Signatures of Adverse Pathological Features, Androgen Insensitivity and Metastatic Potential in Prostate Cancer

YURI TOLKACH, AXEL MERSEBURGER, THOMAS HERRMANN, MARKUS KUCZYK, JÜRGEN SERTH and FLORIAN IMKAMP

Clinic for Urology and Urologic Oncology, Hannover Medical School, Hannover, Germany

Abstract. *Background/Aim: The genetic characterization of prostate tumors is important for personalized therapy. The aim of the present study was to investigate the role of previously described prostate cancer-related genes in the genetic characterization of prostate tumors. Materials and Methods: Forty-two genes were selected for expression analysis (real time-quantitative polymerase chain reaction). One normal prostatic epithelial cell line and three standardized prostate cancer cell lines were used. Twenty-eight patients treated with radical prostatectomy were included in the study. Results: The following genes appeared to be possibly related to the metastatic potential of the tumor: ELOVL fatty acid elongase 7 (ELOVL7), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), gastrulation brain homeobox 2 (GBX2), golgi membrane protein 1 (GOLM1), homeobox C6 (HOXC6), minichromosome maintenance complex component 6 (MCM6), marker of proliferation Ki-67 (MKI67), mucin 1, cell surface associated (MUC1), MYC binding protein 2, E3 ubiquitin protein ligase (MYCBP2), somatostatin receptor 1 (SSTR1), topoisomerase (DNA) II alpha 170 kDa (TOP2A) and exportin 6 (XPO6). Six genes were differentially expressed in patients with localized and locally advanced cancer (GOLM1, GBX2, XPO6, SSTR1, TOP2A and cell division cycle associated 5, CDCA5) and three genes (HOXC6, Cyclin-dependent kinase inhibitor 2A (CDKN2A) and MYC binding protein 2, E3 ubiquitin protein ligase, MYCBP2) in patients with a low vs. high Gleason grade/sum. Conclusion: Some of the investigated genes show promising prognostic and classification features, which might be useful in a clinical setting, warranting for further validation.*

Correspondence to: Dr. Florian Imkamp, Clinic for Urology and Urologic Oncology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany. Tel.: +49 5115323449, Fax: +49 511532163449, e-mail: imkamp.florian@mh-hannover.de

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The natural course of prostate cancer, its aggressiveness, invasiveness and metastatic potential cannot be determined and predicted by clinical parameters alone (1, 2). Thus, genetic characterization of prostate tumours is considered an essential step in the development of individualized therapy (3). All tumors are separate entities, which, nevertheless, share some common genetic changes (4). The identification of these would have a major clinical impact on everyday practice.

In recent years, multiple gene expression analysis (mRNA expression) of prostate cancer has been the focus of intensive study. Previous attempts led to the identification of genes specific for high-grade tumours (5), aggressive prostate cancer with a tendency towards recurrence (6-12), lethal tumours (10, 13) and hormone-refractory (14, 15) and metastatic prostate cancer (10, 16). Nevertheless, many questions remain unresolved, mainly due to the variability of the genomic landscape of prostate cancer (4), the huge number of affected genes and unclear interactions between certain genes, tumour multifocality and inter- and intra-tumoural heterogeneity (17).

The aim of the present study was to use multiple gene-expression data on prostate cancer cell lines and on cancerous tissue from radical prostatectomy specimens to identify gene signatures associated with specific clinical or pathomorphological events or parameters.

Materials and Methods

Design of the study. This study was a single-site prospective pilot study of multiple gene expression patterns in malignant tissues of patients with prostate cancer and cancer cell lines with different metastatic and malignant potential (androgen-insensitive or -sensitive). It aimed to determine the variations in expression profiles that could be further used for approbation in a bigger clinical cohort.

Selection of genes for analysis. Forty-two genes (Table I) were selected for expression analysis based on evidence from published studies of genes that tended to be affected in prostate cancer (5-9). The expression levels of two housekeeping genes [5'-aminolevulinate synthase 1 (*ALAS1*) and hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) served as an endogenous control and were used for normalization.

