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Heparanase-1 gene expression in normal, hyperplastic and neoplastic prostatic tissue

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Abstract

Heparanase-1 (Hpa-1) has been implicated in tumour invasion and metastasis. In the present study, we evaluated the clinicopathological significance of Hpa-1 mRNA expression in prostate cancer and non-cancerous prostatic tissue by one-step polymerase chain reaction (PCR) of laser microdissected prostatic gland cells. In addition, cell type-specific expression of Hpa-1 mRNA in prostatic tissue was analysed by *in situ* hybridisation. Hpa-1 mRNA expression was found in 50% of normal and 40% of hyperplastic prostatic tissue. *In situ* hybridisation showed that Hpa-1 mRNA was strongly expressed in prostate gland cells. Of the 26 prostate carcinomas tested, 42% were positive for Hpa-1 mRNA. However, in non-cancerous prostatic tissue, Hpa-1 mRNA was significantly more often expressed than in less differentiated or more invasive prostate cancers ($P<0.05$). *In situ* hybridisation revealed only focal Hpa-1 mRNA expression in the neoplastic gland cells. Hpa-1 mRNA expression in the tumours significantly correlated with tumour differentiation and tumour stage ($P<0.05$). Our data indicate that Hpa-1 gene expression may be lost during dedifferentiation of prostatic gland cells.

Keywords: Heparanase-1; Hpa-1; Benign prostatic hyperplasia; Prostate cancer; Prostate

1. Introduction

Prostate cancer is the second leading cause of cancer-related death in men in Western industrialised countries. Its incidence is countinuously rising, with over 200 000 new cancers and 35–40 000 deaths each year [1]. Despite extensive research the oncogenesis and the mechanisms influencing the progression of prostate cancer are still not completely understood. Currently used tumour markers, such as prostate-specific antigen (PSA), p53 or vascular endothelial growth factor (vEGF), have not been successful at predicting the development and progression of prostate cancer [2,3]. It is therefore of great importance to understand factors involved in prostate carcinogenesis to identify new prognostic tumour markers.

Prostate carcinogenesis and progression is a multistep process involving both genetic insults to epithelial cells and changes in epithelial–stromal interactions [4]. Recent studies have shown that alterations of the heparan sulphate proteoglycans (HSPGs) on the cell surface of epithelial cells are associated with the malignant transformation of cells [5]. In addition to their role of initial cell transformation, HSPGs bind growth factors and cytokines that are involved in the modulation of tumour cell growth [5,6]. Degradation of HSPGs—which are the main components of the extracellular matrix (ECM)—has been demonstrated to play an important role in signal transduction resulting in the inhibition of tumour cell growth [7]. Heparanase-1 (Hpa-1) is an endo-beta-D-glucuronidase that specifically cleaves the heparan sulphate chain of HSPGs [8–10]. Hpa-1 is expressed on the cell surface of a wide

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range of benign and neoplastic cells and tissues including inflammatory cells, fibroblasts, hepatocellular carcinoma, pancreatic adenocarcinoma, breast cancer and prostate carcinoma cells *in vitro* [8,9,11–13]. Various studies have demonstrated a correlation between the expression of Hpa-1 mRNA and protein in cancer cells and the metastatic potential of various tumours *in vitro* and *in vivo* [8,9,12,13]. These observations suggest that enhanced Hpa-1 mRNA expression could be a new biological marker in prostate cancer. However, in prostate carcinoma, the role of Hpa-1 gene expression remains unclear.

Thus, in the present study we evaluated the clinicopathological significance of Hpa-1 gene expression in prostate carcinoma and non-cancerous prostatic tissue by one-step polymerase chain reaction (PCR) and analysed its cell-specific distribution by *in situ* hybridisation.

2. Materials and methods

2.1. Patients

Routinely processed formalin-fixed and paraffin-embedded prostate specimens from 40 patients were evaluated according to standard criteria as shown in Table 1. The specimens included four probes of normal prostatic tissue from young males that were obtained during autopsy (median age 40 years, range 29–44 years), 10 cases of hyperplastic prostatic tissue (median age 68 years, range 61–87 years), and 26 probes of adenocarcinoma of the prostate (median age 61 years, range 48–74 years). Histological grading and tumour staging was done by one pathologist. The prostate cancers were graded using the Gleason score [14]. Tumour staging was according to the TNM classification [15]. Nine tumours were graded as Gleason score <7, 12 tumours were graded as Gleason score $=7$, and five tumours were graded as Gleason score >7. There were 13 cases staged as pT2 and 13 cases staged as pT3.

