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Gene expression of forkhead transcription factors in the normal and diseased human prostate

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OBJECTIVE

To assess the expression of forkhead transcription factors (FOX) in normal prostate and prostate diseases, as since the first *FOX* was identified, its family members have been implicated in a variety of cellular processes, including embryonic development and disease.

MATERIAL AND METHODS

We analysed a set of 12 different FOX genes by quantitative reverse transcription-polymerase chain reaction in prostate zones, prostate cancer, lymph node metastases, benign prostatic hyperplasia (BPH), xenografts and several prostate cell lines.

RESULTS

There were striking differences among the expression of various FOX family members; most prominent were the high expression of *FOXF1* and *FOXF2* in the normal prostate transition zone and BPH, and their decreased expression in prostate cancer. Interestingly, although the *FOXF* genes are stroma-specific, some of the androgen-independent prostate cancer xenografts uniquely express these two genes. *FOXD1* and *FOXD2* were more highly expressed in prostate cancer and lymph node metastases. *FOXA1* and *FOXC1* have an opposite expression pattern for androgen-dependent growth of prostate cancer cell lines and xenografts.

CONCLUSIONS

Various members of the FOX family are differentially expressed in the zones of the normal prostate and in benign and malignant outgrowths. The expression profiles of *FOXF1* and *FOXF2* suggest a role in epithelial to mesenchymal transition, while *FOXA1* and *FOXC1* expression is linked to androgen-associated growth status of cancer.

KEYWORDS

Forkhead transcription factors, *FOXF1*, *FOXF2*, *FOXD1*, prostate cancer, benign prostate hyperplasia

INTRODUCTION

It has been more than a decade since the discovery of the *Drosophila* transcription factor forkhead (FOX) and subsequent identification of the mammalian orthologues of the FOX DNA binding domain [1]. FOX genes encode a subgroup of helix-turn-helix class of proteins. The arrangement of loops connecting the β strands that flank one of the three α helices, gives rise to a butterfly-like appearance, hence the name 'winged-helix' transcription factors [2]. It is a relatively invariant structure, with most amino acids being conserved between family members. This has made it difficult to understand the molecular mechanisms underlying the sequence specificity of the DNA-binding domains. All FOX genes can bind DNA and the functional effect of this can be either the activation or the inhibition of gene transcription. In contrast to the DNA-binding domains, there is almost no sequence homology between the transactivation or repression domains of members of the FOX family, and little is known about their interactions with the transcriptional machinery [3]. The FOX family has been implicated in various cellular processes and they are important in embryonic development and disease. Several FOX factors, like *FOXP3*, *FOXN1*, *FOXJ1* and members of the *FOXO* subfamily, have crucial roles in various aspects of the immune system [4,5]. Lehmann *et al.* [6] suggested the importance of FOX in disease and development, and speculated that soon regulators and downstream target genes of FOX will be discovered to explain a range of human diseases.

Little is known about the role of the FOX family in the developing and adult prostate. Immunohistochemical localization of *FOXA1* and *FOXA2* revealed epithelial nuclear staining of both members in the developing mouse prostate, but only *FOXA1* in the adult mouse prostate [7,8]. *FOXA1* is essential for full prostate ductal morphogenesis as was shown using *FOXA1*-deficient mice [8]. During prostate cancer progression, *FOXA1* remains highly expressed in the cancer epithelium, while *FOXA2* is turned on in neuroendocrine small cell-type carcinomas [9]. A direct link between prostate cancer progression and *FOXM1* was established by the observation that transgenic *FOXM1* mice, crossbred with the TRAMP and LADY prostate cancer mouse models, had accelerated development and growth of prostatic tumours [10].

