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Overview

Pathophysiology of hormone-resistant prostate cancer

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Key words: metastatic prostate cancer, androgen receptor (AR), castration-resistant prostate cancer (CRPC), androgen deprivation therapy (ADT)

Abbrevations:
AR = androgen receptor
CRPC = castration resistant prostate cancer
PC = prostate carcinoma
ADT = androgen-deprivation therapy

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Abstract

Androgen-deprivation therapy (ADT) has been for many years the cornerstone of metastatic prostate carcinoma (PC) treatment. It is very well documented that the majority of cancer cells in prostate gland remain androgen responsive what was the basis for androgen suppression in treatment. Nevertheless, after some time or sometimes initially, PCa cells become resistant to castration serum level of testosteron, because of some genetic and epigenetic changes that make them independent from hormone activation. There are two therories – the clonal theory and the adaptation theory of how some carcinoma cells become castration-resistant. It has been shown recently, that PCa cells can still use androgen receptor as a major signaling pathway, by activation of enzyme machinery or de novo production of androgen within the cells.

INTRODUCTION

Prostate cancer is the second most common cancer in male in Croatia and the third most common cause of death by cancer (Figure 1) *(1).* In many western countries due to earlier detection and widely used PSA in screening, prostate cancer became the most common cancer in male. Although a great progress has been achieved in the last two decades regarding screening, early detection and treatment, a large number number of patients eventually have advanced (metastatic) disease. One of the most crucial discoveries in the area of hormone treatment of tumors generally was the one of Charles Huggins. Owing to his work we understood androgen deprivation in prostate cancer which became the cornerstone of hormonal treatment until nowdays *(2).* Nevertheless, it was indicative the comment dr Huggins put in his Nobel Prize lecture in 1966: „....*it is obvious that there are many failures of endocrine therapy to control the disease*". Today, it is very well known and experienced that despite inducing a regression of a large magnitude, androgen deprivation therapy (ADT) is usually followed by regrowth of the tumor. This phenomenon is known as androgen independence (AI) or, recently, the term castration resistance prostate cancer has been introduced (CRPC) *(3).*

Initially, the progression of prostate cancer in castration setting was not clearly understood. However, two different theories have emerged to explain the mechanism by which prostate cancer cells become androgen-insensitive. The first one is assuming the existence of pre-existing clones of CRPC cells that escape any hormonal manipulation at any time (*clonal selection theory*); the second theory, known as *adaptive the-*

