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## Original Paper

# Expression of CD44 Isoforms in Human Skin Cancer

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**In animal models, isoforms of CD44 (CD44v) containing sequences encoded by one or several of ten different exons (v1–v10) contribute to tumour metastasis. In certain human cancers, CD44v6 expression is associated with poor prognosis. This paper examines CD44v expression in skin carcinogenesis and skin cancer metastasis. CD44v expression was studied in basal cell carcinoma (BCC), squamous cell carcinoma (SCC), primary malignant melanoma (PMM), metastases of MM (MMM), benign melanocytic naevi (BMN) and normal skin (NS) by immunohistochemistry and reverse transcript polymerase chain reaction (RT-PCR). BCC, SCC and NS expressed several CD44v, including v6, albeit in different distributions and intensities. PMM, MMM and BMN expressed isoforms containing v7/8 and v10, but failed to express epitopes encoded by v5 or v6. Thus, different CD44 isoforms are found in human skin cancers and are modulated during carcinogenesis. However, we did not observe a correlation of CD44v6 expression with metastatic potential. Copyright © 1996 Published by Elsevier Science Ltd**

**Key words: CD44 isoforms, basal cell carcinoma, squamous cell carcinoma, malignant melanoma**
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## INTRODUCTION

The incidence of melanoma and non-melanoma skin cancer is increasing rapidly and thus any research which will help elucidate the biology of these common neoplasms is clearly welcome [1, 2]. All of these tumours can grow invasively, but they differ with respect to their metastatic potential (reviewed in [1, 2]). Basal cell carcinomas (BCC) grow destructively, but almost never metastasise; squamous cell carcinomas (SCC) grow invasively and can metastasise to the regional lymph nodes, but rarely to distant locations; finally, malignant melanomas (MM) grow rapidly and invasively and readily metastasise via lymphogenic spread to the regional lymph nodes as well as via haematogenic spread to distant sites [3]. The mechanisms that govern growth and metastasis of these skin cancers are presently unknown.

Certain isoforms of the cell surface glycoprotein CD44 have recently been shown to play a crucial role in tumour growth and metastasis formation (reviewed in [4, 5]). The smallest CD44 isoform, CD44s, is expressed in a variety of different tissues, whereas expression of larger CD44 isoforms (CD44v), which are generated by alternative splicing, is restricted mainly to epithelial tissues, including the epidermis and cells of the immune system [5, 6]. These CD44v can be assembled from at least ten different exons, designated v1–v10 [7]. In humans, exon v1 seems to be non-functional due to a nonsense codon. Recently, it was demonstrated that CD44v, containing the exon v6, is necessary and sufficient to confer metastatic behaviour on a rat pancreatic adenocarcinoma cell line as well as to a rat fibrosarcoma cell line [5, 8].

Several laboratories have reported changes in CD44 isoform expression during the development and progression of human cancers [9, 10]. For example, expression of alternatively spliced CD44, carrying protein sequences encoded by the exons v5 and v6, correlates with advanced stages of human breast and colon cancers and non-Hodgkin's lymphoma [6, 11–15]. Furthermore, uterine cervical cancers can express a unique epitope (formed by sequences encoded by two exons v7/v8), not present in normal cervical epithelium, that may be suitable for screening early stages of this malignancy [16]. Whether CD44 splice variants, particularly those carrying sequences of v6, are of relevance for the growth and metastasis of human skin cancer is presently unknown. To address this issue, we studied the expression of CD44s and CD44v in BCC, SCC, primary MM (PMM), metastatic MM (MMM), benign melanocytic naevi (BMN) and in normal skin (NS).

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## PATIENTS AND METHODS

*Patients*

Following informed consent, 4–6 mm punch biopsies were obtained at the time of surgery from the tumours of patients with BCC, SCC, PMM, MMM, BMN and NS (Table 1). None of the patients had received prior chemotherapy or immunotherapy. Samples were snap frozen immediately and stored in liquid nitrogen until use.