The role of various FOX genes in prostate cancer progression might be explained by their interaction with the androgen receptor (AR) pathway. The AR is a nuclear receptor that is activated upon testosterone or dihydrotestosterone binding and generally signals growth of prostate cancer cells [11]. Besides the above mentioned *FOXA1* and *FOXA2*, also *FOXG1*, *FOXH1*, *FOXO1* and *FOXO3* affect the AR cascade. The general theme is that these FOX proteins (all except *FOXO3*) repress AR activity by directly binding the AR

TABLE 1 Clinical characteristics and follow-up of patients

Variable	Normal prostate	Organ-confined prostate cancer	
		no metastases	metastases
No. patients	17	36	10
Median (range):			
age at treatment, years	61 (54–72)	61.5 (49–73)	64 (49–70)
PSA at diagnosis, ng/mL	–	9.2 (0.3–181)	32 (0.5–64.3)
Gleason score, *n*			
6	–	23	4
7	–	9	2
8–10	–	2	4
Mean (range):			
Epithelium in tissue, %	68 (60–80)	80 (60–100)	81 (70–90)
Cancer in tissue, %	0	85 (70–100)	90 (70–100)

protein. Takayama *et al.* [12] supported this idea and showed that *FOXP1* is an androgen-responsive transcription factor that negatively regulates AR signalling in prostate cancer cells.

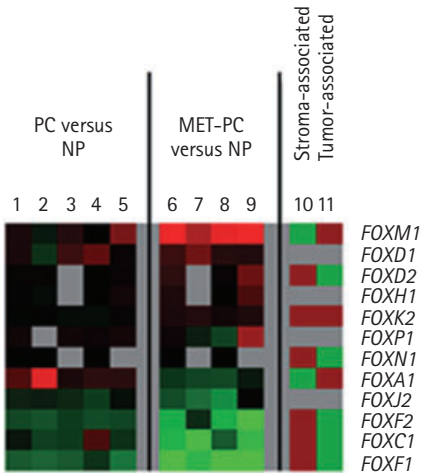
In our previous study, we found that FOX genes are differentially expressed between the prostate zones [13]. *FOXF1* and *FOXF2* are more highly expressed in the transition zone (TZ) than the peripheral zone (PZ). This observation initiated our interest in the potential role of FOX genes in prostate disease development, as BPH and prostate cancer preferentially occur in the TZ and PZ, respectively. To identify which FOX genes are expressed in the prostate and relate their expression to prostate diseases and cell types, we investigated the expression of 12 different FOX genes using by real-time reverse transcription (RT)-PCR.

MATERIALS AND METHODS

The prostate specimens were obtained from patients who had a radical prostatectomy at the Department of Urology, Erasmus MC. The experimental protocols were approved by the Erasmus MC Medical Ethics Committee according to the Medical Research Involving Human Subjects Act. Prostate samples included normal tissue from the TZ and PZ, and BPH, using a special protocol described previously [13]. Prostate cancer samples from the prostate and from lymph node metastases (LNM, Table 1) were obtained from the frozen tissue bank of the Erasmus Medical Center, as described by Hendriksen *et al.* [14]. The prostate samples used by different groups [15–19] in Fig. 1 were all macrodissected; this generally implies that immediately after surgery the pathologist macroscopically isolated different prostate samples. The presence and number of cancer cells and other cell types were then evaluated using histology. It is impossible to macrodissect prostate cancer samples without normal epithelium or stroma contaminating the sample; samples would need to be microdissected to enrich for cancer tissue and to separate cancer cells from normal epithelium and the stromal compartment. However, as the stroma has been indicated to play a major role in cancer initiation and progression, it is important to study prostate cancer cells in context with their surrounding stroma. In most gene-expression studies, including the present, macrodissected prostate cancer tissue was used to examine expression profiles of the whole tumour tissue, including cancer cells and their supporting environment such as fibroblasts, smooth muscle cells, endothelium, etc. In the present study we show that the stroma-specific genes *FOXF2* and *FOXF1* are implicated in human prostate disease and if these samples were cancer-cell enriched by microdissection, we would not have found this association. It is essential when studying gene expression changes in cancer development, that all different cell types present in the tumour are considered. Several types of arrays were used by the different groups: Singh *et al.* [15] and Stuart *et al.* [16] used Affymetrix U95Av2 arrays for their experiments; Lapointe *et al.* [17] used cDNA array containing 26 260 probes, Varambally *et al.* [18] used Affymetrix U133 2.0 arrays and Yu *et al.* [19] used Affymetrix HG_U95A,B,C. Primary human prostate epithelial cells (PrEC) and primary human prostate stromal cells (PrSC) were purchased and cultured according to manufacturer's guidelines (Clonetics Human and Animal Cell Systems, Cambrex Bio Science Walkersvill, IN, USA). To minimize adaptation and variation of cells, only early passages 5 and 7 were used for RNA isolation when they were 70% confluent.

