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Reconsideration of progression to CRPC during androgen deprivation therapy

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Abstract

Androgen blockade-naïve prostate cancer (PCa) develops into CRPC during androgen deprivation therapy (ADT) by various genetic actions. The androgen-AR signaling axis plays a key role in this development. PCa cells mainly adapt themselves to the environment of lower androgen concentrations and change into androgen-hypersensitive cells or androgen-independent cells. Androgens of adrenal origin and their metabolites synthesized in the microenvironment in an intracrine/paracrine fashion act on surviving PCa cells and secrete prostate specific antigen (PSA). Total androgen deprivation (TAD) (castration, antiandrogen, and CYP17A1 inhibitor) can become an effective therapeutic strategy concerning the androgen signaling axis-related pathway. However, it is important to ascertain whether elevation of serum PSA results from AR activation or from an androgen-independent tumor volume effect. Then, clinicians can judge it adequately using the imaging studies such as CT or bone scan as well as PSA and bone metabolic markers, an approach which is necessary to judge which treatment is most suitable for the CRPC patients.

Mechanisms of progression into CRPC related to an androgen-independent pathway

Multiple molecular mechanisms which could account for the development of castration-resistant prostate cancer (CRPC) have been proposed [1] (Figure 1). One mechanism is an androgen receptor (AR)-independent pathway. When we look at many published papers on the immunohistochemistry of AR, the AR expression level is heterogeneous in high Gleason grade prostate cancer (PCa) tissue [2]. Androgen-independent growth might result from clonal expansion of such androgen-independent cells at the latter stage [3]. Even if AR is expressed in PCa cells, other factors, such as BCL-2 and EZH2, might dominantly act on cell proliferation irrespective of androgen deprivation therapy (ADT) [4, 5].

Mechanisms of progression into CRPC related to adrenal androgen precursors

Recent clinical evidences that CYP17A inhibitors and second generation antiandrogens are very effective in CRPC after docetaxel treatment have clearly proven

that the androgen-AR signaling axis is an important pathway in the progression of PCa (Figure 1) [6] [7, 8]. The evidence obtained indicates that alterations of AR itself, which is either absent or at low concentration in the original androgen-dependent state, results in an androgen-hypersensitive situation where stimulation of PCa growth occurs at castrate levels of androgens [9, 10]. One of the AR alterations is AR mutation that results in promiscuous ligand specificity [11]. Therefore, in addition to its normal ligands, namely testosterone (T) and dihydrotestosterone (DHT), both androstenediol, estradiol, and pregnenolone, a common precursor of many steroids, can activate the AR and stimulate the proliferation of LNCaP cells that have such a mutated AR [12-14].

Even if ADT is applied, the concentration of a precursor of T, namely androstenediol in PCa tissue is almost the same [14]. Once AR mutation occurs at T877A androstenediol will activate AR. The activation of AR is also a very critical factor for progression into CRPC. Overexpression of AR by gene amplification and enhanced transcription induces androgen-hypersensitivity [15, 16]. AR coactivators such as TIF2 and ARA55 also enhance androgen-hypersensitivity [17, 18]. Even if ADT decreases serum T to castration levels, androgen-hypersensitive PCa will adapt to the circumstance at the low

level of T in serum, begin to reproduce, and secrete prostate specific antigen (PSA) again.

Synthesis of intratumoral androgens

Not only AR alteration that adapts to the circumstance at the low level of serum T occurs after ADT in PCa, but also residual androgens in PCa tissue and metastasis sites affect altered AR. However, physiological concentrations of the adrenal androgen precursors dehydroepiandrosterone (DHEA) cannot activate AR nor stimulate proliferation of PCa [14]. It is necessary for DHEA to be converted into T or DHT in order to activate AR. Interestingly, DHT is present in PCa tissue after ADT. Specifically, when PCa patients are treated with ADT, serum T and DHT decreases to less than one-tenth of pretreatment levels [19]. However, T and DHT in PCa tissue are still present at 20 to 40% of pretreatment values [14, 19-23]. These residual androgens in PCa tissue after ADT continue to promote AR activation. This is accounted for by the observation that combination therapy with a LH-RH agonist, to block androgen production, and an antiandrogen, to block ligand binding to the AR, is more effective

for PCa treatment than either therapy alone [19, 24] [25]. Moreover, recent clinical trials using abiraterone acetate for post-docetaxel chemotherapy revealed that CYP17 blockade by abiraterone acetate results in decline in PSA [26], suggesting androgen synthesis from adrenal precursors stimulates cancer progression even after docetaxel treatment.

T and DHT in PCa tissue after medical or surgical castration are synthesized locally in the prostate from DHEA of adrenal origin [14, 19-23]. The metabolism from DHEA to DHT in peripheral target tissues depends upon the level of expression of various steroidogenic enzymes in the specific cell types of these tissues [27]. Adrenal DHEA is converted to T by 17 β -hydroxysteroid dehydrogenase (HSD17B) and 3 β -hydroxysteroid dehydrogenase (HSD3B). T is then converted to DHT by 5 α -steroid reductase (SRD5A) in the prostate. HSD3B catalyzes almost exclusively the oxidation of 3-hydroxy-into 3-keto-5-androstene steroids (DHEA and 5-androstenediol are converted into androstenedione and testosterone by HSD3B, respectively) [28]. On the other hand, the HSD17Bs are responsible for the formation and inactivation of all active androgens: types 1, 3, 5, 7, 12 and 13 HSD17B catalyze the reductive reaction (DHEA

and androstenedione are converted into androstenediol and T, respectively) while types 2, 4, 6 and 8 HSD17B catalyze the oxidative reaction (reverse conversion). SRD5A catalyzes the 5-reduction of 4-dione, T and other 4-ene-3-keto-steroids to the corresponding 5-dihydro-3-keto-steroids. These enzymes are localized in various peripheral tissues, including the prostate, with specific expression patterns in each tissue. For example, HSD3B and type 5 HSD17B were localized in basal cells of alveoli, stromal cells and endothelial cells of blood vessels of the prostate [29]. Various androgen-metabolising enzymes were also expressed in prostate cancer [30-33].

