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ORIGINAL RESEARCH

Immunohistochemical expression of PDGFR, VEGF-C, and proteins of the mToR pathway before and after androgen deprivation therapy in prostate carcinoma: significant decrease after treatment

Nicolas Kozakowski · Caroline Hartmann · Hans Christoph Klingler · Martin Susani · Peter R. Mazal · Anke Scharrer · Andrea Haitel

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Abstract Targeted therapy in hormone refractory prostate cancer (HRPC) is currently under evaluation in many trials. The effect of androgen deprivation therapy (ADT) on many targets in prostate cancer is incompletely known. For the first time, immunohistochemical expression of the platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), vascular endothelial growth factor C (VEGF-C), mammalian target of rapamycin (mToR), p70 ribosomal protein S6 kinase 1 (PS6K), human epidermal growth factor receptor 2 (c-erbB-2), and carbonic anhydrase IX (CA9) was evaluated in 44 patients with prostate carcinoma treated with or without ADT, at biopsy time and after radical prostatectomy. PDGFR, VEGF-C, mToR, and PS6K expression was significantly reduced ($p=0.002$, $p=0.035$, $p=0.025$, and $p=0.033$, respectively) after ADT, whereas expression of EGFR, c-erbB-2, and CA9 was not influenced by ADT. In conclusion, targeting PDGFR, VEGF-C, mToR, or PS6K after ADT should be considered with precaution, as those targets can severely be altered or functionally deregulated by ADT.

Keywords Prostate cancer · Targeted therapy · Hormone resistance · Immunohistochemistry

N. Kozakowski · C. Hartmann · M. Susani · P. R. Mazal · A. Scharrer · A. Haitel (✉)
Clinical Institute for Pathology, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria
e-mail: andrea.haitel@meduniwien.ac.at

H. C. Klingler
Department of Urology, Medical University of Vienna, Vienna, Austria

Introduction

Androgen deprivation therapy (ADT) in prostate carcinoma (PC) is indicated in metastasized disease and patients with PSA relapse after radical prostatectomy or radiation. In exceptional clinical situations, it is applied as neoadjuvant therapy before surgery or concomitant to radiation. Thereby, it has been associated with decreased incidence of positive surgical margins, fewer lymph node metastases, and reduced tumor size [1, 2], although not with better outcome [3]. Nevertheless, a lot of patients under ADT develop hormone refractory disease. Possible alternative treatments are second-line chemotherapy with docetaxel, second-line hormonal schemes, or targeted therapies. Docetaxel is currently a standard therapy after the development of hormone insensitivity in metastasized disease and is associated with a decrease of PSA and increased survival [4–7]. Targeted therapy is still experimental, but in hormone refractory prostate cancer (HRPC), and in many other neoplastic diseases, it yields new therapeutic modalities. Inhibitors of tyrosine kinase, of growth factors, and of angiogenesis have already been used in neoadjuvant and adjuvant chemotherapy schemes in a wide range of carcinomas including urologic tumors as bladder or kidney cancers [8, 9].

Many studies investigated the pathway of important tyrosine kinase receptors, the epidermal growth factor receptor (EGFR) and human EGFR 2 (Her-2) in PC. In these studies, expression of EGFR was reported not only in cell lines but also in human tissue in different settings of the disease (with or without hormone therapy, in hormone refractory disease or in metastasized disease) [10–13]. Consequently, other authors studied the effects of EGFR inhibitors in PC cell cultures

[14, 15]. EGFR inhibitors have already been tested in clinical phases I and II studies in patients with HRPC, alone or in combination with other cytostatic drugs, with encouraging outcome [16, 17].

Moreover, several publications demonstrated the influence of Her-2 on the proliferation of PC cells and disease progression [18–20]. These results led to further in vitro inhibition studies [21, 22] or preclinical research, using specific inhibitors such as pertuzumab or lapatinib [17, 23–25].

