SPANDIDOS PUBLICATIONS

The influence of CD44v3-v10 on adhesion, invasion and MMP-14 expression in prostate cancer cells

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Received March 21, 2005; Accepted April 22, 2005

Abstract. The expression of certain CD44 variants has been linked with metastasis and tumour progression. In particular, high molecular weight forms of CD44 show restricted expression in tumours and may correlate with tumour development and metastasis. In this study, we examined the expression of CD44 variants in prostate cancer cell lines: the invasive PC-3 and DU-145, low invasive LNCaP, and two non-invasive prostate epithelial cell lines. PC-3 prostate cancer cells were transfected with a high molecular weight CD44 variant isoform, CD44v3-v10, isolated from non-invasive prostate epithelial cell lines. These transfected cells (PC-NIVO) were assessed using *in vitro* invasion, tumour-endothelial, growth, and migration assays. The expression of MMP-14 was examined using SDS-PAGE and Western blot analysis. Transfected PC-3 cells (PC-NIVO) were found to be less adherent to endothelial cells and had significantly reduced invasiveness compared to wild-type PC-3 or control cells. In addition, tumour cell adhesion to endothelial cells and invasiveness was increased after exposure to HGF/SF, and can be blocked by the presence of anti-CD44 antibodies. Further investigation revealed a reduction in the expression of MMP-14 in PC-NIVO cells, but not in PC-3 or control cells. In conclusion, non-invasive prostate epithelial cells express a high molecular weight CD44 isoform, CD44v3-v10, which may counteract the standard isoform function of CD44 by reducing adhesion and invasion of endothelium by prostate tumour cells through negation of the MMP-14 function.

Introduction

CD44, a family of transmembrane glycoprotein molecules that has been discovered on many different cell types from several mammalian species, is also known as Ly-24, lymphocyte homing receptor (gp90^{Hermes}), phagocytic glycoprotein (Pgp-1), extracellular matrix receptor III (ECMRIII), hyaluronate receptor (H-CAM; homing cellular adhesion molecule) and HUTCH-1. CD44 is expressed on a variety of cells including T-lymphocytes, B-cells, monocytes, granulocytes, erythrocytes, many epithelial cell types, and mesothelial and some endothelial cells (1-5). It has functional roles in T- and B-lymphocyte homing and adhesion (6-8), cell proliferation (9), cell migration (10), lymphocyte and monocyte activation, cell migration and metastasis (1,3,11-14). This versatility in function is not only due to the amino acid sequence of isoforms, but also to differences in post-translational modifications (1,15-17). CD44 is also a member of all hyaladherins that share a limited (approximately 30%) sequence homology and act mainly as receptors for hyaluronic acid (18,19). However, CD44 is the major leukocyte and epithelial cell surface hyaladherin.

In humans, the CD44 family is encoded by a single gene located on chromosome 11p13 and comprises at least 20 exons (20). Exons 1-5, 16-18, and 20 are spliced together to form a CD44 transcript that has become known as the standard isoform (abbreviated to CD44s). At least ten exons can be alternatively spliced and inserted into CD44s at an insertion site between exons 5 and 16 to give rise to variant isoforms of CD44. Thus, exons 6-15 are variant exons and typically identified as v1-v10. The standard isoform of human CD44 is a type 1 transmembrane molecule composed of 341 amino acids and can be subdivided into three major domains: a 70 amino acid (aa) C-terminal cytoplasmic domain, a 23 aa transmembrane domain, and a 248 aa N-terminal extracellular domain. The 70 aa cytoplasmic region (residues 272-341) is encoded by part of exon 18 (three amino acids) and by exon 20. The standard isoform of CD44 undergoes extensive post-translational modification resulting in the attachment of numerous carbohydrates to N- and O-linked glycosylation sites of the extracellular domain (1,12,15-17,21,22). The variable exon products, as well as the increasing size of the CD44 variant molecules, also introduce extra target sites for glycosylation and broaden the ligand specificity. There are at least four additional potential N-glycosylation sites and a large number of potential O-glycosylation sites in variant exons. In particular, CD44 isoforms containing the product encoded by the variant exon v3 can be modified with heparan sulphate chains and have been shown to bind heparin-binding growth factors and chemokines. These high molecular weight isoforms of CD44 show restricted expression in tumours and may correlate with tumour development and metastasis.

