definitive advantage for the highly mobile human species. This is because it enables reproduction to occur year-round despite environment restrictions, allowing man to populate all of the biological niches throughout the world as opposed to “locking” humans to specific indigenous breeding ranges, like other mammals.

The price of such reproductive “freedom”, however, is an increase probability of developing prostate cancer by the human male due to two related reciprocal effects. The first effect is the loss of prostate protection provided by seasonal breeding. This is because seasonal breeding induces episodic cycles of male accessory sex gland regression from their hyperplastic state via apoptotic epithelial cell death when serum testosterone returns to nadir values as breeding season ends. Such apoptotic death “prunes” epithelial cells within these glands, sup-

pressing the possibility of their accumulating the multiple genetic errors needed for malignant transformation. Without such pruning, such multi-step prostatic carcinogenesis is much higher in humans than any other mammalian species [35, 36].

The second effect is that during human prostatic carcinogenesis, there is a conversion from AR-regulated stromal paracrine dependency by normal prostate epithelium to cancer cells acquiring autonomous stromal cell-independent AR-stimulated malignant growth [19, 21, 22, 37]. Such cell autonomous growth involves losing normal AR function as a growth suppressor and instead acquiring the ability to act as an oncogenic gain-of-function stimulator of malignant growth [19, 21, 22]. These oncogenic acquisitions "addict" prostate cancer cells to cell autonomous AR signaling. This addiction involves cancer cells acquiring cell autonomous AR transcription, preventing their apoptotic cell death while also inducing proliferation, making these cancers AR-dependent for their lethal growth [37].

Prospectus for the future

The loss of seasonal episodic cycling in serum testosterone prevents regressive pruning in the human prostate and instead chronically maintains the gland in a fully stimulated "hyperplastic" state, which optimally stimulates the outgrowth of initiated prostate cancer cells. In this regard, the loss of seasonal breeding functions in humans as a "promoter" of prostate carcinogenesis. This predicts that a reduction in prostate androgen levels should decrease the prevalence of prostate cancer development. This hypothesis was initially tested clinically using 5α-reductase inhibitors to lower prostatic DHT levels, since this is the major androgen in the normal prostate. Two large randomized prospective clinical trials documented that oral treatment with 5α-reductase inhibitors (i.e., finasteride in the PCPT trial and dutasteride in the REDUCE trial) lowered the incidence of pathologically detectable, low grade (i.e., Gleason 6) prostate cancer by ~25% over a 10-year period [38].

The two large-scale 5α-reductase inhibitor cancer prevention trials demonstrate, however, that there is not a prostate cancer-specific mortality benefit from the use of 5α-reductase inhibitors to prevent prostate cancer; conversely, strong data demonstrate that there is also no excess mortality [38]. This result is consistent with the fact that while chronic treatment with the 5α-reductase inhibitor, finasteride, decreases prostatic DHT (i.e., from 18.6 +/- 1.4 nM to 0 1.7 +/- 07 nM [39]), it also causes a reciprocal significant increase in prostatic testosterone (i.e., from 1.1 +/- 0.2 nM to 8.3 +/- 0.7 nM [39]). This decrease of prostate cancer DHT coupled with a significant increase in prostate cancer testosterone is also produced by dutasteride; thus inhibiting cancer growth, but not with the same efficacy as castration [40]. These results support that, although testosterone is not as potent as DHT, it is still efficacious in stimulating malignant prostate growth.

These combined results support the rationale that chemoprevention of prostate cancer requires blocking not only DHT, but also testosterone-induced AR transcriptional signaling. This can be achieved if prostate-specific expression of the AR is selectively inhibited. Such selective targeting is possible using the sequiterpene-lactone, thapsigargin (TG) **Figure 4A**. TG is very lipophilic, and thus highly cell-penetrant, and once inside cells inhibits (IC_{50}: 10 nmol/L) the critically important housekeeping SERCA 2b calcium pumps in the endoplasmic reticulum [41]. TG-dependent inhibition of SERCA pumps results in depletion of the endoplasmic reticulum Ca^{+2}, coupled with μmol/L elevation in the intracellular free Ca^{+2}, which initiates a molecular cascade that inhibits Cap-dependent AR protein synthesis, resulting in depletion of both full-length AR protein and truncated variants (i.e., AR-V7), inducing their apoptotic death [41-43]. Due to its highly lipophilic, cell permeant nature, TG is not deliverable as a systemic agent without causing host toxicity.

