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High expression of indoleamine 2,3-dioxygenase gene in prostate cancer ☆

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ABSTRACT

Arginase 2, inducible- and endothelial-nitric-oxide synthase (iNOS and eNOS), indoleamine 2,3-dioxygenase (IDO) and TGF-β, might impair immune functions in prostate cancer (PCA) patients. However, their expression was not comparatively analysed in PCA and benign prostatic hyperplasia (BPH). We evaluated the expression of these genes in PCA and BPH tissues.

Seventy-six patients (42 BPH, 34 PCA) were enrolled. Arginase 2, eNOS and iNOS gene expression was similar in BPH and PCA tissues. TGF-β1 gene expression was higher in BPH than in PCA tissues ($p = 0.035$). IDO gene expression was more frequently detectable ($p = 0.00007$) and quantitatively higher ($p = 0.00001$) in PCA tissues than in BPH. IDO protein, expressed in endothelial cells from both BPH and PCA, was detectable in tumour cells in PCA showing evidence of high specific gene expression. In these patients, IDO gene expression correlated with kynurenine/tryptophan ratio in sera.

Thus high expression of IDO gene is specifically detectable in PCA.

1. Introduction

Prostate cancer (PCA) is the second leading cause of cancer-related death in men reaching 31.5% of annual newly diagnosed cancers.[1] In order to improve the understanding of PCA induction and progression, a contemporary model should include a detailed analysis of mechanisms possibly inhibiting immune responsiveness to cancer.

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A number of factors potentially impairing immune responses in PCA patients, such as arginase 2,[2] inducible nitric-oxide synthase (iNOS)[3], endothelial nitric-oxide synthase (eNOS)[4] and TGF-β[5], have been detected in surgical specimens. Interestingly, some of these immuno-suppressive factors have been suggested to be able to inhibit anti-CD3 or PHA induced T cell proliferation.[2] However, little is known about their comparative expression in PCA and benign prostatic hyperplasia (BPH). Thus, still open is the issue of their specificity as cancer signature.

Indoleamine 2,3-dioxygenase (IDO) eventually produced within tumours initiates the degradation of tryptophan along the kynurenine pathway[6] resulting in the production of immuno-suppressive catabolites known to inhibit T cell stimulation *in vitro* and to cause T cell apoptosis.[7] IDO expression has thus been proposed as a possible mechanism facilitating induction of immune tolerance towards cancer.[8] Interestingly, metabolites generated by IDO mediated tryptophan digestion have been shown to inhibit cytokine induced homeostatic T cell proliferation.[7] IDO expression has indeed been reported in PCA.[8] However, no comparison with BPH has been provided.

In this study, we comparatively evaluated the expression of genes encoding potential immuno-suppressive factors including IDO in PCA and BPH tissues.

Our results indicate that, amongst genes encoding factors potentially affecting immune response in PCA, IDO gene expression is characterised by the highest specificity for PCA tissues.

2. Materials and methods

2.1. Patients and samples

We investigated a consecutive series of 76 specimens from men diagnosed for BPH or PCA at the Department of Urology of the University Hospital of Basel (Switzerland) in 2007. BPH patients underwent conventional transurethral resection (TUR-P), whilst PCA patients underwent either palliative TUR-P or endoscopic extraperitoneal radical prostatectomy (EERP). Relevant clinical data were collected by reviewing patients' files.

Written informed consent was obtained from patients in accordance with the requirements of the Ethical Committee of Basel (EKBB, Ref. No. EK: 176/07).

2.2. Quantification of gene expression in prostatic tissues by quantitative real-time PCR

Prostatic tissues resected during surgical procedures were screened for the presence of tumours by experienced pathologists at the moment of collection, immediately submerged in RNAlater (Ambion, Foster City, CA) and stored at –80 °C until further processing. Total RNA was extracted by using RNeasy® Mini Kit protocol (Qiagen, Basel, Switzerland), treated by Deoxyribonuclease I (DNase I) (Invitrogen, Carlsbad, CA) and reverse transcribed by using the Moloney murine leukaemia virus reverse transcriptase (M-MLV RT, Invitrogen, Carlsbad, CA). Quantitative real-time PCR was performed by a ABI prism™ 7700 sequence detection system, using the TaqMan® Universal PCR Master Mix, No AmpErase® UNG (both from Applied Biosystems, Foster City, CA).

The following primer sequences were derived from the existing literature, as indicated below

GAPDH[9]
Forward ATGGGGAAGGTGAAGGTCG
Reverse TAAAAGCAGCCCTGGTGACC
Probe FAM-CGCCCAATACGACCAAATCCGTTGAC-TAMRA

AMACR A[10]
Forward CGGTTAGCTGGCCACGAT
Reverse GATTCTCACCACTTCTGCCAATT
Probe FAM-TCAACTATTTGGCTTTGTCAGGTGTTCTCTCAA-TAMRA

IDO[8]
Forward GGTCATGGAGATGTCCGTAA
Reverse ACCAATAGAGAGACCAGGAAGAA
Probe FAM-CTGTTCCTTACTGCCAACTCTCCAAGAAACTG-TAMRA

IL-6[11]
Forward CAGCCCTGAGAAAGGAGACATG
Reverse GGTTCAGGTTGTTTTCTGCCA
Probe FAM-AGTAACATGTGTGAAAGCAGCAAAGAGGCAC-TAMRA

TGF-β1[12]
Forward CGA GAA GCG GTA CCT GAA C
Reverse TGA GGT ATC GCC AGG AAT TGT
Probe FAM-CAG CAC GTG GAG CTG TAC CAG AAA TAC AGC-TAMRA

Arginase 2, iNOS, eNOS, IL-23p19 and IL-17 primers and probes were provided by Assays-on-Demand, Gene Expression Products (Applied Biosystems, Foster City, CA).

