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Institut für Pathologie

Arbeit unter der Leitung von Prof. Dr. med. G. Sauter

High Nr-CAM expression is associated with favorable phenotype and late PSA recurrence in prostate cancer treated by prostatectomy

Dissertation

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Abkürzungsverzeichnis

CAM	cell adhesion molecule
CHL	Close Homolog to L1 Protein
EGFR	epidermal Growth Receptor
ICH	Immunhistochemie
NgCAM	Neuroglia cell adhesion molecule
NrCAM	Neuron glia-related cell adhesion molecule
PCa	Prostatakarzinom
PSA	Prostataspezifisches Antigen
TMA	tissue microarray
TNM	Tumor-,Nodulus,-Metastase-Klassifikation
VEGF	Vascular Endothelial Growth Factor

1. Nr-CAM (Publikation-Originalarbeit)

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2. Zusammenfassung: Nr-CAM beim Prostatakarzinom

2.1. Einleitung

Das Prostatakarzinom ist mit 25,4% die häufigste Krebserkrankung des Mannes in Deutschland (Robert-Koch Institut 2008). Jährlich erkranken ca. 58.000 Männer neu an dieser Erkrankung (Robert-Koch Institut 2008). Dia Anzahl der diagnostizierten Neuerkrankungen in Deutschland steigt (Robert Koch Institut 2007). Verantwortlich dafür ist einerseits die Einführung des PSA Tests als Screening Methode und andererseits die gestiegene Lebenserwartung von Männern in den Industrieländern (Greenlee et al. 2000; Wetterauer 2002). Ca. 11.000 Männer sterben jährlich in Deutschland an einem Prostatakarzinom (Robert-Koch Institut 2008). Die meisten Prostatakarzinome zeigen ein indolentes biologisches Verhalten, jedoch gibt es auch viele Prostatakarzinome mit einem aggressiven biologischen Verhalten. Um eine Übertherapie zu vermeiden, ist es wichtig die biologische Aggressivität eines Prostatakarzinoms zum Zeitpunkt der Diagnose einschätzen zu können und somit eine richtige therapeutische Entscheidung treffen zu können. Etablierte Prognosefaktoren sind bislang der Gleason Grad und die Tumorausdehnung in den Stanzbiopsien, das klinische Stadium und das präoperative PSA. (Bostwick et al. 2003). Es bleibt zu hoffen, dass die zunehmenden Kenntnisse über die molekulare Biologie des Prostatakarzinoms zu besseren Prognosefaktoren führen. Ein für das Prostatakarzinom interessantes Protein ist Nr-CAM (neuron-glia-related cell adhesion molecule).

Nr-CAM ist ein Zelladhäsionsmolekül, welches zur Immunglobulin Superfamilie (Ig) gehört (Grumet 1997). Zusammen mit L1, CHL1 und Neurofascin bildet es die L1 Familie der IgCAMs (Falk et al. 2004). Nr-CAM spielt eine wichtige Rolle bei der Entwicklung von Nerven (Grumet et al. 1991; Glienke et al. 2000). Immer mehr Daten zeigen, dass Nr-CAM auch in nicht-neuronalen Geweben eine Rolle spielt. In verschiedenen Tumortypen, wie zum Beispiel beim Pankreaskarzinom, Melanom, Kolonkarzinom, und papillären Schülddrüsenkarzinom wurde eine Expression von Nr-CAM gefunden (Dhodapkar et al. 2001; Conacci-Sorrell et al. 2002; Gorka et al. 2007). Darüber hinaus existieren Berichte, welche eine besonders hohe Nr-CAM Expression in manchen Tumorarten mit einer ungünstigen Prognose assoziieren (Sehgal et al. 1998; Gorka et al. 2007). Über die Expression von Nr-CAM bei normalem Prostataepithel und in Prostatakarzinomen ist bisher wenig bekannt. Normales Prostataepithel wurde in einer Studie als ein Gewebe mit besonders geringer Expression von Nr-CAM beschrieben (Sehgal et al. 1998). Eine herabgesetzte Expression von Nr-CAM wurde ebenfalls in langsam wachsenden Prostatakarzinomen von Mäusen beobachtet (Jennbacken et al. 2009).

In der vorliegenden Studie verwendete ich einen vorbestehenden Prostatakarzinom Tissue Microarray (TMA), bestehend aus Gewebeproben von mehr als 3000 Patienten, um die klinische Bedeutung der veränderten Nr-CAM Expression beim Prostatakarzinom zu überprüfen.

