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Journal of Steroid Biochemistry & Molecular Biology 92 (2004) 255–264
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Alterations of androgen receptor in prostate cancer

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Abstract

The significance of androgens in the development of prostate cancer has been known for more than half century. During the last decade, a lot of effort has been put to study the significance of the specific nuclear receptor of the hormone, androgen receptor (AR). It has been suggested that polymorphisms, especially the length of CAG repeat in exon 1 of the gene, are associated with the risk of prostate cancer. However, not all studies have confirmed the association. Most surprisingly, it has now become clear that prostate carcinomas emerging during the androgen withdrawal therapy (i.e. hormone-refractory tumors) are capable of reactivating the AR-mediated signalling despite of the low levels of androgens. In addition, it has been shown that AR gene itself is genetically targeted. One-third of the hormone-refractory prostate carcinomas contains amplification of the gene. In addition, 10–30% of prostate carcinomas treated by antiandrogens acquire point mutation in the AR gene. The genetic alterations in AR indicate that receptor should be considered as putative treatment target. Evidently, the currently available antiandrogens are not capable to abolish the AR-mediated signalling efficiently enough.

Keywords: Prostate cancer; Androgen receptor; AR; Amplification; Mutation; Predisposition; Polymorphism

1. Introduction

The growth of prostate cancer is highly dependent on androgens. And, already in early 1940s, Huggins and Hodges showed that castration is effective treatment in prostate cancer [1]. Subsequently, hormonal therapy has become the standard therapy for advanced stage of the disease. More than 90% of patients show biochemical response to the therapy [2], and clinical response rates of 80% have been reported [3]. However, during the therapy, hormone-refractory tumor cells eventually emerge leading to clinical progression. Since there are no effective treatments for hormone-refractory prostate carcinoma, the prognosis after progression is poor. The average survival time of patients with hormone-refractory prostate cancer is only about six months [3].

Androgen action takes place through a specific nuclear androgen receptor (AR). Thus, it is natural that the role of AR in the development and progression of prostate cancer has widely been studied. Especially, the significance of AR in the development of hormone-refractory prostate cancer has become evident during the last decade. Although, it was earlier believed that other than androgen-related signalling pathways become the primary growth stimulatory factors in recurrent prostate cancer evidence indicating that actually AR-mediated signalling pathways are reactivated during the progression of the disease has mounted up [4]. In this review article, the alterations in the AR during the development and progression of prostate cancer are discussed in details.

2. Androgen receptor

Androgen receptor is a member of steroid hormone receptor transcription factor superfamily. The activation of AR from inactive, chaperone–protein bound state requires the binding of androgens, which induces a conformational change in the receptor structure. That leads to dissociation of chaperone proteins and receptor dimerization. In the nucleus, dimerized receptor complex regulates the transcription of target genes by binding to its response element in DNA [5,6].

AR activates the expression of target genes and gene networks by facilitating transcriptional initiation. In general, androgen receptor mediated transcription requires several

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doi:10.1016/j.jsbmb.2004.10.012

auxiliary protein complexes. Such proteins are categorized into co-activators and co-repressors. Their function and significance in the prostate cancer are discussed in the review article by Culig et al. [7].

Androgens (like progesterone and estrogen) can also exert effects, which are considered to be nongenomic because they can occur in the presence of transcription inhibitors or they are too fast occurring to involve changes in gene transcription [8]. The nongenomic actions include stimulation of mitogen-activated protein kinase (MAPK) pathway via c-Src tyrosine kinase and induction of cAMP second messenger and protein kinase A (PKA) due to the SHBG binding to its receptor and testosterone [8]. In addition, the existence of a novel, cell membrane bound androgen receptor has been suggested [9], but remains to be identified.