Specific gene expression was quantified by using the $2^{-\Delta\Delta C}{}_T$ method. Normalisation of gene expression was performed using GAPDH as reference gene, and samples were considered positive for a $\Delta C_T < 20$ cycles. Data were expressed as ratio to reference samples represented by lipopolysaccharide (LPS) matured dendritic cells (mDC) or phytohemagglutinin (PHA) activated CD4+ T cells, as indicated in specific tests.

2.3. Immunohistochemistry

Indoleamine 2,3-dioxygenase protein was detected on paraffin-embedded prostate section by a standard protocol[13] using a mouse anti-IDO monoclonal antibody (AbD Serotec, Oxford, UK).

2.4. Measurement of tryptophan and kynurenine concentrations in serum from BPH and PCA patients

Measurement of tryptophan and kynurenine concentrations in sera from BPH and PCA patients was performed as previously described[14] by high-pressure liquid chromatography (HPLC) and fluorimetric detection. Sera were deproteinised by mixing them with an equal volume of 10% (w/v) trichloroacetic acid. Measurement of tryptophan by HPLC separation was obtained with a reverse-phase column (Spherisorb S5

ODS2, 25 cm) and a mobile phase (1 ml/min flow rate) composed of 5% acetonitrile, 100 mM phosphate buffer, pH 3.6, and 1 mM EDTA. Detection was performed with a Perkin–Elmer (Foster City, CA) model LC 240 fluorimeter. Excitation and emission wavelengths were 313 and 420 nm, respectively.

Kynurenine was measured using HPLC and UV detection. Briefly, HPLC separation was obtained with a reverse-phase column (Spherisorb S5 ODS2, 10 cm) and a mobile phase (1 ml/min flow rate) composed of 2% acetonitrile 0.1 mM ammonium acetate and 100 mM acetic acid. Kynurenine was detected at 365 nm with a UV detector (Perkin–Elmer model LC 90).

2.5. Statistical analysis

SPSS software (Version 14.0, SPSS Inc., Chicago, IL) was used for statistical analyses. Skewness and kurtosis parameters were used to test the 'normality' of the populations of each group. Mann–Whitney non-parametric test was used to compare means for independent samples and Wilcoxon non-parametric test for paired samples was used on 'non-normal' populations. The frequencies of specific gene expression in two groups were assessed by χ^2 evaluation. p values lower than 0.05 were considered to be statistically significant.

3. Results

3.1. Clinical profiles of the patients

Average age of the patients enrolled in the study ($n = 76$) was 66.8 +/− 7.7 years (range 49–90). Forty-two of them (55.26%) were diagnosed with benign prostatic hyperplasia (BPH) and 34 (44.74%) with prostate cancer (PCA) (Table 1). Average age was 67.6 +/− 7.4 years (range 50–81) and 65.8 +/− 8.0 years (range 49–90) for BPH and PCA patients, respectively.

Of 34 PCA patients, 2 (5.9%) were diagnosed with stage pT1, 1 with stage pT1a, 1 with stage pT1b, 5 (14.7%) with stage pT2a, 1 with stage pT2b, 20 (59.0%) with stage pT2c, 2 (5.9%) with stage pT3a and 1 with stage pT3b (TNM staging[15]) (Table 1). One PCA patient had a tumour whose stage at diagnosis could not be assessed.

Average serum prostate specific antigen (PSA) value in the 34 PCA patients under investigation was 17.93 +/− 37.62 ng/ml (median: 6.62 ng/ml; range 1.43–206.00) and average Gleason score was 6.55 +/− 1.26 (median: 7.00; range 3–9). Average serum PSA value in BPH patients ($n = 34$) was 4.82 +/− 5.84 ng/ml (median: 2.30 ng/ml; range 0.30–26.00).

3.2. Expression of genes encoding immunosuppressive enzymes from the L-arginine metabolic pathway in PCA and BPH tissues

A number of reports suggest that enzymes involved in L-arginine metabolism may favour cancer growth and development. In particular, arginases were suggested to play a potentially decisive role in PCA induction by enhancing cell proliferation.[16] Similarly, inducible NOS (iNOS) has been shown to be expressed in prostate cells[3] and endothelial NOS (eNOS) is known to protect prostate cancer cells from apoptosis.[4] Finally, it has been suggested that arginase, possibly in synergy with NOSs may be able to inhibit T cell response to antigens, thereby favouring tumour escape from recognition by the immune system.[17]

We quantitatively analysed arginase 2, iNOS and eNOS gene expression in our series of PCA and BPH tissues. As shown in Fig. 1, arginase 2 gene expression was detectable in 22/33 (66.7%) and in 18/24 (75.0%) BPH and PCA specimens, respectively ($p = 0.50$). Furthermore, under a quantitative point of view, in positive cases, arginase 2 gene expression did not significantly differ in PCA or BPH ($p = 0.232$).

iNOS gene was found to be significantly ($p = 0.04$) more frequently expressed in PCA specimens (26/32, 81.3%) as compared to BPH (23/39, 59.0%). However, no significant differences in the extent of specific gene expression in positive cases were observed ($p = 0.111$). On the other hand, eNOS was similarly expressed in 28/39 (71.8%) and in 26/32 (81.3%) of BPH and PCA specimens, respectively ($p = 0.35$). Furthermore, no significant quantitative differences in eNOS gene expression between BPH and PCA positive cases were detectable ($p = 0.515$).

