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Expression patterns of potential therapeutic targets in prostate cancer

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Androgen withdrawal is the only effective therapy for patients with advanced prostate cancer, but progression to androgen independence ultimately occurs in almost all patients. Novel therapeutic strategies targeting molecular mechanisms that mediate resistance to hormonal and chemotherapeutic treatment are highly warranted. Here, we aimed to evaluate the expression of potential therapeutic targets in advanced prostate cancer. A tissue microarray (TMA) containing samples from 535 tissue blocks was constructed, including benign prostatic hyperplasia as controls (n = 65), prostatic intraepithelial neoplasia (PIN; n = 78), clinically localized prostate cancers (n = 181), as well as hormone-refractory local recurrences (n = 120) and distant metastases (n = 91). The expression of 13 different proteins was analyzed using immunohistochemistry (Bcl-2, p53, ILK, Syndecan-1, MUC-1, EGFR, HER2/neu, HSP-90, Ep-CAM, MMP-2, CD-10, CD-117 and Ki67). Significant overexpression in hormone-refractory prostate cancer and metastatic tissue compared to localized prostate cancer was found for Ki67 (64% *vs*. 9%), Bcl-2 (11% *vs*. 1%), p53 (35% *vs*. 4%), Syndecan-1 (38% *vs*. 3%), EGFR (16% *vs*. 1%) and HER2/neu (16% *vs*. 0%). Overexpression of CD-117 was restricted to 1 single metastasis. All other markers did not show relevant differences in expression between subgroups. Taken together, p53, Bcl-2, Syndecan-1, EGFR and HER2/neu are preferentially expressed in hormone-refractory and metastatic prostate cancer. Selected inhibition of these targets might offer a strategy to treat advanced tumors and prevent further progression. Treatment decisions should not be based on findings in primary tumors but rather on tissues from recurrent or metastatic lesions.

Key words: tissue microarray; immunohistochemistry; progression; hormone-refractory; therapy

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer mortality in men in Western industrialized countries.[1] Androgen withdrawal is the only effective therapy for patients with advanced disease. Approximately 80% of patients achieve symptomatic and/or objective response after androgen ablation. However, progression to androgen independence ultimately occurs in almost all patients.[2] Although numerous nonhormonal agents have been evaluated in patients with hormone-refractory prostate cancer, these agents have limited antitumor activity with an objective response rate <20% and no demonstrated survival benefit.[3,4] Therefore, the identification and selected inhibition of molecular targets that mediate the progression of prostate cancer will have great impact on future treatment concepts. Possible targeted therapies include antibodies to inactivate specific proteins, vaccination against tumor-specific antigens, antisense oligonucleotides aimed against messenger RNA, molecules that block specific proteins and pathways or gene therapy for insertion of wild-type genes to restore the function of defective tumor-suppressor genes.[5]

A variety of new therapies for selected inhibition of therapeutic targets are currently under clinical evaluation in advanced prostate cancer, including antisense Bcl-2 therapy (Genasense®, Genta, Inc., Berkeley Heights, NJ). Clinical phase I studies are planned with ZD1839 (Iressa®, AstraZeneca, Macclesfield, UK), which is a potent and specific inhibitor of EGFR tyrosine kinase activity.[6]

The selection of patients for targeted therapies should be based on the presence of specific gene alterations or protein expression profiles in a given tumor. For example, the humanized antibody trastuzumab (Herceptin®, Roche, Basel, Switzerland), which is directed against the HER2/neu receptor, is only effective against breast cancers with HER2/neu amplification and overexpression but not in those with a normal HER2/neu status.[7]

Accordingly, it is not surprising that a series of advanced prostate cancers, which were HER2/neu negative, did not respond to trastuzumab in a phase II clinical trial.[8] Testing the prevalence of targeted molecular alterations in large numbers of tumors is crucial in order to estimate the chance of success of specific therapies in particular tumor types prior to clinical application.

High-throughput analysis of tissue microarrays (TMA) allows rapid molecular profiling of large numbers of tumors in a single experiment.[9,10] Here, we constructed a progression TMA with specimens from 535 patients to analyze the protein expression profiles of potential therapeutic targets across the whole spectrum of prostate cancer progression.

Candidate proteins were chosen depending on the availability of known or suggested targeted therapeutic regimens, including HER2/neu, epidermal growth factor receptor (EGFR), epithelial cellular adhesion molecule (Ep-CAM), Syndecan-1 (CD-138), matrix metalloproteinase 2 (MMP-2), integrin-linked kinase (ILK), urinary mucine 1 (MUC-1), heat-shock protein 90 (HSP90), Bcl-2, p53, CD-10 (neutral endopeptidase) and CD-117 (tyrosine phosphatase) (Table I[6,11–32]). Our data show that several of these proteins are often expressed in advanced prostate cancers, qualifying them as promising therapeutic targets.

Material and methods

Patients and specimens

Formalin-fixed and paraffin-embedded tumor and control specimens were from the archives of the Institute for Pathology, University of Basel and the Tampere University Hospital. All tumors and controls were reviewed by one pathologist (L.B.). The least differentiated tumor area was selected for the tissue microarray. The specimens that were interpretable for immunohistochemistry included a) transurethral resections from 65 patients with BPH as controls; b) samples from 78 high-grade prostatic intraepithelial neoplasias (PIN) and c) 181 clinically localized prostate cancers from transurethral resections (T1a/b; n = 95) and radical prostatectomy specimens (pT2a–pT3b; n = 86); d) transurethral resections from 120 hormone-refractory local recurrences and e) 91 metastases collected at the autopsies from patients who had undergone androgen deprivation by orchiectomy and had subsequently died of end-stage metastatic prostate cancer. Metastatic tissues were from pelvic, paraaortal or mediastinal lymph nodes (n = 24), lung (n = 28), liver (n = 19), adrenal gland (n = 11), pleura (n = 4), kidney (n = 2), dura mater (n = 2) and ureter (n = 1).