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Key words: CD44, prostate cancer, endothelium, invasion, cell adhesion

CD44 proteins have been shown to interact with growth factors and MMPs (23-26). The matrix metalloproteinases (MMPs) are members of a family of at least 15 Zn-dependent endopeptidases that function extracellularly and were initially categorized by their ability to degrade various components of the ECM and basement membrane components. The membrane type (MT)-MMPs are anchored in the plasma membrane by means of a transmembrane and cytoplasmic domain. Expression of MMP-14 or MT1-MMP is associated with invasion and/or metastasis of human cancer (27-30), and mediates cell motility through cleavage of CD44 (31). MMP-14 forms a complex with CD44s via its hemopexin-like (PEX) domain, thus linking MMP-14 to the actin cytoskeleton.

MMP-14 was originally identified as an activator of MMP-2, which plays a role in the degradation of type-IV collagen, a major component of the basement membrane (32). This activation occurs via formation of a membrane-bound trimolecular complex comprising MMP-14, TIMP-2 and inactive MMP-2. MMP-14 also activates proMMP-13 and degrades collagen types I, II, III, V, VII and X, fibronectin, vitronectin, and Laminins 1 and 5 (31,33-40). Thus, MMP-14 triggers MMP activation cascades on the cell surface.

Here, we examine the expression of CD44 variants in prostate cancer cell lines, the invasive PC-3, DU-145 and LNCaP, and two non-invasive prostate epithelial cell lines. In particular, we investigate the role of a high molecular weight CD44 variant isoform that can reduce the adhesion/invasion of prostate tumour cells, and examine its influence on MMP-14 expression.

Materials and methods

Cell lines. Human prostate cancer cell lines, DU-145, LNCaP.FGC, and two non-invasive CA-HPV-10 and PZ-HPV-7, were obtained from the American Type Culture collection (ATCC; MD, USA). PC-3 cells were obtained from the European Collection of Animal Cell Culture (ECACC; Salisbury, UK). HECV, a human umbilical vein endothelial cell line, which has both vascular and lymphatic characteristics (41), was obtained from ICLC (Genova, Italy). These cell lines were routinely maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma Ltd., Poole, Dorset, UK) supplemented with 10% FCS, penicillin and streptomycin.

Materials. Monoclonal antibodies to anti-CD44 were obtained from Autogen Bioclear UK Ltd. (Exeter, UK) and anti-MMP-14 from Insight Biotechnology Ltd. (Middlesex, UK). DiI 1,1'-dioctadecyl-3,3,3'3'-tetra-methylindocarbocyaninine was purchased from Sigma Ltd. and stored in 5 mM stock solution (DMSO) at -20˚C. Human recombinant hepatocyte growth factor (HGF/SF) was a gift from Dr T. Nakamura, Osaka, Japan. Matrigel, a reconstituted basement membrane, was purchased from Becton-Dickinson (Bristol, UK); and G418 was from Invitrogen (Paisley, UK). All other materials were from Sigma Ltd. unless otherwise stated.

RT-PCR and DNA sequencing. RNA extraction was performed using an RNA extraction kit (Advanced Biotechnologies Ltd., UK). RT-PCR was performed using a reverse transcription kit (Advanced Biotechnologies Ltd.) according to the manufacturer's instruction. PCR was carried out using a thermal cycler (Gene Amp 9700; Perking-Elmer) and the following primers (synthesized by Invitrogen): CD44F1 forward primer (5'-3') ACCATGGACAAGTTTTGGTGG CA, and CD44R reverse primer (5'-3') ACCCCAATCTTC ATGTCCACATT [to amplify DNA fragments between 26-1134 of human CD44 (GenBank accession number M59040), and thus amplify all CD44 molecules that exist]; and MT1codeF1 forward primer (5'-3') ATGTCTCCCGCC CCAAG, versus MT1codeR1 reverse primer (5'-3') TCA GACCTTGTCCAGCAG, were used to produce MMP-14 PCR products. Amplification for CD44 PCR was carried out using the following conditions; denaturing at 94˚C for 5 min, followed by 36 cycles at 94˚C for 30 sec, 53.5˚C for 30 sec, 72˚C for 40 sec and a final extension period at 72˚C for 10 min. Amplification for MMP-14 was carried out using the following conditions; denaturing at 94˚C for 5 min, followed by 36 cycles at 94˚C for 45 sec, 58˚C for 45 sec, 72˚C for 80 sec and a final extension period at 72˚C for 10 min. All PCR products, including a 1000-bp ladder (Pharmacia Biotech, USA), were separated on 0.8% agarose gel, and visualised using ethidium bromide and a UV transilluminator (UVP, Inc., Cambridge, UK). Sequencing of PCR products was performed with an MWG Biotech (Milton Keynes, UK) and an Applied Biosystems automated sequencer system (model 377) using a BigDye DNA sequencing kit (Applied Biosystems).