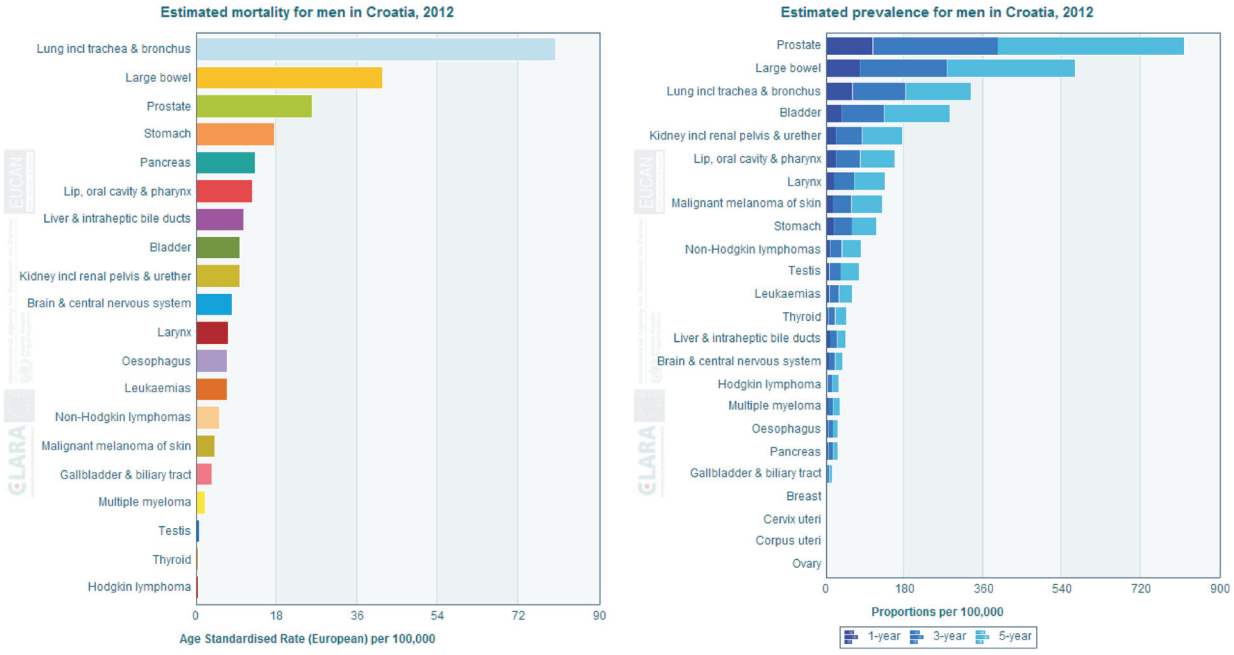

Figure 1. *Estimated mortality and prevalence of cancers for men in Croatia, 2012 (EUCAN).*

ory, postulates the existence of subsequent phases of up- and downregulation or mutations of important genes that enable cancer cells to survive and/or regrow after ADT. We are aware that these two theories coexist in prostate cancer patients (Figure 2) *(4)*.

Androgen dependence of normal and cancerous epithelial cells

The growth and maturation of the normal prostate is dependent on the secretion of androgen by testis. The normal prostatic epithelium is made of different compartments, with different levels of AR expression and therefore different sensitivities to androgens *(5)*. Embryonically, prostate develops from the male urogenital sinus under the stimulation of androgen. This process is under controll of tumor stroma.

Androgen receptors (ARs)

The regulatory effect of androgens is mediated through the androgen receptor (AR). AR is located on X chromosome, spanning about 180 kD od DNA, and is a member of the nuclear steroid hormone receptor super family. It

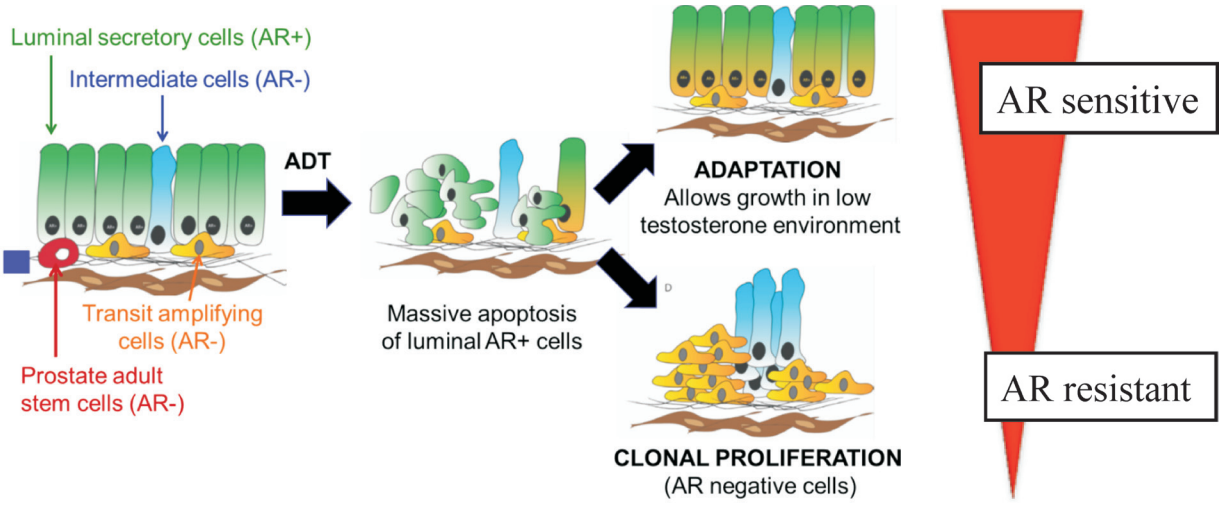

Figure 2. *Two theories explaining mechanisms of CRPC-cells.*

consists of 4 domains: N-terminal domain (NTD), a highly conserved DNA-binding domain (DBD), a short hinge region and a moderately conserved C-terminal domain, the ligand-binding domain (LBD) *(6)*. The DBD contains a classical two zinc-finger motif allowing recognition and binding to specific DNA sequences known as Androgen Response Elements (AREs). The binding of the AR to AREs allows the recruitment of AR co-activators and/or co-repressors that regulate the transcriptional machinery by direct physical interaction with general transcription factors and RNA polymerase, by facilitating AR/ARE binding and chromatin remodelling, and by changing AR folding and AR subcellular localisation *(7)*.