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TABLE I – ANALYZED CANDIDATE MARKERS AND POTENTIAL TREATMENT STRATEGIES

Candidate protein	Synonym(s)	Possible treatment strategy (alone or in combination with chemotherapeutics)	Selected references
Bcl-2	B-Cell CLL/Lymphoma 2	Antisense Bcl-2 oligonucleotides (Genasense®)	11,12
CD-10	Neutral Endopeptidase (NEP), Neprilysin	Monoclonal antibodies	13,14
CD-117	Tyrosine Phosphatase	Imatinib (Glivec®)	15,16
Epidermal Growth Factor Receptor (EGFR)	ErbB1, ErbB Oncogene	ZD1839, gefitinib (Iressa®)	6,17
Epithelial Cellular Adhesion Molecule (EpCAM)	Tumor-associated calcium signal transducer 1	Bispecific antibodies	18,19
HER2/neu	cErbB2	Trastuzumab (Herceptin®)	20,21
Heat-shock Protein 90 (HSP90)	Lipopolysaccharide-associated protein 2	17-allylaminogeldanamycin (17AAG)	22,23
Integrin-linked Kinase (ILK)	p59	Non-steroidal anti-inflammatory drugs (NSAIDs)	24,25
Matrix Metalloproteinase 2 (MMP-2)	Collagenase Type IV A Gelatinase A	MMP-Inhibitor (Roche 28-2653)	26,27
Transmembrane Mucin 1 (MUC-1)	Urinary Mucin 1	Monoclonal antibodies (DF3, B27.29)	28,29
p53	Tumor protein p53 (TP53)	Transfection using wild-type p53, Adenoviral-mediated gene transfer	30,31
Syndecan-1	CD 138	Monoclonal Antibodies	32

Construction of tissue microarrays

The prostate tissue microarray was constructed as described.[9] Briefly, 1 core tissue-biopsy (diameter 0.6 mm) was taken from the least differentiated region of individual paraffin-embedded prostate tumors (donor blocks) and precisely arrayed into a new recipient paraffin block (35 mm × 20 mm) with a semiautomated instrument. After the block construction was completed, 5 μm sections were cut with a microtome using an adhesive-coated tape sectioning system (Instrumedics, Hackensack, NJ) to support the adhesion of the array elements. The presence of tumor tissue on the arrayed samples was verified on a hematoxylin-eosin-stained section (Fig. 1). The number of samples varies slightly between the individual marker analyses because of variability in the number of interpretable specimens on TMA sections.

Immunohistochemistry

TMA sections were used for immunohistochemical staining with 13 different antibodies. Standard indirect immunoperoxidase procedures (ABC-Elite, Vector Laboratories, Burlingame, CA) were used for detection of the secondary antibodies. The primary antibodies, their dilutions and pretreatment conditions are listed in Table II. Diaminobenzidine was used as a chromogen. The primary antibody was omitted for negative controls. All slides were read by 1 pathologist (L.B.). Nuclear Ki67 immunostaining was visually scored and stratified into 6 groups (negative, >5%, 5–9%, 10–20%, 21–50%, and >50%). A high Ki67 Labelling Index (LI) was defined as positivity in >20% of the tumor cell nuclei. The intensity of immunostaining for all other proteins was visually scored and stratified into 4 groups (negative, weak, moderate and strong). As in previous studies, at least a moderate staining intensity was requested in >10% of tumor cells within a tissue spot to define positivity.[33–35] Heterogeneity of marker expression was usually low or absent on the individual TMA spots. Tumor samples without any reactivity for Ki67 LI were excluded from analysis because reduced immunoreactivity was assumed. In Bcl-2 analysis, lymphocytes and basal cells from admixed benign glands served as internal positive staining controls. For Ki67 and p53 analysis, only nuclear staining was considered.

Statistical analysis

Contingency table analysis and chi-square tests were used to study the relationship between the expression of different markers and histological subgroups. The levels of statistical significance were set at least at $p < 0.05$ (2-sided), and all statistical calculations were performed using JMP 3.0 software (SAS Institute, Inc., Cary, NC).

Results

Histology

Gleason grade, assessed in all prostate cancer tissue core specimens,[36] was strongly associated with the stage of progression (Fig.2, $p < 0.001$).

Immunohistochemistry

Representative examples of immunohistochemical staining of BPH, PIN and prostate cancer specimens are shown in Figure 3. A high Ki67 Labelling Index (LI) (>20%) was found in most hormone-refractory and metastatic prostate cancers as compared to BPH, PIN and localized prostate cancer ($p < 0.001$). A high Ki67 LI was significantly associated with recurrent and metastatic disease and high Gleason Grade ($p < 0.0001$; Fig. 4*a*).

The expression profiles of all analyzed candidate markers in BPH, PIN and across prostate cancer progression are shown in Figure 4*a*,*b* (except for CD-117). Significant overexpression in hormone-refractory prostate cancer and metastatic tissue compared to localized prostate cancer was found for Ki67 (64% *vs*. 9%), p53 (35% *vs*. 4%), Bcl-2 (11% *vs*. 1%), Syndecan-1 (38% *vs*. 3%), EGFR (16% *vs*. 1%) and HER2/neu (16% *vs*. 0%). Expression of these proteins was significantly associated with both high Gleason grade and high KI67 LI ($p < 0.01$; Tables III and IV). The expression levels of p53, Bcl-2 and Syndecan-1 were significantly associated with each other ($p < 0.05$, data not shown). There was a higher prevalence of Bcl-2 expression in PIN lesions as compared to localized prostate cancers (13.3 *vs*. 1.3%, $p < 0.05$) as previously shown by other authors.[37,38]

Ep-CAM and HSP-90 were also significantly overexpressed in high-grade PIN and prostate cancer as compared to BPH, consistent with previous reports.[39,40] No evident variability of expression was seen for both proteins across various stages of prostate cancer progression. Similar expression profiles were found for ILK and MMP-2. Overexpression of these proteins was rare (MMP-2) or absent (ILK) in benign prostatic glands, with a marked increase of expression in high-grade PIN and prostate cancer. However, there was no significant increase of ILK or MMP-2 expression with the stage of prostate cancer progression. MUC-1 tended to become gradually more expressed from benign to premalignant and malignant tissue samples ($p > 0.05$.).