The LNCaP, PC3 and DU145 prostate cancer cell lines were maintained in RPMI 1640 with 5% fetal calf serum (FCS) and penicillin/streptomycin (Invitrogen, Merelbeke, Belgium). Before R1881 treatment, LNCaP cells were androgen-deprived for 72 h in medium containing 5% dextran-filtered, charcoal-stripped FCS with the medium replaced after 36 h. After androgen deprivation, the medium was supplemented for 2, 4, 6 or 8 h with 1 nmol/L R1881 or

FIG. 1. In silico *expression analysis of several FOX genes in microarray databases related to normal prostate and prostate diseases. Difference in expression in prostate cancer (PC) and metastasized prostate cancer (Met-PC) vs normal prostate tissue was based on microarray studies by Singh* et al. *[15], column 2; Lapointe* et al. *[17], columns 3 and 7; Varambally* et al. *[18], columns 4 and 8; Yu* et al. *[19], columns 5 and 9. Columns 1 and 6 are averages of these studies of PC vs normal and Met-PC vs normal, respectively. Expression ratios between PC vs normal prostate (NP) and MET-PC vs NP are shown as colours of different intensity. Gene expression was ordered by sorting from highest to lowest expression and illustrated by Treeview. Red indicates higher expression in PC or MET-PC than NP; green indicates the opposite. Stroma- and tumour-associated expression of FOX genes are represented by columns 10 and 11, and based on a study by Stuart* et al. *[16]. Red indicates a significant positive correlation and green a significant negative correlation between gene expression and amount of stroma or tumour.*



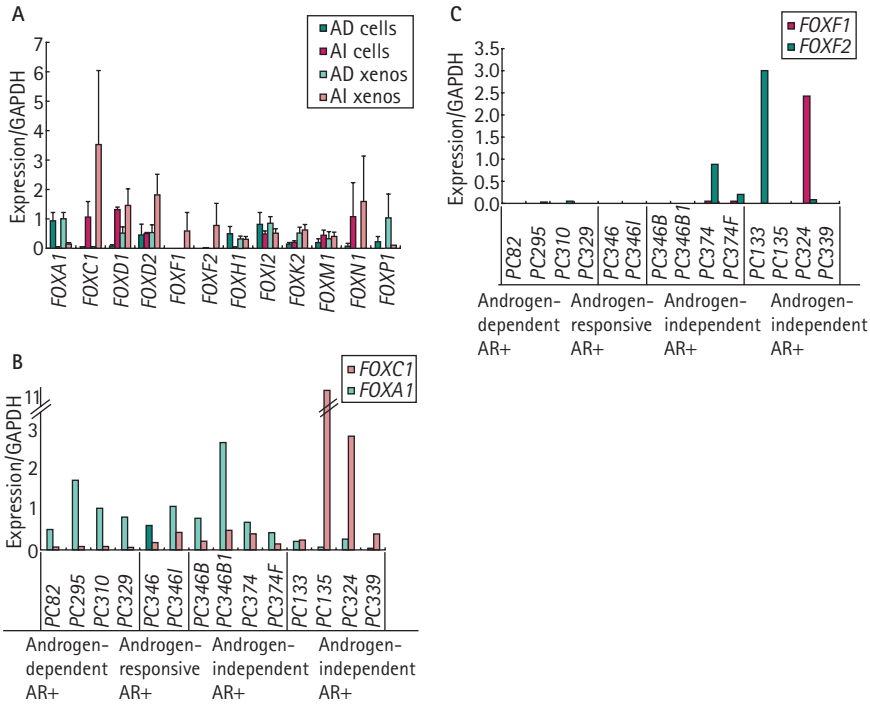
ethanol vehicle. The basic culture medium used in the maintenance of PC346 cell lines consisted of Dulbecco modified Eagle Medium-F12 (Cambrex BioWhittaker, Verviers, Belgium) supplemented with 2% FCS (PAN Biotech, Aidenbach, Germany), 1% insulin-transferrin-selenium (Gibco BRL, Gaithersburg, MD, USA), 0.01% BSA (Boehringer-Mannheim, Mannheim, Germany), 10 ng/mL epidermal growth factor (Sigma-Aldrich, Milan, Italy) and penicillin/streptomycin antibiotics (100 U/mL penicillin, 100 g/mL streptomycin; Cambrex BioWhittaker) plus the following additions: 100 ng/mL fibronectin (Harbor Bio-Products, Tebu-bio, the Netherlands), 20 g/mL fetuine (ICN Biomedicals, Zoetermeer, the Netherlands), 50 ng/mL cholera toxin, 0.1 mM phosphoethanolamine, 0.6 ng/mL triiodothyronine and 500 ng/mL dexamethasone (all from Sigma-Aldrich, St Louis, MO, USA).