Regulation of normal epithelium by androgens

A specific subset of smooth muscle cells expressed both the 5α-reductase (5AR) enzyme and the AR. The 5AR transforms the low level of cirulating testosteron (T) into dyhidrotestosteron (DHT), which stimulates the AR 10 times more than T. DHT binding to the AR stimulates the secretion of paracrine and autocrine growth factors, known as andromedins, such as IGF-1, FGF-7 and -10, and VEGF. These andromedins activate the formation of blood vessels and the maturation of undifferentiated epithelium into a simple stratified glandular circular epithelium composed of a basal layer of cuboidal cells making the epithelial components of the basement membrane separating the epithelium from the stroma. A second layer of columnar secretory-luminal cells forming a glandular lumen is attached to these basal cells. During puberty, under the T serum rise, prostate is maturing into its definitive architecture. After that, because of reciprocal positive feedback loop between stromal and epithelium cells, the epitelial compartment enters a steady-state maintanance phase in which the rate of epithelial proliferation balances the rate of death, such that neither overgrowth nor regression of the gland normally occurs *(8)*.

Different levels of AR expression and therefore different sensitivities to androgens exist in normal prostate epithelium. *Prostate adult stem cells* (PAS) are present in the very small proportion (less than 5%), constituing basal compartment directly separated from the stromal compartment. Nearby, there are also *transit-amplifying* (TA) cells which are progenitor cell type that undergoes a limited number of proliferative replications before terminal differentiation. As in the case of PSA, TA cells do not express AR and are dependent on andromedins produced by stromal cells. After a limited lumber of cell divisions, TA cells mature into *intermediate cells* that express cytokeratins 5, 8, 15 and 18, Prostate Stem Cell Antigen (PSCA), and AR mRNA, but not yet AR protein. Intermediate cells are migrating in the *luminal-secretory layer*. In these cells, AR activation promotes differentiation int secretory cells that will produce prostate-specific antigen (PSA) and other secretion products. AR activation plays, unexpectedly, anti proliferative role in these luminal-secretory cells, by upregulating expression of cyclin-dependent kinase inhibitors p21 and p27. Thus, luminal secretory-cells represent the terminal stage of maturation of hierarchical expanding stem cell units and are quantitatively far the major epithelial genotype in the gland, even though they are non-proliferating.

There has been an intense search to identify the specific epithelial cell subtype in which the initial carcinogenic process could initiate. In two common prostate cancer-precursors, high-grade PIN and prostatic inflam-