Human AR gene is located in chromosome Xq11–12, and it contains eight exons [6]. Transcription of the gene can occur from two different initiation sites, producing two AR transcripts (10 and 7 kb) [10,11]. The gene is almost universally expressed in different tissues [12]. The transactivation domain of AR, containing the ligand independent activation domain AF-1, is encoded by the large first exon [13,14]. Transactivation domain contains also a few homopolymeric amino acid repeats typical for many transcription factors. The most aminoterminal is polyglutamine (Q) repeat, coded by CAG triplets. Like other genes with CAG repeats, the repeat length is very polymorphic, ranging from 14 to 35 [15]. Lengthening of the repeat to 40–62 results in an inherited neuromuscular degenerative disease, Kennedy's disease or spinal and bulbar muscular atrophy, SBMA [16]. The other amino acid repeat encoded by exon 1 is the polyglysine (GGN) repeat, the function of which has remained unclear. The most common glysine repeat allele is 16 repeats [17].

Exons 2 and 3 in the AR gene encode for the DNA binding domain. The amino acid sequence of this domain is most highly conserved region among members of nuclear receptor superfamily. It includes two structures, referred as zinc fingers, which have been shown to be fundamental to the binding to the response element in DNA [18]. The first zinc finger harbors the information for specific recognition of DNA, and the second finger stabilizes the DNA-receptor interaction in contact with DNA backbone [6].

The third domain structure in AR is the carboxy-terminal ligand binding domain, which is encoded by the exons 4–8. It contains the ligand-dependent transactivation function AF-2. As the transactivation function of AR is normally androgen dependent, LBD domain prevents the action of the receptor without the ligand. Deletions in this domain abolish the binding of androgen, which results as constitutive activity of the AR [19].

3. AR in prostate cancer

Several alterations take place in the AR signalling pathway during the development and progression of prostate cancer. First, the action of AR in normal and malignant prostate uses distinct pathways. In normal prostate gland, androgen stimulated proliferation of epithelium requires paracrine involvement of stromal cells expressing AR. In malignant cells the androgen mediated signalling has been converted to autocrine mode and no interaction with stroma is needed [20]. Second, it has been shown that many of the androgen regulated genes become up-regulated during the progression of the disease to a hormone-refractory state [21,22]. Recently, Hara et al. [23] were able to establish a hormone-refractory subline of MDA Pca 2b prostate cancer cell line which showed increased expression of AR and retained androgen-sensitivity. These observations are consistent with the clinical data that prostate cancer patients with hormone-refractory disease benefit from maintenance of androgen withdrawal [24].

Except of the rare small cell form, all untreated prostate carcinomas express AR [25,26]. The expression level does not seem to be associated with phenotype (i.e. histological grade or clinical stage). Somewhat surprisingly, it has now become clear that the expression of AR does not reflect response of the tumor cells to androgen withdrawal since majority of the hormone-refractory prostate carcinomas express AR [26–28]. We and others [29–31] have recently shown that the expression of AR is actually increased in the hormone-refractory compared to the untreated carcinomas. Only in small fraction of hormone-refractory disease the expression of AR is abolished, possibly through hypermethylation of the AR promoter [32]. It is important to notice the fundamental difference between the hormone-refractory forms of breast and prostate cancers. In breast cancer, the loss of responsiveness to the hormonal therapy is associated with the loss of expression of the estrogen receptor (ER) [33], whereas in the prostate cancer the expression of AR is increased during the failure of hormonal therapy. Recently, Chen et al. [34] showed that even a subtle overexpression of AR was sufficient to convert the growth of prostate cancer xenografts from androgen-sensitive to hormone-refractory stage. The progression of the hormone-refractory xenografts was ligand-dependent, and due to the genotropic (nuclear) action of AR. This seminal study, once again, underlined the central role of the AR in the progression of prostate cancer.

4. Genetic alterations in AR

Genetic aberrations underlie the development of malignancies. Some of the alterations maybe be inherited, thus predisposing individual to a cancer. And, most importantly somatic genetic aberrations are the fundamental mechanisms of tumorigenesis. Twin studies have suggested that maybe up to 40% of risk of prostate cancer could be explained by hereditary factors [35]. One of the most intensively studied putative predisposing genes is AR. In addition, somatic mutations in the AR have also been widely analyzed. The following chapters summarizes the current knowledge of AR as susceptibility gene as well as a target for somatic mutations.