The alpha-type platelet-derived growth factor receptor (PDGFR-A) expression has also been shown in prostate cancer epithelial cells [26], and as a consequence, trials were performed with a combined regimen of cytostatic drugs and inhibitors of PDGFR [27–30], however with contradictory results.

Blocking tumor angiogenesis is also an important alternative in cancer treatment. Vascular endothelial growth factor (VEGF) seems to play an important role in metastases of PC cells [31–33]. In addition, blocking the VEGF-C-pathway in PC cell culture leads to tumor suppression [34, 35]. Preclinical trials with bevacizumab in combination with other cytostatic agents in HRPC are currently under investigation, with variable toxicity reports and preliminary results [36–39]. Also, antibodies blocking more than one pathway such as sunitinib (blocking VEGFR, PDGFR, and other kinases) brought uneven results [40, 41].

Furthermore, the mammalian target of rapamycin (mToR) and its activated downstream target p70 ribosomal protein S6 kinase 1 (PS6K), which are involved in cell proliferation and growth, have also been shown to be expressed heterogeneously in PC tissue [42]. In vitro inhibition studies demonstrated the reduction of proliferation of PC cell lines when applying specific inhibitors [43, 44]. Therefore, some authors suggested that the use of mToR inhibitors in combination with other drugs could be a therapeutic option in HRPC, encouraging further clinical trials [45, 46].

Another interesting target, carbonic anhydrase IX (CA9), has been described in various carcinomas as a marker of poor prognosis and possible target for immunotherapy [47]. It has also been described in PC, where it was only occasionally found [48].

All these targeted therapies are (or could be) used as second-line treatments after hormonal deprivation schemes. However, little is known about the possible influence of preemptive hormone therapy of PC on the expression of the molecular targets of these therapies. Reduction of targets could dramatically reduce the efficacy of these aforementioned targeted therapies.

Therefore, we analyzed PC tissue specimens before and after ADT regarding the expression of relevant therapy targets and compared to specimens not influenced by androgen depletion.

Material and methods

Study population

Tissue samples of 44 patients from paraffin-embedded prostate biopsy cores and tumor blocks from subsequent prostatectomies were obtained from the archives of the Clinical Institute of Pathology, University of Vienna, collected between 1993 and 2006.

For all patients, complete follow-up and enough representative tumor tissue for immunohistochemical analysis were available. Twenty-two of then underwent ADT, and in this group, the mean age at time of biopsy was 64.18 years (range 52–74 years), and the mean interval between diagnostic prostate biopsy and prostatectomy was 119 days (range 17–592 days). Neoadjuvant hormonal deprivation was carried out whether with surgical orchiectomy or with chemical castration (Table 1).

As control group, we used biopsy and prostatectomy specimens from 22 age-matched patients with prostatic carcinoma without hormonal therapy. The mean age of patients of this group was 64.68 years (range 50–72 years), whereas the mean interval between biopsy and radical surgery was 41.48 days (range 6–120 days).

Immunohistochemistry

Paraffin-embedded tissue probes from prostate tissue core biopsies with carcinoma and from tumor blocks of radical prostatectomy after ADT (representative cancer tissue of the highest Gleason score) from each patient were selected for immunohistochemical staining. Immunohistochemistry was performed with monoclonal antibodies against EGFR (Zymed Laboratories, San Francisco, CA; antigen retrieval (AR) with enzymatic digestion, dilution of 1:20, incubation for 1 h), mToR, and PS6K (both from Cell Signaling Technology, Danvers, MA; AR with steam autoclave, dilution of respectively 1:50 and 1:100, incubation for 1 h) or polyclonal antibodies against PDGFR-α (Thermo Fisher Scientific, Fremont, CA; AR with microwave oven, dilution of 1:40, incubation for 1 h), VEGF-C (R&D Systems, Germany; AR with microwave oven, dilution of 1:40, incubation 1 h), carbonic anhydrase IX (Abcam, UK; AR with microwave oven, dilution of 1:20,000, incubation for 1 h), and c-erbB-2 (Her-2/neu) (Dako, Denmark; AR with incubator, dilution of 1:100, incubation for 1 h), following the manufacturer's instructions. Slides were then counterstained with Mayer's hemalum, dehydrated, and mounted.