Alteration of androgen metabolism in PCa and CRPC

Fung et al. have observed increased expression of the androgen synthesizing enzyme AKR1C3 (type 5 HSD17B), in PCa tissue [34] while Stanbrough et al. confirmed that ADT-resistant PCa and bone marrow metastases expressed increased levels of multiple genes responsible for androgen metabolism (HSD3B2, AKR1C3, SRD5A1, AKR1C2, AKR1C1 and UGT2B15) [35]. Especially, the mRNAs encoding HSD3B2, AKR1C3, and SRD5A1 that can make DHT from DHEA were overexpressed

by 1.8, 5.3, and 2.1-fold in CRPC, respectively. These studies support the concept that PCa and CRPC tissues can perform local biosynthesis of T and DHT resulting in activation of the AR [36].

Moreover, the activity of the steroidogenic enzymes is also affected by cytokines and growth factors. Activin A up-regulated expression of AKR1C3, inducing local conversion of androstenedione to T in LNCaP and VCaP PCa cell lines. The antiproliferative effects of activin were consequently counteracted in the presence of physiological levels of androstenedione. In addition, the ratio of inhibin A- and B-subunits to follistatin was increased in human PC tissue samples and inversely associated with metastasis-free survival [37]. Treatment of LNCaP cells with IL-6 induced the expression of steroidogenic enzymes including CYP11A, HSD3B2, AKR1C3 and HSD17B3, and increased levels of T in lysates of cells grown in serum free media by 2-fold [38]. In DHEA-treated primary prostate stromal cells, transforming growth factor- β 1 (TGF β 1) induced time- and dose-dependent increases in metabolism of DHEA to androstenedione and testosterone. Moreover, TGF β 1-treated prostate stromal cells exhibited changes in the gene expression level of enzymes involved in

steroid metabolism including up-regulation of HSD3B, and down-regulation of type 5, and type 2 HSD17Bs [39]. Since TGF β 1 is one of the major cytokines that present in bone, androgen biosynthesis might be more increased in bone metastasis sites. IGF2 that is overexpressed in CRPC tissue also increases expression of steroidogenesis enzymes, such as CYP17A1, AKR1C3, and HSD17B3, and increased *de novo* steroidogenesis resulting in AR activation [40].

Microenvironment of PCa cells and steroidogenesis

It remains unclear; however, in which cell types T and DHT are synthesized from DHEA in PCa tissue, although the products from DHEA and the relevant steroidogenic enzymes are definitively present in the prostate. If all steroidogenic enzymes are active in PCa cells, DHEA should be converted into T and DHT locally and activate AR and stimulate PCa cell proliferation. However, treatment with DHEA itself had little effect on AR activation and the proliferation of LNCaP and LAPC-4 cells [14] [41]. Therefore, we needed to pay attention to not only PCa cells but also to the microenvironment of PCa cells, like stromal cells. Stromal cells can be involved in a

paracrine fashion but the main site of conversion of DHEA into T is most likely the epithelial cells (intracrinology?). In vitro, the secreted factors stay around with elevated concentrations but not in vivo? The enzymes transforming DHEA into T and DHT are possibly at a higher concentration in stromal and other cells cocultured with prostatic cell lines having low DHEA-converting ability.

Arnold JT et al. demonstrated the importance of PCa-associated stromal cells on the conversion from DHEA into T [41]. When prostate cancer-associated (6S) stromal cells were added in coculture, DHEA stimulated LAPC-4 cell PSA protein secretion to levels approaching induction by DHT. Also, DHEA induced 15-fold more PSA mRNA in LAPC-4 cocultures than in monocultures. LAPC-4 proliferation was increased 2–3 fold when cocultured with 6S stromal cells regardless of hormone treatment. DHEA-treated 6S stromal cells exhibited an increase in T secretion. We also explored the hypothesis that PCa stromal cells contribute to the biosynthesis of T and DHT in PCa [42]. DHEA alone had little effect on PSA promoter activity and the proliferation of LNCaP cells. However, the addition of normal prostate stromal cells (PrSC) or PCa-derived stromal cells (PCaSC) increased DHEA-induced PSA promoter activity via

AR activation in the LNCaP cells. Moreover, PCa-derived stromal cells and bone-derived stromal cells accelerated DHEA-induced PSA promoter activity 1.1-6.5 fold more than the PrSC. Importantly, physiological concentrations of DHEA stimulate the proliferation of LNCaP cells in the presence of stromal cells, especially PCa-derived stromal cells. Biosynthesis of T and DHT from DHEA coculture of LNCaP cells with normal stromal cells and PCa-derived stromal cells was associated with AR activation (Figure 2, 3).