Table I. *List of the 42 genes analysed in the study.*

Gene	Name, Family, function	Gene	Family, function	Gene	Family, function
ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4 Transport, signal transduction	*ERG*	V-ets avian erythroblastosis virus E26 oncogene homolog Oncogene, cell cycle	*MYO6*	Myosin VI Protein, intracellular transport
AMACR	Alpha-methylacyl-CoA racemase Enzyme, cell metabolism	*ETV1*	Ets variant 1 Protein, cell cycle	*NRP1*	Neuropilin 1 Protein, receptor, cell growth, migration, invasion
ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1 Enzyme, lipid metabolism	*EZH2*	Enhancer of zeste 2 polycomb repressive complex 2 subunit Enzyme, gene silencing	*PLK1*	Polo-like kinase 1 Protein kinase, cell cycle
AURKA	Aurora kinase A Protein kinase, cell cycle	*FGFR2*	Fibroblast growth factor receptor 2 FGF receptor	*PTTG1*	Pituitary tumor-transforming 1 Protein, cell cycle, tumorigenic
BMI1	B lymphoma Mo-MLV insertion region 1 homolog (mouse) Oncogene, cell cycle	*GBX2*	Gastrulation brain homeobox 2 Protein, gene expression regulator	*SMARCA4*	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 Protein, transcriptional factor
BUB1	BUB1 mitotic checkpoint serine/threonine kinase Protein kinase, cell cycle	*GOLM1*	Golgi membrane protein 1 Golgi complex function	*SPINK1*	Serine peptidase inhibitor, Kazal type 1 Protein, tumor associated
BUB3	BUB3 mitotic checkpoint protein Protein, cell cycle	*HOXC6*	Homeobox C6 Transcription factor	*SSTR1*	Somatostatin receptor 1 Somatostatin receptor type 1
CBX3	Chromobox homolog 3 Protein, component of heterochromatin	*KLK3*	Kallikrein-related peptidase 3 PSA coding gene	*TMPRSS2-ERG fusion*	Transmembrane protease, serine 2 and ERG fusion Oncogenic fusion gene
CCNB1	Cyclin B1 Protein, cell cycle	*MCM6*	Minichromosome maintenance complex component 6 Protein, cell cycle	*TOP2A*	Topoisomerase (DNA) II alpha 170kDa Enzyme, transcription
CDC6	Cell division cycle 6 Protein, cell cycle	MKI67	Marker of proliferation Ki-67 Protein, cell cycle, transcriptional factor	*TPD52*	Tumor protein D52 Tumor protein
CDCA5	Cell division cycle associated 5 Protein, cell cycle	MSH2	MutS homolog 2 DNA mismatch repair protein, tumor suppressor	*UBE2I*	Ubiquitin-conjugating enzyme E2I Protein, abnormal protein degradation
CDK4	Cyclin-dependent kinase 4 Protein kinase, cell cycle	*MTA1*	Metastasis associated 1 Transcriptional factor, chromatin remodeling	*UHRF1BP1*	UHRF1 binding protein 1 Protein, chromatin structure, gene expression
CDKN2A	Cyclin-dependent kinase inhibitor 2A Tumor suppressor protein, cell cycle	*MUC1*	Mucin 1, cell surface associated Cell surface glycoprotein	*XIAP*	X-linked inhibitor of apoptosis, E3 ubiquitin protein ligase Protein, inhibitor of apoptosis
ELOVL7	ELOVL fatty acid elongase 7 Protein, lipid metabolism	*MYCBP2*	MYC binding protein 2, E3 ubiquitin protein ligase Cell signalling, pathogen protection via binding	*XPO6*	Exportin 6 Protein, intracellular transport

FGF: Fibroblast growing factor; PSA: prostate-specific antigen.

Description of the patient cohort. Twenty-eight patients diagnosed with clinically localized and biopsy-confirmed prostate cancer in 2010–2011 were included in the study (Table II). All the patients underwent either a laparoscopic (n=1) or open retropubic (n=27) radical prostatectomy. Pelvic lymphadenectomy was performed in 26 cases with a limited or extended volume. Only one patient had a family history of prostate cancer (one first-line relative). Metastatic disease was ruled out by computed (CT) or magnetic resonance tomography (MRT) and a bone scan according to current guidelines of European Association of Urology (EAU).