TOPO TA cloning and electroporation. Cloning was performed using a TOPO TA Cloning® kit in accordance with the manufacturer's instructions. Briefly, the CD44 variant PCR product was first purified from agarose gel and mixed with the TOPO GFP plasmid, which carries a GFP reporter gene and neomycin resistance gene. After a short incubation, the plasmid was transformed into *E. coli* bacteria, incubated and plated onto ampicillin-containing agar plates. Bacterial colonies were screened for a plasmid incorporating the PCR product in the correct orientation. A colony was isolated and cultured to produce sufficient amounts of the plasmid, which was then extracted, purified, and subsequently electroporated into wild-type PC-3 cells using Equibio Easyject Plus electroporation equipment (Flowgen, Leicestershire, UK). Transfected cells were cultured in complete medium for 48 h before culture in medium containing the antibiotic G418 to select cells expressing the plasmid. A GFP expression-control plasmid was also electroporated into wild-type PC-3 cells and the transfected cells, selected using G418-containing media. Transfected cells that survived selection were cultured in media containing G418.

Plasmid digestion. Digestion of the plasmid to be used for transfection was performed by mixing 5 μl of plasmid with 1 μl of each buffered restriction enzyme (*Sna*B1 and *Eco*RV; New England Labs, USA), and incubated for 3 h in a 37˚C water bath. The products were run on 0.8% agarose gel and visualised using ethidium bromide with a UV transilluminator (UVP, Inc.).

Western blot analysis. Prostate cancer cell lines were treated with HGF/SF (40 ng/ml final concentration) for 35 min or left untreated prior to cells being harvested with a disposable

er. Cells were then pelleted at 1600 rpm prior to ion in cell lysis buffer (50 mM Tris, pH 7.8, 150 mM NaCl, 1% Triton, 0.1% SDS, 0.01% sodium azide, 0.05% sodium deoxycholate, and PMSF 100 μg/ml) at 4˚C for 1 h. Cellular debris was removed by centrifugation at 12,000 rpm, and the resulting extracted proteins were quantified using fluorescamine (Sigma Ltd.) and a fluorescent plate reader (Denley, Sussex, UK). After adding an electrophoresis sample buffer (50 mM Tris, 10% glycerol, 2% SDS, 0.1% bromophenol blue, and 5% ß-mercaptoethanol) and boiling for 5 min at 100˚C, equal amounts of protein were resolved on 8% SDS-PAGE and electroblotted onto a nitrocellulose membrane (Hybond; Amersham). Immunoblots were blocked in 10% skimmed milk for 60 min, then probed with anti-MMP-14 antibodies (in TBS buffer containing 3% milk protein and 0.1% Tween-20) for 1 h. Washing was performed with 3% skimmed milk/TBS/0.1% Tween-20 buffer, and primary antibodies were detected by peroxidase-conjugated immunoglobulins (1:1000) and an enhanced chemiluminescence system (KPL; Insight Biotechnologies).

Tumour cell-endothelial cell attachment assay. The assay used in this study was performed as described by Hiscox and Jiang (42). Briefly, tumour cells were first trypsinised to remove them from the culture flask, then centrifuged and resuspended in a 50 μg/ml solution of the fluorescent marker 1,1'-dioctadecyl-3,3,3'3'-tetra-methylindocarbocyaninine (DiI) for 60 min. DiI-labelled tumour cells were washed twice with DMEM (to remove unbound DiI), then seeded onto HGF/SF-treated and untreated (control) endothelial monolayers in 96-well plates at 30,000 cells/well. Tumour cells were cocultured with the endothelial cells for 35 min with either 50 μl of anti-CD44 mAbs (1:100) or 50 μl of DMEM/F12 media. Wells were washed twice with DMEM media, and 4% formaldehyde was added to each well to fix the cells. The plate was incubated at 4˚C overnight before the fixative was removed, then resuspended in 100 μl of 70% glycerol. The plates were then examined using a fluorescent microscope (Leica) under a x20 lens objective. Results are shown as the mean of triplicate counts.