2.2. Material und Methoden

Patienten und Gewebeproben

Das Patientenkollektiv ist in der Publikation dieser Doktorarbeit detailliert beschrieben (Erbersdobler et al. 2002). Mithilfe von einem Tissue Microarray (TMA) konnten gleichzeitig 3.261 Geweproben von 0.6mm Durchmesser auf das Vorhandensein von Nr-CAM untersucht werden. Bei der Herstellung von TMAs werden mit einer Nadel Gewebezylinder aus ausgewählten Tumorgewebeblöcken in Paraffin entnommen und anschließend in ein Loch in einen leeren Paraffinblock eingefügt. Die 3.261 Proben wurden dabei auf 7 TMA Blöcke verteilt. Jeder TMA Block enthält zwischen 129-522 Tumorproben.

Immunhistochemie (IHC)

Die Immunhistochemie macht es möglich spezifische Proteine durch das Verwenden von Antikörpern auf einem Gewebeschnitt nachweisen zu können. Unter geeigneten Umständen werden die primären Antikörper auf den Gewebeschnitt gegeben. In einem weiteren Schritt erfolgt das Visualisieren der gebunden Antikörper mit Hilfe eines sekundären Detektionssystems. Die an ein Visualisierungssystem gekoppelten Antikörper können lichtmikroskopisch - was in unserer Studie der Fall war - oder auch fluoreszenzmikroskopisch dargestellt werden. Wir verwendeten einen Antikörper gegen Nr-CAM, der mit dem Visualisierungssystem DAKO (EnVision) sichtbar gemacht wurde. Die Intensität der Färbung (0, 1+,2+,3+) und eine Abschätzung des Prozentsatzes der gefärbten Tumorzellen wurde für jede Gewebeprobe registriert und in einem kombinierten Score verwendet.

Statistik

Für unsere Untersuchungen haben wir das Statistik-Programm JMP (SAS Istitute, Cary, NC) verwendet. Die Beziehungen zwischen den verschiedenen klinischen Parametern und der Nr-CAM Expression wurden mittels Kreuztabelle über Chi-quadrat-Tests (Likelihood) ausgewertet. Die PSA-Rezidivkurven wurden durch Kaplan-Meier-Kurven dargestellt.

2.3. Resultate

2.883 (88,4%) der 3.261 Gewebeproben waren für die Bestimmung der Nr-CAM Expression auswertbar. Die restlichen 378 Fälle (11,6%) konnten entweder wegen komplett fehlender Gewebeproben oder wegen des Fehlens eindeutiger Tumorzellen in dem Gewebespot nicht ausgewertet werden. Die Ergebnisse des Projektes sind in der beiliegenden Publikation zusammengefasst und in Tabellen und Abbildungen dargestellt. Die wesentlichen Punkte der Arbeit waren:

1. Die Nr-CAM Expression war in der Regel in Prostatakarzinomen höher, als in normalem Prostataepithel.

2. Eine membranöse Nr-CAM Expression fand sich in 1.418 (49.2%) der 2.883 analysierbaren Fälle.

3. Eine besonders hohe Nr-CAM Expression war signifikant mit einem günstigen Gleason Grad (p=0.0003) und einem niedrigen pathologischen Tumorstadium (p=0.0015) assoziiert.

4. Eine geringe Nr-CAM Expression war mit einem erhöhten Risiko für ein frühes biochemisches Rezidiv assoziiert (p=<0.0001).

2.4. Diskussion

Diese Studie untersuchte die Häufigkeit und die potentiell klinische und pathologische Bedeutung der Nr-CAM Expression beim Prostatakarzinom. Die Daten zeigen, dass die Nr-CAM Expression beim Prostatakarzinom häufig vorkommt und mit einem günstigen Tumorphänotyp und einem verlängerten biochemischen Rezidiv-freiem Intervall assoziiert ist.