We fortunately succeeded in culturing recurrent PCa-derived stromal cells (PCaSC-26) from the same primary PCa patient (PCaSC-8). We compared PCaSC-26 with PCaSC-8 AR activity after treatment with DHEA. Recurrent PCaSC-26 enhanced DHEA-induced AR activity almost twice more in a LNCaP coculture system than PCaSC-8 (unpublished data). This data suggests that microenvironment of recurrent PCa tissue after ADT might support androgen metabolism to further enhance AR activity. Finally, the dual 5α -reductase inhibitor dutasteride appears to function not only as 5α -reductase inhibitor but also as HSD3B inhibitor in LNCaP cells. In a Phase II study of 3.5 mg dutasteride daily, an inhibitor of SRDA5, for CRPC during ADT has

shown that serum PSA level in several CRPC patients was improved by dutasteride treatment [43]. Auchus et al. identified the alternative pathway that synthesizes 5α -androstane- $3\alpha,17\beta$ -diol (androstanediol), which is the proximal precursor of DHT in the testes of pouch young of the tammar wallaby and immature postnatal testes of several species. They called this phenomenon the 'backdoor pathway' to DHT formation instead of classical pathway where progesterone is converted to 17α -OH-progesterone, androstenedione, testosterone, and DHT sequentially [44]. Moreover, Locke et al. confirmed that this backdoor pathway as well as classical pathway existed by adding [3 H]-progesterone to LNCaP cells and measuring several steroids that are related with backdoor pathway in LC-MS [45]. It is not clear how much DHT is synthesized through the backdoor pathway in CRPC after ADT when we consider the physiological level of progesterone in serum (\sim nM order) compared with the classical pathway.

We experienced some precious cases in which SRDA5 might be associated with further progression of CRPC with bone metastases in our hospital. One CRPC patient with diffuse bone metastasis was treated 20 times with docetaxel and dexamethasone

(docetaxel treatment more than 10 times is approved in Japan) (Figure 4A). However, since PSA raised gradually and dysuria due to prostate swelling was observed, we treated with 0.5 mg daily dutasteride. Then, serum PSA decreased to 50% of baseline and was kept at a low level for 5 months. The other case was the CRPC patient whose life expectancy was speculated to be within 2 months because he had bulky lymph node mass in pelvis, severe pelvic bone metastasis, right pleural effusion, and dysuria by prostate swelling (Figure 4B). He was also treated with 0.5 mg daily dutasteride. The serum PSA level continued being stable for five months and CRP was decreased dramatically. It was speculated that synthesis of DHT was accelerated as a result of increased expression of SRDA5 and promoted a proliferation of PCa. Progesterone/17 α -OH-progesterone may also be quickly metabolized to a precursor of androsterone through activation of the backdoor pathway after SRDA5 activity is enhanced in some patients with CRPC. These cases suggested that the intracellular DHT concentration was important and not the serum levels.

Reconsideration of the mechanism of progression to CRPC from androgen-naïve

PCa

We would like to consider a mechanism whereby androgen-naïve PCa progression to CRPC at a little more macro level (not molecular level) on the basis of clinical aspects.

PSA is an androgen responsive gene with a promoter that contains 3 androgen-response elements (AREs). An ARE at 4.1 kb upstream of the transcription initiation site is an extremely strong enhancer that reacts with AR [46, 47]. Basically, the PSA gene is regulated merely by androgens and is regulated independently from cell proliferation. Therefore, ADT should cause a rapid decrease of the serum PSA level for one or two months in most PCa patients since the pretreatment concentrations of serum androgen are at less than a one-tenth of the pre ADT levels and can hardly activate AR, and AR cannot bind AREs of the PSA promoter even if PCa cells are not killed by ADT (PSA-androgen response phase) (Figure 5). A few months after initial treatment, many clinicians experience that the serum PSA level gradually decreases for a while (Tumor regression phase). ADT then regulates apoptosis-related genes and cell cycle-related genes through direct or indirect pathway during that period

[48]. Therefore, PSA decreases according to the number of surviving cells which is gradually decreasing. Then, the serum PSA level becomes stable so that the number of cells that are going to die and cells that are going to reproduce again equilibrates (Proliferation quiescent phase). Finally, PCa cells activated during the period of ADT begin to reproduce again (CRPC phase).

As described above, when looking at the immunohistochemistry of AR in advanced PCa, AR expression is heterogeneous: even if there are many PCa cells overexpressing AR, the cells in which expression of AR decreases in PCa tissue. Accordingly, we have classified PCa cells into four types depending on functions of androgen: androgen-dependent cells, androgen-sensitive cells, androgen-hypersensitive cells, and androgen-independent cells (Figure 6A). Androgen-sensitivity and the ratio of each cell population should be different in each PCa patient. When first line ADT is applied, androgen-dependent cells will be killed and the number of androgen-sensitive cells will decrease, and then serum PSA decreases according to the ratio of cell type and AR activity (Figure 6B). However, androgen-hypersensitive cells and androgen-independent cells will survive in the presence of the castration level of

androgens in the serum. In some situations, androgen-sensitive cells may adapt themselves to the environment of low androgen concentrations and change into androgen-hypersensitive cells or androgen-independent cells (Figure 6B). Thus CRPC develops more and shifts to CRPC threatening life whereas what kind of cell types contributes to a development of CRPC predominantly is different in each patient (Figure 6C).