Sample acquisition. All samples for further analysis were extracted in the operation theatre immediately after the removal of a prostate

Table II. *Perioperative characteristics of patients and results of the final pathomorphological evaluation of the prostatectomy specimen (n=28)*

Parameter	Additional information				
	Median	IQR	Range		
Age, years	66	59.5-69.5	49-75		
BMI, kg/m^2	26.7	24.6-30.2	21.7-32		
Total PSA, ng/ml	7.02	5.57-11.0	4.0-64.0		
Diabetes mellitus, n	Yes	No			
	4	24			
	Pathomorphological staging				
pT	pT2a	pT2b	pT2c	pT3a	pT3b
	2	2	15	5	4
pT stage (localized vs. advanced)	Localized cancer			Locally advanced cancer	
	19			9	
pN, n	pN0	pN1			
	26	2			
pR, n	pR0	pR1			
	21	7			
	Gleason 3	Gleason 4	Gleason 5		
Primary Gleason, n	23	5	0		
Secondary Gleason, h	8	18	2		
	6 (3+3)	7 (3+4)	7 (4+3)	8 (4+4)	9 (4+5)
Gleason Sum, n	4	19	3	0	2

BMI: Body mass index, IQR: interquartile range.

specimen in the course of the radical prostatectomy. Based on the biopsy results (and further controlled using the final pathology), a tissue specimen was extracted from regions of the expected location of the prostate cancer in the gland using a standard 16-core needle and an automatic biopsy device. Specimens were also obtained randomly from the entire prostate gland. Two tissue cylinders were extracted from each zone for research purposes and histological control. All the samples were fresh-frozen in liquid nitrogen and stored at −80˚C until the further processing in the laboratory.

Cell line and culture conditions. Three standardized prostate cancer cell lines were used: LNCaP (androgen-sensitive, low metastatic potential), DU145 (androgen-insensitive, moderate metastatic potential) and PC3 (androgen-insensitive, high metastatic potential). As a control, a PreC cell line (normal prostatic epithelial cells) was used. The selected cell lines were received from Cell Line Services (Heidelberg, Germany), cultivated in standard conditions and used for analyses after maximum of 1-2 passages.

Sample processing, RNA extraction and RNA expression analysis. In the analysis, after histopathological verification, total RNA was extracted from the prostate core biopsies according to standard protocols using TRIZol® Reagent (Life Technologies, Carsbad, CA, USA). A high-capacity cDNA reverse transcription kit (Applied Biosystems, Waltham, MA, USA) was used for RNA conversion into cDNA. The expression analysis of all the selected genes was assayed using the TaqMan (Life Technologies) quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and reported as crossing-point values on a continuous scale. In the qRT-PCR, the ABI 7900 Fast Sequence Detection System with universal PCR master mix and TaqMan expression assays (Applied Biosystems) were used according to the manufacturer's instructions with commercially available primers from Applied Biosystems. TaqMan assays were used for all aforementioned genes, including the housekeeping genes, which served as endogenous controls. Relative quantification calculations of gene expression (the quantity of mRNA present in the cytoplasm of the cells) were performed using the absolute RT-PCR CT values of each gene's mRNA, adjusted to the etalon expression data derived from normal epithelial cells of the prostate. In addition, blank, no-template and no reverse transcription controls were included for each measurement. The same extraction, purification and RT-PCR procedures were used for the cell line study.

Data analysis and statistics. DataAssist Software v. 3.01 (Life Technologies) was used for final processing of the mRNA expression data *p*-values were adjusted for multiple hypotheses testing using the Benjamini–Hochberg false discovery rate method. Relative overexpression was considered significant when the fold-change was more than 2.0, or for underexpression, less than 0.5. The statistical analysis was performed with STATISTICA 8.0 software (StatSoft, Tulsa, OK, USA).

Ethical issues. The study was approved by the local University Ethical Committee (Hannover Medical School, Hannover, Germany; HiLF-2010).