3.3. Expression of genes encoding cytokines in PCA and BPH tissues

A number of cytokines have been suggested to be involved in PCA pathogenesis. In particular, IL-6 is known to be a mediator of PCA morbidity and disease activity, and might act as a cell growth factor and protect cancer cells from death. In addition, it has been demonstrated that patients with metastatic PCA have increased IL-6 serum levels.[18] We comparatively evaluated *IL-6* gene expression in BPH and PCA tissues.

As shown in Fig. 2, *IL-6* gene was expressed in 18/39 (46.2%) and in 26/32 (81.3%) of BPH and PCA specimens, respectively ($p = 0.002$). The extent of gene expression in positive cases was significantly higher in PCA as compared to BPH ($p = 0.00018$), indicating that IL-6 gene expression might indeed be of use in discriminating PCA from BPH tissues.

TGF-β1 mRNA levels have been suggested to be increased in PCA.[19] Additionally, patients with metastatic PCA displaying high levels of serum IL-6 have also been reported to present increased serum levels of TGF-β1.[5]

In our series of samples, TGF-β1 gene was expressed in 32/39 (82.1%) and in 30/32 (93.8%) of BPH and PCA specimens, respectively ($p = 0.14$). However, unexpectedly, TGF-β1 gene expression in positive cases was quantitatively significantly lower in PCA as compared to BPH tissues ($p = 0.035$).

The expansion of a newly characterised subset of CD4+ helper T cells specifically secreting IL-17[20] is promoted by IL-23 which shares its p40 chain with IL-12.[21] The role played by these cytokines in tumour growth and immune responsiveness against tumours is debated. IL-23 has been reported to promote tumour incidence and growth[22], but also to induce immune enhancement and anti-tumour activity.[23] No data are available so far regarding IL-23 expression and PCA. Also regarding IL-17, conflicting data have been reported on its capacity to promote or inhibit tumour growth '*in vivo*'[20,24]. Interestingly, IL-17 gene expression has been detected in prostate tissues.[25]

IL-23A gene was only marginally expressed in 7/32 (21.9%) and 6/25 (24%) of the BPH and PCA specimens, respectively

Table 1 – Clinical profiles of the patients included in the study

	BPH patients (n = 42)					PCA patients (n = 34)					
	Patient	Age (year)	Surgical technique	PSA (ng/ml)		Patient	Age (year)	Surgical technique	PSA (ng/ml)	Gleason score	pT stage
	1	72	TUR-P	NA	PCA IDO^{low}	1	60	EERP	6.43	6	2c
	2	75	TUR-P	NA	n=25	2	61	EERP	8.50	6	2a
	3	72	TUR-P	NA		3	59	EERP	7.20	7	2c
	4	78	TUR-P	NA		4	69	EERP	48.00	7	3a
	5	78	TUR-P	18.60		5	74	EERP	1.43	7	2c
	6	75	TUR-P	2.07		6	83	TUR-P	106.00	9	1b
	7	78	TUR-P	NA		7	71	EERP	10.00	5	2a
	8	62	TUR-P	1.26		8	56	EERP	4.40	7	2c
	9	69	TUR-P	3.93		9	64	EERP	6.50	6	2c
	10	67	TUR-P	4.40		10	64	EERP	10.00	6	2c
	11	67	TUR-P	17.00		11	49	EERP	35.00	7	3b
	12	69	TUR-P	1.16		12	90	TUR-P	206.00	3	NA
	13	65	TUR-P	6.40		13	67	EERP	4.40	7	2c
	14	65	TUR-P	1.36		14	74	EERP	17.00	7	3a
	15	73	TUR-P	10.10		15	62	EERP	6.64	6	2c
	16	52	TUR-P	1.66		16	66	EERP	6.20	6	2a
	17	50	TUR-P	1.11		17	67	EERP	13.30	7	2c
	18	61	TUR-P	1.67		18	60	EERP	5.70	9	2b
	19	68	TUR-P	5.40		19	66	EERP	6.04	6	2c
	20	60	TUR-P	2.12		20	54	EERP	10.00	7	2c
	21	62	TUR-P	2.30		21	68	EERP	6.88	7	2c
	22	63	TUR-P	NA		22	68	EERP	3.44	7	2c
	23	59	TUR-P	4.25		23	76	EERP	11.52	6	2c
	24	65	TUR-P	1.24		24	65	TUR-P	6.19	6	1a
	25	58	TUR-P	2.66		25	69	EERP	4.10	7	2c
	26	65	TUR-P	2.96							
	27	65	TUR-P	26.00	PCA IDO^{high}	26	73	TUR-P	6.50	3	1
	28	72	TUR-P	1.79	n = 9	27	72	TUR-P	9.70	NA	1
	29	61	TUR-P	2.30		28	61	EERP	4.78	6	2a
	30	69	TUR-P	8.14		29	66	EERP	4.50	7	2c
	31	61	TUR-P	3.50		30	57	EERP	8.80	7	2c
	32	67	TUR-P	15.70		31	58	EERP	10.90	7	2c
	33	73	TUR-P	1.74		32	66	EERP	4.60	6	2a
	34	65	TUR-P	1.31		33	59	EERP	6.60	7	2c
	35	64	TUR-P	0.90		34	63	EERP	2.44	9	2c
	36	62	TUR-P	2.93							
	37	69	TUR-P	0.30							
	38	78	TUR-P	2.40							
	39	81	TUR-P	NA							
	40	63	TUR-P	1.26							
	41	81	TUR-P	NA							
	42	81	TUR-P	4.00							
Mean		67.6		4.82	Mean		65.8		17.93	6.55	
Standard deviation		7.4		5.84	Standard deviation		8.0		37.62	1.26	

NA, not available.