Systemic delivery of TG and targeting its therapeutic efficacy restrictively to the prostate is possible, however, based upon: 1) the essentially exclusive high level expression of PSA by the prostate among all other normal body tissues **Figure 5A**, despite widespread expression of AR in many benign tissues **Figure 5B** [44]; 2) that PSA is a protease produced by prostate luminal epithelial cells that "leaks" into the extracellular fluid in the stromal compartment from both normal and malignant prostate tissues [45]; 3) that PSA is enzymatically active in

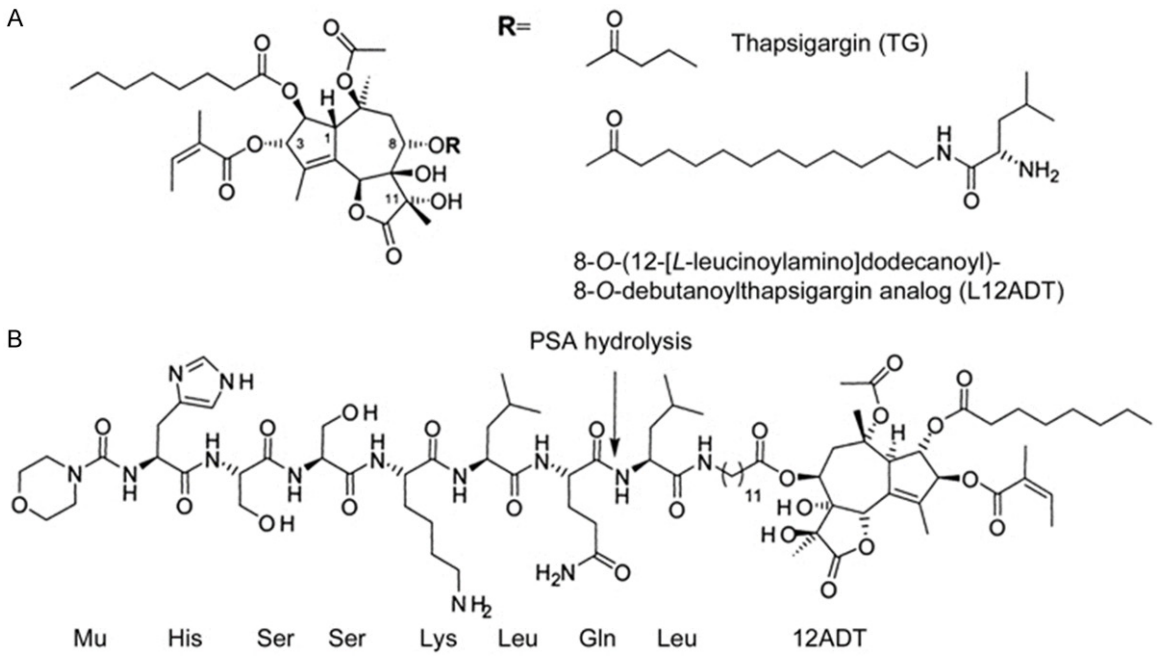

Figure 4. Chemical structures of thapsigargin (TG) and a PSA-activated TG-based prodrug. A. Structures of the highly lipophilic, cell permeant TG and L12ADT; B. Structure of water-soluble, cell impermeant 4-Morpholinecarbonyl (Mu) N-terminal blocked-histidine-serine-serine-lysine-leucine-glutamic acid-leucine-12ADT (i.e., HSSKLQL-12ADT) prodrug. The arrow indicates the site of prostate-specific antigen (PSA) hydrolysis, which liberates the highly lipophilic, cell permeant Leu-12ADT (i.e., L12ADT).