In this aspect, clinicians have to pay attention to the reason why serum PSA level is elevating in CRPC during ADT. It was difficult to monitor the longitudinal change of bone metastases until recently. However, the concept of measuring the quantitative level of bone metastasis by bone scan (Bone Scan Index, BSI) was developed [49]. Further, the software program for calculating BSI using the neural network system has also been developed using whole-body images with a Swedish database [50]. A Japanese multi-center database based on this software program significantly improved the diagnostic accuracy for estimating the quantitative level of bone metastasis [51]. Moreover, the changes in BSI have shown a close relationship with all bone metabolic markers but not with the serum PSA, and the BSI was

confirmed to reflect the activity and extent of bone metastases [52]. We have measured the BSI calculated from bone scan of several patients who were treated with ADT (LH-RH agonist and bicalutamide) and show one case in Figure 7. Baseline serum PSA and BSI of the patient were 2163 ng/ml and 3.9%, respectively, and those declined to 3.2 ng/ml and 0.12% within one year under ADT, respectively. However, the serum PSA level has begun to gradually increase from 15 months of initial treatment, and reached 41.8 ng/ml at 20 months. Although the serum PSA level was still a one-50th of baseline at this point, BSI worsened to a level almost the same as at baseline (4.3%). What does this phenomenon mean? When PCa cells progresses to CRPC, it is considered that the serum PSA level is elevated for two mechanisms mainly. As mentioned above, one mechanism is involvement of the AR-related pathway. Especially, androgens of adrenal origin and their metabolites act on surviving PCa cells that became androgen-hypersensitive by some mechanisms and secretes PSA. In this pathway, strategies that block AR function, such as total androgen deprivation (TAD, castration, blocking adrenal androgen synthesis, and antiandrogens of a new generation) should be effective. Another mechanism is just a tumor volume effect (cell number effect). Even if

one PCa cell hardly secretes PSA (minimum basal level), the serum PSA level as all total amounts should increase if the number of PCa cells increases markedly. In this pathway, TAD will not be effective anymore because the basal PSA expression is not regulated by AR. Instead, chemotherapy or molecular target therapies may become strategies to control androgen-independent CRPC. In the patient of Figure 7, bone metastases suddenly worsened as compared with the increase of serum PSA level, thus, suggesting that the tumor volume effect is the main factor for progression to CRPC. How can clinicians distinguish AR-related pathways and tumor volume effect? One possibility is to conduct alternative antiandrogen therapy when serum PSA level begin to increase again [53]. Overall, PSA significantly decreased (50% or greater) in 83 of the 232 patients (35.8%) and also, a partial PSA response (PSA decrease 0% to 50%) to alternative antiandrogen therapy was observed in 59 of the 232 patients (25.4%). It will be useful to examine responsiveness of serum PSA by the alternative antiandrogen therapy in order to ascertain whether progress to CRPC passes through an AR-related pathway or not. If serum PSA level decreases by the alternative antiandrogen therapy, TAD should be the best way to decline serum PSA level and stabilize the symptom of

the patients for a long time.

Conclusion

It is considered that PCa develops to CRPC during ADT by various genetic actions, but the mechanism playing a key role is the AR-related pathway. PCa cells mainly adapt themselves to the environment of low androgen concentrations and change into androgen-hypersensitive cells or androgen-independent cells. Androgens of adrenal origin and their metabolites synthesized in an intracrine fashion act on surviving PCa cells that became androgen-hypersensitive. Total androgen deprivation (TAD) can become an effective therapeutic strategy to control the androgen-related pathway. Clinically, it is important to ascertain whether elevation of serum PSA results from AR activation or from androgen-independent tumor volume effect.

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Legends

Figure 1 Mechanisms of recurrence focusing on AR during ADT

Blue and purple shapes represent AR-independent pathway and AR-mediated pathways, respectively. The two pathways sometimes overlap.

Figure 2 Up-regulation of DHEA-induced AR activity (A) and activation of DHT biosynthesis by coculture of LNCaP cells with normal prostate-derived stromal cells (PrSC) and PCa-derived stromal cell (PCaSC-8) (B).

Figure 3 Intracrine and paracrine androgen biosynthesis in PCa tissue after ADT

Figure 4 Effect of dutasteride (dual SRDA5 inhibitor) on far advanced CRPC patients in Kanazawa University Hospital

Figure 5 Serum PSA change by hormonal therapy and CRPC

When PCa cells develop in CRPC, serum PSA elevation results from 2 reasons mainly:

involvement of hypersensitive AR and androgens of adrenal precursor origin and tumor volume effect.

Figure 6 Hypothetical mechanisms of development in CRPC from androgen-blockade-naïve PCa.

A. Before ADT. Four kinds of cell populations can exist in PCa tissue.

Androgen-independent cells grow very well regardless of the presence or absence of AR.

The ratio among these kinds of cells can be different in each patient. **B.** After ADT.

Three kinds of cell populations remain in PCa tissue including metastasis sites. **C.**

State of CRPC. Two kinds of cells remain in CRPC tissue. The effect of treatment changes according to which cell population is present predominantly in CRPC tissue.