In vitro invasion assay. The *in vitro* invasion assay used in this study was modified from the procedure described by Albini *et al* (43). Briefly, cell culture inserts (0.8 μm pore size; Becton-Dickinson) were placed into a 24-well plate (Nunclon). A total of 100 μl of pre-cooled Matrigel (250 μg/ml solution) was added to each pre-chilled cell culture insert, and the 24-well plate was placed in a 37˚C incubator overnight to allow the Matrigel to set. After incubation, the Matrigel was rehydrated by adding 100 μl of DMEM/F12 media and incubating for 1 h in a 37˚C/5% CO_2 incubator. Following this step, 50,000 human endothelial cells (HECV) were added to each insert, and the plate was incubated for a further 24 h. After incubation, the HECV endothelial cells formed a confluent monolayer completely covering the Matrigel. PC-3 cell suspensions were prepared and labelled with DiI as described in the tumour cell-endothelial cell attachment assay above, with 30,000 tumour cells added to each insert. Some 100 μl of media containing HGF/SF was added to half of the inserts and DMEM/F12 only was added to the other half. The inserts were further treated with the addition of 100 μl of anti-CD44 mAbs, 100 μl of HA (100 μg/ml) or 100 μl of DMEM/F12 media. The plate was returned to the 37˚C/5% CO_2 incubator for approximately 72 h. Endothelial cells and the Matrigel layer together with the non-invasive cells were removed from the inside of the insert using a cotton swab. The cells were fixed in 4% formaldehyde for 5 min at room temperature, and invading cells were identified and recorded using a fluorescent microscope (Leica). The average number of invading cells from five randomly selected fields/insert was determined using a x20 lens objective.

Migration assay. Tumour cell migration was measured by 'wounding' the cell monolayer and measuring the traversed distance travelled over time as described by Hiscox *et al* (44). Wild-type PC-3 cells and transfected variant CD44, and expression control cells were seeded at 80% confluence into the wells of a 24-well plate and allowed to grow to 100% confluence. A wound was made in the monolayer using a sterile pipette tip. The same area was recorded onto videotape under light microscopy (Leica) for >90 min using a time-lapse video recorder. The 'wound closure' was assessed using Optimas® 6 software (Optimas, UK) over 15-min time points. Cell motility was determined as the average velocity for five measurements for each time point.

Growth assay. Cell growth was assessed using the PicoGreen® dsDNA quantitation reagent (Molecular Probes, OR, USA), which uses an ultrasensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. Briefly, tumour cells were seeded into multiple 96-well plates and, over a period of 5 days, a plate was removed each day, the wells washed with BSS buffer and the cells fixed with 4% formalin. At the end of 5 days, the fixative was discarded from each plate, and the cells were resuspended in 80 μl of 0.1% Triton X-100 for 1 h. Pre-prepared PicoGreen dsDNA quantitation reagent (80 μl) was added to each well, and the fluorescence was detected using a standard spectrofluorometer with 485 nm excitation and 520 nm emission wavelengths.

Statistical analysis. Significance was determined using the Student's t-test and ANOVA analysis using Microsoft Excel with $p<0.05$ set as a significant difference.

Results

Expression of CD44 variants in prostate cancer cells. RT-PCR revealed multiple isoforms of CD44 expressed in PC-3, DU-145 and the two non-invasive cell lines (Fig. 1). All cell lines, with the exception of LNCaP, produced bands >1 Kb, which correspond to the standard and larger variant isoforms of CD44. However, a high molecular weight isoform of just over 2 Kb, identified only from the non-invasive cell lines, was completely absent in invasive PC-3, DU-145 and LNCaP. The other variants were similarly expressed in DU-145 and PC-3 cell lines.