this extracellular prostate fluid, but not when it is enters circulation [45]; and 4) there are unique peptide sequences [e.g., His-Ser-Ser-Lys-Leu-Gln-Leu, (HSSKLQ^{V}L)], which only PSA hydrolyzes (i.e., between Gln and the terminal Leu) efficiently [45]. Based upon these facts, TG analogues containing amino acids [e.g., Leucyl-12-aminododecanoyl-TG, (L12ADT)], **Figure 4B**, have been synthesized, which retain high cell penetrance and high potency (i.e., EC_{50}<50 nM) to deplete AR protein and induce cell death [42]. These have been covalently linked via a peptide bond to such PSA cleavable peptide carriers to produce water-soluble peptide prodrugs (e.g., **Figure 4B**) for systemic delivery and selective targeting of the prostate [46]. When such a prodrug is delivered systemically via the blood, it remains extracellular and is efficiently hydrolyzed for restrictive liberation of the highly lipophilic cell permeant molecules only in sites where enzymatically-active PSA is present (i.e., the prostate cancer tumor microenvironment) [46].

While the previous studies provide the rationale for such a PSA-activated TG-based prodrug approach, long-term chemoprevention testing will require the development of a depot formulation, which can be injected subcutaneously for maintenance delivery. To develop such a depot formulation, advantage is being taken of the fact that human serum albumin (HSA) has a single reactive free cysteine (cys-34) which has been used to produce albumin-coupled prodrugs for clinical trials [47]. Based upon this realization, a second-generation PSA-activated TG prodrug has been synthesized in which cys-34 of HSA is covalently bound to a "stabilized" maleimide (i.e., 2-fluoro-5-maleimidobenzoate) linker [48] that is covalently coupled to the N-terminus of the PSA-activated peptide ending in L12ADT, **Figure 6** [49]. Presently, this HSA-coupled PSA-activated prodrug is being formulated in a hydrogel matrix for subcutaneous injection as a long-term depot form for prostate cancer chemoprevention.

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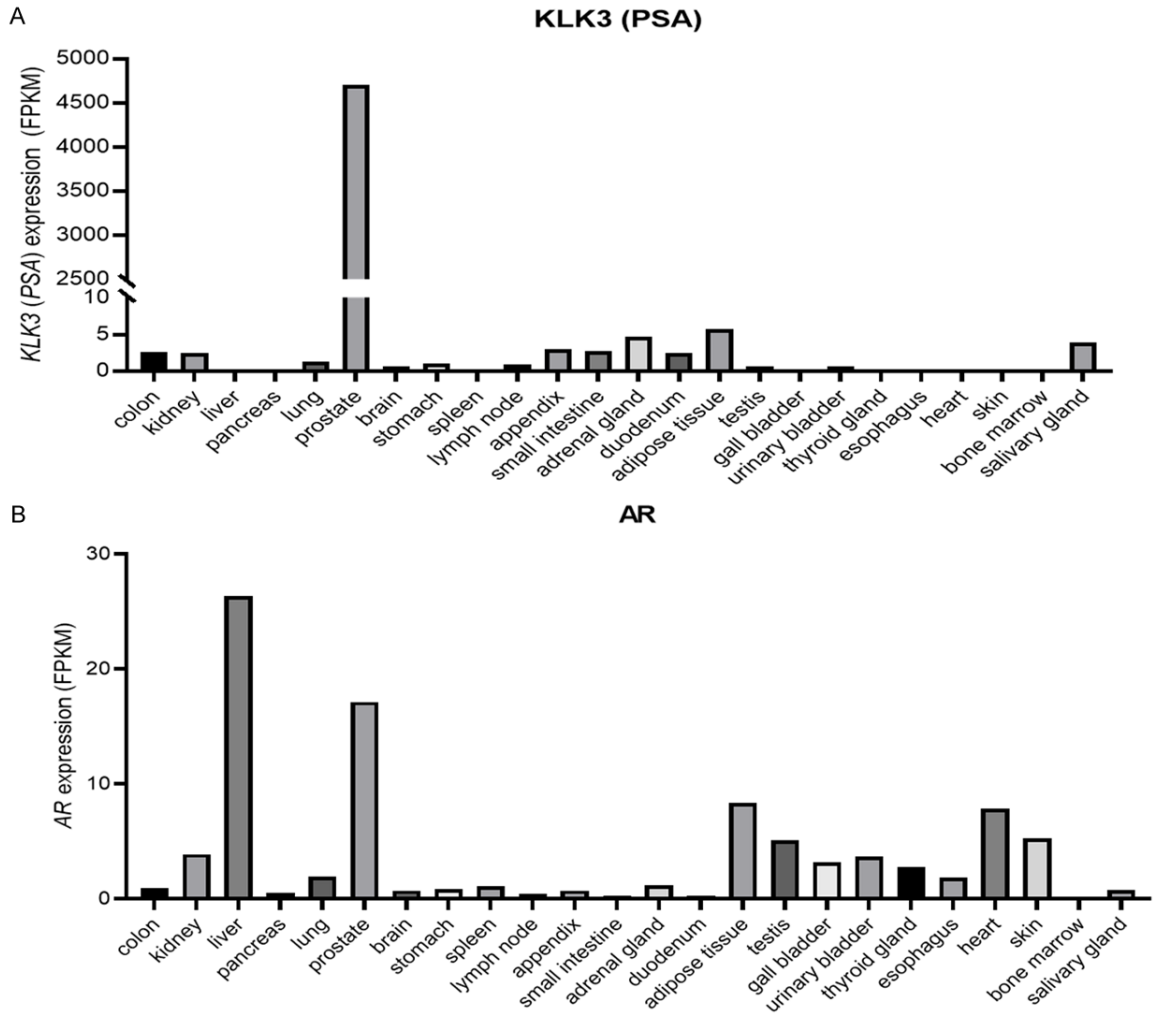