*Monoclonal antibodies and staining reagents*

MAb (monoclonal antibodies) against specific CD44 splice variants used in this study have been described previously [6, 14, 16, 17]. They were generated and kindly provided by Bender & Co. GmbH, Vienna, Austria unless stated differently. Briefly, MAb directed against an epitope encoded by exon v3 (abbreviated anti-v3, VFF-23, mIgM), anti-v4 mAb (VFF-11, $mIgG_1$), two different anti-v5 mAb (VFF-6, VFF-8 both $mIgG_1$), three different anti-v6 mAb (VFF-4 $mIgG_{2b}$, VFF-7, VFF-18 both $mIgG_1$), anti-v7/v8 (VFF17, $mIgG_{2b}$), two anti-v10 mAb (VFF-14, VFF-16, both $mIgG_1$) and the polyclonal serum anti-v3-v10 (rabbit anti-human antiserum [6]) were used. MAb Leu44 ($IgG_1$, specific for the N-terminal portion of CD44, common to all CD44 isoforms) and MAb 3G5 (anti-V3) were purchased from Becton Dickinson, Sunnyvale, California, U.S.A. and R&D Systems, Abingdon, U.K., respectively.

*Immunohistochemistry*

Frozen skin specimens were embedded in Optimum Cutting Medium (OCT, Miles Inc., Elkhart, U.S.A.) and 5 μm serial cryostat sections were prepared using a Crycut 2000 (Reichert & Jung, Nußach, Germany). Air-dried, acetone-fixed frozen sections were stained, as previously described [18], using a four-step immunohistochemical staining protocol (ABC-technique, all reagents from DAKO): (1) primary MAb (mouse IgG, 0.2 μg/ml, 30 min, room temperature (RT); (2) biotin-conjugated goat anti-mouse IgG (1 μg/ml, 15 min, RT); (3) peroxidase-conjugated streptavidin (1:250, 15 min, RT); (4) diaminobenzidine (brown) or AEC (red, in case of heavily pigmented melanoma specimen) as chromogenic substrates (10 min, RT according to the manufacturer's instructions). Finally, sections were counterstained with haemalum. The slides were evaluated by four independent dermatopathologists in a blinded manner using a Zeiss Axioskop, equipped with a MC100 camera system. The intensity of CD44 staining on tumour cells was compared to the epidermal staining in the same section and was scored semiquantitatively on a scale of – to ++: –, no staining; (+), faint immunoreactivity; +, specific staining, weaker intensity than overlying epidermis; ++, specific staining, same intensity as epidermis. Specimens were termed positive when >25% of dermal or epidermal tumour cells had a reactivity of +, or ++ with the relevant MAb.

*Cells*

Cultures of non-transformed melanocytes were established from human neonatal foreskin as previously described [18].

*RNA isolation, oligonucleotides and RT–PCR*

Total cellular RNA was extracted from snap frozen tumour specimens using guanidinium isothiocyanate lysing buffer followed by CsCl gradient purification as previously described [16]. Sense (nucleotides 513–540) and antisense primers (nucleotides 958–934) bordering exons v1–v10 were designed based on the published sequence for human CD44 [20]. A $^{32}P$-labelled, v6-specific hybridisation probe was synthesised using v6-specific primers homologous to positions 357–383/456–482 as described [16]. For glyceraldehyde-3-phos-

*Table 1. Clinical data of patients*

| Tumour | Total no. of patients | Classification | No. of patients* |
|---|---|---|---|
| BCC | 17 | Relapse | |
| | | No recurrence | 10 |
| | | Recurrence† | 7 |
| SCC | 16 | Metastasis | |
| | | No metastasis | 12 |
| | | Locoregional LN‡ | 4 |
| PMM | 16 | Tumour thickness§ | |
| | | 0–0.7 mm | 5 |
| | | 0.7–1.5 mm | 4 |
| | | >1.5 mm | 7 |
| MMM | 18 | Type of metastasis | |
| | | Locoregional LN | 7 |
| | | Distant LN | 4 |
| | | Distant subcutaneous | 5 |
| | | Distant visceral organs | 2 |
| BMN | 13 | Histological grading§ | |
| | | Compound | 11 |
| | | Dysplastic | 2 |

*Number of patients from the total study population classified into each clinical subgroup; †Recurrent BCC requiring multiple surgical excisions; ‡SCC metastasis to cervical lymph nodes at the time of excision of the primary tumour; §As determined by routine H&E histology. LN, lymph nodes; BCC, basal cell carcinoma; PMM, primary malignant melanoma; MMM, metastases of malignant melanoma; BMN, benign melanocytic naevi.

phate dehydrogenase (GAPDH), sense primers (positions 8–29) and antisense primers (positions 362–339) of the published cDNA sequence [21] were used as described [16]. PCR conditions were exactly as described [16]. For each sample, controls were performed without reverse transcriptase and without template, respectively. In all experiments, no amplified fragments due to genomic DNA or cDNA contamination were detected. PCR products were electrophoresed on a 1.2% agarose gel, followed by staining with ethidium bromide (Sigma, Diesenhofen, Germany) and Southern blot transfer to Hybond $N^+$ Nylon membranes (Amersham, Braunschweig, Germany), followed by hybridisation with a $^{32}P$-labelled v6-specific probe.