Table 1
Expression of Hpa-1 mRNA in prostate cancer cells according to the cancer characteristics of patients analysed using a one-step PCR

Parameters	No. of patients	No. overall positive (%)	*P* value	r^a
Tumour grade			0.040*	0.497
Gleason <7	9	5 (56)		
Gleason $=7$	12	6 (50)		
Gleason >7	5	0 (0)		
Tumour stage			0.044*	0.395
pT2	13	8 (62)		
pT3	13	3 (23)		

Hpa-1, heparanase-1; PCR, polymerase chain reaction.
P values were evaluated by the log-likelihood ratio test (G-test), $*P<0.05$. r^a, product–moment correlation coefficient.

2.2. Microdissection

To study the expression of Hpa-1 mRNA in normal, hyperplastic and neoplastic prostatic tissue, pure epithelial cell population were selected by laser-assisted microdissection using a PixCell laser capture microscope with an infrared diode laser (Arcturus Engineering, Santa Clara, CA, USA) as previously described in Ref. [16]. Therefore, formalin-fixed and paraffin-embedded tissue sections were rehydrated following standard protocols. After haematoxylin eosin staining, the sections were fixed in 100% ethanol and air-dried. Thereafter, tissue sections were overlaid with a thermoplastic membrane mounted on optically transparent caps, and cells captured by focal melting of the membrane through laser activation. The captured prostatic gland cells were stored in reaction tubes until the PCR analysis.

2.3. RNA isolation and one-step PCR

Total mRNA was isolated from captured prostatic gland cells (500–1000) using the RNA Isolation Kit from GENTRA (Minneapolis, MN, USA) according to the manufacturer‘s protocol. RNA was precipitated after the addition of 1 μg/ml glycogen (Roche, Basel, Switzerland), resuspended in 10 μl Rnase-free water, and 5 μl was used for one-step PCR with glyceraldehyde-3-phosphodehydrogenase (GAPDH) and Hpa-1, respectively. PCR was performed with the one-step kit from Qiagen (Hilden, Germany) according to the supplier's protocol. The PCR products were size-fractionated in a 2% agarose gel and visualised with ethidium bromide. The following primer pairs were used: GAPDH forward: 5′-GGTGAAG-GTCGGTG TCAA-3′; reverse: 5′-CAAAGTTGTCATGGATGA-3′; heparanase-1 forward: 5′-TTCGATCCCAAGAAGGA ATCAAC-3′; reverse: 5′-GTAGTGATGCCATGTA ACTG-AATC-3′ (results in a 584-bp fragment covering the gene segment from exon 3 to exon 7).

2.4. Tissue processing and in situ hybridisation

To study the distribution of Hpa-1 mRNA in the prostatic tissue, formalin-fixed and paraffin-embedded prostate specimens, from the same patients as used for the PCR analysis, were processed for *in situ* hybridisation. Therefore, a Hpa-1-specific DNA fragment was generated from placental RNA by the primer pair described above, cloned into a pGEM-T easy® vector (Promega) and sequence verified. The vector was linearised and used as a template for *in vitro* transcription of fluorescein-labelled antisense or sense (control) riboprobes using a SP6/T7 transcription kit (Roche, Switzerland). Tissue sections were dewaxed and rehydrated and then digested with proteinase K (20 μg/ml) at room temperature (RT) for 30 min. Slides were prehybridised and

hybridised with the anti-sense probe or sense probe (negative control) at concentrations of 2 μg/ml. After hybridisation washes and incubation with antifluorescein antibodies, an APAAP detection system (Dako, Glostrup, Denmark) was used according to the supplier's protocol. Slides were counterstained with Mayers' haematoxylin.

2.5. Statistical analyses

The difference in the positive staining rate of Hpa-1 mRNA between non-cancerous tissue and prostate cancer, as well as the expression of Hpa-1 mRNA in prostate carcinomas according to their cancer characteristics were analysed by the log-likelihood ratio test (G-test). Results are expressed as positive Hpa-1 mRNA expression in prostatic gland cells and in a percentage of controls. Significance was implied at $P<0.05$.