Figure 5. RNAseq determination of *AR* and *KLK3* (PSA) expression normalized as FPKM in the indicated normal human tissues (*note 167-fold difference in the y-axis scales for the KLK3 vs. AR*). Data from Ref [44].

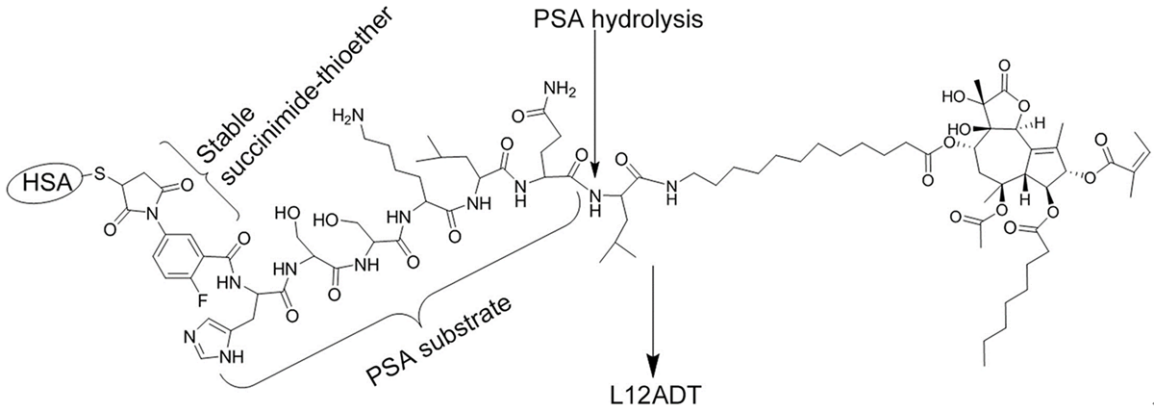

Figure 6. Structure of Human Serum Albumin (HSA) covalently bound via its position 34 cysteine to 2-fluoro-5-maleimidobenzamide linked to the N-terminal of HSSKLQL-12ADT PSA-activated TG prodrug.

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Disclosure of conflict of interest

None.

Address correspondence to: John T Isaacs, Department of Urology, James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. E-mail: isaacjo@jhmi.edu

References

[1] Eick GN and Thornton JW. Evolution of steroid receptors from an estrogen-sensitive ancestral receptor. Mol Cell Endocrinol 2011; 334: 31-8.

[2] Thornton JW. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci U S A 2001; 98: 5671-6.