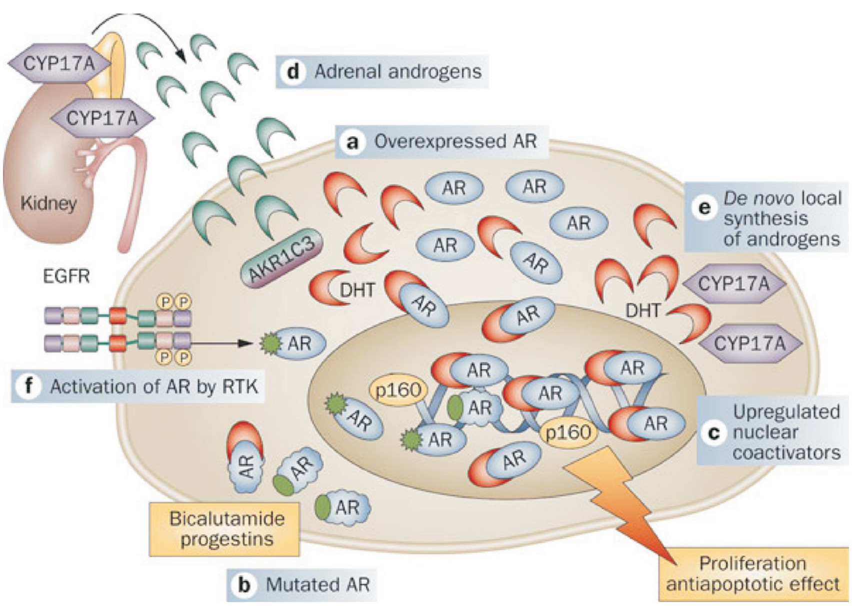

Figure 3. *Mechanisms of castration-resistance.*

matory atrophy (PIA), high levels of AR have been found *(9)*. That means, obviously, that AR is during early phase of carcinogenesis converted from a growth suppressor gene to an oncogene. As the consequence, AR-gain-of-function allows activation of different molecular signaling pathways stimulating the proliferation and survival of these initiated prostatic cells directly *(10)*.

Physiological basis of androgen suppression

In normal physiologic conditions, prostatic cell proliferation is balanced by an equal rate of prostatic cell death such that neither involution nor overgrowth of the gland my occur with time. Following castration, there is rapid decrease of serum T below critical value resulting in loss in the glandular epithelial cells that are androgen dependent and undergo apoptosis *(11)*. By 12 to 24 hours post-castration, the intracellular DHT levels are about 5% of normal, leading to changes in nuclear AR function. This mechanisms is based on stromal-epithelial interactions since it requires paracrine activity of stromal AR, thus leading to decrease of andromedins below critical level *(12)*. Some of andromedins are explained as modulators of DHT-dependent survival, such as the keratinocyte growth factor (KGF), and transformin growth factor β-1 (TGF β-1) *(13,14)*. Once levels of andromedins decrease below critical level, apoptosis is activated. Some authors demonstrated that the apoptosis is activated in prostate cancer cells, even if they are not proliferating. On the other side, in PC cells that are AR-independent apoptosis can not be activated *(15)*.

How do cells progress in a low testosterone environment?

The phenomenon named hormone resistance or androgen independence, nowdays used term castration resistance, means the loss of ADT effect after certain time, in the absence of circulating androgen. It was already described by Huggins, and today this term include two different clinical scenarios. One includes „adaption" model, which means that CRPC cells arise through genetic/epigenetic conversion of previously androgen dependent cells during conditions of ADT. The alternative model is known as „clonal selection", suggesting proliferation of quiscent population of rare castration-resistant cells within an otherwise androgen.dependent tumor. We know that progression to CRPC is a very heterogeneous process between patients and within a patient's own cancer. There are many changes in protein and gene regulation that in part might explain the complex process of castration resistance (Figure 3).

The clonal theory

There are evidences that ADT induces selective outgrowth of aggressive hormone-refractory PCa clones expressing distinct cellular and molecular properties not present in parental androgen-dependent cancer cells *(16)*.

A pool of androgen-independent cells. CRPC may originate from a long-term resident pool of PSA cells and TA cells that are insensitive to AR pathways. The cancer stem cell model is consistent with the observed phenotypic heterogeneity found in many tumors, including prostate adenocarcinoma. Specific features of neuroendocrine carcinoma could be also explained in that sense. Neuroendocrine cells are rare and originate from PAS or intermediate cells located in the luminal layer of the epithelium together with the secretory cells *(17)*. Neuroendocrine cells produce a number of growth factors, including serotonin and bombesin, which promote the growth via paracrine mechanisms. Neuroendocrine cells are AR independent and there is no apoptosis induced by ADT *(18)*.

Acquired mutations in androgen-dependent PCa cells to become androgen-independent. During the process of carcinogenesis, CRPC clones may appear from acquired mutations. There are many pathways to be involved in such mechanism, like *TMPRSS2-ETS* rearrangements, *PI3K/AKT/PTEN* pathways, and *BCL-2*.