4.1. Germ-line alterations of AR

Table 1 summarizes the studies that have evaluated AR as a prostate cancer susceptibility gene. For example, it has been suggested that the short CAG repeat may result in increased risk of prostate cancer and that the length of the repeat could also partly be responsible for the difference in prostate cancer risk in different racial groups [14,36]. For instance, Giovannucci et al. [37] observed that shorter repeat was associated with increased risk for advanced and clinically significant prostate cancer. Short CAG repeat length has also been reported to correlate with young age at diagnosis [38]. However, these observations have not been confirmed by several recent studies [39–47].

The biological significance of polyglycine (GGN) repeat in exon 1 is less clear. Nevertheless, some studies have proposed that the size of glycine repeat might increase the risk for prostate cancer [14,37,48]. A recent study by Chang et al. [45] suggested that alleles of ≤16 GGC repeats are associated with risk of prostate cancer. However, several studies have not found such an association [43,44,49].

Germ-line point mutations in AR gene are not commonly associated with prostate cancer, but they are occasionally found. In Finnish population, Arg726Leu substitution has been reported to increase the risk for prostate cancer [50], but this observation was not confirmed by the study done with North American population [51].

4.2. Somatic aberrations of AR gene in androgen-dependent prostate cancer

Most early studies (Table 2) found only a few somatic mutations of AR in the untreated prostate cancers [26,52–54]. However, two investigations suggested that mutations are present in a substantial fraction of cancers. First, Gaddipati et al. [55] reported that codon 877 mutation (known as LNCaP mutation) was found in 25% of transurethral resection of prostate (TURP) specimens of patients with untreated

Table 1
Reported association studies between germ-line alterations of AR and prostate cancer

Publication	CAG repeat length	GGN repeat length	Number of cases + controls	Comments
Irvine et al. [17]	CAG < 22/GGCnot-16: RR 2.1 ($p = 0.08$)		57 + 37	Repeat lengths correlate with racial risk groups
Hardy et al. [93]			109	Short CAG repeat associated with younger age at diagnosis
Giovannucci et al. [37]	≤18 CAG: RR = 1.52 (0.92–2.49)		587 + 588	Associated with advanced stage
Stanford et al. [48]	<22 CAG, ≤16 GGC: RR = 2.05 (1.09–3.84)	≤16 GGC: OR = 1.60 (1.07–2.41)	301 + 277	
Ingles et al. [94]	<20 CAG: OR = 2.10 (1.11–3.99)		57 + 169	
Hakimi et al. [95]	≤17 CAG: OR = 3.7 (1.31–10.5)	≤16 GGC: OR = 4.6 (1.3–16.1)	59+370	
			54 + 110	
Platz et al. [49]		GGN 23 OR = 1.2 (0.97–1.49)	582 + 794	
Correa-Cerro et al. [39]	No association	No association	105 + 132	
Bratt et al. [38]	No association		190 + 186	Short CAG repeat associated with younger age at diagnosis
Edwards et al. [40]	No association	No association	178 + 195	Long GGC associated with poor prognosis
Lange et al. [41]	No association		226 + 305	Familial cases included
Hsing et al. [96]	<23 repeat: OR = 1.65 (1.14–2.39)	<23 repeat: OR = 1.12 (0.71–1.78)	190 + 304	
Latil et al. [42]	No association		256 + 156	
Miller et al. [43]	No association	No association	140 + 70	Familial cases
Mononen et al. [97]	≤18 CAG: OR = 1.47 (1.00–2.16)		461 + 574	No association in familial
Chen et al. [44]	No association	No association	300 + 300	
Chang et al. [45]	No association	≤16 GGC: OR = 1.58 (1.08–2.32)	327 + 174	Included 129 familial cases
Suzuki et al. [47]	No association		88 + 53	
Gsur et al. [46]	No association		190 + 190	

Publication	R726L missense alteration	Number of cases + controls	Comments
Mononen et al. [50]	In sporadic cancer OR = 5.8 (1.5–22.1)	418 + 900	Mutation frequency
	In familial cancer: OR = 5.8 (0.95–34.8)	106 + 900	1.91% among Finnish cancer patients
Gruber et al. [51]	No association	548	No R726L mutation found

RR: relative risk, OR: odds ratio, both followed by 95% confidence intervals in brackets.