[3] Lahn BT and Page DC. Four evolutionary strata on the human X chromosome. Science 1999; 286: 964-7.

[4] Watson JM, Spencer JA, Riggs AD and Graves JA. Sex chromosome evolution: platypus gene mapping suggests that part of the human X chromosome was originally autosomal. Proc Natl Acad Sci U S A 1991; 88: 11256-60.

[5] Hughes JF, Skaletsky H, Pyntikova T, Minx PJ, Graves T, Rozen S, Wilson RK and Page DC. Conservation of Y-linked genes during human evolution revealed by comparative sequencing in chimpanzee. Nature 2005; 437: 100-3.

[6] Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, Platzer M, Howell GR, Burrows C, Bird CP, Frankish A, Lovell FL, Howe KL, Ashurst JL, Fulton RS, Sudbrak R, Wen G, Jones MC, Hurles ME, Andrews TD, Scott CE, Searle S, Ramser J, Whittaker A, Deadman R, Carter NP, Hunt SE, Chen R, Cree A, Gunaratne P, Havlak P, Hodgson A, Metzker ML, Richards S, Scott G, Steffen D, Sodergren E, Wheeler DA, Worley KC, Ainscough R, Ambrose KD, Ansari-Lari MA, Aradhya S, Ashwell RI, Babbage AK, Bagguley CL, Ballabio A, Banerjee R, Barker GE, Barlow KF, Barrett IP, Bates KN, Beare DM, Beasley H, Beasley O, Beck A, Bethel G, Blechschmidt K, Brady N, Bray-Allen S, Bridgeman AM, Brown AJ, Brown MJ, Bonnin D, Bruford EA, Buhay C, Burch P, Burford D, Burgess J, Burrill W, Burton J, Bye JM, Carder C, Carrel L, Chako J, Chapman JC, Chavez D, Chen E, Chen G, Chen Y, Chen Z, Chinault C, Ciccodicola A, Clark SY, Clarke G, Clee CM, Clegg S, Clerc-Blankenburg K, Clifford K, Cobley V, Cole CG, Conquer JS, Corby N, Connor RE, David R, Davies J, Davis C, Davis J, Delgado O, Deshazo D, Dhami P, Ding Y, Dinh H, Dodsworth S, Draper H, Dugan-Rocha S, Dunham A, Dunn M, Durbin KJ, Dutta I, Eades T, Ellwood M, Emery-Cohen A, Errington H, Evans KL, Faulkner L, Francis F, Frankland J, Fraser AE, Galgoczy P, Gilbert J, Gill R, Glöckner G, Gregory SG, Gribble S, Griffiths C, Grocock R, Gu Y, Gwilliam R, Hamilton C, Hart EA, Hawes A, Heath PD, Heitmann K, Hennig S, Hernandez J, Hinzmann B, Ho S, Hoffs M, Howden PJ, Huckle EJ, Hume J, Hunt PJ, Hunt AR, Isherwood J, Jacob L, Johnson D, Jones S, de Jong PJ, Joseph SS, Keenan S, Kelly S, Kershaw JK, Khan Z, Kioschis P, Klages S, Knights AJ, Kosiura A, Kovar-Smith C, Laird GK, Langford C, Lawlor S, Leversha M, Lewis L, Liu W, Lloyd C, Lloyd DM, Loulseged H, Loveland JE, Lovell JD, Lozado R, Lu J, Lyne R, Ma J, Maheshwari M, Matthews LH, McDowall J, McLaren S, McMurray A, Meidl P, Meitinger T, Milne S, Miner G, Mistry SL, Morgan M, Morris S, Müller I, Mullikin JC, Nguyen N, Nordsiek G, Nyakatura G, O'Dell CN, Okwuonu G, Palmer S, Pandian R, Parker D, Parrish J, Pasternak S, Patel D, Pearce AV, Pearson DM, Pelan SE, Perez L, Porter KM, Ramsey Y, Reichwald K, Rhodes S, Ridler KA, Schlessinger D, Schueler MG, Sehra HK, Shaw-Smith C, Shen H, Sheridan EM, Shownkeen R, Skuce CD, Smith ML, Sotheran EC, Steingruber HE, Steward CA, Storey R, Swann RM, Swarbreck D, Tabor PE, Taudien S, Taylor T, Teague B, Thomas K, Thorpe A, Timms K, Tracey A, Trevanion S, Tromans AC, d'Urso M, Verduzco D, Villasana D, Waldron L, Wall M, Wang Q, Warren J, Warry GL, Wei X, West A, Whitehead SL, Whiteley MN, Wilkinson JE, Willey DL, Williams G, Williams L, Williamson A, Williamson H, Wilming L, Woodmansey RL, Wray PW, Yen J, Zhang J, Zhou J, Zoghbi H, Zorilla S, Buck D, Reinhardt R, Poustka A, Rosenthal A, Lehrach H, Meindl A, Minx PJ, Hillier LW, Willard HF, Wilson RK, Waterston RH, Rice CM, Vaudin M, Coulson A, Nelson DL, Weinstock G, Sulston JE, Durbin R, Hubbard T, Gibbs RA, Beck S, Rogers J and Bentley DR. The DNA sequence of the human X chromosome. Nature 2005; 434: 325-37.