PI3K/AKT/PTEN pathway has been implicated in CRPC. PI3K are involved in the phosphorylation of membrane inositol lipids, mediating cellular signal transduction *(19)*. One of the most important downstream proteins is mTORcomplex 1, a serine/threonine kinase that plays a critical role in protein synthesis, angiogenesis, and cell cycle progression. AKT is negatively regulated by tumor suppressor protein PTEN, which dephosphorylates PIP3. Several observations suggest that PI3K/AKT/PTEN signaling pathways are playing an important role in CRPC by helping cells to maintain continued proliferation in low-androgen environments. Functional loss of PTEN is associated with increased AKT-1 phosphorylation and appears to be involved in resistance to castration *(20)*. Immunohistochemical studies have indicated a loss of PTEN expression in 79% of CRPC samples *(21)*. Furthermore, about 30% of the chemo-naive patients and 24% of the chemo-resistant patients had homozygous or heterozygous loss of PTEN *(22)*.

BCL-2 in an antiapoptotic gene, and its overexpression might mediate development of CRPC. It is expressed in CRPC, but usually not in secretary prostate epithelial cells. Some IHC studies demonstrated increased BCL-2 expression in CRPC specimens compared to hormone-naive prostate cancer tissues *(23)*.

The adaptation theory and the role of AR

The most important argument in favour of the „adaptation" model is the evidence of retention of AR signaling in CRPC. Even when PCa progresses to CRPC, AR activation and signaling remains sustained through a variety

of mechanisms *(24, 25).* Several mechanisms have been proposed to explain the reactivation of an AR-dependent pathway: amplification of AR gene copy, gain-of-function mutations of AR, deregulation of the AR/co-regulator/co-respressor control, intracellular steroidogenesis and ligand-dependent activation of AR.

Amplification of AR gene. Amplification of AR-gene copy number means higher sensitivity and responsiveness to low levels of circulating or intracellular androgens, resulting in survival and growth advantage upon castration. It is seen in about 30% of CRPC *(26).* It is more likely that cells with an AR gene amplification will respond to ADH several times better after progressing. It is also interesting, that increased AR levels can convert an antiandrogen from na AR antagonist to an AR agonist, what is known mechanism of antiandrogen-withdrawal response *(27, 28).*

Gain-of-function mutations of AR. Several mutations have been identified that may confer increased AR protein stability, greater sensitivity to androgens, aberrant responses to antiandrogens or other steroid hormones, ligand-independent activity, or increased recruitment of AR co-activator proteins *(29).* In some cases, mutated AR may lack the entire LBD, leading to an auto-activated mutant that mediates AR signaling independent of any ligands. That means, that such an AR can be constitutively active *(30).*

Deregulation of the AR/co-regulator/co-respressor. Some ligands can affect receptor activity at the transcriptional level, with subsequent effects on target gene regulation. AR overexpression increases the expression of androgen-stimulated co-activators such as MAK, BRCA1, AIB1, or CB *(29).*

Intracellular steroidogenesis. One of the most exciting observations in the last several years is the ability of CRPC to synthesise androgen de novo or convert adrenal androgens into T and DHT by expressing or up-regulating steroidogenic enzymes including CYP17A1, HSD3B1, CYP19A1. It was observed almost 30 years ago that adrenal androgenes could induce AR signaling after intraprostatic conversion despite low levels of circulating testosterone *(31, 32).* It was also discovered that CRPC cells were capable of synthetising de novo androgens from membranes' cholesterol molecules. T levels within metastases can be several times higher than levels in the primary untreated PCa *(33).*

Ligand-dependent activation of AR. The activation of different signal transduction pathways in CRPC cells can enhance the activity of the AR or its co-activators in the presence of low levels or even in the absence of androgens. In CRPC there are many molecules overexpressed leading to AR stimulation – for example, IL-6, KGF, EGF, IGF-1 etc. *(34).*

CONCLUSION

CRPC is one of the greatest challenges in the treatment of advanced prostate cancer. Although ADT is still the golden standard of the treatment of PCa, treatment paradigm is changing due to new insights in pathophysiology of prostate cancer. Today, we already use our knowledge of AR-active signaling pathway even after progressing prostate cancer, in treatment prostate cancer patients, before or after chemotherapy. In the future, hopefully, we'll be able to inhibit growth of prostate cancer cells based on more individual base than it is nowdays.

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