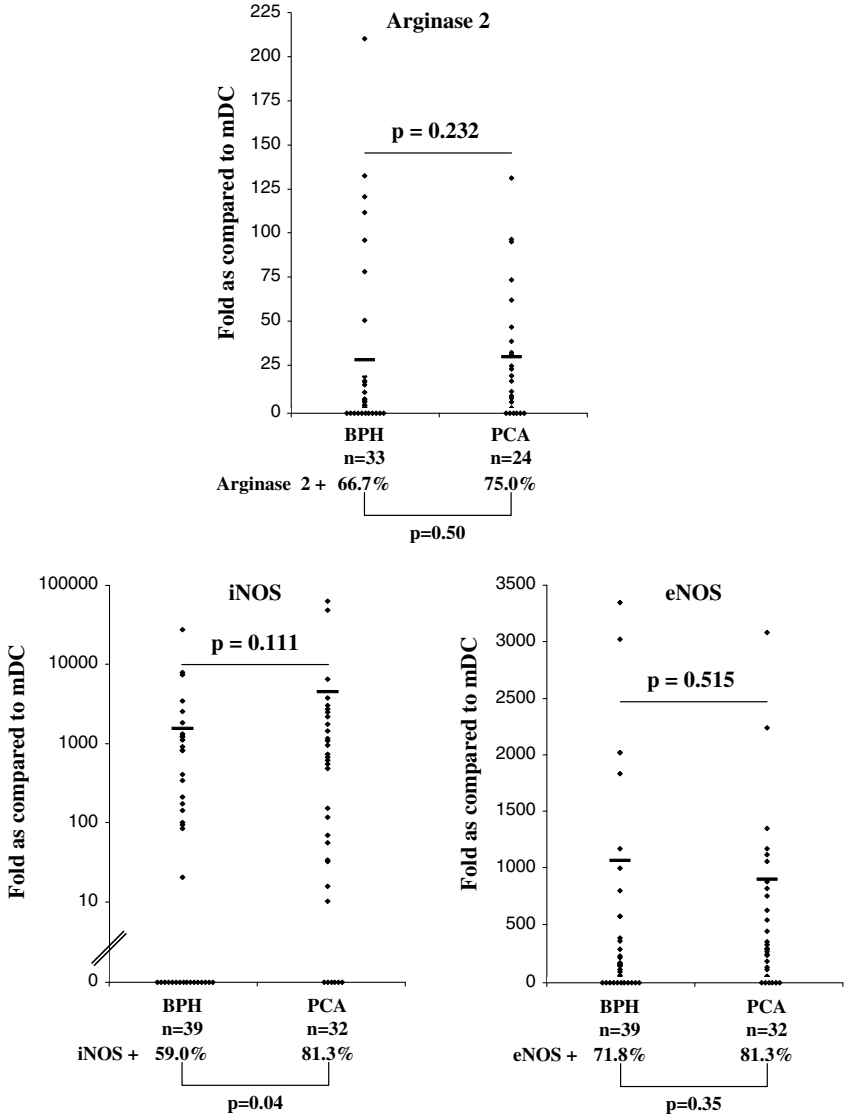

Fig. 1 – Expression of genes encoding immunosuppressive enzymes from the L-arginine metabolic pathway in PCA and BPH tissues. Total RNA was extracted from BPH and PCA tissues, DNAse treated, reverse transcribed and analysed by quantitative real-time PCR for arginase 2 (BPH: *n* = 33, PCA: *n* = 24), iNOS and eNOS (BPH: *n* = 39, PCA: *n* = 32) specific gene expression. Data are expressed as ratio to a positive control reference sample (cDNA from LPS matured dendritic cells – mDC).

($p = 0.85$), under investigation (Fig. 2). No significant quantitative differences were detectable between positive samples of the two groups ($p = 0.784$).

Regarding IL-17 (Fig. 2), only 6/39 (15.4%) and 8/32 (25%) of BPH and PCA specimens, respectively ($p = 0.31$), were positive for specific gene expression, and no significant quantitative differences could be observed between positive PCA and BPH tissues ($p = 0.343$).

3.4. *IDO expression in prostatic tissues*

Tryptophan degradation by IDO has been proposed as a mechanism favouring tumour escape from immune response[8].

We investigated IDO gene expression in our series of specimens. As shown in Fig. 3 (panel 1), IDO gene expression was detectable in 12/42 (28.6%) and in 24/32 (75%) of BPH and PCA samples, respectively ($p = 0.000075$). In addition, under a quantitative point of view, IDO gene expression in positive cases was significantly higher in PCA as compared to BPH ($p = 0.00001$).

Interestingly, the existence of two subpopulations of PCA showing evidence of different IDO gene expression clearly emerged. Indeed, 9/24 (37.5%) PCA were high and 15/24 (62.5%) were low IDO expressers, with a difference in gene expression of $\geqslant$100 times. Furthermore, IDO gene expression in PCA and BPH tissues was significantly ($p = 0.004$, data not shown) correlated with the expression of the gene encoding alpha-methyl coenzyme-A racemases A (AMACR A), known to be over-expressed in prostatic adenocarcinoma as compared to normal tissue.[26]

Prompted by these data, we investigated IDO expression at the protein level by immunohistochemical analysis on