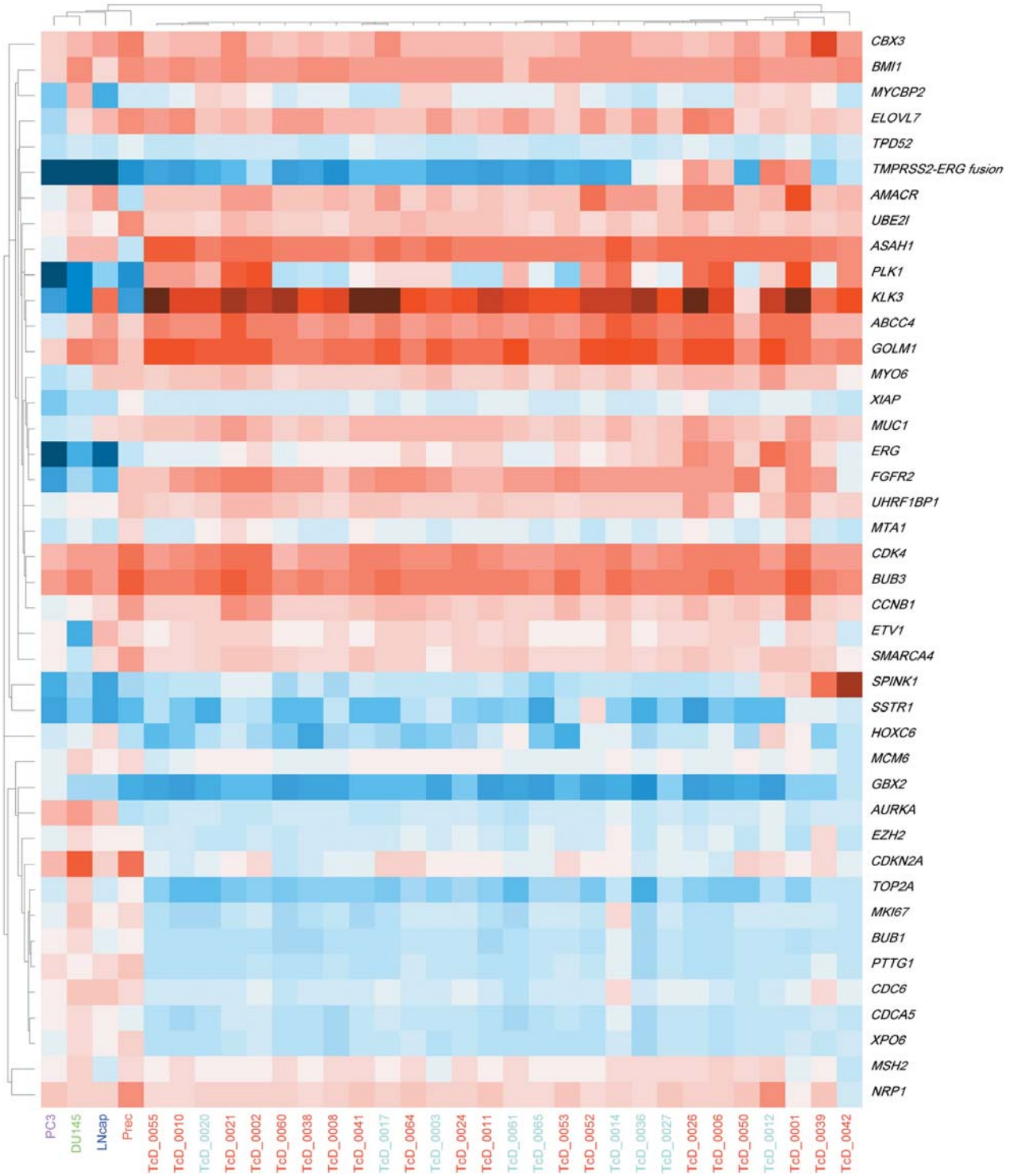

Figure 1. *Heat map of the expression of the 42 investigated genes in cell lines and human prostate cancer tissue with unsupervised clustering. The cell lines are: PreC: normal epithelial cells of the prostate, LNCaP: androgen-sensitive prostate cancer cells with low metastatic potential, DU145: androgen-insensitive cells with moderate metastatic potential, PC3: androgen-insensitive cells with high metastatic potential. The TcD classifier represents a tumor sample obtained from a patient. Blue-coloured font: samples obtained from patients with a locally advanced tumour; red-coloured font: samples obtained from patients with localized disease. Heat map colours: The red colour represents the overexpression of genes, with various intensity, and the blue colour represents the underexpression of genes, with various intensity. See Table I for full gene details.*