The expression profile of CD-10 (neutral endopeptidase) differed from the other marker profiles in this study. CD-10 was overexpressed in all BPH samples and approximately half of the PIN and prostate cancer samples with no significant difference between hormone-refractory and metastatic prostate cancers as compared to androgen-dependent localized tumors. CD-117 (c-kit

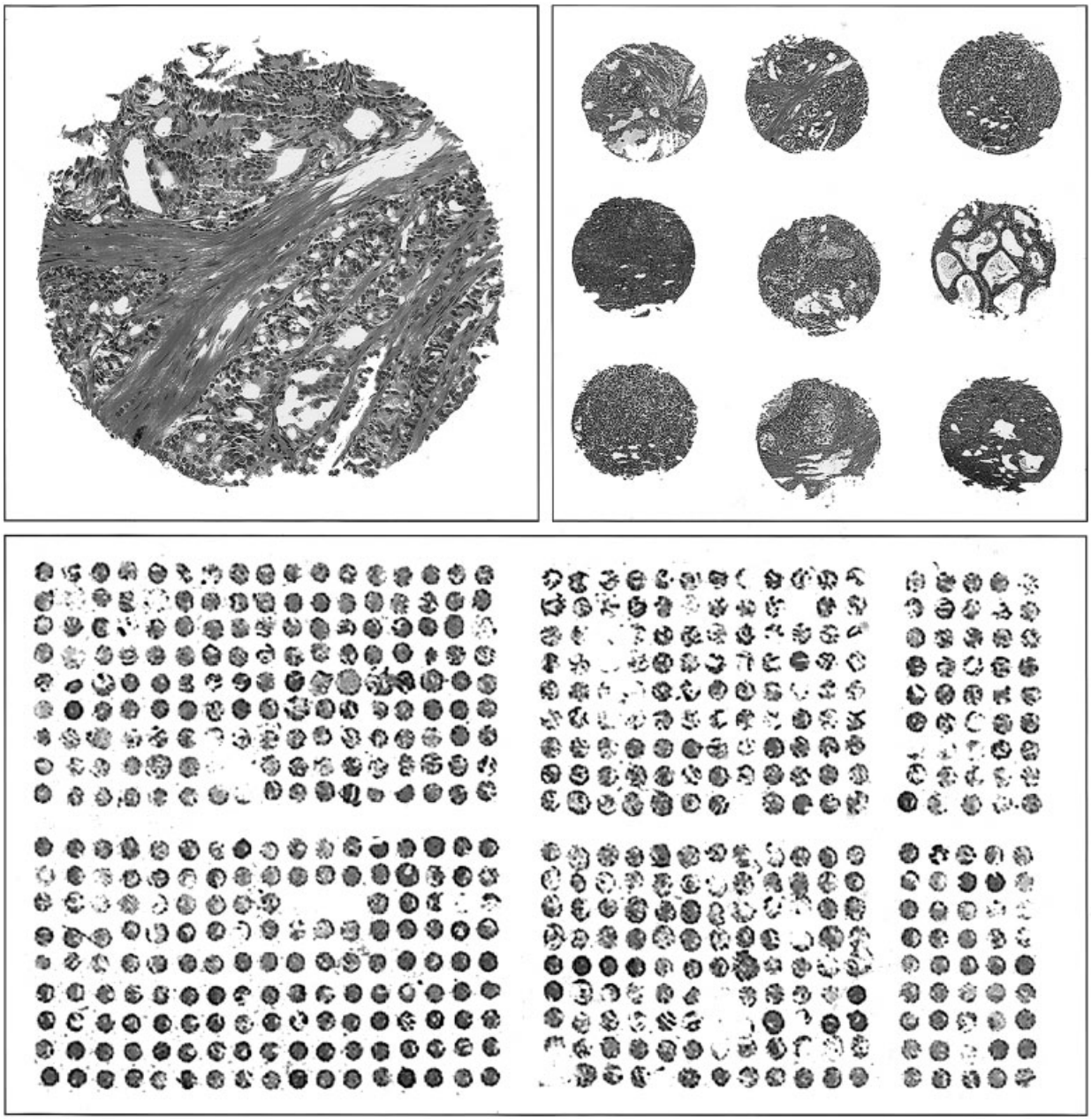

FIGURE 1 – Prostate progression tissue microarray containing specimens from 470 tumors and 65 benign controls (hematoxylin-eosin staining).