Evaluation of the immunohistochemistry

Expression of targets in biopsy specimens and in representative blocks of following prostatectomies with or without ADT was compared. Assessment was considered positive in PC

Table 1 Patient data with hormone deprivation and results of the immunohistochemical study

Patient nr.	Age (years)	Therapy	Time interval (days)	Gleason score		EGFR		PDGFR		VEGF-C		mTOR		PS6K		CA9		Her-2/neu	
				Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	70.88	Bicalutamide, goserelin	60	7	9	−	+	+	+	+	−	+	+	+	+	+	+	−	−
2	52.33	Goserelin	149	9	10	−	−	+	−	−	−	+	+	+	−	−	−	−	−
3	59.19	Goserelin	184	5	7	+	+	+	−	+	−	+	+	−	+	−	+	−	−
4	60.22	Flutamid	82	5	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	56.44	Orchiektomie	146	8	8	−	−	+	−	−	−	+	+	+	−	−	−	−	−
6	66.82	goserelin	225	7	9	−	+	+	−	+	+	+	+	+	+	−	−	−	−
7	70.7	AA	61	5	5	−	+	+	−	−	−	+	+	−	+	−	−	−	−
8	71.5	Flutamid, goserelin	41	9	9	+	+	+	+	−	−	+	+	+	−	−	+	−	−
9	64.3	Bicalutamide, leuprorelin	29	7	9	−	+	+	−	−	−	+	+	−	−	+	+	−	−
10	63.29	Flutamid	48	7	9	+	+	+	−	−	−	+	−	+	−	−	−	−	−
11	68.41	Flutamid	17	5	7	−	+	+	+	+	−	+	+	−	+	−	−	−	−
12	66.72	Flutamid	20	5	8	−	−	+	−	+	−	+	+	+	−	−	−	−	−
13	52.62	Flutamid	28	6	8	−	−	+	+	−	−	+	−	+	+	−	−	−	−
14	66.52	Goserelin	194	5	5	−	−	+	+	+	−	+	−	+	+	−	−	−	−
15	63.72	Flutamid	18	6	6	−	−	+	−	+	−	+	−	+	+	+	−	−	−
16	62.3	Goserelin	94	5	7	−	−	+	−	+	−	+	+	+	−	+	−	−	−
17	63.35	Flutamid, AA	592	5	8	−	−	+	−	−	−	+	+	+	+	−	−	−	−
18	63.48	Antiandrogen	57	6	6	+	−	+	+	−	+	+	+	+	−	−	−	−	−
19	62.49	Goserelin	106	6	6	+	−	+	−	+	−	+	−	+	−	−	−	−	−
20	60.36	AA	200	5	6	−	−	+	+	+	−	+	+	+	−	−	−	−	−
21	74.32	Goserelin	159	6	6	+	−	+	+	−	−	+	+	+	−	+	−	−	−
22	71.98	Leuprorelin	103	8	10	−	−	−	+	−	+	+	+	+	−	−	−	−	−
				$p=0.001$		$p=0.002$		ns		$p=0.035$		$p=0.025$		$p=0.033$		ns		ns	

AA androgen ablation (not otherwise specified), *ns* not significant

cells when cytoplasmic and/or cell membrane signal for PDGFR (negative=− vs. weak to strong positive=+), cytoplasmic signal for VEGF-C (negative and focal positive=− vs. strong and diffuse positive=+) and mToR (negative and focal positive=− vs. strong and diffuse positive=+), nuclear and cytoplasmic signal for PS6K (negative and focal positive=− vs. strong and diffuse positive=+), and membrane signal for CA9 (negative=− vs. weak to strong positive=+), EGFR (negative or focal positive=− vs. strong or diffuse positive=+), and c-erbB-2 (Her-2/neu, following the HercepTest) were present. Positive controls for Her-2 were carried out on Her-2-positive mammary carcinoma tissue.