Table 2
Somatic genetic alterations of AR in prostate cancer

Gene amplification	Frequency	Comments
Visakorpi et al. [27]	7/23 (30%) in HR	
Koivisto et al. [29]	15/54 (28%) in HR	
Bubendorf et al. [60]	11/47 (23%) in locally recurrent and in 12/59 (20%) in metastatic HR	
Miyoshi et al. [98]	1/5 (20%) in HR	
Hernes et al. [99]	10/18 (56%) in HR	
Palmberg et al. [61]	10/77 (13%) in HR	Associated with response to second-line MAB
Edwards et al. [100]	3/20 (15%) in HR	
Haapala et al. [73]	0/11 (0%) in HR	Patients treated with combination of orchiectomy and bicalutamide
Linja et al. [31]	4/13 (31%) in HR	Also two xenografts containing the amplification identified
Hyytinen et al. [101]	4/16 (25%) in HR	
Brown et al. [102]	9/18 (50%) in HR	

AR mutation	Frequency	Comments
Newmark et al. [52]	1/26 (4%) in AD	
Suzuki et al. [103]	0/7 (0%) in AD, and 1/8 (13%) in HR	
Culig et al. [54]	1/7 (14) in metastatic HR	
Gaddipati et al. [55]	6/24 (25%) in AD	All T877A mutations in advanced tumors
Schoenberg et al. [69]	1/40 (3%) in AD	CAG repeat contraction 24 → 18
Ruizeweld de Winter et al. [26]	0/18 (0%) in HR	
Visakorpi et al. [27]	0/23 in HR	Only T877A mutation analysed
Taplin et al. [70]	5/10 (50%) in metastatic HR	Patients treated with flutamide
Elo et al. [104]	1/23 (4%) in AD, and 0/6 (0%) in HR	Germ-line mutation
Suzuki et al. [53]	0/30 (0%) in AD, and 3/22 (14%) in HR	
Evans et al. [105]	1/31 (3%) in AD, and 0/13 in HR	
Tilley et al. [56]	11/25 (44%) in AD	
Koivisto et al. [29]	1/13 (8%) in HR	All samples AR amplified
Watanabe et al. [106]	5/36 (14%) in AD	
Taplin et al. [71]	5/16 (31%) in HR	Patients treated with flutamide
Wallén et al. [68]	2/32 (6%)	Patients treated with monotherapy
Marcelli et al. [57]	11/137 (8%) in AD	All mutations found in stage D1 disease
Haapala et al. [73]	4/11 (36%) in HR	Patients treated with bicalutamide
Hyytinen et al. [101]	7/21 (33%) in HR	
Segawa et al. [107]	3/45 (7%) in AD	All mutations silent
Taplin et al. [108]	5/48 (10%) in HR	Patients treated with antiandrogens
Lamb et al. [109]	1/10 (10%) in HR	Exon 1 included, both AD and HR tumors analysed from each patient
Thompson et al. [110]	5/21 (24%) in AD	14 poorly differentiated primary tumors, and 7 metastases

AD: androgen dependent prostate cancer, HR: hormone-refractory prostate cancer; MAB: maximal androgen blockade.

metastatic prostate cancer. Second, Tilley et al. [56] reported that about 50% of cancers including early stages of the disease contain mutated AR. It was suggested that the reason why most of the investigations failed to find mutations was the methodological problems related to normal cell contamination. However, in a recent study Marcelli et al. [57] found no mutations in 99 prostate cancer using either microdissected or nonmicrodissected samples. Therefore it is now generally accepted that AR mutations are rare in untreated prostate cancer.

Of the prostate cancer models only few have been established from untreated prostate carcinomas. CWR22 is an androgen-dependent prostate cancer xenograft derived from untreated tumor [58]. It contains H874Y (histide to tyrosine) mutation in the ligand binding domain of AR enabling the receptor to bind adrenal androgen dehydroepiandrosterone in addition to several other steroid hormones and hydroxyflutamide [59].