Der immunhistochemische Vergleich von normalem Prostataepithel und Prostatakarzinomen, zeigte, dass fast 50% der Prostatakarzinome Nr-CAM während der Tumorentwicklung und/oder Progression hochregulieren. Es ist denkbar, dass das Vorhandensein von hohen Mengen eines Zelladhäsionsmoleküls, wie zum Beispiel Nr-CAM mit einer reduzierten Fähigkeit der Zellen für Invasion, Migration und Metastasierung einhergeht. Dafür, dass ein Zelladhäsionsmolekül im Vergleich zu nicht neoplastischem Gewebe in malignem Gewebe hochreguliert sein kann und dass dies zu einem „günstigeren“ Tumorphänotyp führt, gibt es mindestens zwei denkbare Erklärungen. Einerseits könnte Nr-CAM bei Zellen, die eine oder mehrere bestimmte tumorassoziierte zelluläre Alterationen aufweisen, als protektiver Mechanismus aktiviert werden. Wenig differenzierte Prostatakarzinome würden diese Fähigkeit verlieren was zu einem aggressiveren Verhalten dieser Prostatakarzinome mit beitragen würde. Weiter wäre es auch denkbar, dass Nr-CAM keine spezifische Funktion in der Biologie des Prostatakarzinoms hat. Die unerwartete Überexpression eines Zelladhäsionsmoleküls in manchen Prostatakarzinomen könnte einfach nur einen „bystander Effekt“ repräsentieren. Expressionsstudien zeigen, dass tausende von Genen in Karzinomzellen dysreguliert sind (Lockhart et al. 2000; Perou et al. 1999; Alon et al. 1999).

Dementsprechend wäre die erhöhte Expression von Nr-CAM ein unspezifisches Ereignis, welches bevorzugt in klinisch weniger aggressiven Prostatakarzinomen vorkommt.

Daten über die Nr-CAM Expression in Prostatakarzinomen waren bisher nicht bekannt. Ähnliche Beobachtungen wie die unseren sind jedoch in Pankreaskarzinomen beschrieben worden. Dhodapkar et al (Dhodapkar et al. 2001) haben eine erhöhte Nr-CAM Expression in wenig und mäßig differenzierten Karzinomen gefunden, während die meisten wenig differenzierten Läsionen keine Nr-CAM Expression zeigten. Im Gegensatz dazu haben andere Autoren eine Assoziation zwischen hoher Nr-CAM Expression und schlechtem Tumorphänotyp oder schlechtem Überleben gefunden (Sehgal et al. 1998; Gorka et al. 2007; Lukashova-v Zangen et al. 2007).

Diese unterschiedlichen Daten sprechen für Gewebe- oder Zelltyp abhängige Wirkungsmechanismen von Nr-CAM. Möglicherweise spielen dabei variable Interaktionen mit anderen Molekülen eine Rolle (Gibson et al. 2011; Grumet 1997). Denburg et al (Denburg et al. 1995) beschreiben das Vorkommen von unterschiedlichen Konformationen dieses Zelladhäsionsmoleküls als mögliche Ursache.

Das vermehrte Vorkommen von Nr-CAM in Prostatakarzinomen ist auch deshalb interessant, weil Nr-CAM ein potentielles Therapieziel darstellt. Sehgal et al (Sehgal et al. 1999) konnten mit Antisense Nr-CAM bespielsweise eine Reduktion der Nr-CAM Expression sowie Reduktion der Proliferation von Nr-CAM positiven Glioblastomzellen in Mäusen erzielen.

Zusammenfassend zeigen unsere Daten, dass Nr-CAM häufig bei Prostatakarzinomen exprimiert ist. Eine hohe Expression von Nr-CAM ist mit

einem guten Phänotyp und einem verlängerten PSA Rezidiv-freiem Intervall assoziiert. Es bleibt zu hoffen, dass die Untersuchungen von Nr-CAM in Kombination mit anderen molekularen Markern in Zukunft zur besseren Einordnung der biologischen Aggressivität eines Prostatakarzinoms zum Zeitpunkt der Diagnose führen können.

2.5. Zusammenfassung

Das Prostatakarzinom ist der häufigste Tumor des Mannes in den wesentlichen Ländern. Um eine Übertherapie zu vermeiden, ist es von großer Bedeutung neben den bereits etablierten Prognoseparametern, Faktoren zu finden, um die biologische Aggressivität eines Prostatakarzinoms zum Zeitpunkt der Diagnose besser einschätzen zu können. Ziel dieser Studie war es, die klinische Relevanz der Nr-CAM Expession beim Prostatakarzinom zu untersuchen. Ein bereits bestehender Tissue Microarray (TMA), mit über 3.000 Proben radikaler Prostatatektomien wurde dazu verwendet. Insgesamt waren 88.4% der Fälle auswertbar wobei 1.418 (49.2%) der Tumoren eine membranöse Nr-CAM Färbung zeigten. Eine hohe Nr-CAM Expression war mit einem günstigen Tumorphänotyp und verlängertem PSA Rezidiv-freiem Intervall assoziiert. Die häufige Präsenz von hohen Mengen an Nr-CAM als auch die Assoziation zu klinisch-pathologischen Parametern machen Nr-CAM sowohl zu einem interessanten Prognoseparameter, als auch zu einem interessanten Therapieziel.