Cell line	Prec		LNCaP		DU145		PC3	
Gene								
ABCC4	1		3.88	↑	1.02		0.16	↓↓
AMACR	1		85.41	↑↑↑	24.16	↑↑	4.89	↑
ASAH1	1		40.51	↑↑↑	50.65	↑↑↑	4.13	↑
AURKA	1		44.79	↑↑↑	135.53	↑↑↑	68.21	↑↑↑
BMI1	1		0.09	↓↓↓	0.80		0.14	↓↓
BUB1	1		0.63		1.65		0.90	
BUB3	1		0.13	↓↓	0.29	↓	0.12	↓↓
CBX3	1		0.41	↓	0.35	↓	0.11	↓↓
CCNB1	1		0.20	↓↓	0.13	↓↓	0.05	↓↓↓
CDC6	1		1.91		1.79		0.60	
CDCA5	1		1.37		1.91		1.28	
CDK4	1		0.28	↓	0.26	↓	0.19	↓↓
CDKN2A	1		0.06	↓↓↓	1.76		0.15	↓↓
ELOVL7	1		0.53		0.11	↓↓	0.0033	↓↓↓
ERG	1		0.0006	↓↓↓	0.02	↓↓	0.0003	↓↓↓
ETV1	1		5.68	↑↑	0.002	↓↓↓	0.80	
EZH2	1		0.98		1.50		0.39	↓
FGFR2	1		0.0019	↓↓↓	0.01	↓↓↓	0.0002	↓↓↓
GBX2	1		9.46	↑↑	11.81	↑↑↑	150.97	↑↑↑
GOLM1	1		3.31	↑	5.77	↑↑	0.46	
HOXC6	1		16.77	↑↑↑	6.02	↑↑	3.04	↑
KLK3	1		32254.0	↑↑↑	0.27	↓	0.71	
MCM6	1		0.81		1.74		0.43	↓
MKI67	1		0.56		2.16	↑	0.37	↓
MSH2	1		0.21	↓	1.47		0.54	
MTA1	1		0.09	↓↓↓	0.26	↓	0.09	↓↓↓
MUC1	1		0.63		0.14	↓↓	0.06	↓↓↓
MYCBP2	1		0.01	↓↓↓	15.66	↑↑↑	0.03	↓↓↓
MYO6	1		1.17		0.07	↓↓↓	0.02	↓↓↓
NRP1	1		0.18	↓↓	0.17	↓↓	0.28	↓
PLK1	1		25.32	↑↑↑	0.34	↓	0.04	↓↓↓
PTTG1	1		0.42	↓	0.26	↓	0.40	↓
SMARCA4	1		0.16	↓↓	0.02	↓↓↓	0.15	↓↓
SPINK1	1		0.03	↓↓↓	0.69		0.06	↓↓↓
SSTR1	1		0.33	↓	3.62	↑	0.40	↓
TOP2A	1		0.37	↓	2.47	↑	0.36	↓
TPD52	1		0.29	↓	0.57		0.10	↓↓↓
UBE2I	1		0.10	↓↓↓	0.10	↓↓↓	0.07	↓↓↓
UHRF1BP1	1		0.29	↓	0.26	↓	0.17	↓↓
XIAP	1		0.10	↓↓↓	0.09	↓↓↓	0.02	↓↓↓
XPO6	1		0.53		0.86		0.33	

Underexpression		*Overexpression*	
↓	2-5 fold	↑	2-5 fold
↓↓	5-10 fold	↑↑	5-10 fold
↓↓↓	>10 fold	↑↑↑	>10 fold

Figure 2. *Relative expression (fold change) of the gene panel (42 genes) in four cell lines. Normal prostatic epithelia of cell line PreC was used as the reference sample (calibrator) to which relative expression was measured for other cell lines. Significant overexpression was defined as a fold change of more than 2 and significant underexpression as a fold change of less than 0.5. See Table I for full gene details. Characteristics of the cell lines: PreC - normal epithelia; LNCaP - low invasiveness, low metastatic potential, androgen naïve; DU145 - moderate invasiveness, moderate metastatic potential, androgen insensitive, no PSA expression; PC3 - high invasiveness, high metastatic potential, androgen insensitive, no PSA expression.*

A

Gene	Localized cancer		Locally advanced cancer	p-value
	Fold change	Colour code	Fold change	
GOLM1	1		2.21	0.03
GBX2	1		0.01	0.04
XPO6	1		0.20	0.04
SSTR1	1		0.06	0.05
TOP2A	1		0.09	0.05
CDCA5	1		0.31	0.05
TPD52	1		0.35	0.06
MKI67	1		2.1	0.06
XIAP	1		0.47	0.07
AURKA	1		0.44	0.07
BUB1	1		0.26	0.08
PTTG1	1		0.31	0.08

B

	Groups according to primary Gleason score and Gleason sum			
	Primary Gleason score			p-value
Gene	3		4	
HOXC6	1		3.84	0.05
	Gleason sum			
	6		≥7	
CDKN2A	1		2.36	0.03
MYCBP2	1		2.5	0.01