4.3. Somatic aberrations of AR gene in hormone-refractory prostate cancer

While investigating the putative target genes for commonly amplified chromosomal region Xq11–q13, we [27] found high-level AR amplification in 30% of hormone-refractory tumors but in none of the specimens taken from the same patients prior to therapy. The finding has subsequently been confirmed by several other studies (Table 2). For example, Bubendorf et al. [60], found AR gene amplification in 23% of the 54 locally recurrent and 22% of the 62 metastases of hormone-refractory disease. Amplification of the gene leads to the increased expression as expected from

the target gene of the amplification [29,31]. The findings suggest that the amplification of AR gene is selected for by the hormonal therapy and that it is one of the mechanisms by which the prostate tumors acquire growth advantage in androgen depleted environment. The amplification of AR may sensitize the prostate cancer cells to minimal amounts of androgens [27]. Indeed, we have now shown that patients with AR gene amplification respond more often to the second-line maximal androgen blockade (MAB), combining antindrogens to castration, than patients whose tumors do not contain the amplification [61]. The finding demonstrates that the amplified AR is truly functional and that tumors with amplification are hypersensitive to the androgens. Unfortunately, the treatment response-time to the second-line MAB is short and benefit of the therapy is marginal. Recently, the functionality of the AR in hormone-refractory tumors has also been directly demonstrated using LAPC-9 in vivo xenograft model [62].

The first thoroughly studied point mutation in hormone-refractory prostate cancer was the one discovered in LNCaP prostate cancer cell line [63]. LNCaP cell line was originally established from lymph node metastases of patient treated with hormonal therapy [64]. In the cell line the mutation T877A in the ligand binding domain of AR enables the receptor to be activated by other steroid hormones such as estradiol and progesterone, and even by antiandrogen flutamide. Recently, it was shown that cell lines MDA-Pca 2a and 2b, which were established from a prostate cancer bone metastasis developed after orchiectomy, also harbor mutations in androgen receptor gene. The AR gene of the cell lines contains two mutations, T877A (threonine to alanine) and L701H (leucine to histidine), also located in the ligand binding domain [65,66]. These mutations reduce affinity for androgens, but enhance binding of adrenal corticosteroids. The two mutations have high synergistic effect in promoting promiscuous ligand binding. Together they increase the affinity of AR for glucocorticoids by 300% more than the L701H mutation alone [66]. It has also been shown that AR point mutations occur spontaneously in transgenic adenocarcinoma of the prostate mouse model (TRAMP), and certain mutations are selected for by the changes (castration) in androgen environment [67].

Recently, we screened 32 prostate carcinomas from patients treated with castration alone for AR mutations using single strand conformation polymorphim (SSCP) analysis and sequencing [68]. Only two tumors showed mutations, one point mutation ($Gly^{674} \rightarrow Ala$) and a contraction of CAG repeat in exon 1. The point mutation had previously been shown not to alter the transactivation properties of the receptor [29]. The somatic contraction of CAG repeat has also been previously reported in a prostate cancer sample [69]. Since, the CAG repeat length affect to the transactivational properties of AR, the contraction could theoretically have functional significance.

The finding of low mutation frequency in tumors treated by castration alone is contradictory to what has been found in patients treated by antiandrogens. For example, several reports have suggested that the use of AR antagonist flutamide is associated with the frequency of mutations in AR [70–72]. Taplin et al. [70,71] found mutated AR in 31% (5 out of 16) of patients receiving MAB with flutamide compared to only 6% of patients (1 out of 17) treated with monotherapy. The mutated ARs found from the flutamide-treated patients were also shown to be stimulated by flutamide. Most of these mutations are located in the ligand-binding domain (LBD) and the most frequently found mutation was identical to the mutation in the LNCaP (T877A) [70,71]. Recent studies have shown that also tumors from patients treated with another antiandrogen, bicalutamide, often contain AR mutations. Haapala et al. [73] found mutated AR in 36% (4 out of 11) of orchiectomy and bicalutamide treated patients. It has been suggested that some of these mutations could lead to stimulation of AR by bicalutamide, in similar fashion than the AR containing the LNCaP-mutation is transactivated by flutamide [74].