[7] Wilson MA and Makova KD. Evolution and survival on eutherian sex chromosomes. PLoS Genet 2009; 5: e1000568.

[8] Spencer JA, Watson JM, Lubahn DB, Joseph DR, French FS, Wilson EM and Graves JA. The androgen receptor gene is located on a highly conserved region of the X chromosomes of

marsupial and monotreme as well as eutherian mammals. J Hered 1991; 82: 134-9.
[9] Chen J, Okimura K and Yoshimura T. Light and hormones in seasonal regulation of reproduction and mood. Endocrinology 2020; 161: bqaa130.
[10] Dardente H and Simonneaux V. GnRH and the photoperiodic control of seasonal reproduction: delegating the task to kisspeptin and RFRP-3. J Neuroendocrinol 2022; 34: e13124.
[11] Rohrer CWG. A contribution to comparative anatomy of the prostate. PhD thesis: Illinois Wesleyan University; 1909.
[12] Brennen WN and Isaacs JT. Mesenchymal stem cells and the embryonic reawakening theory of BPH. Nat Rev Urol 2018; 15: 703-715.
[13] O'Hara L and Smith LB. Androgen receptor roles in spermatogenesis and infertility. Best Pract Res Clin Endocrinol Metab 2015; 29: 595-605.
[14] Walker WH. Androgen actions in the testis and the regulation of spermatogenesis. Adv Exp Med Biol 2021; 1288: 175-203.
[15] Russell DW and Wilson JD. Steroid 5 alpha-reductase: two genes/two enzymes. Annu Rev Biochem 1994; 63: 25-61.
[16] Isaacs JT and Coffey DS. Changes in dihydrotestosterone metabolism associated with the development of canine benign prostatic hyperplasia. Endocrinology 1981; 108: 445-53.
[17] Isaacs JT, Brendler CB and Walsh PC. Changes in the metabolism of dihydrotestosterone in the hyperplastic human prostate. J Clin Endocrinol Metab 1983; 56: 139-46.
[18] Page ST, Lin DW, Mostaghel EA, Marck BT, Wright JL, Wu J, Amory JK, Nelson PS and Matsumoto AM. Dihydrotestosterone administration does not increase intraprostatic androgen concentrations or alter prostate androgen action in healthy men: a randomized-controlled trial. J Clin Endocrinol Metab 2011; 96: 430-7.
[19] Litvinov IV, De Marzo AM and Isaacs JT. Is the Achilles' heel for prostate cancer therapy a gain of function in androgen receptor signaling? J Clin Endocrinol Metab 2003; 88: 2972-82.
[20] Isaacs JT. Prostate stem cells and benign prostatic hyperplasia. Prostate 2008; 68: 1025-34.
[21] Vander Griend DJ, Litvinov IV and Isaacs JT. Conversion of androgen receptor signaling from a growth suppressor in normal prostate epithelial cells to an oncogene in prostate cancer cells involves a gain of function in c-Myc regulation. Int J Biol Sci 2014; 10: 627-42.