Figure 7 Result of serum PSA and bone scan index by bone scan

A. Normalized bone scan. **B.** Graph of serum PSA and bone scan index. Flu (flutamide), EMP (estramustine phosphate).

Fig. 1

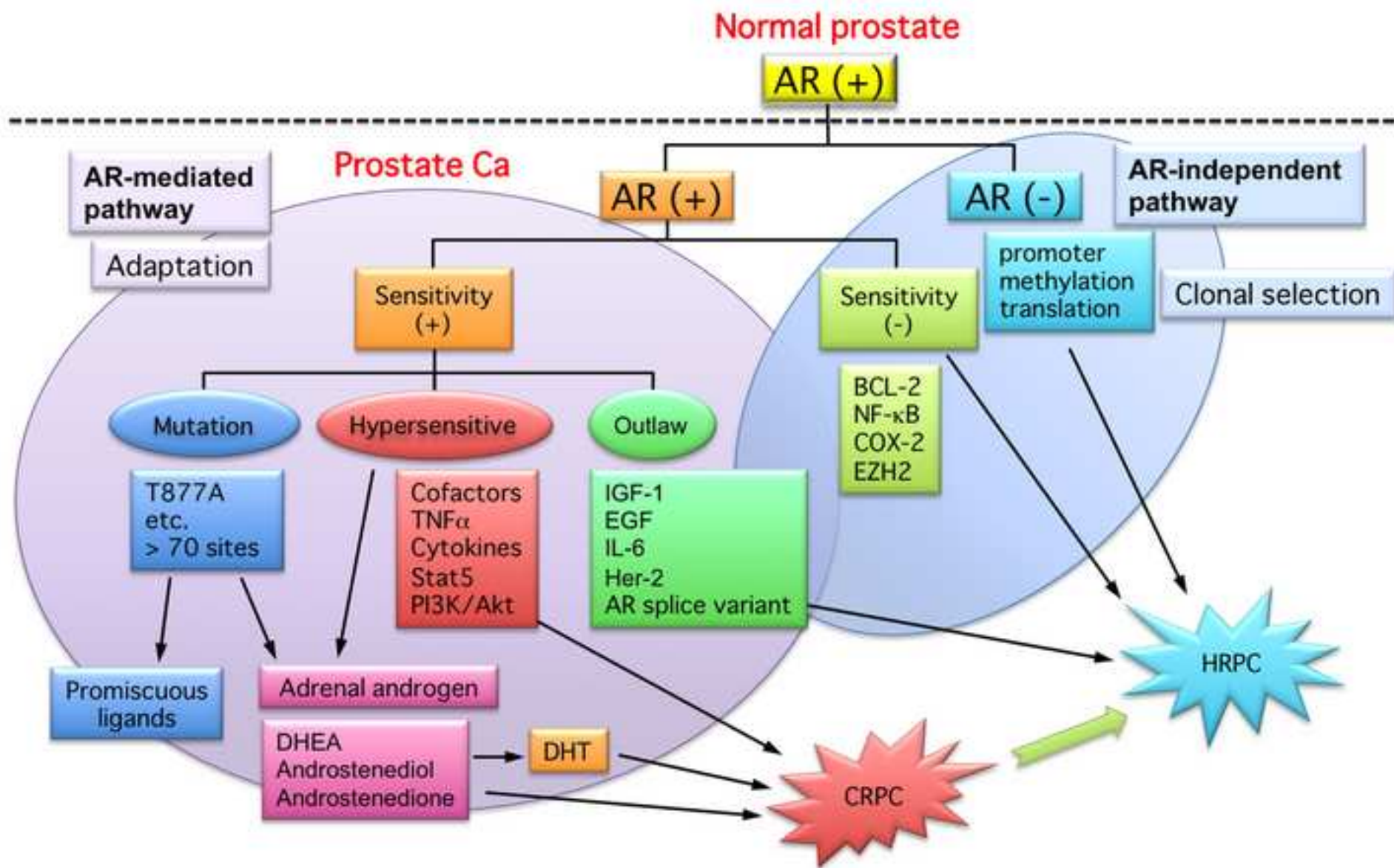


Fig. 2

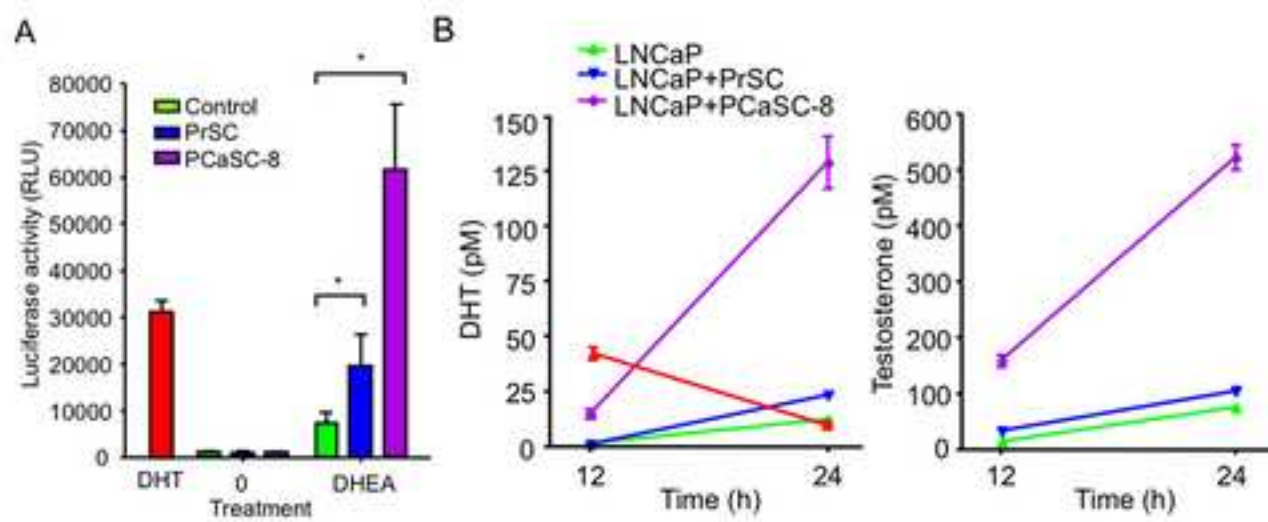


Fig. 3

Intracrine and paracrine androgen synthesis

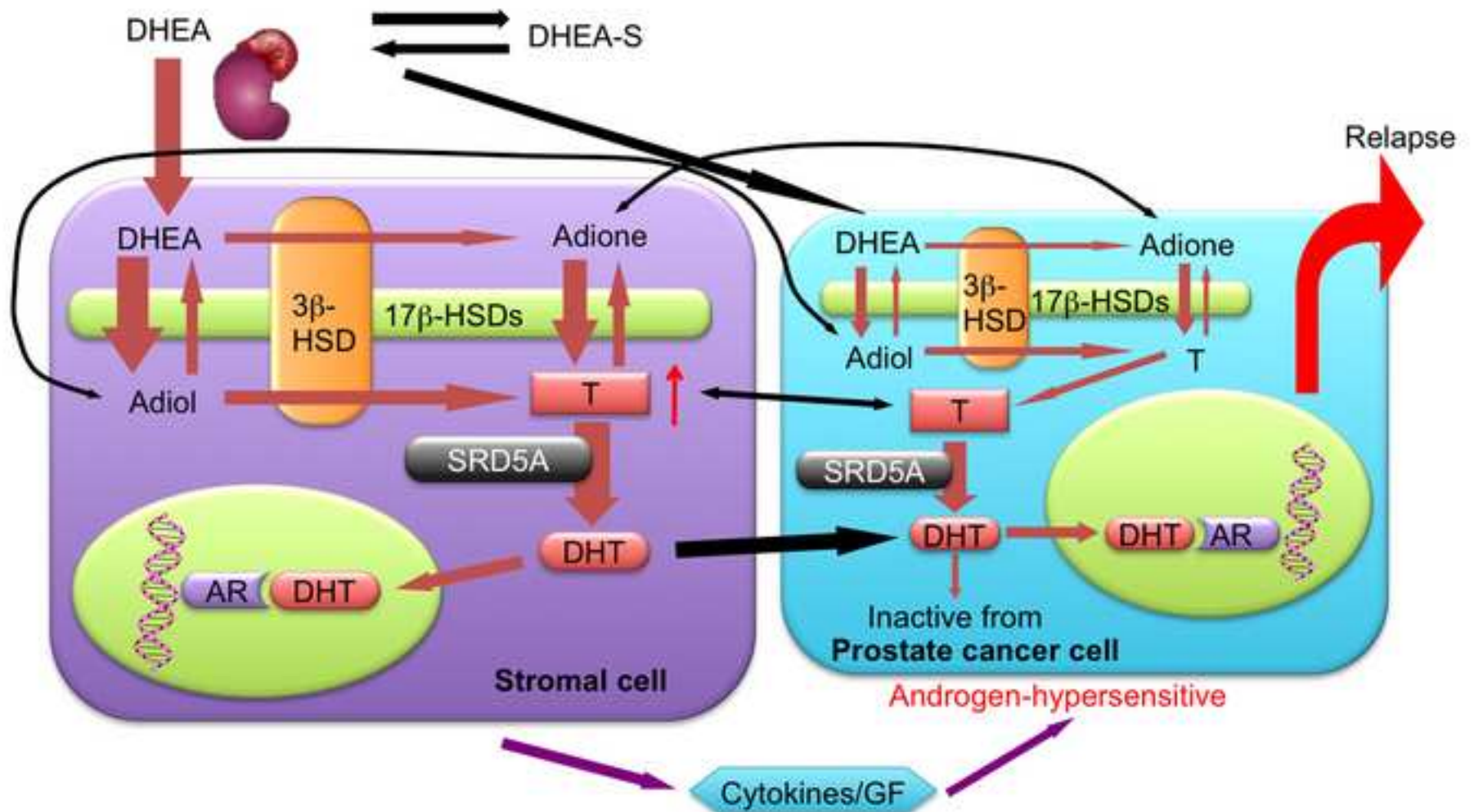
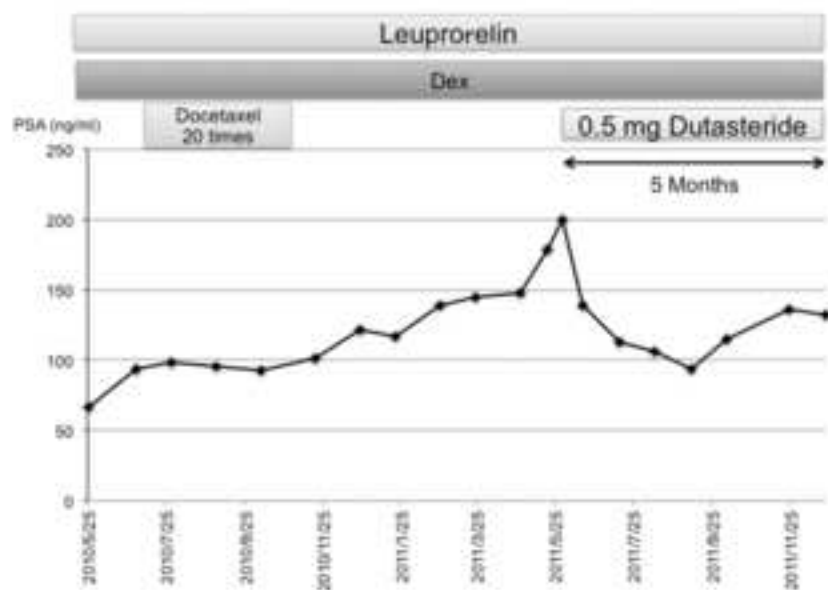