It is likely that mutations in the AR gene are selected for by antiandrogens. However, it is difficult to evaluate the significance of each of these mutations. No functional studies have been performed on all mutations detected. And, only a subset of all the mutant AR forms identified demonstrate expanded ligand spectrum or increased activities in the absence of ligand. Recently, Shi et al. [75] investigated the functional status of 44 missense mutations previously identified in prostate cancers. Twenty out of 44 mutations (45%) studied had gains of function, and five of them (11%) showed promiscuous activity, transactivation by nonandrogens. Combination of estradiol and progesterone, at physiological concentrations, weakly activated additional seven mutated receptors. Thus, it clearly seems that in some circumstances, AR mutations allow the tumors to adapt to low androgen conditions enabling them to progress despite the androgen withdrawal.

5. Interaction of AR with other signalling pathways

It has been proposed that in the absence of ligand, AR activation could take place by cross-talk with various growth factor pathways. For example, it has been demonstrated that epidermal growth factor (EGF), epidermal growth factor receptor-2 (ERBB2/Her-2), keratinocyte growth factor (KGF/FGF-7), insulin-like growth factor-1 (IGF-1), protein kinase A, mitogen-activated protein kinase, as well as IL-6 could activate AR signalling [76–80]. Additional mechanism underlying ligand dependent activation of the AR by these alternative pathways may involve phosphorylation of either AR or its associated proteins [79,81].

One of the most interesting suggested AR-interacting pathways is ERBB2-mediated signalling. It has been shown that ERBB2 enhances signalling activity of AR in the absence or in low levels of androgens in two xenograft models LAPC-4 and LNCaP [80,82]. Forced overexpression of ERBB2 in androgen-dependent prostate cancer cells was demonstrated to cause androgen-independent growth in castrated animals.