Sequencing of variant CD44 PCR products. Direct DNA sequencing, performed on the major PCR product at

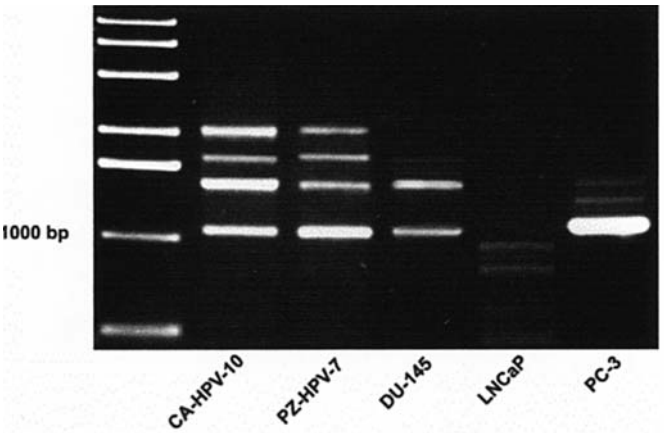

Figure 1. RT-PCR products revealed multiple isoforms of CD44 in prostate cell lines.

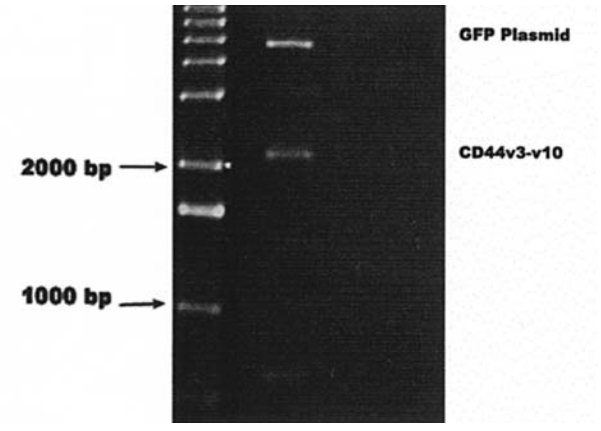

Figure 2. Plasmid digestion confirmed the presence of the full-length CD44 variant.

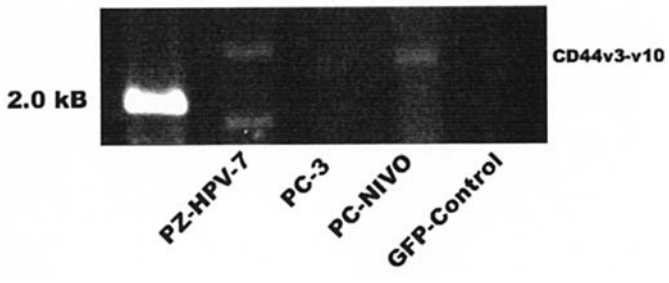

Figure 3. CD44 RT-PCR products of the transfected cells and wild-type PC-3 cells.

approximately 1 Kb, confirmed the identity of the CD44 standard (CD44s) isoform. The band above the standard isoform, strongly visible in DU-145, was identified as CD44E. The largest isoform of >2 Kb, present only in non-invasive cell lines, was sequenced and determined to be CD44v3-v10 isoform. Our data indicate that both DU-145 and PC-3 express variant isoforms of CD44 including CD44E, as well as CD44s at the mRNA level. The non-invasive cell lines expressed both the standard CD44s and CD44E, but also CD44v3-v10.

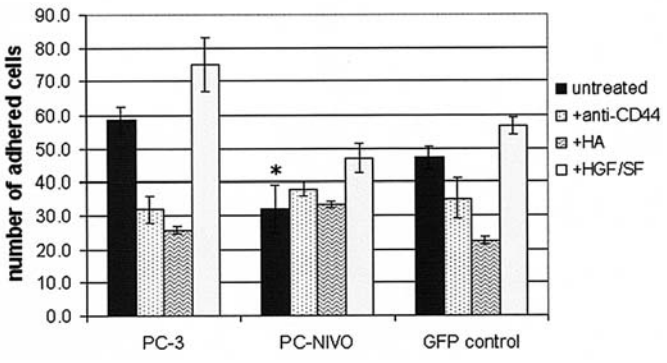

Figure 4. Effect of HGF/SF, anti-CD44 and hyaluronic acid on the cell adhesion to endothelium as seen in wild-type PC-3 cells, PC-NIVO cells and PC-3 cells transfected with control plasmid. *p<0.05.