The *in vivo* growing xenografts PC82, PC133, PC135, PC295, PC310, PC324, PC329, PC339, PC346, PC346I, PC346B, PC346BI, PC374 and PC374F were propagated by serial transplantation on male nude mice as described [20,21]. PC82, PC295, PC310, and PC329, derived from primary tumours or local metastases, are androgen-dependent (AD). PC133, PC324, PC339, PC346, PC346I, PC346B, PC346BI, PC374 and PC374F are derived from distant metastases or local progressive disease and are androgen-independent (AI) (PC133, PC324 and PC339) or androgen-sensitive (PC346 and PC374). PC135 is AI and is derived from a LNM.

RNA from prostate specimens and cell lines was isolated using RNAbee reagent as described by the manufacturer (Tel-Test Inc. Friendswood, TX, USA). The RT reaction was performed with 1 μg RNA from the samples with oligo T12 primer and pre-incubated for 10 min at 70 °C. First-strand buffer, dithiothreitol, dNTPs and RNAsin were added and incubated for 2 min at 37 °C. The RT reaction was initiated by MMLV-RT and incubated for 1 h at 37 °C. After this, the reaction was maintained for 10 min at 90 °C and immediately thereafter frozen.

Quantitative real-time RT-PCR analysis was done with a ABI Prism 7700 Sequence Detection System using AmpliTaq Gold according to the manufacturer's specifications (Applied Biosystems, Foster City, CA, USA). The probes and primers for

FIG. 2. ***A****, Expression of FOX genes was investigated by quantitative RT-PCR analyses of RNA extracted from AD cell lines (LNCaP and PC346c), AI cell lines (DU145 and PC3), AD xenografts (PC82, PC295, PC310 and PC329) and AI xenografts (PC133, PC135, PC324 and PC339). Data are represented as mean ratio of* FOX *expression/*GAPDH *expression (± SEM).* ***B,*** *Gene expression analyses of* FOXA1 *and* FOXC1 *in human prostate cancer xenografts; AD, AR positive (AR+); androgen-responsive, AI, AR+ and AR-negative. Expression is represented as the ratio of* FOXA1 *or* FOXC1 *expression/*GAPDH *expression.* ***C,*** *Gene expression analyses of* FOXF1 *and* FOXF2 *by quantitative RT-PCR in several types of human prostate cancer xenografts. Expression is represented as ratio of* FOXF *gene expression/*GAPDH *expression.*



Taqman Gene Expression Assays were obtained from Applied Biosystems. The amount of target gene expressed was normalized to an endogenous reference and relative to a calibrator. The endogenous reference was glyceraldehyde-3-phosphate dehydrogenase (GAPDH); a mixture of cDNAs of the prostate carcinoma xenografts was used as the calibrator. The following primers were used: *FOXA1*, Hs00270129_m1; *FOXC1*, Hs00559473_s1; *FOXJ2*, Hs00218236_m1; *FOXK2*, Hs00189612_m1; *FOXN1*, Hs00186096_m1; *FOXM1*, Hs00153543_m1; *FOXF1*, Hs00230962_m1; *FOXF2*, Hs00230963_m1; *FOXP1*, Hs00415004_m1; *FOXD1*, Hs00270117_s1; *FOXD2*, Hs00270129_s1; *GAPDH*, Hs99999905_m1. The RT-PCR data were analysed statistically using the paired *t*-test.