3. Results

To study the expression of Hpa-1 mRNA in prostatic glands, one-step PCR of laser microdissected prostatic gland cells was performed. Positive Hpa-1 mRNA expression in the epithelial cells was detected in 50% of the normal prostatic tissue and 40% of the hyperplastic prostatic tissue. 42% of the prostate carcinomas showed a positive Hpa-1 mRNA expression that was not significantly different from the expression in benign prostatic tissue (Fig. 1). However, Hpa-1 mRNA expression in less differentiated ($P<0.05$) or more invasive prostate cancers ($P<0.05$) was significantly lower than in non-cancerous prostatic tissue (Fig. 1). The expression of Hpa-1 mRNA in prostate carcinoma related to cancer characteristics is shown in Table 1. Hpa-1 mRNA expression was significantly associated with tumour grade ($P<0.05$) or tumour stage ($P<0.05$). Fifty-six percent of the prostate cancers graded as Gleason <7, 50% of the tumours graded as Gleason $=7$, but none of the tumours graded as Gleason $\geqslant 7$ showed a positive Hpa-1 mRNA expression. Moreover, Hpa-1 mRNA was expressed in 62% of stage pT2 tumours, but only 23% of pT3 prostate cancers.

To study the cell-specific expression and distribution of Hpa-1 mRNA in prostatic tissue, *in situ* hybridisation was performed. In normal and hyperplastic prostatic tissue, the vast majority of the Hpa-1 mRNA was expressed at the luminal part of the prostatic gland cells (Fig. 2a and b). Only occasional and focal Hpa-1 mRNA expression could be detected in the surrounding stroma that was confined to single fibroblasts, endothelial cells, and intra- or extravascular granulocytes (Fig. 2a). Benign hyperplastic glands adjacent to neoplastic glands showed a comparatively stronger expression of Hpa-1 mRNA compared with the invasive cancer cells (Fig. 2c). In contrast to the non-cancerous prostatic tissue, prostate cancer cells only focally expressed Hpa-1 mRNA (Fig. 2d) with also focal labelling of the surrounding desmoplastic stromal cells (Fig. 2c and d).

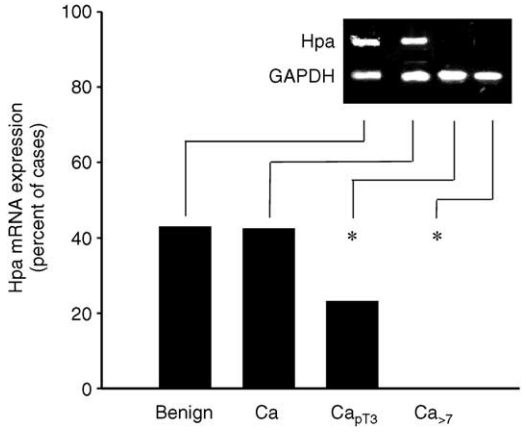

Fig. 1. Detection of heparanase-1 (Hpa-1)-specific mRNA transcripts by one-step polymerase chain reaction (PCR). Total RNA was isolated from laser-captured prostatic gland cells and used for one-step PCR with glyceraldehyde-3-phosphodehydrogenase (GAPDH) and Hpa-1, respectively. Hpa-1 mRNA expression in non-cancerous tissue (Benign) is not significantly different from prostate cancer (Ca). However, Hpa-1 mRNA in non-cancerous prostatic tissue is significantly more often detected than in less differentiated ($Ca_{>7}$) or more invasive prostate cancers (Ca_{pT3}). *$P<0.05$.

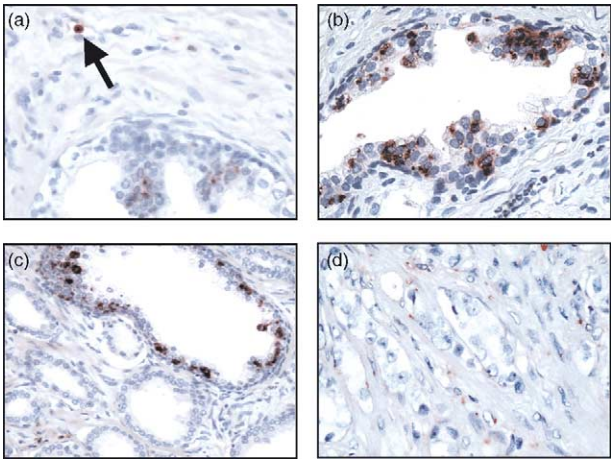

Fig. 2. Heparanase-1 (Hpa-1) mRNA (*in situ* hybridisation) expression in prostatic tissue. Positive labelling is red, nuclei counterstain is blue. Normal prostate: (a) Hpa-1 mRNA expression in prostatic gland cells; neutrophils serve as positive internal controls (arrow). Benign prostatic hyperplasia: (b) Strong expression of Hpa-1 mRNA in the majority of prostatic gland cells whereas (c) adjacent neoplastic epithelial tissue is often negative for Hpa-1 mRNA. Prostate cancer: (d) Only focal expression of Hpa-1 mRNA in the majority of less differentiated or more invasive neoplastic gland cells with (c, d) positive expression also of the surrounding desmoplastic stroma. (a, b, d, original magnifications 40×; c, original magnification 20×).