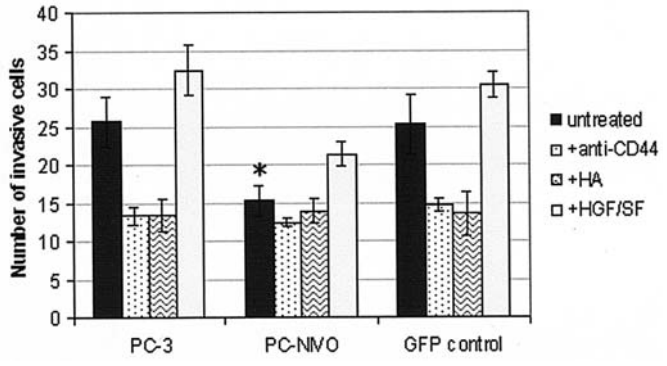

Figure 5. Effect of HGF/SF, anti-CD44 and hyaluronic acid on the cell invasion of endothelium as seen in wild-type PC-3 cells, PC-NIVO cells and PC-3 cells transfected with control plasmid. *p<0.05.

Cloning expression of CD44v3-v10. A large DNA fragment from Pz-HP-V10 was isolated from the agarose gel and cloned into a pcDNA3.1 GFP vector. Plasmid digestion was carried out to confirm the presence of the full-length CD44 variant isoform prior to its transfection into wild-type PC-3 cells (Fig. 2). After G418 selection, the RNA was extracted, reverse transcription performed and RT-PCR carried out to reveal the larger variant isoform (Fig. 3). After culture, the CD44v3-v10-transfected cells, now termed PC-NIVO, were selected for use in cell function assays.

Transfected tumour cells show reduced adhesion to endothelium. Results from a tumour-endothelial adhesion assay (Fig. 4) found PC-NIVO cells to be less adhesive to endothelial cells than wild-type PC-3 cells ($p<0.05$). The addition of hepatocyte growth factor/scatter factor (HGF/SF), a cytokine known to enhance cancer invasion and migration, resulted in increased adhesion in the wild-type PC-3 and PC-NIVO cells. The inclusion of anti-CD44 antibodies, or addition of an excess of hyaluronic acid (HA) in the medium, reduced adhesion to endothelial cells in the wild-type PC-3 ($p<0.05$) and in PC-3-transfected cells with control plasmid ($p<0.05$), but not in PC-NIVO.

Transfected cells have reduced invasiveness. Using a modified *in vitro* endothelial invasion assay (Fig. 5), we showed a

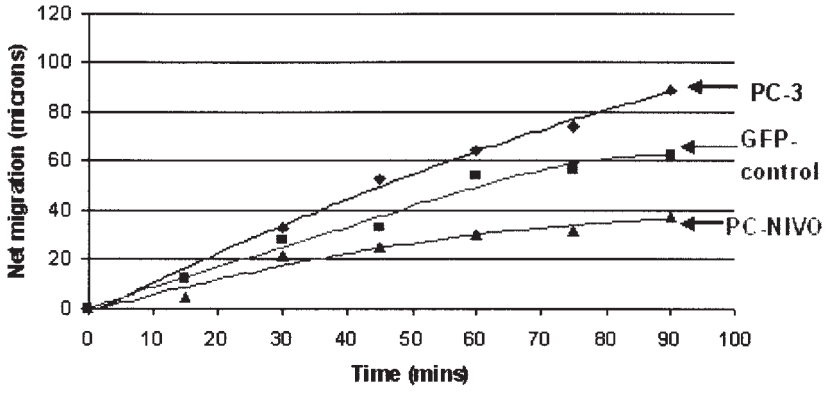

Figure 6. Migration rates of the transfected and wild-type PC-3 cells.