Fig. 4

A



B

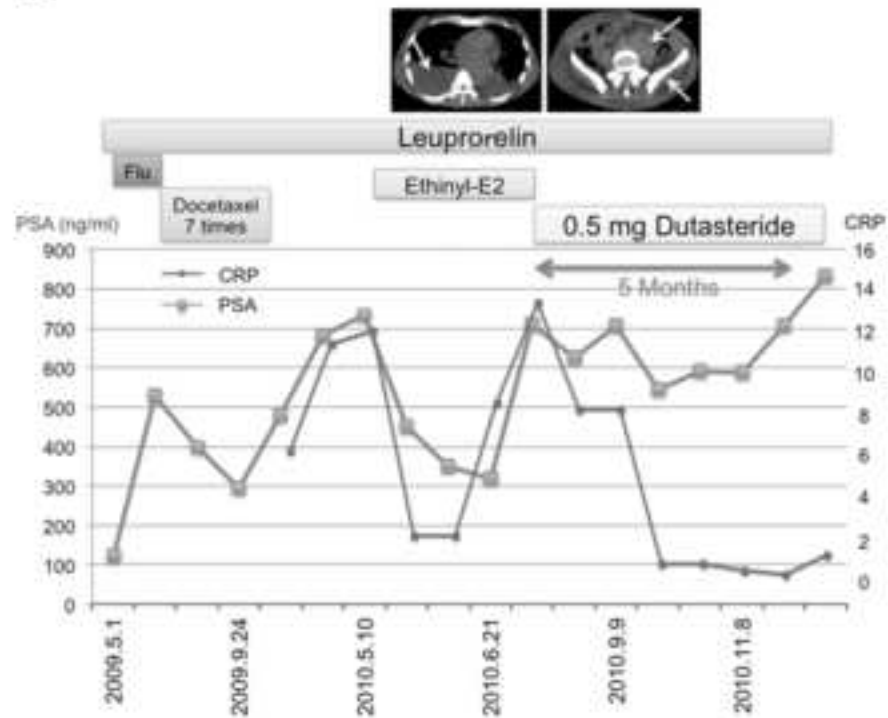


Fig. 5