RESULTS

Twelve different FOX genes were analysed by quantitative RT-PCR in prostate cancer cell lines, xenografts, normal prostate tissues, BPH and prostate cancer tissue. FOX genes were selected based on differential gene expression in various microarray studies and previous studies for cancer and AR interaction (Fig. 1) [15–19].

The expression of the 12 FOX genes was investigated in different prostate cancer cell lines, including the AR-positive LNCaP and PC346C, and the AI PC3 and DU145 (Fig. 2A). In addition, expression was determined in our panel of prostate cancer xenografts, consisting of AD, androgen-sensitive and AI growing tumours [22,23].As the prostate cancer xenografts contain mouse contaminants such as stroma, endothelium and blood cells, each quantitative RT-PCR FOX gene assay was checked for cross-reactivity with RNA extracted from mouse sarcoma tissue. None of the RT-PCRs gave detectable signals on mouse tissues within the first 35 PCR cycles, showing the detection of human-specific gene expression (data not shown).

*TABLE 2 Ratio data for real-time PCR (expression/*GAPDH*), showing the expression of 12 different* FOX *in AD cell lines (LNCaP and PC346C), AI cell lines (PC3 and DU145), AD xenografts (PC82, PC295, PC310 and PC329) and AI xenografts (PC133, PC135, PC324 and PC339). LNCaP data shown are the means of two independent studies. The origin of cell lines and xenografts, and AR expression are indicated*

Sample	Origin	Androgen response	AR expression	*FOX* *F2*	*F1*	*A1*	*C1*	*H1*	*M1*	*N1*	*P1*	*J2*	*D1*	*D2*	*K2*
Cell line															
LNCaP	Lymph node	AD	Yes	0.00	0.00	0.69	0.03	0.28	0.05	0.01	0.36	0.51	0.12	0.16	0.14
PC346C	Primary PC346P xenograft	AD	Yes	0.00	0.00	1.40	0.07	0.92	0.48	0.23	0.00	1.46	0.05	1.06	0.25
PC3	Bone	AI	No	0.00	0.00	0.07	0.56	0.10	0.34	0.00	0.00	0.38	1.42	0.54	0.21
DU145	Brain	AI	No	0.01	0.00	0.07	1.61	0.00	0.62	2.23	0.00	0.59	1.28	0.49	0.28
Xenografts															
PC82	Primary	AD	Yes	0.00	0.00	0.50	0.04	0.00	0.11	0.00	0.11	0.28	0.17	0.78	0.26
PC295	Lymph node	AD	Yes	0.01	0.00	1.72	0.07	0.78	1.06	0.00	3.56	1.17	0.83	1.17	0.78
PC310	Primary	AD	Yes	0.04	0.00	1.01	0.08	0.30	0.25	0.00	0.20	1.27	1.06	0.25	0.12
PC329	Primary	AD	Yes	0.00	0.01	0.79	0.03	0.06	0.03	0.00	0.26	0.74	0.03	0.04	0.98
PC133	Bone	AI	No	3.05	0.02	0.18	0.22	0.35	0.63	0.23	0.21	0.86	2.60	0.95	0.70
PC135	Primary	AI	No	0.00	0.00	0.07	10.81	0.62	0.22	6.22	0.01	0.65	0.78	2.49	0.46
PC324	Primary	AI	No	0.13	2.46	0.27	2.78	0.08	0.70	0.00	0.00	0.43	2.26	3.48	1.13
PC339	Primary	AI	No	0.00	0.00	0.00	0.39	0.06	0.06	0.00	0.07	0.12	0.26	0.46	0.28