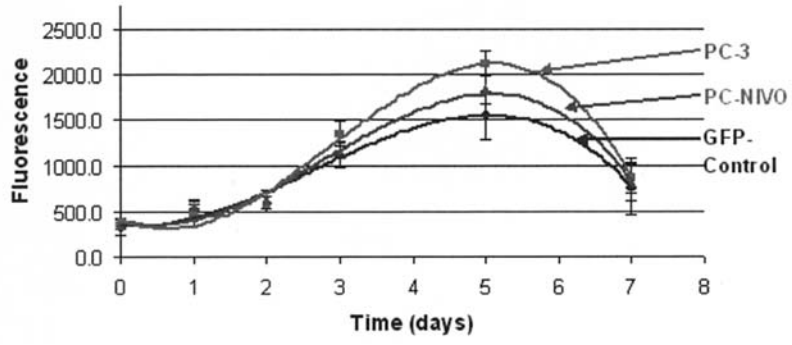

Figure 7. Growth rates of the transfected and wild-type PC-3 cells.

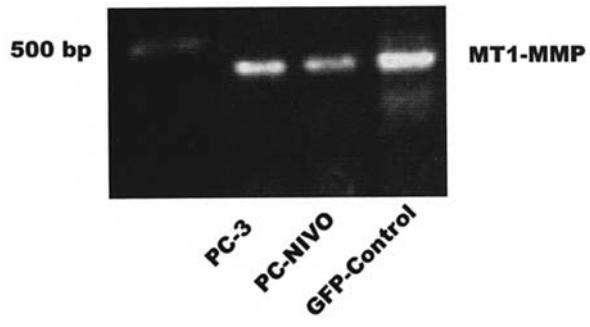

Figure 8. RT-PCR products of MMP-14 amplification in wild-type PC-3 cells and transfected cell lines.

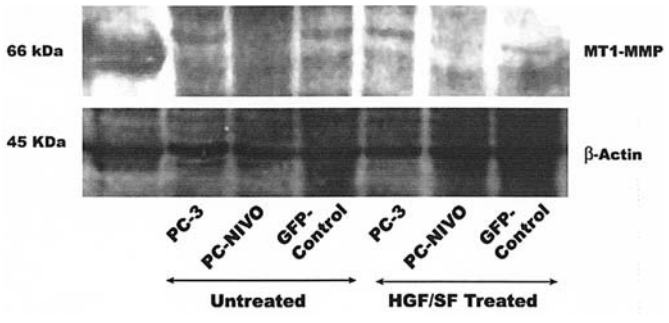

Figure 9. Western blot analysis revealed MMP-14 protein expression in wild-type PC-3 cells and control plasmid cells, but only weak expression in the CD44v3-v10-transfected cells (PC-NIVO).

significant reduction in the invasive potential of PC-NIVO compared to wild-type PC-3 cells ($p<0.05$) and PC-3-transfected cells with control plasmid ($p<0.05$). Furthermore, invasiveness was reduced to the same levels as in PC-NIVO by the inclusion of anti-CD44 antibodies or excess hyaluronic acid (HA) in PC-3 cells. Results from the invasion assay confirm a significant increase in the invasive potential of HGF-treated cells. More importantly, it was shown that the presence of anti-CD44 antibodies or excess hyaluronic acid was able to significantly reduce the number of invasive cells to similar levels whether they were HGF-activated or untreated.

Variant CD44-transfected cells did not significantly reduce motility. Analysis of the migration assay (Fig. 6) showed a reduction in the migration capacity of PC-NIVO compared to the wild-type PC-3 cells and PC-3-transfected cells with control plasmid. However, this was not found to be significant ($p=0.26$)

Cell proliferation of wild-type and transfected cells. Using the PicoGreen assay, growth rates were plotted over 5 days (Fig. 7). Statistical analysis (ANOVA) indicates that the transfected PC-3 cells (PC-NIVO) showed no significant difference in their growth rates compared with the wild-type PC-3 ($p=0.91$).

MMP-14 expression in wild-type and transfected cells. RT-PCR revealed equivalent levels of MMP-14 expression in the

wild-type PC-3 and transfected cells at the RNA level (Fig. 8). However, Western blot analysis revealed a reduction in MMP-14 in PC-NIVO cells, but not in wild-type PC-3 and PC-3-transfected cells with control plasmid (Fig. 9). This level of expression was unchanged after HGF/SF treatment.