## RESULTS

*Immunohistochemical detection of CD44 variants in basal cell carcinoma and squamous cell carcinoma*

In BCC, a non-metastasising skin cancer of keratinocyte origin, the pan CD44 epitope was expressed weakly in the centre of the tumour (Figure 1a). Similarly, epitopes indicating the presence of one or several CD44 splice variants were expressed only weakly and focally, i.e. in the centre of the tumour nests in the majority of BCC. Staining revealed the presence of all epitopes detectable by the MAbs, i.e. those encoded by exons v3, v4, v5, v6, v10 and of the composite epitope formed by v7 and v8 (Figure 1b, c, d, Table 2). This would be compatible with the expression of the keratinocyte isoform CD44v3–v10. BCC thus resemble in CD44 expression non-transformed keratinocytes [19], except that the tumour cells in the periphery tend to lose this expression.

In SCC, a skin cancer which also originates from keratinocytes, a different pattern of CD44 expression was observed. The common CD44 epitope recognised by Leu 44 was clearly detected on 16/16 SCC, although it was found predominantly in the invasive periphery of the tumour (Figure 1e, Table 2). When CD44v epitopes were examined, the same tumour cells at the edges expressed epitopes encoded by variant exons: epitopes covering the sequences v3–v10 were expressed exclusively in the periphery of 16/16 SCC (Figure 1f–h, Table 2). The intensity of this CD44v staining was identical to that of unaffected epidermis (Figure 1a–h). Furthermore, it should be emphasised that all of the SCC examined expressed the CD44v6 epitope on their surface (Figure 1g, Table 2). Thus, in contrast to non-metastasising BCC, the SCC maintain the CD44 expression pattern of their parent cells particularly at the invasive edges.

*Immunohistochemical detection of CD44 variants in benign and malignant tumours of melanocytic origin*

The pan CD44 MAb Leu 44 stained all melanoma cells in 16/16 primary tumours (PMM) and 18/18 metastases (MMM) (Figure 2a, f, Table 2) as well as naevus cells in 13/13 BMN (Figure 2l, Table 2). In addition, this epitope was expressed in all layers of the epidermis and in dermis by stromal cells and infiltrating lymphoid cells, as described above for BCC and SCC (Figure 2a, f, l). The majority of PMM and MMM were found to express epitopes encoded by the exons v7/v8 and v10 (Figure 2d, e, j, k, Table 2). However, we failed to detect any expression of v5 or v6 epitopes on the surface of 16/16 PMM and 18/18 MMM (Figure 2b, c, h, i), using five different anti-v5 or anti-v6 MAb (VFF-4, VFF-6, VFF-7, VFF-8, VFF-18). However, in the same sections, strong epidermal v5 and v6 expression was observed (Figure 2b, c, h, i). BMN and cultured non-transformed