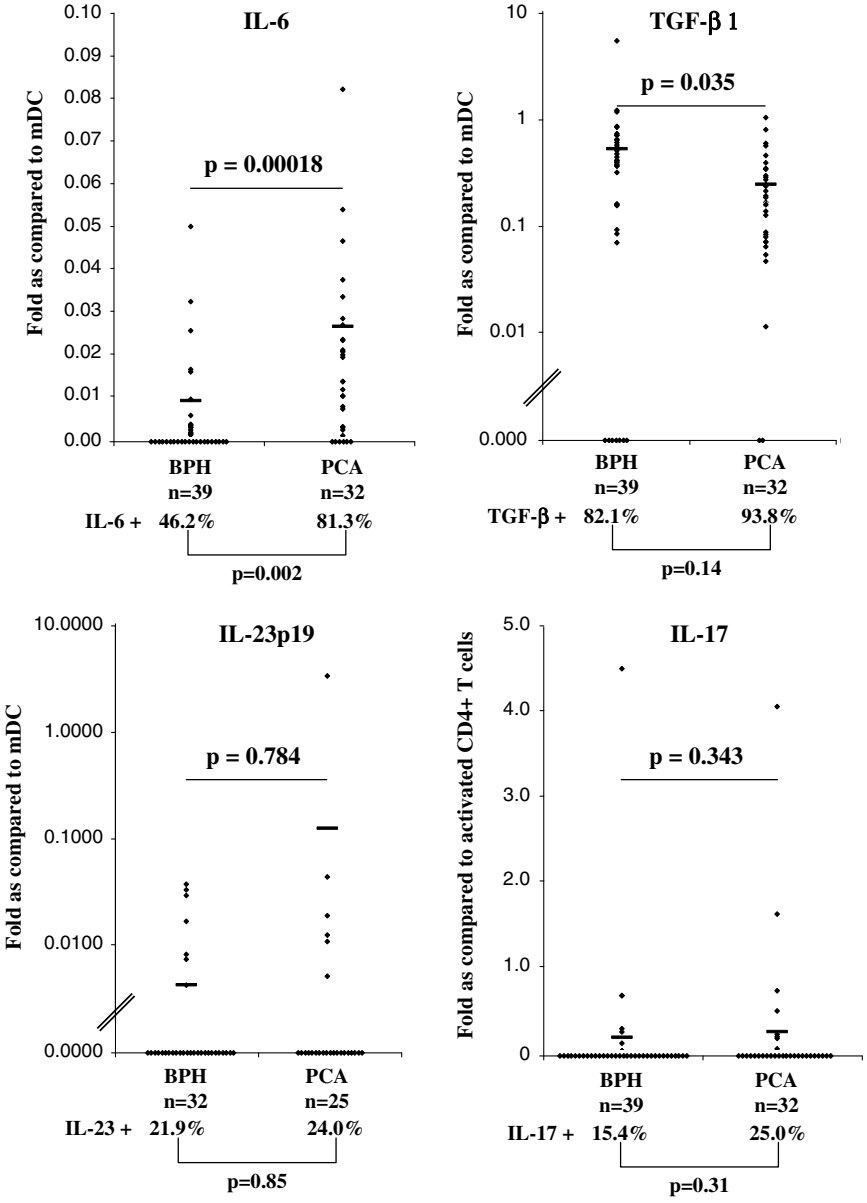

Fig. 2 – Expression of genes encoding cytokines in PCA and BPH tissues. Total RNA was extracted from BPH and PCA tissues, DNAse treated, reverse transcribed and analysed by quantitative real-time PCR for IL-6, TGF-β1, IL-17 (BPH: *n* = 39, PCA: *n* = 32) and IL-23p19 (BPH: *n* = 32, PCA: *n* = 25) gene expression. Data are expressed as ratio to a positive control reference sample represented by LPS matured dendritic cells (mDC) and PHA activated CD4+ T cells for IL-6, TGF-β1 or IL-23p19, and IL-17, respectively.

paraffin-embedded PCA and BPH tissues. Surprisingly, as illustrated in Fig. 3 (panel 2: c and d), IDO protein was almost exclusively detectable in endothelial cells in BPH and PCA tissues displaying low levels of IDO gene expression. However, in PCA displaying high levels of IDO gene expression, focal IDO specific staining in 2–5% of tumour cells was clearly detectable (Fig. 3, panel 2: e and f).

To determine if local IDO expression in PCA could have a systemic impact, we measured the concentrations of tryptophan and of its IDO dependent metabolite kynurenine in sera from BPH ($n = 33$) and PCA ($n = 34$) patients, as described in materials and methods. Overall, no significant differences ($p = 0.111$) were detected in kynurenine/tryptophan ratios in sera from PCA as compared to BPH patients (data not shown). However, in the subgroup of PCA specimens displaying a high level of IDO gene expression, a significant correlation between the expression of this gene and kynurenine/tryptophan ratios in sera ($p = 0.0045$, $R^2 = 0.8406$) was observed, thus suggesting an increased metabolism of the target amino acid (Fig. 3, panel 3).

In these patients, a highly significant correlation between IDO and TGF-β1 gene expression was also detectable (Table 2). In contrast IDO gene expression in PCA tissues did not correlate with Gleason score, pT stage or PSA levels.

Table 2 – Correlation between IDO gene expression in PCA tissues and clinical profiles

		Total PCA patients (n = 34)		PCA patients IDO^{high} expressers (n = 9)		PCA patients IDO$^{low/neg}$ expressers (n = 25)	
		p	R^2	p	R^2	p	R^2
Correlation of IDO gene expression with	PSA	0.5459	–0.1108	0.5545	–0.2284	0.7408	–0.0730
	Gleason score	0.1197	0.2854	0.1310	0.5810	0.7464	0.0713
	pT stage	0.2044	–0.2305	0.5477	–0.2322	0.9834	–0.0046
	Kyn/Trp[a]	0.3889	0.1577	**0.0045**	**0.8406**	0.7254	–0.0774
	IL-6[b]	0.3263	–0.1792	0.3238	–0.3723	0.9906	–0.0026
	TGF–pi[b]	0.1284	0.2745	**0.0028**	**0.8627**	0.6256	–0.1074

a Kynurenine/tryptophan ratios in sera.
b Gene expression in tissues.