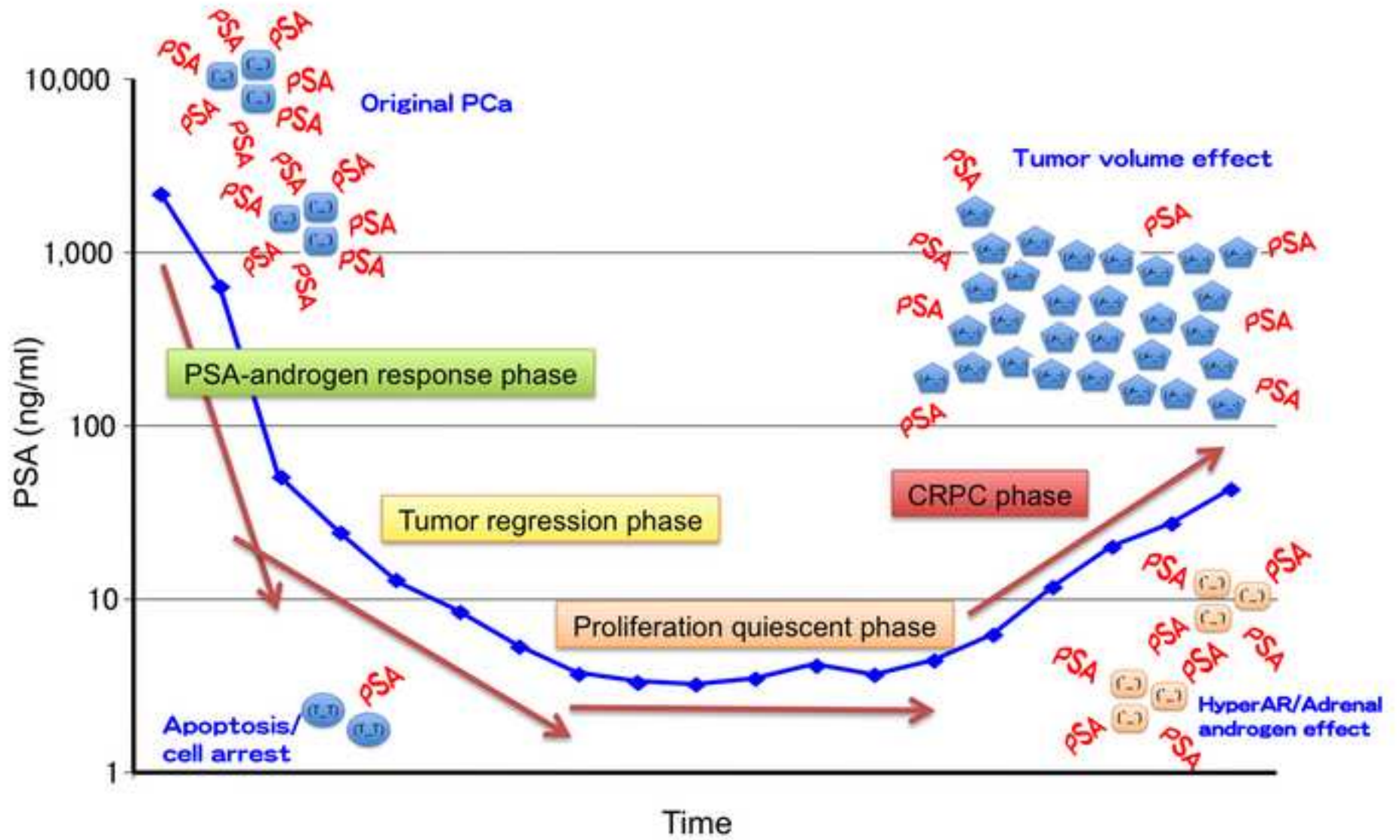


Fig. 6

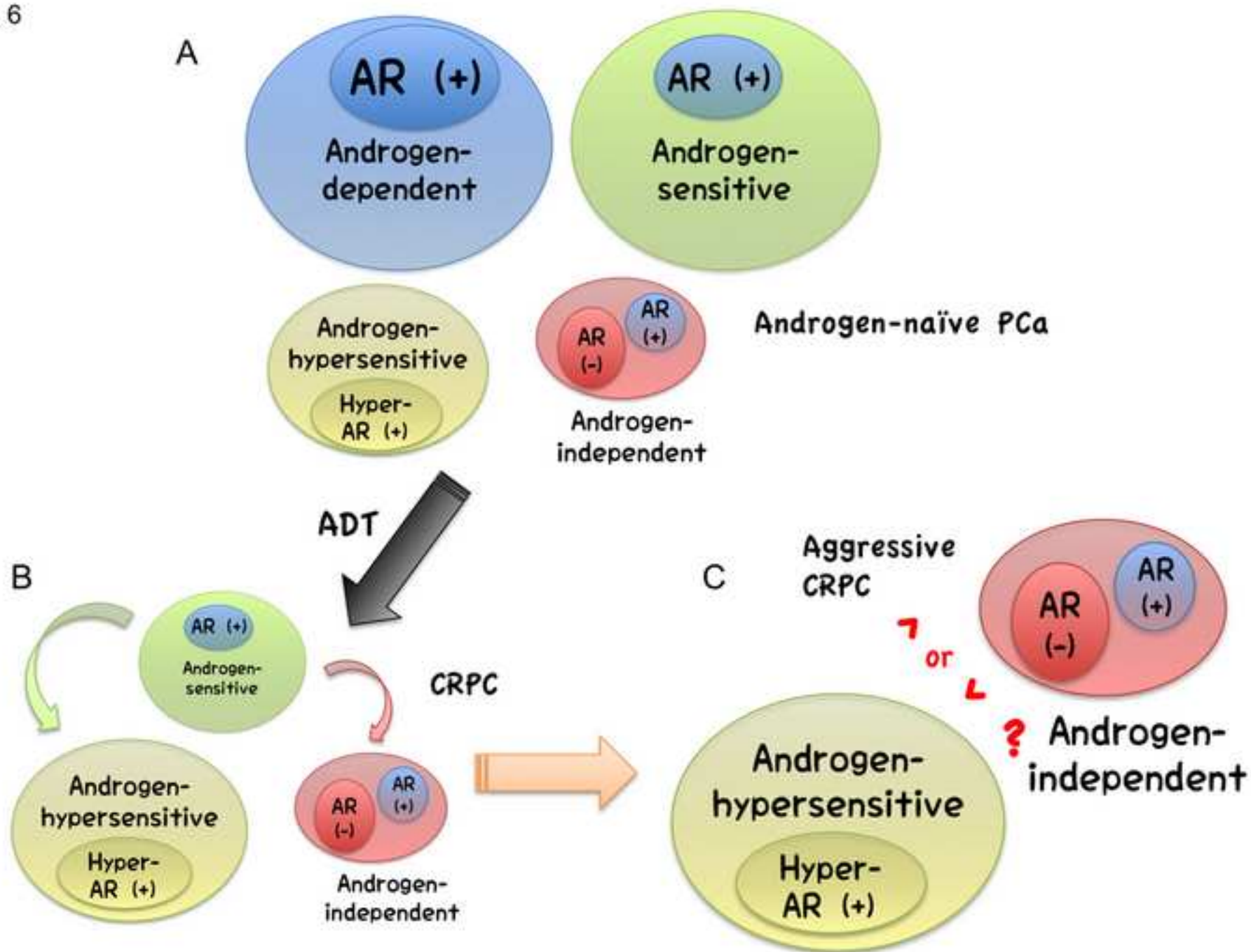


Fig. 7