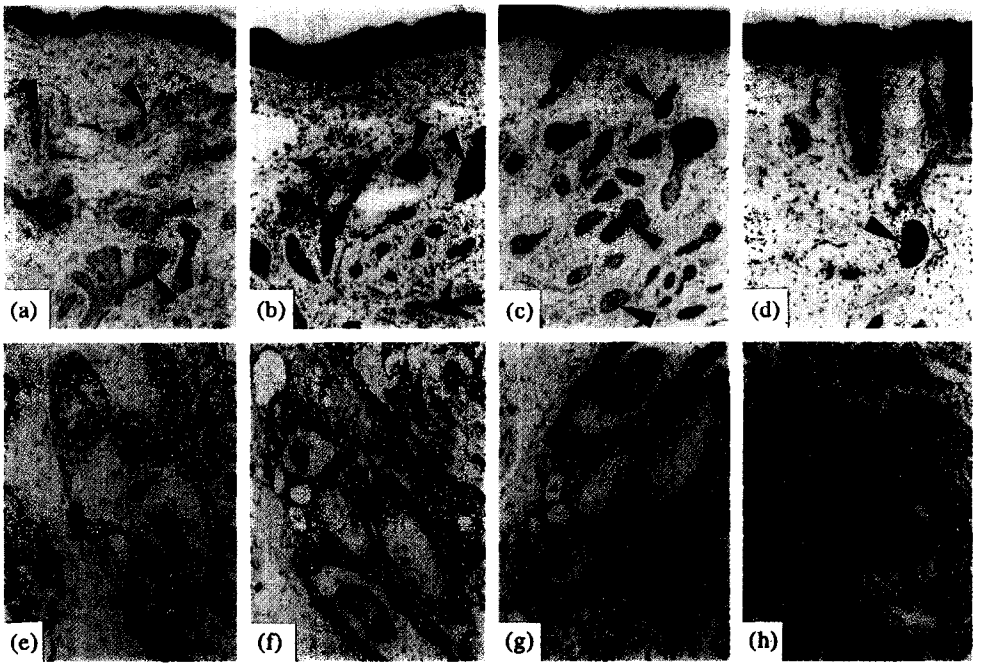


**Figure 1. Immunohistological detection of CD44 isoforms in basal cell carcinoma and squamous cell carcinoma: (a) BCC: Leu 44 staining epidermal cells, dermal stromal cells, and weakly in the centre of the tumour (arrowheads) (100×); (b) BCC (same as in a): CD44v5 is expressed by all layers of the epidermis, but only in the centre of the tumour islets (arrowheads) (VFF8, 100×); (c) BCC (same as in a): CD44v6 is expressed in the centre of BCC (arrowheads) (VFF7, 100×); (d) BCC (same as in a): CD44v10 is expressed in the centre of BCC (VFF16, 400×); (e) SCC: Leu 44 staining in the periphery of SCC (arrowheads) (100×); (f) SCC (same as in e): CD44v5 is expressed in the periphery of SCC (arrowheads) (VFF8, 100×); (g) SCC (same as e): CD44v6 is expressed in the periphery of SCC (arrowheads) (VFF7, 100×); (h) SCC (same as e): CD44v10 is expressed in the periphery of SCC (VFF16, 100×).**

*Table 2. Summary of immunohistological detection of CD44 isoforms in human skin cancer*

| MAb<br>Epitope | Leu 44<br>panCD44 | VFF23, 3G5<br>v3 | VFF11<br>v4 | VFF8,6<br>v5 | VFF4,7,18<br>v6 | VFF17<br>v7 + v8 | VFF14/16<br>v10 |
|---|---|---|---|---|---|---|---|
| Tumour type | | | | | | | |
| BCC | 14/17*,c† | 12/17, c | 17/17, c | 13/17, c | 17/17, c | 17/17, c | 17/17, c |
| SCC | 16/16, p† | 16/16, p | 16/16, p | 16/16, p | 16/16, p | 16/16, p | 16/16, p |
| PMM | 16/16 | 4/16 | 3/16 | 0/16 | 0/16 | 15/16 | 14/16 |
| MMM | 18/18 | 3/18 | 2/18 | 0/18 | 0/18 | 15/18 | 16/18 |
| BMN | 13/13 | 4/13 | 1/13 | 0/13 | 0/13 | 11/13 | 11/13 |

*Number of tumours with positive staining/or total number of tumours studied. Specimens were termed positive when >25% of dermal or epidermal tumour cells revealed a reactivity of +, or ++ with the relevant MAb, as detailed in Patients and Methods.

†c, staining in the centre of the tumour; p, staining in the periphery of the tumour.

Abbreviations as in Table 1.