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3. Erklärung des Eigenanteils an der Publikation

- Erweiterung der bestehenden Tissue Microarrays
- Auswertung der gefärbten Präparate zusammen mit einem Pathologen Literaturrecherche
- Schreiben der Publikation zusammen mit Dr. Maria Christina Tsourlakis, Prof. G. Sauter und seinem Team
- Schreiben der Zusammenfassung

4. Danksagung

Zunächst möchte ich mich bei Prof. Dr. med. G. Sauter für das interessante Thema meiner Doktorarbeit und seine gute Betreuung bedanken!

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Liebe Tina, danke für Deine liebevolle Unterstützung!

6. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

7. Anhang

Prostate Cancer and Prostatic Disease (2013) 16, 159–164

www.nature.com/pcan

ORIGINAL ARTICLE

High Nr-CAM expression is associated with favorable phenotype and late PSA recurrence in prostate cancer treated by prostatectomy

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BACKGROUND: Neuron-glia-related cell-adhesion molecule (Nr-CAM) is another potential membrane-bound target molecule for specific prostate cancer therapy. The role of Nr-CAM in normal and neoplastic prostate tissue has not been extensively studied. The aim of our study is to evaluate the prevalence of Nr-CAM expression in prostate cancer and to explore its association with phenotype and clinical disease course.
METHODS: A preexisting tissue microarray including more than 3000 prostate cancers that underwent prostatectomy at our center with clinical follow-up data was used. The tissue microarray (TMA) was immunhistochemically stained for Nr-CAM.
RESULTS: A total of 2883 (88.4%) of tumor samples were interpretable in our TMA analysis. Membranous Nr-CAM staining was seen in 1418 (49.2%) of 2883 analyzable cases. According to predefined criteria, staining was considered weak in 778 (27.0%), moderate in 412 (14.3%) and strong in 228 (7.9%) cancers. Significant associations were found with pathological tumor stage ($P = 0.0015$), Gleason grade ($P = 0.0003$), nodal stage ($P = 0.0061$), preoperative PSA ($P = 0.0138$) and prolonged PSA recurrence-free survival ($P < 0.0001$).
CONCLUSIONS: Nr-CAM expression is frequent in prostate cancer. High level of Nr-CAM expression is associated with favorable tumor phenotype and reduced risk of PSA recurrence. The abundant presence of Nr-CAM in prostate cancer epithelium makes Nr-CAM a potential target of therapy.

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Keywords: Nr-CAM; tissue microarray; prognosis

INTRODUCTION

Worldwide more than 220 000 men die annually of prostate cancer, usually as a result of metastases.[1] Today, androgen ablation (hormonal therapy) is the first-line therapy for metastatic prostate cancer, resulting in a transient reduction of symptoms and tumor progression until these cancers become androgen-independent and rapidly progressive. Androgen-independent prostate cancer is only moderately sensitive to chemotherapy.

The striking success of new targeted drugs raise the hope that prostate cancer patients might eventually also benefit from such an approach.[2] However, the few clinical trials in prostate cancer using drugs targeting epidermal growth factor receptor,[3,4] vascular endothelial growth factor,[5] prostate stem cell antigen[6] and HER2[7–9] were discouraging so far. Neuron-glia-related cell-adhesion molecule (Nr-CAM) is another potential membrane-bound target molecule for specific prostate cancer therapy. Nr-CAM belongs to the immunglobulin (Ig) superfamily.[10] Together with L1, CHL1 and neurofascin, it builds the L1 family of IgCAMs.[11] Nr-CAM acts as an adhesion molecule and has a role in axonal guidance and growth.[12] Accumulating data suggest that Nr-CAM has also relevance in non-neuronal tissues. It was suggested that Nr-CAM expression in endothelium may have a role in mediating capillary outgrowth.[13] Nr-CAM is also expressed in a variety of neoplastic tissues and cancer-derived cell lines, including pancreatic cancer,[14] melanoma,[15] colon carcinoma,[15] glioblastoma multiforme,[16] astrocytoma,[16] glioma[16] and papillary thyroid carcinoma.[17] For some cancer types, an association between Nr-CAM expression and unfavorable prognosis/phenotype was suggested, including cerebral and thyroid cancers.[16,17] Functional data also support a role of Nr-Cam in tumor biology. In melanoma cell lines, an association of high Nr-CAM expression with increased tumorigenicity was shown in one study.[15] The role of Nr-CAM in normal and neoplastic prostate epithelium has not been extensively studied. Normal prostate epithelium was described as a tissue with a relatively low expression level in one study.[16] A reduced expression of Nr-CAM has also been described in slow-growing prostate cancer in mice.[18]