TABLE II – ANTIBODIES AND ANTIGEN-RETRIEVAL TECHNIQUES APPLIED[1]

Protein (antibody)	Retrieval	Source	Dilution
Bcl-2	MW, 98°C, 60 min	DAKO	1:400
CD-10	MW, 98°C, 60 min	Novocastra	1:50
CD-117	PK, 121°C, 5 min	DAKO	1:300
Epidermal Growth Factor Receptor (EGFR)	Pronase, 37°C, 30 min	Zymed	1.200
Epithelial Cellular Adhesion Molecule (EpCAM)	MW, 80°C, 30 min	Novocastra	1:800
HER2/neu	MW, 98°C, 60 min	DAKO	1:2000
Heat-shock Protein 90 (HSP90)	MW, 98°C, 60 min	Novocastra	1:400
Integrin-linked Kinase (ILK)	MW, 98°C, 60 min	Upstate Biotech.	1:3200
Ki67 (Mib1)	PK, 121°C, 5 min	DAKO	1:800
Matrix Metalloproteinase 2 (MMP-2)	MW, 98°C, 30 min	Neomarkers	1:200
Transmembrane Mucin 1 (MUC-1)	PK, 121°C, 5 min	Transgene	1:1800
p53	MW, 98°C, 60 min	DAKO	1:200
Syndecan-1	MW, 98°C, 60 min	DAKO	1:200

[1]For all proteins (except Ki67) positivity was defined as at least moderate staining intensity in >10% of tumor cells. MW = microwave, PK = pressure cooker.

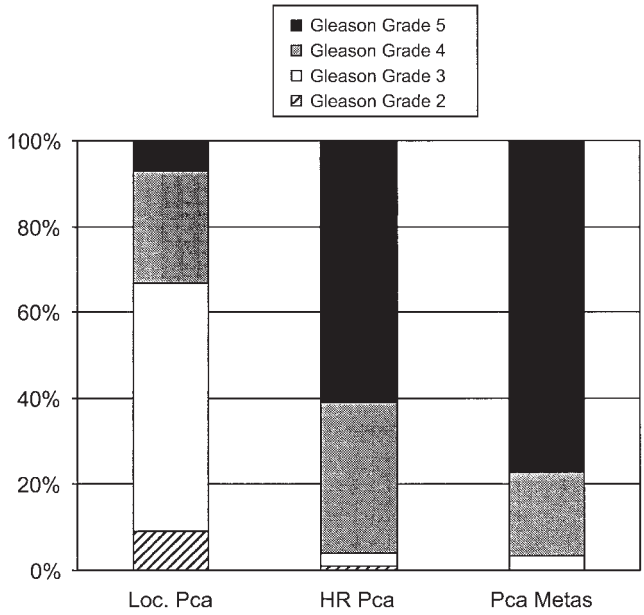

FIGURE 2 – Gleason grades in localized, hormone-resistant and metastatic prostate cancer ($p < 0.001$). Loc.Pca, localized prostate cancer; HR Pca, hormone-refractory prostate cancer; Pca Metas, prostate cancer metastases.

tyrosine phosphatase) expression was found in 1 single distant metastasis but not in any other specimen. The reactivity of this antibody was verified in mast cells serving as internal controls.

Discussion

TMA technology is a powerful tool for rapid testing of molecular markers in hundreds of tumors in a single experiment.[9,10] Previous studies from our laboratory have shown that the analysis of 1 minute tissue sample (diameter 0.6 mm) per tumor provides highly representative epidemiologic information.[41] The strong confirmation of the previously known association of high expression levels of Ki67, Bcl-2 and p53 with advanced prostate cancer stage[42–45] provide again strong support for our experimental approach for the identification of progression associated markers in our study.

This project was focused on potential and established therapeutic targets, several of which have previously not been examined in a large number of prostate cancers. A significant overexpression in hormone-refractory and metastatic tissue as compared to localized prostate cancer was found for p53, Bcl-2, Syndecan-1, EGFR and HER2/neu. Except for Syndecan-1, which has previously not been analyzed in advanced prostate cancers, these results confirm earlier findings from smaller cohorts.[46–50] Our data add a further argument to the growing evidence that these proteins may contribute to the progression to late-stage, prostate cancer and encourage further experimental and clinical trials aiming at their inhibition. The low prevalence of Bcl-2 overexpression in 105 hormone-refractory cancers (10.5%) conflicts with the results from 2 other studies where Bcl-2 overexpression was reported in almost 100% of small series of hormone-refractory prostate cancers.[48,51] The reasons for this striking difference remains unclear. Most likely it is due to