Expression is high
Expression is low

Figure 3. *A: Relative differences (fold change) in gene expression patterns of cancerous tissues between the groups of patients with localized (n=19) and locally advanced (n=9) prostate cancer. The levels of gene expression in the group with localized prostate cancer were used as a reference sample (calibrator). Here all genes are included with expression difference of 2 or more (i.e. >2 or <0.5) and given that this difference was statistically significant or had a clear trend towards significance. Under the bold line – the genes with trend to clinical significance, which have not reach the level of 0.05. B: Relative differences (fold change) in gene-expression patterns in cancerous tissues with different primary Gleason score and Gleason sum. As reference sample (calibrator) were chosen the levels of the gene expression in the groups with primary Gleason score 3 and Gleason sum 6, correspondingly. See Table I for full gene details.*

Results

Multiple gene expression in normal epithelial and prostate cancer cell lines. The patterns of the gene expression for 42 selected genes in cell lines are shown in Figure 1, which presents a heat map of the whole panel of genes both for the cell lines and for the human-derived tumor tissue.

Almost all the selected genes, with the exception of cell division cycle 6 (CDC6), cell division cycle-associated 5 (CDCA5) and BUB1 mitotic checkpoint serine/threonine kinase (BUB1), were differentially expressed in one or several of the cell lines (Figure 2). All the genes analyzed in the cell lines could be placed into one of three groups: genes without significant differences between two or more cell lines and genes that were significantly overexpressed, or underexpressed in certain cell lines. In Figure 2, the 42 genes are arranged according to the level of their relative expression.

Common changes among all tumor cell lines in comparison with the culture of the normal epithelial cells were relatively homogenous overexpression of alpha-methylacyl-CoA racemase (*AMACR*), N-acylsphingosine amidohydrolase (acid ceramidase) 1 (*ASAH1*) and aurora kinase A (*AURKA*) and underexpression of BUB3 mitotic checkpoint protein (*BUB3*), cyclin B1 (*CCNB1*), cyclin-dependent kinase 4 (*CDK4*), V-ets avian erythroblastosis virus E26 oncogene homolog (ERG), fibroblast growth factor receptor 2 (*FGFR2*), metastasis-associated 1 (*MTA1*), neuropilin 1 (*NRP1*), pituitary tumor-transforming 1 (*PTTG1*), SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4 (*SMARCA4*), ubiquitin-conjugating enzyme E2I (UBE2I), UHRF1 binding protein 1 (*UHRF1BP1*) and X-linked inhibitor of apoptosis, E3 ubiquitin protein ligase (*XIAP*). Other genes in the prostate cancer cell lines also exhibited differential expression (see Discussion).

Multiple gene expression in patients with prostate cancer. One of the main aims of the present study was to determine the associations between the multiple gene-expression profiles of the tumours and important clinical and pathomorphological parameters. The main parameters of interest were the pT and pN stages, surgical margin status (R-status), Gleason grade (primary Gleason grade and Gleason sum) and preoperative level of prostate-specific antigen (PSA). The pN stage was eventually excluded from the analysis, as only two patients had regional lymph node metastases.

All the patients were divided into two groups according to the final pathomorphological examination: those with localized disease (pT2a-c, n=19) and those with locally advanced disease (pT3a-b, n=9). It was assumed that the possible differences in gene expression in these two groups could help to identify the genes that are responsible for the invasive phenotype of the tumour. The results are presented in Figure 1. All the genes that were statistically significantly differentially expressed are outlined in Figure 3A. The Figure also contains the names of genes that seemed to display a trend towards statistical significance. These genes may be found to be of importance when applied to larger cohorts of patients.

Six genes were considered significant, and another six were considered possible predictors of local disease (pT). Importantly, only two of these (golgi membrane protein 1, GOLM1, and marker of proliferation Ki-67, MKI67) were overexpressed in patients with locally advanced prostate cancer. All others were underexpressed.

When the patients were divided according to the primary Gleason grade and Gleason sum of the final specimen pathology, the expression of three other genes homeobox C6 (*HOXC6*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and MYC binding protein 2, E3 ubiquitin protein ligase (*MYCBP2*) differed significantly between the investigated groups (Figure 3B). These three genes tended to be overexpressed in patients with a higher Gleason grade or sum.

The analysis of the R-status after prostatectomy failed to identify any correlation with gene expression. The R-status was not linked with clinical parameters, such as the pT stage, Gleason grade or PSA level, which means that other factors influence this parameter. Similarly, the serum level of total PSA was not associated with the expression of the genes.