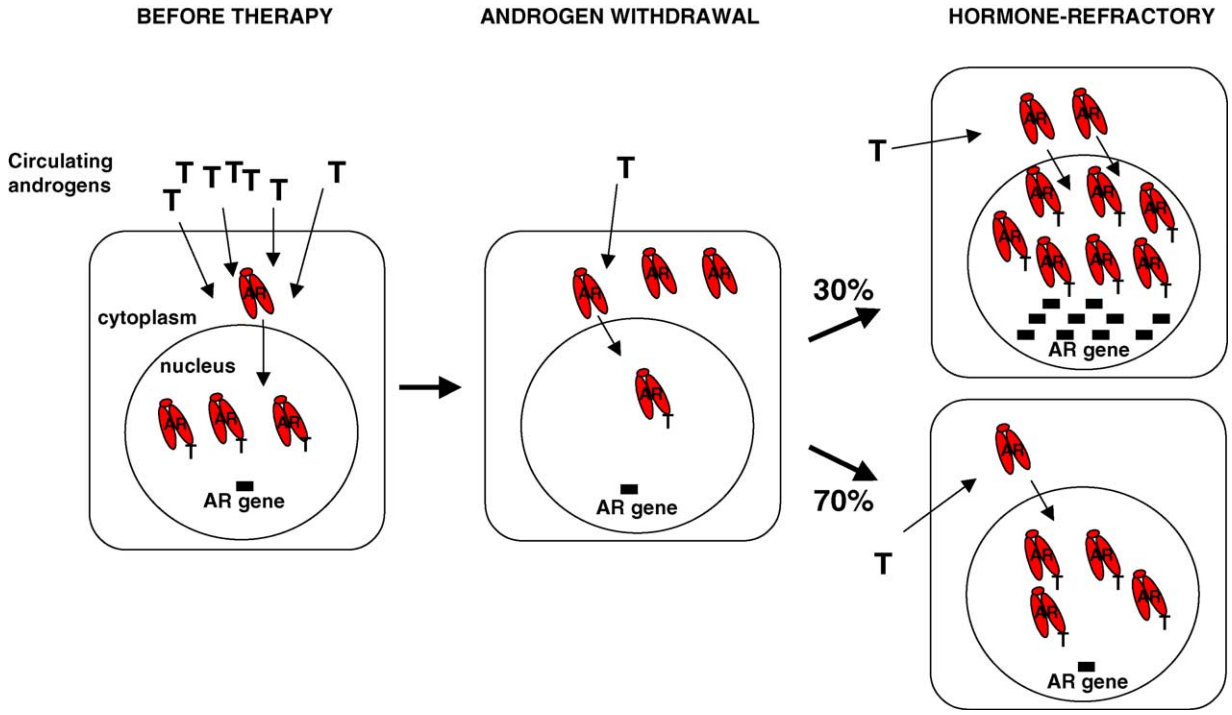

Fig. 1. Model for progression of prostate cancer during the hormonal therapy. Androgen withdrawal decreases the level of testosterone in serum, whereas adrenal androgens remain intact. In hormone-refractory prostate carcinomas, emerging during treatment, the expression of AR is increased allowing the activation of the receptor by the remaining androgens or by other ligands. In one-third of the hormone-refractory tumors high-level overexpression of AR is caused by the amplification of the gene.

ERBB2 gene (HER-2/neu) encodes for a transmembrane glycoprotein that contains tyrosine kinase activity and belongs to the epidermal growth factor receptor family [83]. Amplification of the ERBB2 gene, leading to overexpression, has been found, e.g. in 25–30% of human breast and ovarian cancers and the amplification is associated with poor prognosis in these malignancies [84]. The development of anti-ERBB2 antibody (trastuzumab) based strategy for treatment of ERBB2 overexpressing breast carcinomas has raised the question whether ERBB2 could be a useful target in treatment of prostate cancer too. However, most investigations have not found high-level amplification of the ERBB2 gene in prostate cancer [60,85]. In addition, no high-level overexpression of ERBB2 has been demonstrated [85,86]. Thus, it is unlikely that therapies based on overexpression of ERBB2, such as trastuzumab, are useful in treatment of prostate cancer, as the first clinical trials have also now demonstrated [87].

6. Conclusions

Although the importance of androgens in the development of early prostate cancer is evident, the role of AR is less clear. The data from molecular epidemiological studies on AR as predisposing factor have been confusing. Some providing evidence for the significance of AR polymorphisms as risk factor, others not confirming such associations. Also somatic mutations in the untreated prostate cancers seems to be rare. It is obvious that larger and more conclusive studies for investigation of AR polymorphisms as risk factor should be carried out. And, such allelic association studies would probably be more informative if performed in combination with analyses of environmental risk factors.

The significance of AR in the emergence of hormone-refractory prostate cancer has become better understood (Fig. 1). It seems that majority of the hormone-refractory clinical prostate tumors overexpress AR. In about one-third of the cases the overexpression maybe explained by the amplification of the gene. In rest of the cases the mechanism of overexpression remains to be solved. In addition, it has recently been shown that disruption of AR by hammerhead ribozyme or antisense oligonucleotides inhibits proliferation of both androgen sensitive and hormone-refractory prostate cancer cell lines indicating directly the significance of the AR [88–90].

The finding that AR gene amplification is found only in patients with hormone-refractory prostate cancer but not in tumors from same patients prior to therapy suggests that the amplification of AR gene is selected for by the therapy. Similar type of gene amplifications related to treatment relapse have now been reported also in other malignancies. For example, one of mechanism for failure of treatment with STI571 kinase inhibitor in leukemia seems to be amplification of the BCR-ABL fusion gene encoding the target of the drug [91]. The amplified and overexpressed BCR-ABL gene reactivate the ABL signalling despite the ST571 therapy. The finding

of AR gene amplification suggests that hormone-refractory tumor cell, instead of being androgen-dependent might actually be androgen hypersensitive. The critical question is can better drugs be developed to suppress the AR activity also in the hormone-refractory prostate cancer. The currently available antiandrogens are clearly not enough. Compounds, such as sulindac sulfide, that decrease the expression and function of AR have been demonstrated [92]. Such molecules may form bases for novel drugs for treatment of hormone-refractory prostate cancer. It should also be noticed that the down-stream genes of AR signalling that are important for tumor progression are not well known. Such genes would also be putative treatment targets and thus should be identified.

Acknowledgements

Supported by the Cancer Society of Finland, the Academy of Finland, the Reino Lahtikari Foundation, the Medical Research Fund of Tampere University Hospital, the Finnish Life and Pension Insurance companies, and the Sigrid Juselius Foundation.

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