Statistical analysis

Differences between groups were evaluated with the nonparametric Wilcoxon test, using SPSS 15.0. The test was considered significant when $p \leq 0.05$.

Results

All PCs were of the usual adenocarcinoma type. Mean Gleason score (GS) at primary diagnosis was 6.23 (range 5–9) for the ADT group and 6.65 for the ADT-free group (range 6–9); although the value of Gleason grading after therapy is problematic, we applied Gleason grading to compare Gleason score at biopsy diagnosis with that of RPE for the ADT group. At the time of radical operation after ADT, the mean Gleason score was higher—7.5 (range 5–10)—as expected. This difference was statistically significant ($p=0.001$). The mean Gleason score for the ADT-free group in the operation specimens was 6.77 which, however, was not significantly different ($p=0.41$).

In the ADT group, there was a statistically significant decrease of expression regarding PDGFR ($p=0.002$), VEGF-C ($p=0.035$), mToR ($p=0.025$), and PS6K ($p=0.033$) after hormonal deprivation therapy. In the ADT group, 12/20 carcinomas positive for PDGFR at the time of biopsy displayed loss

of expression in the following prostatectomy, while only one tumor initially negative for PDGFR showed positivity after ADT (Fig. 1a, b). Ten PC expressed VEGF-C in core biopsies; nine of them lost expression after hormonal therapy, and two PCs initially negative for VEGF-C became then positive (Fig. 1c, d). Of 21 cases initially positive for mToR, five exhibited a loss of expression in the prostatectomy (Fig. 1e, f). The 12 cases initially positive for PS6K almost entirely lost specific expression, and only one tumor remained positive. Three initially negative PC developed PS6K positivity after hormonal deprivation (Fig. 1g, h) (Table 1).