Discussion

The main goal of the present study was to investigate the expression of 42 genes which have been shown to be important in prostate cancer (5-9) in a small cohort of patients and in cells lines and to select the most relevant genes for extension studies with archived and prospectively collected material.

We analyzed gene expression in three prostate cancer cell lines and one normal epithelial cell line. These cell lines partly represent the natural course of prostate cancer from healthy epithelial cells to androgen-insensitive tumors with high metastatic potential. This set of cell lines could provide insight into genetic changes taking place in tumors treated with androgens and representing different patterns of invasiveness and metastatic potential. Almost all selected genes, with the exception of *CDC6, CDCA5* and *BUB1*, exhibited differential expression in one or several cell lines (Figure 2), thereby partly confirming previous findings.

Keeping in mind restrictions based on cell line-based studies, we attempted to draw conclusions linking the gene-expression profiles of the tumors to their phenotypes. From a clinical point of view, it is important to be aware of the gene alterations associated with metastatic behaviour. Such knowledge has direct clinical applications (*e.g.* evaluation of biopsy samples with the aim of individualized treatment). However, many of the detected changes are overcast by the manifestation of androgen insensitivity at the genetic level, which has iatrogenic roots. In the present study, among the genes possibly reflecting the metastatic potential of tumours, the following appeared important: ELOVL fatty acid elongase 7 (*ELOVL7*), enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*), gastrulation brain homeobox 2 (*GBX2*), *GOLM1*, *HOXC6*, minichromosome maintenance complex component 6 (*MCM6*), *MKI67*, mucin 1, cell surface associated (*MUC1*), *MYCBP2*, somatostatin receptor 1 (*SSTR1*), topoisomerase (DNA) II alpha 170 kDa (*TOP2A*) and exportin 6 (XPO6). Some of these genes have been previously linked to prostate tumors. ELOVL7 is an important lipid metabolism enzyme, which has been shown to be overexpressed in hormone-naive prostate cancer (18). Our results demonstrated moderate and prominent underexpression of this gene in DU145 (moderate metastatic potential) and PC3 (high metastatic potential) cells, respectively. Thus, this could

be related to progressive changes in tumor cells, leading to more aggressive tumoral behaviour. EZH2 is a histone transferase and gene silencer, that is overexpressed in metastatic prostate cancer (19). However, in the present study, it was depressed in the highly metastatic PC3 tumour cell line and unaffected in the DU145 cell line, possibly indicating that the *EZH2* gene has an oppositional role in cancer growth (20). The GBX2 homeobox gene, which is necessary for cancer growth (21), was dramatically overexpressed in the PC3 cells in our study. Given that this gene is targetable (22), it might be considered a potential therapeutic target in prostate cancer. GOLM1 could also be a target for silencing due to its overexpression in prostate cancer (23). However, according to our data, it is not overexpressed in metastatic prostate cancer in 100% of cases. *HOXC6* has been shown to be associated with adverse cancer features and reduced apoptosis of cancer cells (24, 25). In our study, *HOXC6* was uniformly overexpressed in the cancer cell lines. Its expression was significantly lower in cancer cells with higher metastatic potential, indicating the possible prognostic role of this marker. In our study, *MKI67* was overexpressed in the DU145 cell line and suppressed in the PC3 cell line. *MKI67* is a well-known proliferation marker (26), which warrants further investigation with regard to prostate cancer aggressiveness. Previous research demonstrated that *MUC1*, an androgen receptor-regulated gene, was related to more aggressive behaviour of prostate tumours (27). In our study, it was underexpressed both in cell lines with moderate and high metastatic potential. However, we found no significant changes in the expression of *MUC1* in our clinical cohort, thereby casting doubt on its role in the prognosis of hormone-naive cancer (28). *MYCBP2* has not been previously reported to have any significant role in prostate cancer. Our results show that *MYCBP2* could be a promising prognostic/therapeutic target and warrants further investigation, especially in view of the fact that it is directly associated with the *MYC* oncogene and that its overexpression played a discriminatory role in our clinical cohort (Figure 1 and 3B). The *SSTR1* gene, encoding the somatostatin type 1 receptor, exhibited interesting bi-directional expression in the androgen-insensitive cell lines. In the DU145 cells, *SSTR1* was overexpressed, which is atypical in castration-refractory prostate cancer (29). Nevertheless, if confirmed in human-derived material, this finding might be useful for individualized therapy. The *TOP2A* gene displayed almost the same pattern of expression, which could be of prognostic relevance. Although recent studies demonstrated overexpression of this gene in aggressive prostate cancer (30, 31), *TOP2A* was underexpressed in our clinical series and, therefore, potentially linked to advanced local-stage tumors (Figure 3A). Again, further studies might confirm its prognostic importance.