Discussion

CD44 is a multifunctional cell surface adhesion molecule involved in cell-cell and cell-matrix interaction. The expression of CD44 variants (CD44v) on the surface of tumour cells appears to be associated with invasive and metastatic behaviour. Gunthert and colleagues were the first to show a CD44 variant protein as being responsible for metastatic behaviour in rat pancreatic carcinoma cells. They transfected non-metastatic cells with CD44v4-7 isoforms and were able to confer metastatic behaviour that could be blocked with monoclonal antibodies specific to CD44v6. The decreased expression of CD44s has been shown to be involved in the progression of prostatic cancer to a metastatic state (45). CD44 variant proteins have also been shown to be involved in tumour metastasis (46,47) and may not only promote tumour growth, but can also suppress it under certain conditions (48,49).

A previous study using prostate tumour cells has shown that the up-regulation of CD44 by HGF/SF treatment caused increased adhesion and invasiveness (50). In this study, we have shown that the expression of one CD44 isoform, CD44v3-v10, distinctly expressed by non-invasive cells, can cause a significant reduction in adhesion to endothelium and invasiness through a Matrigel matrix, compared to the controls, when expressed in the invasive PC-3 cell line. The more aggressive cancer cells did not show expression of these larger variant CD44 isoforms, with PC-3 cells showing only strong expression of CD44s. CD4v3-v10 is similar to Epican, a protein expressed by keratinocytes that is particularly important in epidermal differentiation (51) and a major heparin-binding proteoglycan in the human epidermis (52). The use of *in vitro* assays to investigate PC-NIVO cells demonstrated a significant reduction in cell adhesion and invasion compared to the wild-type and control plasmid cells. Data from the functional assays suggest that CD44v3-v10 can reduce cell adhesion and invasion to near background levels compared to the use of blocking anti-CD44 antibodies and the CD44 ligand, hyaluronic acid. However, the PC-NIVO-transfected cells showed increased cell adhesion and invasion after HGF/SF treatment to the same degree as wild-type PC-3 cells and control-plasmid PC-3 cells. Furthermore, CD44v3-10-expressing cells did not display a change in cell proliferation compared with the PC-3 cells, suggesting that the mechanism of metastatic suppression in PC-NIVO cells is unlikely to be that of reduced cell proliferation. In most tumours, CD44 acts as a metastasis-promoting molecule whereas CD44 can function as a metastasis suppressor in prostate carcinomas (48). This is the first study to demonstrate that expression of the full-length CD44v3-10 variant isoform can confer a non-metastatic phenotype on human prostate cancer cells, and that CD44 isoforms functionally contribute as tumour suppressors in prostate cancer cells.

Previous studies have demonstrated that CD44v3-expressing isoforms can bind MMPs and growth factors, and this may be the mechanism whereby this variant CD44 isoform functions (24,53). MMP-14 is a key enzyme in tumour cell invasion and metastasis (31,35). Indirect immunofluorescence found MMP-14 to be localised at the leading edge, and this would be appropriate for promoting cell invasion and migration. Expression of MMP-14 and MMP-2 in human prostate tissue has been shown to be associated with tumour progression (54). A reduction in MMP-14 expression would therefore reduce cellular invasion, and its reduction in PC-NIVO cells may explain the reduced adhesion and invasion seen in these experiments.

An alternative mechanism for the observed behaviour might be through signal transduction (55,56), with one study reporting the up-regulation of MMP2 synthesis by CD44 antibodies (57). It is clear that CD44v3-v10 influences pathways associated with metastatic behaviour, possibly those pathways associated with the regulation of MMP-14 expression. The exact mechanism through which CD44v3-v10 isoform is able to function as a metastasis suppressor is being investigated. If CD44v3 exon or other exons are involved in conferring this phenotype on PC-NIVO cells, the use of ribozyme knockout technology should confirm their role. The use of ribozyme-mediated knockout of the variant exon 3, which would prevent the expression of the CD44v3 gene and its normal regulatory elements, would not only be useful in identifying the exon or exons involved, but may increase their invasiveness by using these ribozymes on the non-invasive cell lines.

In conclusion, non-invasive prostate cancer cells express a high molecular weight CD44 isoform, CD44v3-v10, that may counteract the CD44 standard isoform function by reducing adhesion and invasion of endothelium by prostate tumour cells through negation of the MMP-14 function.

Acknowledgements

We would like to thank Cancer Research Wales (CRW) for its support.

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