[22] Antony L, van der Schoor F, Dalrymple SL and Isaacs JT. Androgen receptor (AR) suppresses normal human prostate epithelial cell proliferation via AR/beta-catenin/TCF-4 complex inhibition of c-MYC transcription. Prostate 2014; 74: 1118-31.
[23] Kyprianou N and Isaacs JT. Activation of programmed cell death in the rat ventral prostate after castration. Endocrinology 1988; 122: 552-62.
[24] Zhou Q, Nie R, Prins GS, Saunders PT, Katzenellenbogen BS and Hess RA. Localization of androgen and estrogen receptors in adult male mouse reproductive tract. J Androl 2002; 23: 870-81.
[25] Maddocks S, Hargreave TB, Reddie K, Fraser HM, Kerr JB and Sharpe RM. Intratesticular hormone levels and the route of secretion of hormones from the testis of the rat, guinea pig, monkey and human. Int J Androl 1993; 16: 272-8.
[26] Wright WW and Frankel AI. Endogenous androgen concentrations in nuclei isolated from seminiferous tubules of mature rat testes. J Steroid Biochem 1979; 10: 633-40.
[27] Turner TT, Jones CE, Howards SS, Ewing LL, Zegeye B and Gunsalus GL. On the androgen microenvironment of maturing spermatozoa. Endocrinology 1984; 115: 1925-32.
[28] Zirkin BR, Santulli R, Awoniyi CA and Ewing LL. Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentration within the testis. Endocrinology 1989; 124: 3043-9.
[29] Kyprianou N and Isaacs JT. Quantal relationship between prostatic dihydrotestosterone and prostatic cell content: critical threshold concept. Prostate 1987; 11: 41-50.
[30] Cao Z and Kyprianou N. Mechanisms navigating the TGF-beta pathway in prostate cancer. Asian J Urol 2015; 2: 11-18.
[31] Ewing LL, Schanbacher B, Desjardins C and Chaffee V. The effect of subdermal testosterone filled polydimethylsiloxane implants on spermatogenesis in rhesus monkeys (Macaca mulata). Contraception 1976; 13: 583-96.
[32] Lee TN, Buck CL, Barnes BM and O'Brien DM. A test of alternative models for increased tissue nitrogen isotope ratios during fasting in hibernating arctic ground squirrels. J Exp Biol 2012; 215: 3354-61.
[33] Buck CL and Barnes BM. Androgen in free-living arctic ground squirrels: seasonal changes and influence of staged male-male aggressive encounters. Horm Behav 2003; 43: 318-26.
[34] Zornitzki T, Tshori S, Shefer G, Mingelgrin S, Levy C and Knobler H. Seasonal variation of testosterone levels in a large cohort of men. Int J Endocrinol 2022; 2022: 6093092.
[35] Isaacs JT. Prostatic structure and function in relation to the etiology of prostatic cancer. Prostate 1983; 4: 351-66.
[36] Carter HB, Piantadosi S and Isaacs JT. Clinical evidence for and implications of the multistep