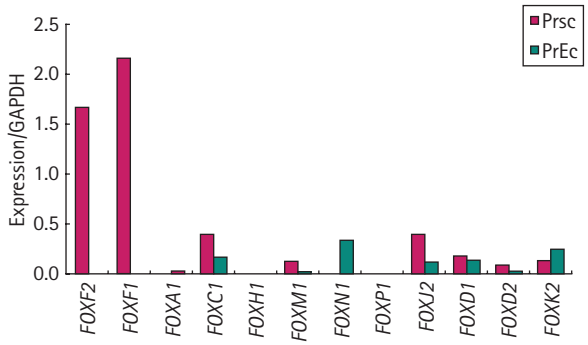


FIG. 3. Gene expression analyses of several FOX genes in PrSC and PrEC; expression is represented as the ratio of FOX *expression/* GAPDH *expression.*

In Fig. 2A, the strict AD lines PC82, PC295, PC310 and PC329 were compared to the AR-negative PC133, PC135, PC324 and PC339 lines. Some of the FOX genes have a xenograft or cell line-specific expression pattern, explaining the large error bars. *FOXC1* was notably expressed in all AI xenografts, but was extremely high in PC135. Also *FOXN1* was mostly undetectable, except for high expression in DU145 and PC135. *FOXP1* was a unique outlier with high expression in PC295 (more information on individual cell lines and xenografts is shown in Table 2).

One of the most striking observations in the comparison between AD and AI lines was the inverse expression pattern of *FOXA1* and *FOXC1*. While *FOXA1* was more highly expressed in AD cell lines and xenografts, *FOXC1* had the opposite association (Fig. 2B). Also, the expression of *FOXF1* and *FOXF2* stands out by the unique expression in some AI xenografts (Fig. 2C).

Whether any of the FOX genes is directly androgen-regulated was determined by gene expression analysis of LNCaP cells cultured for 8 h in the absence or presence of 1 nM R1881. None of the 12 FOX genes showed a significant difference on androgen treatment, which is in agreement with published expression microarray data that to date have identified no FOX genes as potential AR targets in LNCaP cells [24]. By contrast, Takayama *et al.* [12] recently reported that *FOXP1* is an androgen-responsive transcription factor that negatively regulates AR signalling in prostate cancer cells.

PrSC and PrEC were used to study the expression of FOX genes in normal prostate stroma and epithelium (Fig. 3). *FOXF1* and *FOXF2* were highly expressed in PrSC, but not in PrEC, which confirms previous findings that these genes are highly stroma-specific [25]. *FOXH1*, *FOXA1* and *FOXP1* were not expressed, while the other FOX genes tested showed low expression with no or minor differences between PrSC and PrEC.

To identify differential expression of the selected FOX genes between the normal prostate, BPH and prostate cancer, RNA was extracted from the normal prostate TZ and PZ of seven radical prostatectomy samples and from five samples of BPH, 10 samples of organ-confined cancer and six LNM (Fig. 4). The expression of *FOXH1* and *FOXM1* was in all cases very low and not significantly different among the various groups (data not shown).

The quantitative RT-PCR confirmed our previous finding that *FOXF1* and *FOXF2* are more highly expressed in the TZ than the PZ [12]. *FOXA1*, *FOXC1*, *FOXJ2* and *FOXK2* were equally expressed between the prostate zones. *FOXP1* was more highly expressed in the PZ, while *FOXD1* and *FOXD2* were expressed at a very low level in the normal prostate.