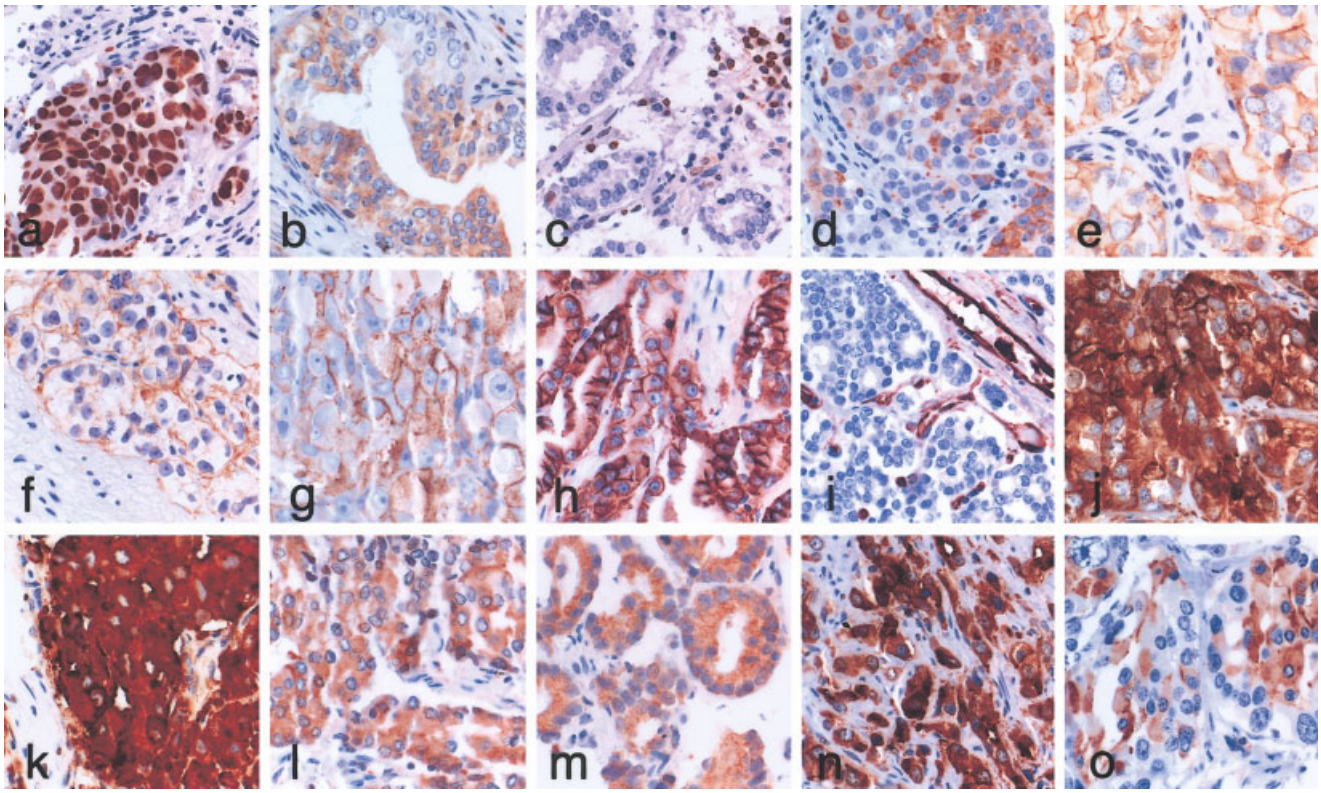

FIGURE 3 – Representative immunostainings (×400): (*a*) p53, (*b*) Bcl-2, PIN, (*c*) Bcl-2 negative prostate cancer (Pca) with lymphocytes as positive internal control, (*d*) Bcl-2 positive Pca. (*e*) Pca's positive for CD-138 (Syndecan), (*f*) HER2/neu, (*g*) EGFR, (*h*) Ep-CAM. (*i*) MMP-2 negative Pca with endothelial cells as internal positive control, (*j*) MMP-2 positive Pca. Pca's positive for (*k*) MUC-1, (*l*) HSP-90, (*m*) ILK, (*n*) CD-10 and (*o*) CD-117.

differences in the experimental conditions or cut-offs used. The paramount influence of experimental conditions on the reported prevalences of immunohistochemical Bcl-2 expression is illustrated by variations described from 46–81% in breast cancer,[52,53] 15–59% in squamous cell carcinoma of the lung[54,55] and 2–38% in clinically localized prostate cancers.[56,57] It is unlikely that a sampling error due to the TMA approach is responsible for the low incidence of positive cases in our study. Also in our routine diagnostic prostate core needle biopsy specimens less than 5% of the prostate cancers are Bcl-2 positive (own unpublished data). A proper immunohistochemical reaction in our study was confirmed by the presence of Bcl-2 positive lymphocytes or basal cells from adjacent benign glands, which were present in most of TMA specimens. Our data suggest that response to therapies directed against Bcl-2 can only be expected in a minor fraction of hormone-refractory prostate cancers.

The preferential overexpression of HER2/neu in hormone-refractory and metastatic prostate cancers is in accordance with previous immunohistochemical studies and supports experimental evidence, suggesting that HER2/neu might be involved in hormone-refractory growth of prostate cancer.[49,50,58] Accordingly, HER2/neu overexpression has been implicated in the ligand independent activation of the androgen receptor and in the development of hormone-refractory tumor growth *in vitro*.[50,59,60] However, treatment with the anti-HER2/neu antibody trastuzumab (Herceptin®), which is efficient in breast cancers with amplification-driven HER2/neu overexpression,[61] has failed in advanced prostate cancers despite experimental evidence of favorable response of prostate cancer xenograft models.[8,62] The reasons for treatment failure of Herceptin® in advanced prostate cancer is not entirely clear. It might be explained be the lack of HER2/neu gene amplification in prostate cancer[63,64] and the low prevalence of HER2/neu overexpression, when the standardized Hercep-Test® kit to predict therapy response is used.[65] In the absence of gene HER2/neu amplification, protein expression may not reach the high levels that are required for response to Herceptin® therapy. The broad range of HER2/neu overexpression in prostate cancer reported in the literature may be due to technical factors such as different antibodies, protocols, fixations and different scoring procedures.[58,66]

EGFR, another member of the transmembrane tyrosine kinase receptor family, revealed a similar expression pattern as HER2/neu. In preclinical studies, ZD1839 (Iressa®, AstraZeneca Pharmaceuticals, Macclesfield, UK), a selective inhibitor of epidermal growth factor receptor-tyrosine kinase, produced growth inhibition in a wide variety of common solid tumor types including human prostate tumor xenografts.[17,67,68] Early results from clinical phase I trials in patients with advanced prostate cancers suggest that ZD 1839 offers an acceptable tolerability profile and promising clinical efficacy.[69,70] Most recent studies in nonsmall cell lung cancers suggest that activating EGFR mutations can predict the sensitivity to ZD 1839.[71,72] Further studies are needed to investigate whether such mutations of the EGFR gene are also present in a subgroup of advanced prostate cancers.