development of prostate cancer. J Urol 1990; 143: 742-6.
[37] Vander Griend DJ, D'Antonio J, Gurel B, Antony L, Demarzo AM and Isaacs JT. Cell-autonomous intracellular androgen receptor signaling drives the growth of human prostate cancer initiating cells. Prostate 2010; 70: 90-9.
[38] Liss MA and Thompson IM. Prostate cancer prevention with 5-alpha reductase inhibitors: concepts and controversies. Curr Opin Urol 2018; 28: 42-45.
[39] Norman RW, Coakes KE, Wright AS and Rittmaster RS. Androgen metabolism in men receiving finasteride before prostatectomy. J Urol 1993; 150: 1736-9.
[40] Xu Y, Dalrymple SL, Becker RE, Denmeade SR and Isaacs JT. Pharmacologic basis for the enhanced efficacy of dutasteride against prostatic cancers. Clin Cancer Res 2006; 12: 4072-9.
[41] Furuya Y, Lundmo P, Short AD, Gill DL and Isaacs JT. The role of calcium, pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent prostatic cancer cells induced by thapsigargin. Cancer Res 1994; 54: 6167-75.
[42] Vander Griend DJ, Antony L, Dalrymple SL, Xu Y, Christensen SB, Denmeade SR and Isaacs JT. Amino acid containing thapsigargin analogues deplete androgen receptor protein via synthesis inhibition and induce the death of prostate cancer cells. Mol Cancer Ther 2009; 8: 1340-9.
[43] Isaacs JT, Brennen WN, Christensen SB and Denmeade SR. Mipsagargin: the beginning-not the end-of thapsigargin prodrug-based cancer therapeutics. Molecules 2021; 26: 7469.
[44] Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjöstedt E, Lundberg E, Szigyarto CA, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F and Uhlén M. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics 2014; 13: 397-406.
[45] Denmeade SR, Lou W, Lövgren J, Malm J, Lilja H and Isaacs JT. Specific and efficient peptide substrates for assaying the proteolytic activity of prostate-specific antigen. Cancer Res 1997; 57: 4924-30.
[46] Denmeade SR, Jakobsen CM, Janssen S, Khan SR, Garrett ES, Lilja H, Christensen SB and Isaacs JT. Prostate-specific antigen-activated thapsigargin prodrug as targeted therapy for prostate cancer. J Natl Cancer Inst 2003; 95: 990-1000.
[47] Kratz F. A clinical update of using albumin as a drug vehicle - a commentary. J Control Release 2014; 190: 331-6.
[48] Akinboye ES, Rogers OC and Isaacs JT. 2-fluoro-5-maleimidobenzoic acid-linked albumin drug (MAD) delivery for selective systemic targeting of metastatic prostate cancer. Prostate 2018; 78: 655-663.
[49] Akinboye ES, Brennen WN, Denmeade SR and Isaacs JT. Albumin-linked prostate-specific antigen-activated thapsigargin- and niclosamide-based molecular grenades targeting the microenvironment in metastatic castration-resistant prostate cancer. Asian J Urol 2019; 6: 99-108.
[50] Turner JW Jr and Kirkpatrick JF. Androgens, behaviour and fertility control in feral stallions. J Reprod Fertil Suppl 1982; 32: 79-87.
[51] Tomiyasu J, Kayano M, Hazano K, Matsui M, Nemoto Y, Naganuma T, Koike S and Yamazaki K. Associations between plasma testosterone levels and season, nutritional status, age, and body size in free-ranging male Asian black bears (Ursus thibetanus) in central Honshu, Japan. Gen Comp Endocrinol 2021; 309: 113794.
[52] Liu Q, Yu W, Fan S, Zhuang H, Han Y, Zhang H, Yuan Z and Weng Q. Seasonal expressions of androgen receptor, estrogen receptors, 5alpha-reductases and P450arom in the epididymis of the male muskrat (Ondatra zibethicus). J Steroid Biochem Mol Biol 2019; 194: 105433.
[53] Schradin C. Seasonal changes in testosterone and corticosterone levels in four social classes of a desert dwelling sociable rodent. Horm Behav 2008; 53: 573-9.
[54] Gomes MA, Ditchkoff SS, Zohdy S, Gulsby WD and Newbolt CH. Patterns of testosterone in male white-tailed deer (Odocoileus virginianus): seasonal and lifetime variation. Ecol Evol 2021; 11: 5320-5330.
[55] Minter LJ and DeLiberto TJ. Seasonal variation in serum testosterone, testicular volume, and semen characteristics in the coyote (Canis latrans). Theriogenology 2008; 69: 946-52.
[56] Racey PA. Seasonal changes in testosterone levels and androgen-dependent organs in male moles (Talpa europaea). J Reprod Fertil 1978; 52: 195-200.
[57] Howell-Skalla LA, Cattet MR, Ramsay MA and Bahr JM. Seasonal changes in testicular size and serum LH, prolactin and testosterone concentrations in male polar bears (Ursus maritimus). Reproduction 2002; 123: 729-33.
[58] Okuyama MW, Shimozuru M, Takahashi N, Fukui D, Nakamura R and Tsubota T. Seasonal changes in spermatogenesis and peripheral testosterone concentration in raccoons (Procyon lotor) in Hokkaido. J Vet Med Sci 2012; 74: 727-32.