4. Discussion

Neoplastic cells are detectable with high frequency in prostates from aged males. However, only in a minority of cases outgrowth of clinically relevant cancers can be observed. Mechanisms governing this transition are unclear, but it is tempting to speculate that defective tumour specific immune responsiveness could be involved.

Indeed, a number of factors have been suggested to be able to impair the functions of the immune system in PCA. However, the expression of genes encoding these factors in PCA and in BPH has not been comparatively addressed so far.

In this study, we show that PCA and BPH specimens display a similar expression of several genes encoding potentially immuno-suppressive factors, such as arginase 2, iNOS and eNOS. Conflicting data have been reported regarding the capacity of IL-23 and IL-17 to favour or inhibit cancer growth.[22,24] We found that the overall expression of the genes encoding these factors is weak in prostate specimens, and nevertheless similar in BPH and PCA.[25] Interestingly, a significant decrease in TGF-β1 gene expression in PCA as compared to BPH was concomitantly detected. Notably, serum levels of TGF-β proteins have been found to be significantly increased in PCA patients.[5] However, levels of TGF-β1 mRNA have also been shown to be reduced in PCA tissues.[27] Recent evidence suggests that disruption of TGF-β signalling in tumour cells through loss of TGF-β type I[28], II[29] and III[30] receptors, rather than increased amounts of protein, plays a role in the metastatisation process in prostate cancer.

Although the specimens under investigation were carefully screened for the presence of tumours by experienced pathologists at the moment of tissue collection, we cannot formally exclude that they did not actually contain malignant cells. Remarkably, however, the expression of the gene encoding IL-6, previously indicated as a possible mediator of prostate cancer morbidity[18], was found to be highly significantly enhanced in PCA as compared to BPH tissues under investigation. These data help validating the integrity of our technical approach.

Indoleamine 2,3-dioxygenase (IDO) has been proposed to induce tumoural immune resistance.[8] Whilst expression of IDO gene has been detected in PCA[8], its cancer specificity has not been investigated so far.

We found that although IDO gene expression is also detectable in BPH specimens, it is strongly enhanced in a sizeable percentage of PCA specimens. Notably, overall IDO gene expression is significantly correlated with the expression of the gene encoding AMACR A, a candidate biomarker for PCA.[31] Most interestingly, in the subgroup of PCA patients whose tumours express high levels of IDO gene, a significant increase of the kynurenine/tryptophan ratio is detectable in the sera. These data suggest that the impact of IDO gene expression could extend beyond tumour microenvironment and result in systemic effects.

Interestingly, in our study, IDO protein was frequently expressed by endothelial cells rather than by tumour cells in BPH and PCA tissues expressing low level of IDO gene. Notably, IDO has been shown to be expressed at the protein level mainly by vascular endothelial cells in term placenta[32] and in renal cell carcinoma.[33] Furthermore, inhibition of IDO activity is known to improve the ability of HUVEC cells to stimulate allogenic T cell responses, and HUVEC cells transfected with the IDO gene induce anergy in allospecific T cells.[34]

However, in PCA specimens of our series where high levels of IDO gene expression were observed, IDO protein was also focally detectable in tumour cells. Remarkably, prostate cancer cells expressing IDO protein were often located in areas of inflammation and atrophic glands and ducts were found to be positive for IDO specific staining in PCA.

Taken together, our findings indicate that whilst arginase 2, eNOS and iNOS are similarly expressed in both BPH and PCA, high IDO gene expression is typically detectable only in a subgroup of PCA, but not in BPH. In patients bearing these tumours a high kynurenine/tryptophan ratio can also be observed in sera.

The finding that TGF-β1 gene expression is significantly correlated with IDO gene expression in PCA expressing high levels of IDO gene contributes to the identification of a peculiar subset of tumours. Whilst mechanisms underlying the concomitant expression of these genes are still unclear, it is tempting to speculate that their products might synergise in down regulating tumour specific immune responses. Further studies with adequate follow-up are warranted to address the potential diagnostic and/or prognostic significance of high IDO gene expression in PCA.

Notably, IDO might eventually represent an attractive target for the development of new drugs of potential use in the