Overexpression of syndecan-1 in recurrent hormone refractory prostate cancer confirms previous results from DNA microarray studies in the CWR22 xenograft model system where syndecan-1 was strongly upregulated in the hormone-refractory xenograft CWR22R as compared to the hormone-sensitive parent tumor CWR22.[73] We recently found a prognostic importance for Syndecan-1 in prostate cancers from 551 patients with clinical long-time follow-up information.[35] Syndecan-1 (CD-138) binds to various matrix proteins and to a large number of heparin-binding polypeptide growth factors.[74–76] Syndecan-1 might contribute to prostate cancer progression by providing heparan sulfate chains to both fibroblast growth factor receptor-1 (FGFR1)- and FGFR2-signaling complexes.[77] Recent experiments show that coating myeloma cells with anti-Syndecan-1 antibodies promotes cross-presentation of cellular antigens by dendritic cells to autologous T cells from healthy donors, thereby offering one possible strategy for immunotherapy.[32]

Proteins with enhanced expression in advanced stages are preferable therapeutic targets, since the abortion of their progression promoting properties may lead to regression or inhibit tumor growth. However, also proteins that were not directly associated with progression in this study such as MUC-1, ILK, HSP-90, MMP-2, CD-10 and Ep-CAM could be of therapeutic interest, as they might still be part of critical pathways sustaining a basic level of tumor growth. Expression of human MUC-1 (urinary mucin 1), a membrane bound glycoprotein, slowly but gradually increased from BPH to PIN and throughout the various stages of prostate cancer. MUC-1 overexpression in cancer cells interferes with cell adhesion and shields the tumor cell from immune recognition, thus favoring tumor progression and development of metastases.[78] It has previously been suggested that overexpression of MUC-1 correlated with higher Gleason grades and advanced pathologic stage.[79] Several clinical phase I/II trials were recently announced using interleukin-2 based vaccines targeting the MUC-1 peptide for the treatment of recurrent or progressive prostate cancer.[80,81]

As previously suggested, overexpression of integrin-linked kinase (ILK) was restricted to high-grade PIN and invasive cancer but did not occur in benign prostatic hyperplasia.[82] This is in accordance with earlier experimental evidence indicating that ILK might act as a proto-oncogene by inducing tumorigenicity of epithelial cells in nude mice.[83] In prostate cancer, ILK is negatively regulated by the tumor suppressor PTEN and has been shown to be upregulated in tumors with PTEN mutation, which frequently occurs in late-stage prostate cancers.[84,85] ILK is therefore being considered as a promising therapeutic target in tumors with PTEN mutations, and selective small molecule inhibitors of ILK have been developed.[25] ILK is also involved in the Wnt-1 signaling pathway, leading to stabilization of beta-catenin and enhanced gene transcription, which is a critical early mechanism of colorectal carcinogenesis.[24,86] Interestingly, the nonsteroidal anti-inflammatory drugs acetylsalicylic acid and sulindac have been shown to inhibit ILK signaling in the colon carcinoma cell line Caco-2.[24]

We previously found that CD-10 expression is suppressed after neoadjuvant androgen withdrawal of prostate cancer,[35] suggesting that CD-10 is androgen-regulated, possibly through a androgen response element of the CD-10 gene.[87] Retained expression in about half of hormone-refractory prostate cancers in our study could reflect a nonligand induced restoration of the androgen-receptor pathway, which has been suggested as a possible mechanism of hormonal therapy escape.[73,88]

The tyrosine phosphatase inhibitor imatinib (STI 571, Glivec®, Novartis) leads to dramatic treatment responses in chronic myelogeneous leukemias and gastrointestinal stromal tumors, which show strong expression of CD-117, a product of the proto-oncogene c-kit.[89,90] In contrast, CD-117 was not expressed in any prostatic tissue except for 1 metastasis. To evaluate the therapeutic potential of imatinib for the treatment of advanced prostate cancer, further studies are required investigating the expression profiles of other tyrosine kinases inhibited by Imatinib such as platelet derived growth factor receptor (PDGFR).

Despite the apparent advantages of TMA analyses, several technical aspects still need to be considered.[10] The prevalence of marker positivity from TMA experiments can be an underestimate of the true prevalence due to heterogeneity. However, this sampling bias affects different prognostic groups or tumor stages to an equal degree. Therefore, differences in prognosis or stage distribution can still reliably be detected using 1 single tissue core per tumor, as long as the TMA contains a sufficiently high number of different samples.[10,34] The results of molecular analyses can be affected by the degree of tissue preservation in different types of tissue specimens. In our study, the expression profiles of most analyzed proteins were unexpectedly lower in the metastases from autopsies than in the hormone-refractory local recurrences from resection specimens. This phenomenon is most likely due to an increased decay of some proteins in postmortem autopsy tissues as compared to biopsies or surgical