The differential expression of some other genes in the present study may also be clinically relevant and warrant further investigation. For example, previous studies confirmed that the multi-drug resistance protein 4 (ATP-binding cassette, sub-family C (CFTR/MRP), member 4; *ABCC4*) was important in estimating the efficacy of chemotherapy (32) and in determining the prognosis, with the protein gradually down-regulated in accordance with an increase in the malignant potential of tumour cells (33). We confirmed this finding in our cell line study. From a clinical point of view, our small cohort of patients could be dichotomized according to the expression of *ABCC4*. The prognostic relevance of this finding remains unclear.

In the clinical arm of our study, the primary aim was to identify which of the selected genes might have prognostic value with regard to adverse pathological features after a radical prostatectomy. Although our cohort was relatively small, we identified six genes which discriminated locally advanced disease and three genes which were differentially expressed in the tumors dependent on the Gleason score (Figure 3). Interestingly, five out of the six genes in the first group and two out of the three genes in the second group seemed to be linked with more aggressive disease in our cell line study. Five out of 28 tumors in our series were positive for gene fusion between transmembrane protease, serine 2 (*TMPRSS2*) and *ERG* (TMPRSS2:ERG) positive. *TMPRSS2–ERG* positivity was not related to any of the clinical or pathological parameters. Four tumors overexpressed serine peptidase inhibitor, Kazal type 1 (*SPINK1*). Three of these also showed overexpression of ERG, and two of the latter were *TMPRSS2–ERG* fusion-positive. This confirms that ERG positivity and overexpression of *SPINK1* are not mutually exclusive (34). The precise role of overexpression of *SPINK1* was not found in our study (too few cases). Previous studies are contradictory, considering the overexpression of *SPINK1* as a predictor of aggressive disease and of an increased risk of biochemical recurrence (34, 35).

This study was a pilot and validation study, which aimed to select for genes with previously demonstrated relevance, and only a small group of patients and cells lines were used for the analysis and further validation. The clinical significance of our findings are as follows: some of the genes seem to represent promising candidates in the estimation of tumor aggressiveness. These genes warrant further consideration as tissue biomarkers. Based on an analysis of biopsy material (*i.e.* after the first minimally-invasive contact with the prostate tumor), these biomarkers could be helpful in the selection of patients for radical treatment or active surveillance. The pool of data on some of the genes (cell line data) related to the androgen-insensitivity of the tumor in the castration-refractory prostate cancer stage of the disease could be valuable for future therapeutic trials.

Our study has certain limitations, which should be considered when interpreting the results. All the cell cultures were cultivated and maintained *in vitro* for a long time. The passive evolution of tumour cells might result in some changes of the genome, and such changes are hard to predict.

Another limitation for the cell lines is the absence of a typical prostate stromal environment. Our clinical cohort was small and not prospectively followed to account for any other hard endpoints, rather than adverse pathology at final investigation after prostatectomy. Nevertheless, the local stage (pT) is considered prognostically relevant (36). Therefore, gene-expression information is important in the prognosis with regard to other hard endpoints. One other limitation is that this study did not consider the intra- and inter-focal heterogeneity of the tumours. An additional limitation of the present study was the absence of a control group of normal tissues, which could be compared to cancerous tissues, obtained from the patients. In the study, we used results derived from a gene-expression analysis of a PreC cell line, which is a pure line of prostate epithelial cells.

Despite the aforementioned limitations, the data obtained from this study could be successfully used for further studies with large cohorts of patients with prostate cancer, both prospectively and with the use of archive material.

In this confirmatory study, a multiple gene-expression analysis was performed of normal prostatic epithelial, prostate cancer cell lines and cancerous tissues of prostatic tumors removed during radical prostatectomy in patients with biopsy-confirmed clinically-localized prostate cancer. The present study identified a set of genes that may have potential relevance for prognosis and tumor characterization. These genes should be included in further large-scale studies of the genetic characterization of prostate tumors that include survival-based end-points.

Conflicts of Interest

The Authors declare no conflicts of interest with regard to this study.

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