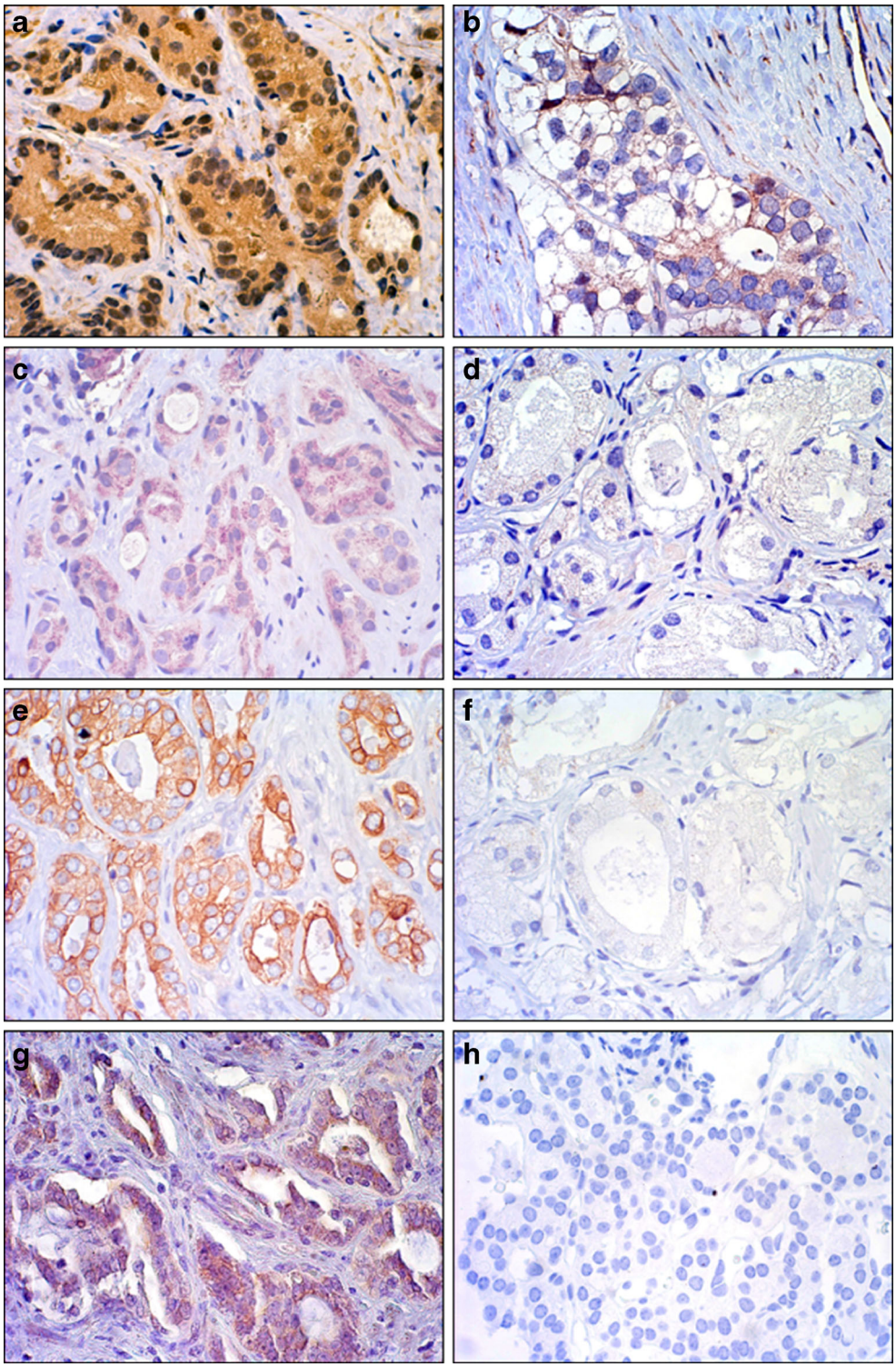

Fig. 1 Immunohistochemical staining for PDGFR, VEGF-C, mToR, and PS6K. **a** PDGFR-positive PC, **b** PDGFR-negative PC, **c** VEGF-C-positive PC, **d** VEGF-C-negative PC, **e** mToR-positive PC, **f** mToR-negative PC, **g** PS6K-positive PC, **h** PS6K-negative PC (original magnification, ×400)

There was no statistically significant difference regarding the expression of EGFR, CA9, and Her-2 before and after ADT.

Loss of EGFR expression was seen in three of six initially positive tumors, and there was an increase of expression in six other cases, but this result was not statistically significant. Three of five carcinomas expressing CA9 were negative in the following prostatectomy. An increase of expression of CA9 was observed in two PC. Positive expression of Her-2 was not seen in any PC, neither prior to nor after treatment. Conversely in the control group without ADT, there was no significant difference in expression at biopsy and at prostatectomy time neither for PDGFR ($p=1$), VEGF-C, and mToR nor for PS6K.

Discussion

ADT is the treatment of choice for advanced PC [49, 50]. Nevertheless, after a while, the majority of patients receiving ADT develop hormone refractory disease. A growing number of alternative secondary therapeutic trials, among others targeted therapy, are under evaluation. However, little is known about the influence of ADT on numerous proteins and receptors, which are potential targets of targeted therapy. In this study, we document the influence of ADT on the expression of different tumor targets such as EGFR, PDGFR, VEGF-C, mToR, PS6K, CA9, and Her-2, and most of which were significantly decreased after ADT.