The aim of our study is to evaluate the prevalence of Nr-CAM expression in prostate cancer and to explore its association with phenotype and clinical disease course. The analysis of a preexisting tissue microarray (TMA) including more than 3000 prostate cancers with clinical follow-up data demonstrated that Nr-CAM expression is frequent in prostate cancer, and that high level of Nr-CAM expression is associated with favorable tumor phenotype and reduced risk of PSA recurrence.

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MATERIALS AND METHODS

Patients

Radical prostatectomy specimens were available from 3261 patients, consecutively treated at the Department of Urology, University Medical Center Hamburg-Eppendorf between 1992 and 2005 (Table 1). Follow-up data were available for 2891 patients, ranging from 0.03 to 219 months (mean, 72.1 months). None of the patients received neo-adjuvant or adjuvant therapy. Additional (salvage) therapy was only initiated after a biochemical relapse, the clinical endpoint of this study. In all patients, PSA values were measured quarterly in the first year, followed by biannual measurements in the second and annual measurements after the third year following surgery. Recurrence was defined as a postoperative PSA of 0.1 ng ml^{-1} and rising. The first PSA values above or equal to 0.2 ng ml^{-1} were used to define the time of recurrence. Patients without evidence of tumor recurrence were censored at last follow-up. All prostatectomy specimens were analyzed according to a standard procedure. All prostates were completely paraffin-embedded, including whole-mount sections as previously described.[19] One 0.6-mm tissue core was punched out from the diagnostically relevant cancer area of each case and transferred in a TMA format as described.[20] The 3261 cores were distributed among seven TMA blocks each containing 129–522 tumor samples. Each TMA block also contained various control tissues, including normal prostate tissue and other normal tissues. The utilization of tissues and clinical data was approved by our local Ethical Committee.

Table 1. Clinicopathological features of 3261 arrayed prostate cancers

Characteristics	*No. on TMA* n = *3261*	*No. (%) with complete follow-up* n = *2891*
Follow-up (months)		
Mean		72.1
Median		68.9
Range		0.03–219
Age (years)		
<50	83	78 (94.0)
50–60	998	912 (91.4)
60–70	1807	1699 (94.0)
>70	175	169 (96.6)
Preteatment PSA (ng ml^{-1})		
<4	513	478 (93.2)
4–10	1673	1544 (92.3)
10–20	641	608 (94.9)
>20	225	212 (94.2)
pT category (AJCC 2002)		
pT2	2080	1907 (91.7)
pT3a	609	579 (95.1)
pT3b	372	361 (97.0)
pT4	42	42 (100.0)
Gleason grade		
⩽3+3	1426	1307 (91.7)
3+4	1311	1238 (94.4)
4+3	313	297 (94.9)
⩾4+4	55	49 (89.1)
pN category		
pN0	1544	1492 (96.6)
pN+	96	93 (96.9)
pNx	1457	1298 (89.1)
Surgical margin		
Negative	2475	2295 (92.7)
Positive	627	594 (94.7)

Abbreviations: AJCC, American Joint Committee on Cancer; TMA, tissue microarray.
Patients with PSA recurrence: $n = 728$ (25.2%), median PSA recurrence: 29.0 months. Numbers do not always add up to 3261 in the different categories because of cases with missing data.

Immunhistochemistry

Freshly cut TMA sections were stained in 1 day during a single experiment. High-temperature pretreatment of slides was done in an autoclave in citrate buffer, pH 9. Nr-CAM immunostaining was performed using a commercial polyclonal antibody (goat antihuman Nr-CAM antibody; AF2034, R & D Systems, Minneapolis, MN, USA). The specifity to the antibody was determined by the manufacturer. Normal pancreatic tissue was used as a positive control as previously described.[14] Lymphoid tissue was used as a negative control. The Envision system (DAKO, Glostrup, Denmark) was used to visualize the immunostaining. Only membranous staining was evaluated because cytoplasmatic staining—if present—was usually linked to a particularly strong membranous staining. The staining intensity (0, 1+, 2+ and 3+) and the fraction of positive tumor cells were recorded for each tissue spot. A final score was built from these two parameters according to the following: negative scores had staining intensity of 0, weak ones had a staining intensity of 1+ in ⩽70% of tumor cells or 2+ in ⩽30% of tumor cells; moderate had staining intensity of 1+ in >70% of tumor cells, staining intensity of 2+ in >30% and ⩽70% of tumor cells or staining intensity of 3+ in ⩽30% of tumor cells and strong scores had staining intensity of 2+ in >70% of tumor cells or staining intensity of 3+ in >30% of tumor cells.