4. Discussion

Hpa-1 has been recognised as a key enzyme in cancer cell invasion and metastasis by cleaving HSPGs from the basement membrane and the ECM [8–10,17]. Recently, mRNA or protein expression of Hpa-1 has been identified in various cancer cells, and the overexpression of Hpa-1 in tumour cells has been reported to correlate with the metastatic potential of cancers [8,9,12,13].

However, results from our study are completely different to those previously reported. In the present study, we demonstrate that Hpa-1 mRNA is expressed in benign as well as malignant prostatic tissue. However, Hpa-1 mRNA in non-cancerous prostatic tissue was significantly more frequently expressed than in advanced prostate cancers as confirmed by PCR analysis. The Hpa-1 mRNA expression in neoplastic gland cells significantly correlated with tumour differentiation and tumour stage.

The results from the PCR analysis were confirmed by *in situ* hybridisation studies of benign and neoplastic prostatic tissue. Although one-step PCR analysis proved to be very sensitive at detecting Hpa-1 mRNA, the message about Hpa-1 mRNA distribution in the prostatic tissue was limited due to heterogenic expression in the prostatic glands and to invading inflammatory cells expressing Hpa-1 mRNA. *In situ* hybridisation of prostatic tissue allowed the accurate identification of the benign prostatic gland cell as a major site of Hpa-1 synthesis. In contrast, the neoplastic gland cells displayed only focal Hpa-1 mRNA expression with positive labelling of the surrounding stromal cells. Together with the results of the PCR studies, these findings indicate that Hpa-1 gene expression may be lost during the progression of prostate cancer.

Changes in the expression pattern of Hpa during cancer development and progression has been described in various tumours including gastric, pancreatic, colon, bladder and breast carcinoma [13,18–21]. Most of the studies have demonstrated an increased Hpa gene and/or protein expression in carcinoma cells compared with normal or dysplastic gland cells. However, recent studies from Ikeguchi and coworkers [7] have shown significantly lower Hpa mRNA levels in zirrhotic hepatocellular carcinomas than those of normal livers. In contrast to our study, Hpa mRNA expression in the hepatocellular carcinomas did not correlate with tumour differentiation or with tumour stage [7]. They concluded that Hpa gene expression may be lost during the malignant transformation of hepatocytes through alterations of their cell surface HSPGs profile.

Recent evidence highlights the fact that changes in the HSPGs expression on the cell surface or in the ECM modulates the cellular phenotype including transformation of a normal cell to a tumour cell and tumour growth kinetics [5]. Hpa-1 that specifically cleaves HSPGs may therefore be involved in the initial oncogenic transformation of cells and the control of tumour growth. However, recent studies reported that treatment with Hpa reduces tumour volume and the number of lung metastasis in mice injected with melanoma cells [22]. These findings are supported by Ikeguchi and collegues [7] which demonstrated a significant positive correlation between Hpa mRNA expression levels and the percentage of apoptotic hepatic carcinoma cells. Thus, Hpa-activity may control growth-signal transduction essential for cell growth. The mechanisms by which Hpa-1 can modulate tumour cell transformation and proliferation are not completely understood. Results from recent studies suggest that Hpa-1 may act through two different pathways: (1) alteration of HSPGs on the epithelial cell surface resulting in the transformation of a normal cell into a tumour cell, and/or (2) cleaving of HSPGs-bound growth factors in the ECM such as transforming growth factor-beta (TGF-β), basic fibroblast growth factor (bFGF) or vascular endothelial growth factor (vEGF) that promote or inhibit cell growth [5–7]. However, Hpa-1 expression in tissue stroma in close apposition to tumour glands may contribute to this process by means of releasing HSPGs-binding cytokines and growth factors that are stored in the ECM [10]. It is tempting to suggest that Hpa-1 expression by stromal cells may be induced by tissue remodelling during tumour progression and may thus reflect a physiological response to alterations in the local tissue caused by the tumour growth [18].

In summary, we demonstrated Hpa-1 mRNA expression in non-cancerous and cancerous prostatic tissue. The present study points to the benign prostatic gland cell as a major site of Hpa-1 expression. Our results indicate that Hpa-1 expression in normal prostatic gland cells is important for the regulation of their growth properties. We conclude that loss of Hpa-1 gene expression in epithelial gland cells may result in abnormal cell proliferation and prostate cancer progression.

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