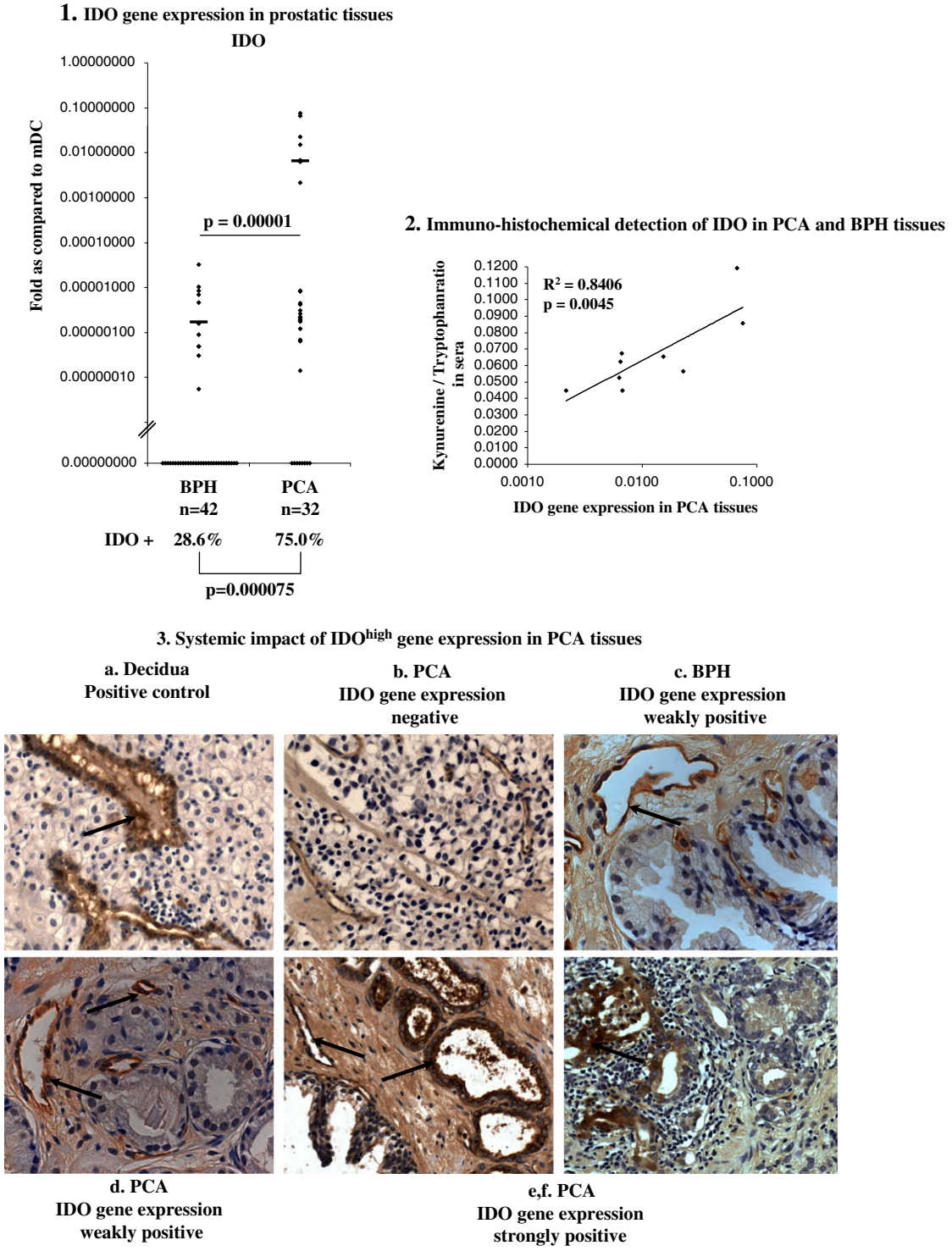

Fig. 3 – IDO expression in prostatic tissues. (Panel 1) IDO gene expression in prostatic tissues. Total RNA was extracted from BPH (n = 42) and PCA (n = 32) tissues, DNAse treated, reverse transcribed and analysed by quantitative real-time PCR for IDO gene expression. Data were expressed as ratio to a reference positive control represented by LPS matured dendritic cells (mDC). (Panel 2) Immunohistochemical detection of IDO in PCA and BPH tissues. (a) Decidua was used as positive control tissue and stained with IDO specific antibodies (400X). (b) PCA tissue negative for IDO gene expression (400X). (c) BPH tissue showing weak IDO gene expression and (d) PCA tissue showing weak IDO gene expression and displaying IDO^+ endothelial cells in capillaries and IDO^- tumour cells (630X). (e, f) PCA tissues expressing high levels of IDO gene expression and showing IDO^+ tumour cells, especially in inflamed regions (panel f) (400X). Arrows indicate IDO^+ cells. (Panel 3) Systemic impact of IDO^{high} gene expression in PCA tissues. A subgroup of PCA (n = 9) tissues highly positive for IDO gene expression (see panel 1) was identified. IDO gene expression in these tumours was correlated to kynurenine/tryptophan ratio in sera from the corresponding patients sampled simultaneously to collection of surgical specimens.

treatment of PCA. Indeed, IDO inhibition is not intrinsically cytotoxic, and potent bioactive IDO inhibitors such as 1-methyl-DL-tryptophan (1-MT) and methyl-thiohydantoin-tryptophan (MTH-trp) have been shown to cooperate with diverse chemotherapeutic agents to effectively promote regression of established tumours in experimental models.[35]

Conflict of interest statement

None declared.