Statistics

Statistical calculations were performed using JMP statistical software (SAS Institute, Cary, NC, USA). Contingency tables were calculated with the χ^2 test to analyze the relationship between categorical parameters. Survival curves were calculated by the Kaplan–Meier method and compared with the log-rank test.

RESULTS

Technical issues

A total of 2883 (88.4%) of tumor samples were interpretable in our TMA analysis. Reasons for non-informative cases (378 spots; 11.6%) included lack of tissue samples or absence of unequivocal cancer tissue in the TMA spot.

Immunohistochemistry

Nr-CAM staining always showed strong membrane predominance in our tissues. Although some cytoplasmatic staining was sometimes seen, this was mostly associated with a much higher staining level on the membranes. Examples of Nr-CAM-positive and -negative cancers are shown in Figure 1a and b. Membranous Nr-CAM staining was seen in 1418 (49.2%) of 2883 analyzable cases. According to our predefined criteria, staining was considered weak in 778 (27.0%), moderate in 412 (14.3%) and strong in 228 (7.9%) cancers. Low or no expression of Nr-CAM was observed in benign prostate epithelium compared with many invasive cancers (Figure 1c and d).

The relationship between Nr-CAM immunostaining and tumor phenotype is given in Table 2. In general, positive Nr-CAM expression was associated with favorable tumor phenotype. Significant associations were found with pathological tumor stage ($P = 0.0015$), Gleason grade ($P = 0.0003$), nodal stage ($P = 0.0061$), preoperative PSA ($P = 0.0138$) and prolonged PSA recurrence-free survival ($P < 0.0001$, Figure 2a). This held true if all tumors were analyzed but also for the subgroup of Gleason ⩽3+4 cancer (Figure 2b). The association was lost in high-grade (Gleason ⩾4+3) cancers (Figure 2c). The massive association between Gleason grade and PSA recurrence is also given as a reference for the quality of clinical follow-up data (Figure 2d).

DISCUSSION

The results of this study show that many prostate cancers show elevated Nr-CAM expression as compared with normal epithelium and that such high Nr-CAM expression is associated with favorable tumor phenotype and prolonged PSA recurrence-free survival in prostate cancer patients.

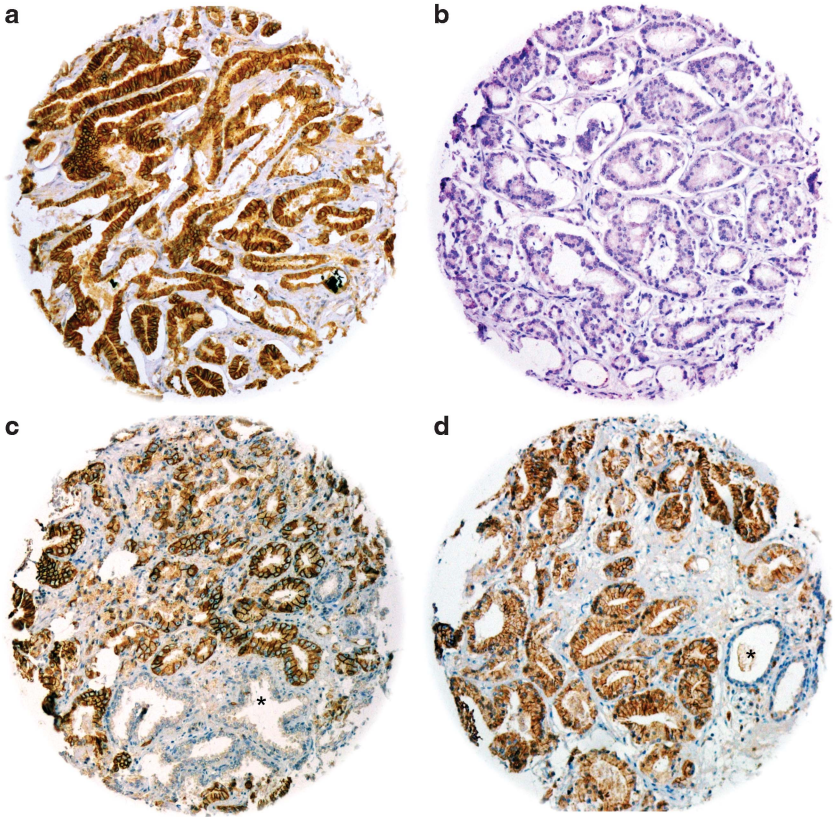

Figure 1. Representative images of neuron-glia-related cell-adhesion molecule (Nr-CAM) immunostainings. (**a**) Positive staining in prostate cancer, (**b**) negative staining in prostate cancer and (**c, d**) intense positive staining in prostate cancer as compared with the lack of staining in non-neoplastic prostate epithelium (marked by an asterix).