Immunohistochemical expression of PDGFR in PC cells has been correlated with PSA levels in serum and Gleason score [10]. Blocking PDGFR with specific antibodies is an efficient treatment in mouse models of PC bone metastases [30, 51]. However, clinical testing of the combination of docetaxel and imatinib (PDGFR inhibitor) did not show convincing results, neither in neoadjuvant setting with concomitant use of LHRH agonists [28] nor in patients with metastasized HRPC after androgen withdrawal [29]. None of these studies investigated PDGFR expression before and after therapy. In these studies, PDGFR levels were measured in serum, but not directly in tissue samples of PC. The conflicting results of these studies could be explained by our present results, showing a significant reduction of PDGFR in tumor tissue after ADT. This should prompt caution in selecting PC patients for PDGFR-targeted therapy.

In addition, angiogenesis seems to play a role in PC progression, which has been shown in studies concerning VEGF-C in PC metastases [31, 32, 52]. Blocking angiogenesis can have an influence on PC. In migration and growth assay, inhibition of VEGF-C activity has proved to be effective when used in combined targeted therapy [53]. Moreover, antibodies directed against VEGF demonstrated cytostatic effects and reduced lymph node metastases in mouse xenograft models [34, 54]. In addition, early clinical trials applying combined therapy with bevacizumab (anti-VEGF antibody) showed encouraging results and led to further trials [37]. In regard to our results showing significantly reduced VEGF-C expression after ADT, selecting PC patients for therapy with bevacizumab after measuring VEGF-C expression levels can lead to more effective individual treatment.

The role of mToR and one of its downstream pathways—PS6K—has been demonstrated in androgen-resistant PC cells [55]. Comparing other solid tumors, rapamycin therapy in PC patients has only been tested recently [46, 56]. Heterogeneity of mToR expression in individual PC is known [42]. We found a decreased expression of mToR after ADT in the present study. Therefore, measuring mToR expression prior to targeted therapy seems to be a reasonable method to select patients.

Other potential targets like EGFR, Her-2, and CA9 did not display a significant modification of their expression after ADT in our study.

EGFR expression in PC before ADT has already been observed in 41.4 % [10] and in 23 % [11]. In our study, the mean expression level of EGFR in diagnostic prostate biopsy specimens was 27 %, which is between the levels of these two studies. Di Lorenzo [10], who examined only prostatectomy specimens, found in his study an expression of EGFR in PC in 75.9 % after ADT and in 100 % in cases of HRPC. Hernes [11] found an increase of EGFR (c-erbB-1) in 31 of 82 initially negative cases and a decrease of EGFR in 9 of 24 initially positive cases. In contrast, we did not find a significant increase of expression after ADT. The difference between the two observations might consist in the fact that we used representative tissue from prostatectomy specimens after ADT, whereas Hernes et al. [11] used biopsy cores, which might lack of representativeness.

Some studies reported rare and variable overexpression of c-erbB-2 (Her-2), another member of the EGFR family, in PC with respectively 13.3, 29, and 31 % positive cases [57–59]. The study of Neto at al.—a review of 83 published studies dealing with Her-2 expression in PC between 1991 and 2008—reported a significant increase of Her-2 overexpression in advanced disease [59], which suggests a similar increase also in HRPC. Nevertheless, no information about the relation between Her-2 expression and development of HRPC has been available. In our study, we could not demonstrate the modification of Her-2 expression after ADT, since all of our cases were initially negative and remained negative after ADT. It seems that Her-2 does not play a key role in the biology of PC, which was also concluded in the review of Neto et al. [59].

In our study, CA9 was only infrequently expressed before (22 %) and after ADT (18 %) without a statistically significant influence of the therapy. This is in accordance with the only other study investigating CA9 in PC, which also described only occasional CA9 expression in PC [48].

In conclusion, we demonstrate a significant loss of expression of PDGFR, VEGF-C, mToR, and PS6K after ADT in PC, a fact that should be taken into consideration before starting targeted therapy. This also motivates further research on targeted therapy, which could be more efficient in patients without preceding ADT.

Conflict of interest All authors declare no conflict of interest.

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