For prostate diseases, most FOX genes are more highly expressed in BPH than in

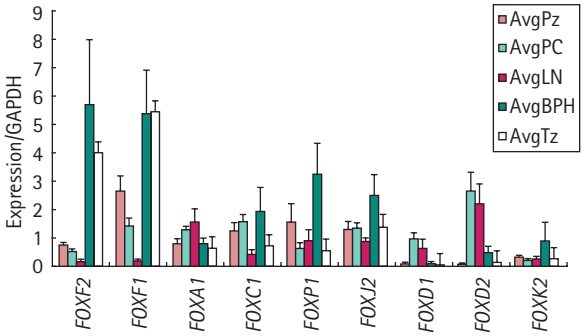
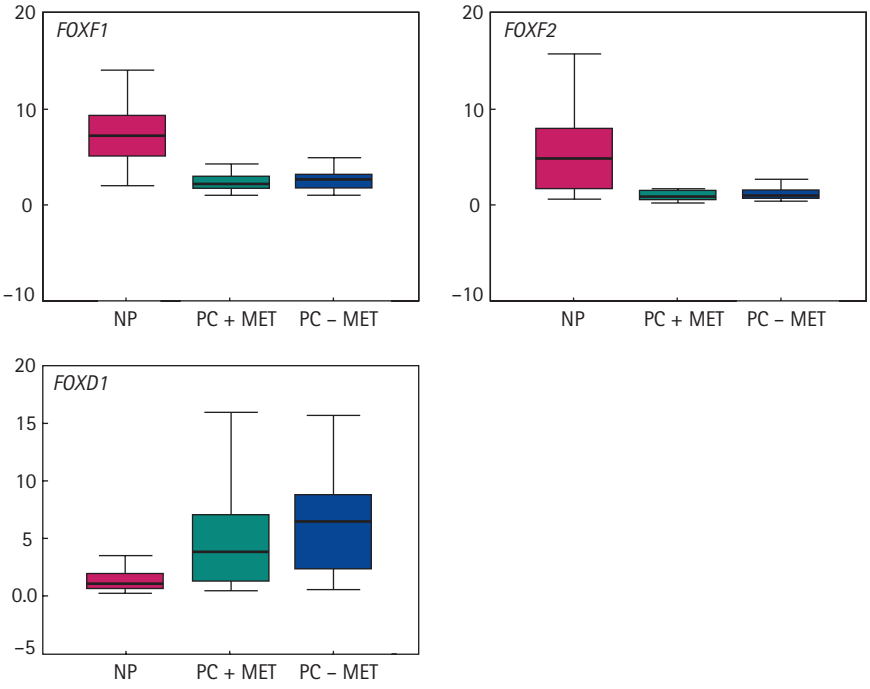


FIG. 4.
Gene expression of several FOX genes in prostate cancer (PC), BPH, LNM, PZ and TZ samples.

FIG. 5. Comparison of FOXF1, FOXF2, FOXD1 *expression in normal prostate (NP) and organ-confined prostate cancer in patients who did (PC + MET) or did not (PC − MET) develop metastases after radical prostatectomy.* FOXF1 *and* FOXF2 *had a significantly higher expression in NP than PC ± MET.* FOXD1 *expression was higher in PC + MET than NP and in PC − MET than NP. In each comparison NP was significantly different from PC + and − MET ($P < 0.05$).*



normal TZ in which the benign enlargement originates (*FOXC1*, *FOXP1*, *FOXJ2* and *FOXK2*; Fig. 4). Both *FOXF1* and *FOXF2* remained highly expressed in BPH but were no different from TZ. The malignant growths showed a more differential pattern. *FOXD1* and *FOXD2* were up-regulated in cancer and LNM than in the normal PZ. However, *FOXF1* and *FOXF2* showed a consistent down-regulation on progression of the malignant disease.

As the expression pattern of *FOXF1* and *FOXF2* in the PZ, prostate cancer and LNM were in contrast to that of *FOXD1*, we investigated if any of these genes or their combination could predict whether local cancer after radical surgery recurs (Fig. 5). *FOXF1* and *FOXF2* had significantly higher expression in normal prostate (17 samples) than in local prostate cancer (46), confirming the data shown in Fig. 4. Also the higher expression of *FOXD1* in prostate cancer than in normal prostate was substantiated. However, there was no difference in expression of these three FOX genes between men who did (10) or did not (36) develop metastases after radical prostatectomy.

DISCUSSION

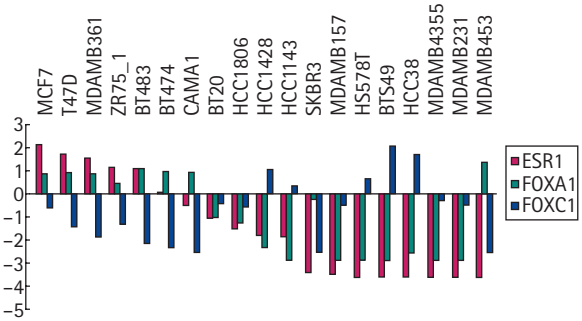
The aim of the present study was to better understand the expression and potential roles of FOX expression in the healthy and diseased prostate. Although the importance of FOX genes in development and disease is well established, their potential role in prostate development and outgrowths is poorly studied [4,5,26]. Twelve different Fox genes were selected and gene expression was studied by quantitative RT-PCR analyses in various prostate tissues.