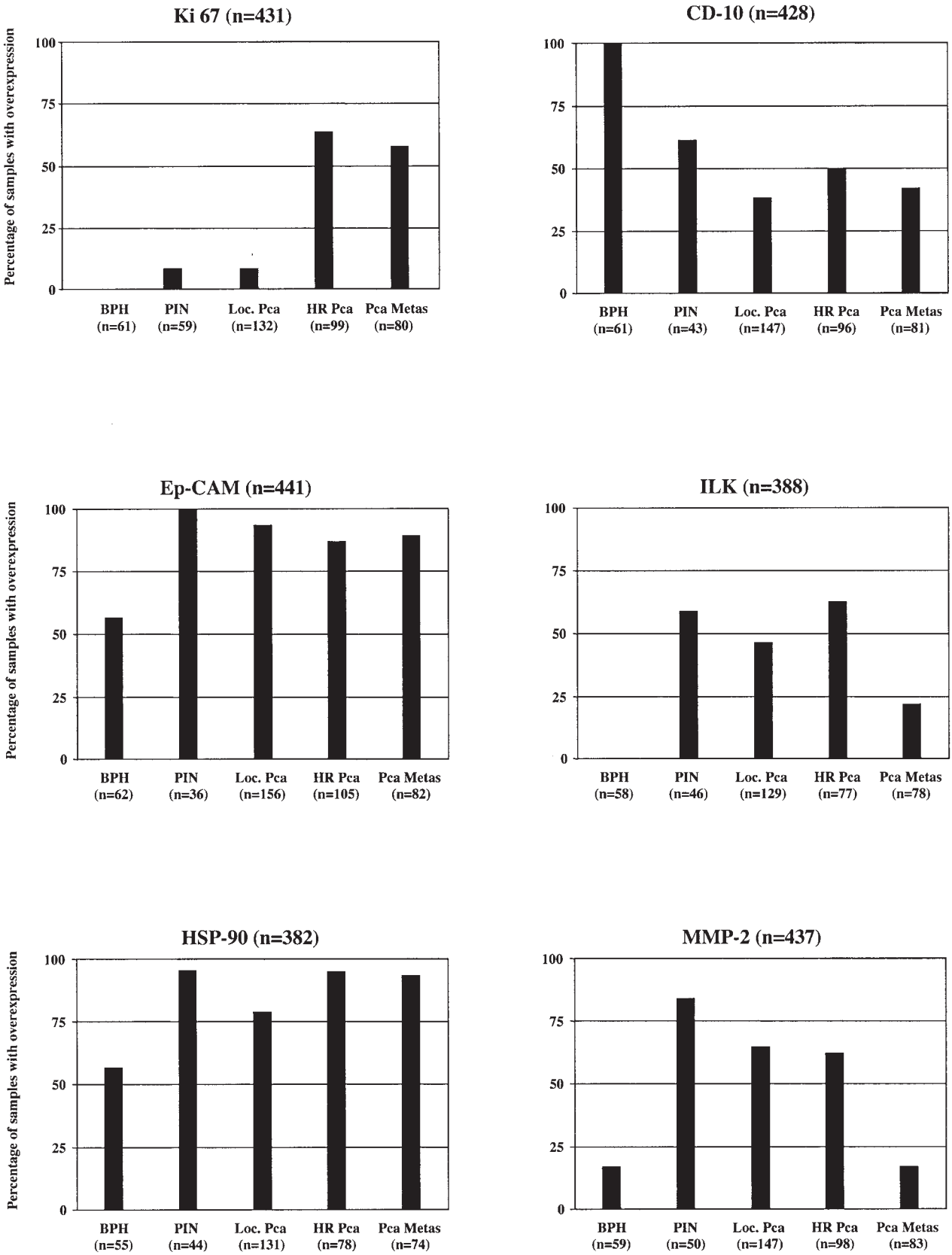

FIGURE 4 – (*a*,*b*) Growth fraction (Ki67 Labelling Index) and expression profiles of candidate markers in BPH, PIN and across prostate cancer progression. A Ki67 LI > 20% was defined as high. For the other markers, positivity was defined as at least moderate staining intensity in >10% of the tumor cells. BPH = benign prostatic hyperplasia, PIN, high-grade prostatic intraepithelial neoplasia; Loc. Pca, localized prostate cancer; HR Pca, hormone-refractory prostate cancer; Pca Metas, prostate cancer metastases. Note that that for better visualization, scales in (*b*) have been changed to a maximum of 50% on the y-axis.