Table 2. Associations between Nr-CAM expression and tumor phenotype

				Nr-CAM expression				
	Variable	n *on TMA*	n *Analyzable*	*Negative %*	*Weak %*	*Moderate %*	*Strong %*	P-*value*
	All cancers	3261[a]	2883	50.8	27.0	14.3	7.9	
pT category	pT2	2080	1812	48.2	28.2	15.7	7.9	0.0015
	pT3a	609	555	52.3	26.8	12.1	8.8	
	pT3b	372	339	60.8	21.5	11.2	6.5	
	pT4	42	38	63.2	26.3	5.2	5.3	
Gleason grade	$\leqslant 3+3$	1426	1224	46.1	28.6	16.5	8.8	0.0003
	$3+4$	1311	1190	52.9	26.9	13.1	7.1	
	$4+3$	313	282	60.7	22.3	9.2	7.8	
	$\geqslant 4+4$	55	50	60.0	18.0	16.0	6.0	
pN category	pN0	1544	1382	51.8	26.2	13.0	9.0	0.0061
	pN1	96	89	67.4	23.6	4.5	4.5	
Preoperative	<4	513	439	46.9	26.4	17.3	9.4	0.0138
PSA (ng ml^{-1})	4–10	1673	1475	49.4	27.5	15.2	7.9	
	10–20	641	583	56.1	26.1	11.5	6.3	
	>20	225	202	54.5	27.7	8.9	8.9	

Abbreviations: Nr-CAM, neuron-glia-related cell-adhesion molecule; TMA, tissue microarray.
[a]Numbers do not always add up to 3261 in the different categories because of cases with missing data.

Our immunohistochemical comparison of normal prostate epithelium and prostate cancer demonstrates that almost 50% of prostate cancers upregulate membranous Nr-CAM protein during tumor development and/or progression. It is conceivable that high availability of a cell-adhesion molecule such as Nr-CAM goes along with decreased capabilities of cells for invasion,

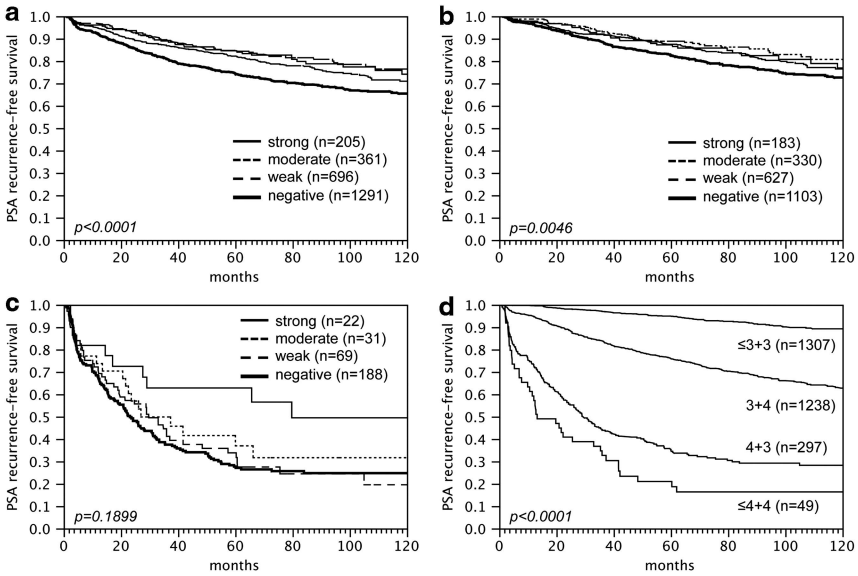

Figure 2. Association between neuron-glia-related cell-adhesion molecule (Nr-CAM) expression and patient prognosis (endpoint: biochemical (PSA) recurrence). (**a**) All cancers, (**b**) subset of cancers with Gleason $\leqslant 3+4$, (**c**) subset of cancers with Gleason $\geqslant 4+3$ and (**d**) association between Gleason grade and patient prognosis.