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REFERENCES

1. Edwards BK, Brown ML, Wingo PA, et al. Annual report to the nation on the status of cancer, 1975–2002, featuring population-based trends in cancer treatment. *J Natl Cancer Inst* 2005;**97**(19):1407–27.
2. Bronte V, Kasic T, Gri G, et al. Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers. *J Exp Med* 2005;**201**(8):1257–68.
3. Wang J, Torbenson M, Wang Q, Ro JY, Becich M. Expression of inducible nitric oxide synthase in paired neoplastic and non-neoplastic primary prostate cell cultures and prostatectomy specimen. *Urol Oncol* 2003;**21**(2):117–22.
4. Tong X, Li H. eNOS protects prostate cancer cells from TRAIL-induced apoptosis. *Cancer Lett* 2004;**210**(1):63–71.
5. Adler HL, McCurdy MA, Kattan MW, Timme TL, Scardino PT, Thompson TC. Elevated levels of circulating interleukin-6 and transforming growth factor-beta1 in patients with metastatic prostatic carcinoma. *J Urol* 1999;**161**(1):182–7.
6. Stone TW, Darlington LG. Endogenous kynurenines as targets for drug discovery and development. *Nat Rev Drug Discov* 2002;**1**(8):609–20.
7. Weber WP, Feder-Mengus C, Chiarugi A, et al. Differential effects of the tryptophan metabolite 3-hydroxyanthranilic acid on the proliferation of human CD8+ T cells induced by TCR triggering or homeostatic cytokines. *Eur J Immunol* 2006;**36**(2):296–304.
8. Uyttenhove C, Pilotte L, Theate I, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat Med* 2003;**9**(10):1269–74.
9. Martin I, Jakob M, Schafer D, Dick W, Spagnoli G, Heberer M. Quantitative analysis of gene expression in human articular cartilage from normal and osteoarthritic joints. *Osteoarthr Cartilage* 2001;**9**(2):112–8.
10. Mubiru JN, Shen-Ong GL, Valente AJ, Troyer DA. Alternative spliced variants of the alpha-methylacyl-CoA racemase gene and their expression in prostate cancer. *Gene* 2004;**327**(1):89–98.
11. Hartwig D, Hartel C, Hennig H, Muller-Steinhardt M, Schlenke H, Kluter H. Evidence for de novo synthesis of cytokines and chemokines in platelet concentrates. *Vox Sang* 2002;**82**(4):182–90.
12. Mocellin S, Ohnmacht GA, Wang E, Marincola FM. Kinetics of cytokine expression in melanoma metastases classifies immune responsiveness. *Int J Cancer* 2001;**93**(2):236–42.
13. Bubendorf L, Sauter G, Moch H, et al. Ki67 labelling index: an independent predictor of progression in prostate cancer treated by radical prostatectomy. *J Pathol* 1996;**178**(4):437–41.
14. Chiarugi A, Rovida E, Dello SP, Moroni F. Tryptophan availability selectively limits NO-synthase induction in macrophages. *J Leukoc Biol* 2003;**73**(1):172–7.
15. Wittekind C, Meyer HJ, Bootz F. *UICC (2002) TNM Klassifikation maligner Tumoren, 6. Auflage*. Berlin: Springer; 2003.
16. Keskinege A, Elgun S, Yilmaz E. Possible implications of arginase and diamine oxidase in prostatic carcinoma. *Cancer Detect Prev* 2001;**25**(1):76–9.
17. Rodriguez PC, Quiceno DG, Zabaleta J, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* 2004;**64**(16):5839–49.
18. Twillie DA, Eisenberger MA, Carducci MA, Hseih WS, Kim WY, Simons JW. Interleukin-6: a candidate mediator of human prostate cancer morbidity. *Urology* 1995;**45**(3):542–9.
19. Merz VW, Arnold AM, Studer UE. Differential expression of transforming growth factor-beta 1 and beta 3 as well as c-fos mRNA in normal human prostate, benign prostatic hyperplasia and prostatic cancer. *World J Urol* 1994;**12**(2):96–8.
20. Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. *J Leukoc Biol* 2002;**71**(1):1–8.
21. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;**24**(2):179–89.
22. Langowski JL, Zhang X, Wu L, et al. IL-23 promotes tumour incidence and growth. *Nature* 2006;**442**(7101):461–5.
23. Hao JS, Shan BE. Immune enhancement and anti-tumour activity of IL-23. *Cancer Immunol Immunother* 2006;**55**(11):1426–31.
24. Numasaki M, Fukushi J, Ono M, et al. Interleukin-17 promotes angiogenesis and tumor growth. *Blood* 2003;**101**(7):2620–7.
25. Steiner GE, Newman ME, Paikl D, et al. Expression and function of pro-inflammatory interleukin IL-17 and IL-17 receptor in normal, benign hyperplastic, and malignant prostate. *Prostate* 2003;**56**(3):171–82.
26. Rubin MA, Zhou M, Dhanasekaran SM, et al. alpha-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. *JAMA* 2002;**287**(13):1662–70.
27. Soulitzis N, Karyotis I, Delakas D, Spandidos DA. Expression analysis of peptide growth factors VEGF, FGF2, TGFB1, EGF and IGF1 in prostate cancer and benign prostatic hyperplasia. *Int J Oncol* 2006;**29**(2):305–14.
28. Kim IY, Ahn HJ, Zelner DJ, et al. Loss of expression of transforming growth factor beta type I and type II receptors correlates with tumor grade in human prostate cancer tissues. *Clin Cancer Res* 1996;**2**(8):1255–61.
29. Tu WH, Thomas TZ, Masumori N, et al. The loss of TGF-beta signaling promotes prostate cancer metastasis. *Neoplasia* 2003;**5**(3):267–77.
30. Turley RS, Finger EC, Hempel N, How T, Fields TA, Blobe GC. The type III transforming growth factor-beta receptor as a novel tumor suppressor gene in prostate cancer. *Cancer Res* 2007;**67**(3):1090–8.
31. Xu J, Stolk JA, Zhang X, et al. Identification of differentially expressed genes in human prostate cancer using subtraction and microarray. *Cancer Res* 2000;**60**(6):1677–82.
32. Ligam P, Manuelpillai U, Wallace EM, Walker D. Localisation of indoleamine 2,3-dioxygenase and kynurenine hydroxylase in the human placenta and decidua: implications for role of the kynurenine pathway in pregnancy. *Placenta* 2005;**26**(6):498–504.

33. Riesenberg R, Weiler C, Spring O, et al. Expression of indoleamine 2,3-dioxygenase in tumor endothelial cells correlates with long-term survival of patients with renal cell carcinoma. *Clin Cancer Res* 2007;**13**(23):6993–7002.
34. Beutelspacher SC, Tan PH, McClure MO, Larkin DF, Lechler RI, George AJ. Expression of indoleamine 2,3-dioxygenase (IDO) by endothelial cells: implications for the control of alloresponses. *Am J Transpl* 2006;**6**(6):1320–30.
35. Muller AJ, DuHadaway JB, Donover PS. Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat Med* 2005;**11**(3):312–9.