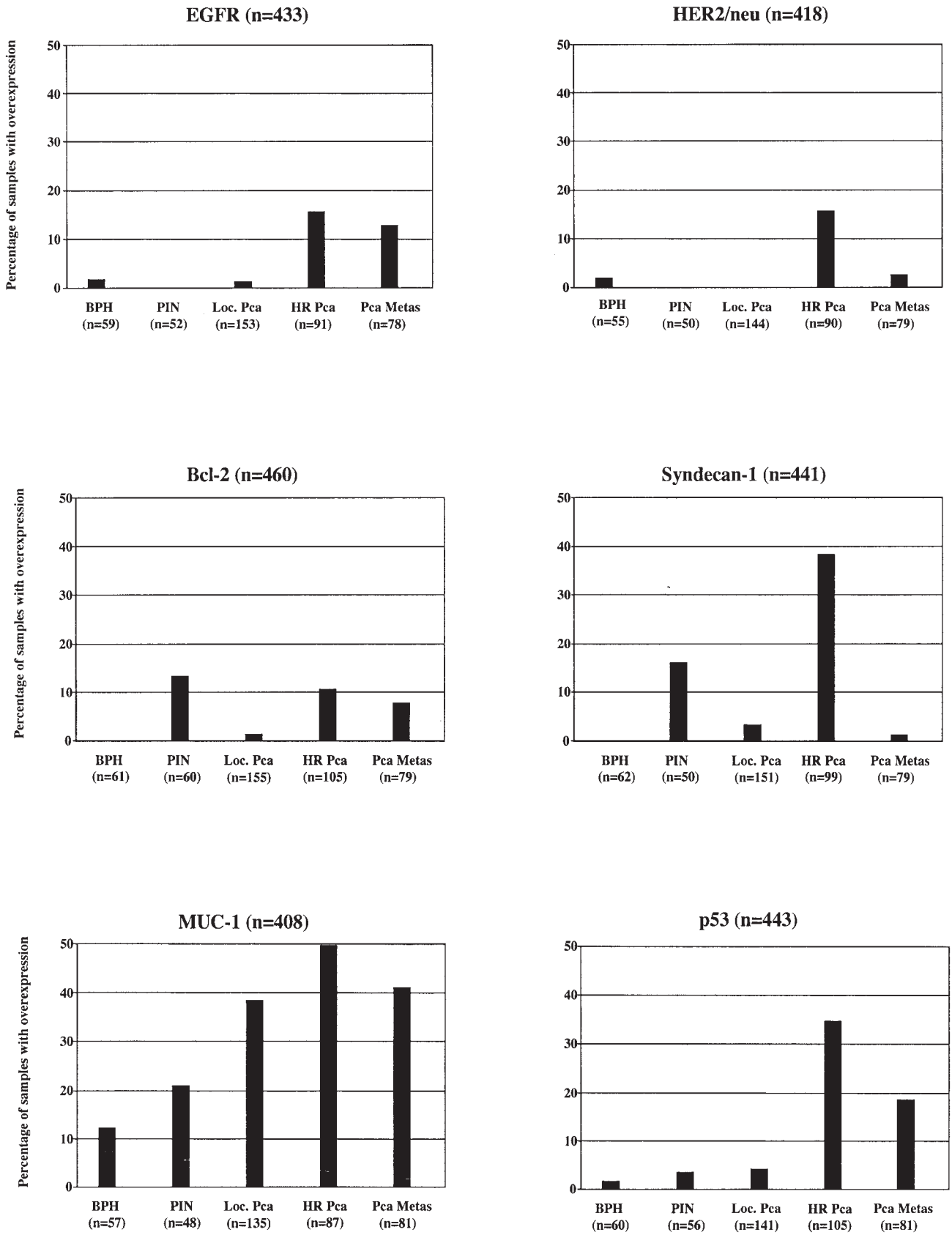

FIGURE 4 – CONTINUED.

TABLE III – ASSOCIATION BETWEEN PROTEIN EXPRESSION AND GLEASON GRADES

Protein	Gleason 2	Gleason 3	Gleason 4	Gleason 5	*p*-value
Bcl-2 pos ($n = 16$)	0	0	4	12	0.0017
Bcl-2 neg ($n = 201$)	14	3	79	105	
CD-10 pos ($n = 116$)	7	29	33	47	0.2
CD-10 neg ($n = 160$)	5	50	44	61	
CD-117 pos ($n = 1$)	0	0	1	0	0.45
CD-117 neg ($n = 292$)	14	85	78	115	
EGFR pos ($n = 25$)	0	1	7	17	0.000001
EGFR neg ($n = 253$)	14	82	69	88	
EpCAM pos ($n = 265$)	13	79	79	94	0.000001
EpCAM neg ($n = 29$)	0	3	4	22	
HER2/neu pos ($n = 15$)	0	0	9	6	0.0007
HER2/neu neg ($n = 294$)	11	77	67	94	
HSP 90 pos ($n = 211$)	4	62	53	92	0.000001
HSP 90 neg ($n = 32$)	5	11	10	6	
ILK pos ($n = 113$)	0	37	36	40	0.0036
ILK neg ($n = 126$)	8	33	30	55	
Ki67 high ($n = 42$)	0	0	2	40	0.000001
Ki67 low ($n = 171$)	12	75	44	40	
MMP-2 pos ($n = 157$)	5	51	48	53	0.000001
MMP-2 neg ($n = 122$)	8	29	28	57	
MUC-1 pos ($n = 108$)	2	28	35	43	0.0008
MUC-1 neg ($n = 148$)	8	44	37	59	
P53 pos ($n = 46$)	0	1	8	37	0.00001
P53 neg ($n = 241$)	13	79	70	79	
Syndecan-1 pos ($n = 41$)	0	2	12	27	0.000001
Syndecan-1 neg ($n = 245$)	14	81	67	83	

TABLE IV – ASSOCIATION BETWEEN PROTEIN EXPRESSION LEVELS (POS/NEG) AND Ki67 LI (LOW/HIGH)

Protein	Low Ki67 LI (0–20% pos. cells)	High Ki67 LI (21–100% pos. cells)	*p*-value
Bcl-2 pos ($n = 27$)	19	8	0.001
Bcl-2 neg ($n = 390$)	353	37	
CD10 pos ($n = 204$)	184	20	0.09
CD10 neg ($n = 178$)	155	23	
CD117 pos ($n = 1$)	1	0	0.4
CD117 neg ($n = 395$)	350	45	
EGFR pos ($n = 26$)	17	9	0.0013
EGFR neg ($n = 361$)	328	33	
EpCAM pos ($n = 338$)	299	39	0.27
EpCAM neg ($n = 57$)	51	6	
HER2/neu pos ($n = 14$)	13	1	0.225
HER2/neu neg ($n = 353$)	312	41	
HSP90 pos ($n = 280$)	238	42	0.000001
HSP90 neg ($n = 60$)	60	0	
ILK pos ($n = 131$)	110	21	0.22
ILK neg ($n = 205$)	183	22	
MMP-2 pos ($n = 196$)	178	18	0.36
MMP-2 neg ($n = 190$)	164	26	
MUC1 pos ($n = 128$)	110	18	0.1
MUC1 neg ($n = 229$)	204	25	
P53 pos ($n = 55$)	36	19	0.000001
P53 neg ($n = 348$)	322	26	
Syndecan-1 pos ($n = 49$)	36	13	0.000001
Syndecan-1 neg ($n = 344$)	313	31	

resections.[91,92] Therefore, data from autopsy tissues should generally be interpreted with caution.

In conclusion, our study shows that p53, Bcl-2, Syndecan-1, EGFR and HER2/neu are predominantly expressed in hormone-refractory and metastatic prostate cancer, thus being valuable targets for further therapeutic evaluations. However, since these markers are only expressed in a fraction of advanced tumors, it will be necessary to assess the efficacy of targeted therapies in relation to the marker expression in the individual patients rather than looking at a raw overall efficacy. Once such therapies reach clinical routine, the patients will need to be selected based on the marker profile of their tumors, which can easily be assessed using immunohistochemistry on biopsies from local recurrences or distant metastases. It remains to be shown if some targeted therapies against progression-related markers may also be effective in tumors in an adjuvant setting, *before* development of hormone-refractory growth or metastases, *e.g.*, in asymptomatic patients with rising PSA-values after radiotherapy or radical prostatectomy.

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