migration and metastasis. However, the observation of a cell-adhesion molecule being upregulated in cancer as compared with normal prostate epithelium and at the same time conferring a favorable prognosis to affected tumors is counterintuitive. There are at least two different conceptual explanations for such a scenario. First, it is possible that Nr-CAM may represent a protective mechanism in cells with a cancer-prone metabolic alteration. In such a case, Nr-CAM protein expression would be activated in case of cellular alterations that are typically associated with cancer. Poorly differentiated prostate cancers may be more likely to loose the functionality of such a mechanism, which could then contribute to the more aggressive behavior of these neoplasms. Alternatively, our Nr-CAM data are also consistent with a complete lack of a specific functional role of Nr-CAM overexpression in prostate cancer biology. The unexpected overexpression of a cell-adhesion molecule in some prostate cancer 'with the purpose of making the cancer more benign' may just represent a simple 'bystander effect'. It is well known from expression screening studies that hundreds or thousands of genes are dysregulated in cancer cells.[21–23] Accordingly, high levels of Nr-CAM expression might represent an unspecific event in a clinically less-aggressive molecular prostate cancer subgroup that has no direct effects on a cells cancer properties.

Data from other groups on Nr-CAM expression in prostate cancer are currently lacking, but similar observations have been described for pancreatic carcinoma.[14] Dhodapkar *et al*[14] found increased Nr-CAM expression in areas of well and moderately differentiated carcinoma (54%), while most poorly differentiated lesions did not exhibit Nr-CAM surface expression. In the same study, a lack of Nr-CAM expression is described in three of five patients with poorly differentiated tumors who presented with lymph node metastasis not only in the poorly differentiated but also in the well-differentiated areas.[14]

However, other authors reported a worse tumor phenotype and unfavorable clinical outcome to be associated with high Nr-CAM protein levels in several other tumor entities.[16,17,24] For example, Sehgal *et al*.[16] found significantly more Nr-CAM expression in astrocytoma IV and glioblastoma cell lines as compared with cell lines from astrocytoma III, and concluded that overexpression of Nr-CAM is a late event in this tumor entity. Gorka *et al*.[17] found higher amounts of Nr-CAM mRNA in pT3/pT4N1 papillary thyroid carcinomas than in low stage tumors, but these differences were not statistically significant. In the same study, the authors could not find any association between Nr-CAM expression and regional lymph node metastasis. Overexpression of Nr-CAM in ependymomas was associated with poor outcome.[24]

These controversial data argue for different mechanisms of action for Nr-CAM in different cell types/tissues. It is possible that these variations may be owing to variable interactions with other molecules as suggested by Gibson *et al*.[25] Denburg *et al*[26] suggest that the existence of multiple conformation states of this cell-adhesion molecule might be the reason for its different functions.

In this study, a small but significant prognostic effect of Nr-CAM expression was demonstrated using a TMA approach. This observation further corroborates our approach of utilizing just one 0.6 mm tissue spot per cancer in prostate cancer TMA studies to identify prognostic molecular features. While it is possible or even likely that our approach of analyzing one TMA spot per cancer will have missed several cancers with heterogeneous Nr-CAM positivity, it appears unlikely that a better identification of a few additional Nr-CAM-positive cancers would have lead to a markedly different outcome of our analysis. The TMA used for this project was earlier successfully used to identify a prognostic relevance of multiple biomarkers on the protein and DNA level.[27–36] Some investigators had proposed that multiple tissue spots per prostate cancer would be advantageous for the identification of clinically relevant biomarkers.[37–44] However, we recently showed for several biomarkers that the number of cores per tumor has no or little impact on study results in the case of large-scale TMAs.[45]

The abundant presence of Nr-CAM in prostate cancer epithelium makes Nr-CAM a potential target of therapy. This notion is supported by experiments by Sehgal *et al*.[46] showing that antisense Nr-CAM causes a reduction in the native Nr-CAM expression and proliferation of Nr-CAM-positive glioblastoma cells in mice.

In summary, our data show that Nr-CAM is abundantly expressed in prostate cancer. High levels of Nr-CAM expression are linked to favorable prostate cancer phenotype and characterize a subset of tumors with a prolonged PSA recurrence-free interval. It can be hoped that biomarkers such as

Nr-CAM expression levels in combination with other molecular features will substantially improve the individual prediction of prostate cancer aggressiveness.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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