Several of the selected FOX genes have a clear differential expression pattern in the present comparisons. *FOXF1* and *FOXF2* were selected based on their association with stroma, lower expression in prostate cancer (Fig. 1) and their significantly higher expression in TZ than PZ [13]. We confirmed that the expression was lower in prostate cancer and LNM, and showed that *FOXF* expression is stroma-specific. Taken together, it appears that *FOXF1* and *FOXF2* are involved in regulating normal prostate homeostasis through the stromal compartment, particularly in the TZ. This role for *FOXF* genes was already suggested for mice in murine gut development, and during mouse and rat embryogenesis [27]. As for mouse gut development, we suggest that these genes are involved in stromal–epithelial interaction. Importantly, the *FOXF* genes might also play a role in epithelial-mesenchymal transition, as the stroma-specific *FOXF* genes are expressed uniquely in several late stage AI xenografts.

FOXD1 had higher expression in prostate cancer and LNM than in normal prostate. We investigated whether this opposite expression pattern of *FOXF1/FOXF2* and *FOXD1* would be different between primary tumours that will or will not form metastatic disease after radical prostatectomy with intent to cure. A more extensive quantitative RT-PCR analysis clarified that their expression had no prognostic value.

It has been suggested that FOXA proteins play a role in the development and progression of prostatic adenocarcinoma [9]. Average *FOXA1* expression was higher in the PZ, prostate cancer and LNM than in BPH and the TZ. Due to considerable variability between the patient samples, this difference was not statistically significant. There was an inverse expression pattern of *FOXA1* and *FOXC1* between AD and AI lines. *FOXA1* had higher

*FIG. 6. Expression of oestrogen-receptor alpha (*ESR1*), *FOXA1 *and* FOXC1 *in breast cancer cell lines. Affymetrix gene expression data was retrieved from Bild* et al. *[28] and was normalized and mean-centred for each of the genes. Cell lines were ordered from highest to lowest* ESR1 *expression.* FOXA1 *is always highly expressed in* ESR1*-positive cell lines and poorly expressed in* ESR1*-negative cells.* FOXC1 *showed the opposite expression pattern. As shown in Fig. 2B, this expression pattern was very similar to the correlation of the AR and* FOXA1/FOXC1 *in prostate cancer xenografts.*



expression in AR-positive, AD cell lines and xenografts, while *FOXC1* had the opposite expression pattern. We extracted data from Bild *et al.* [28] and identified the same inverse expression pattern of *FOXA1* and *FOXC1* in oestrogen-receptor-positive vs -negative breast cancer cell lines (Fig. 6). In agreement, *FOXA1* was shown to be a prognostic marker for luminal subtype breast cancers by Thorat *et al.* [29]. Also our data indicate a potential role for *FOXA1* and *FOXC1* as markers for AD growth of prostate cancer.

This inventory of the expression of 12 FOX genes in several prostate tissues provides further evidence for a role of FOX in prostate maintenance and abnormal outgrowths. *FOXF* and *FOXD1* are differentially expressed between normal tissue and cancer, while *FOXA1* and *FOXC1* expression changes during progression of prostate cancer, indicating their involvement in regulating the balance between normal and diseased tissue.

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CONFLICT OF INTEREST

None declared.

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Abbreviations: **FOX**, forkhead transcription factor; **PZ**, peripheral zone; **TZ**, transition zone; **PrSC**, primary human prostate stromal cells; **PrEC**, primary human prostate epithelial cells; **LNM**, prostate cancer lymph node metastases; **AR**, androgen receptor; **AD**, androgen-dependent; **AI**, androgen-independent; **GAPDH**, glyceraldehyde-3-phosphate dehydrogenase; **RT**, reverse transcription; **FCS**, fetal calf serum