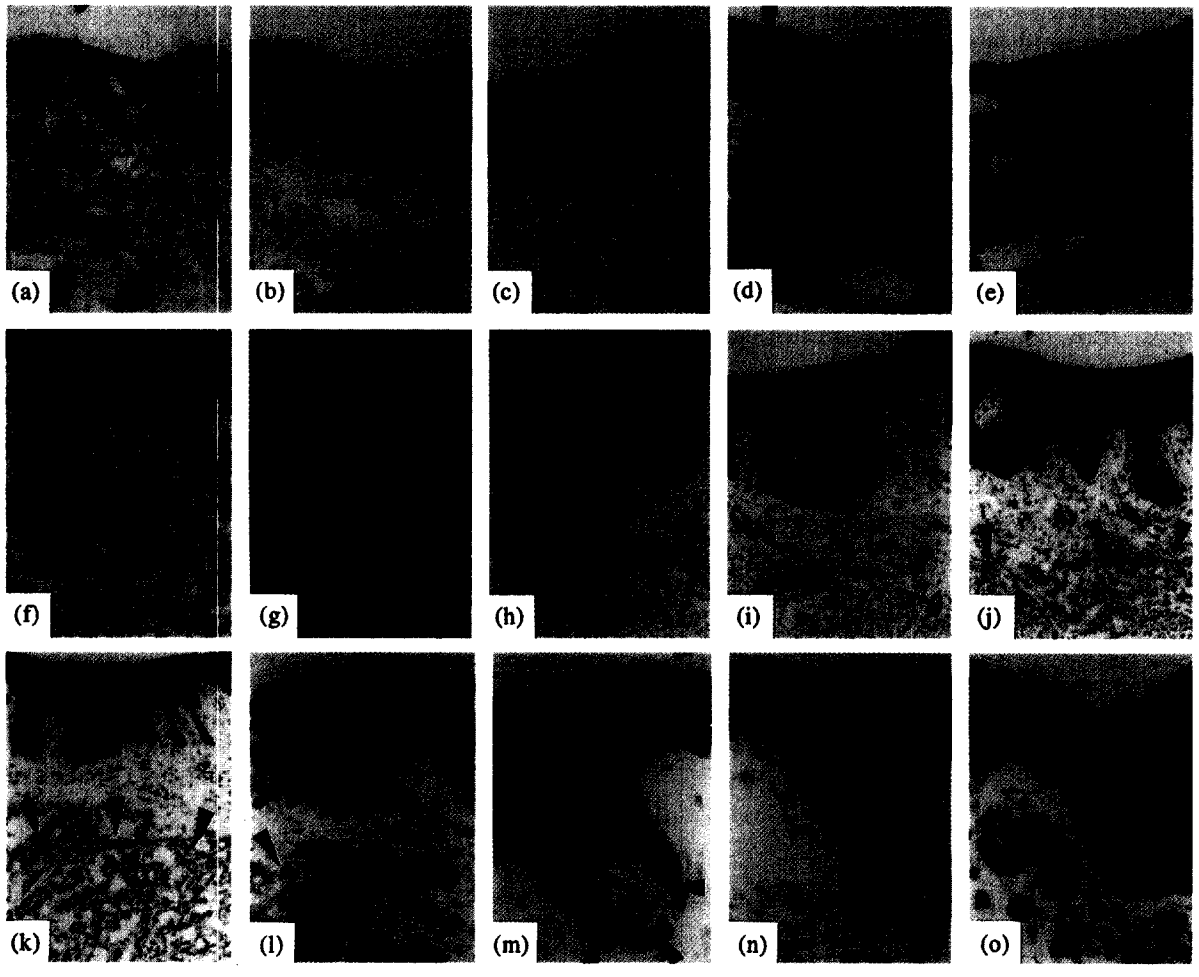


**Figure 2. Immunohistological detection of CD44 isoforms in malignant melanoma and in benign melanocytic naevi: (a) PMM (level III, tumour thickness 3.4 mm): Leu 44 staining on epidermal cells, dermal stromal cells, infiltrating inflammatory cells and on MM cells (arrowheads) (100×); (b) PMM (same as in a): CD44v5 expressed by epidermal cells but not by MM cells (arrowheads) (MAb VFF8, 100×); (C) PMM (same as in a): CD44v6 is not expressed by MM cells (arrowheads) (MAb VFF7, 100×); (d) PMM (same as in a): CD44v7/v8 is expressed by MM cells (arrowheads) (MAb VFF17, 100×); (e) PMM (same as in a): CD44v10 is expressed by MM cells (arrowheads) (MAb VFF16, 100×); (f) MMM (subcutaneous metastasis): Leu 44 staining on epidermal cells, dermal stromal and inflammatory cells and on MM cells (arrowheads) (100×); (g) MMM (same as in f): CD44v3 (MAb 3G5, 100×) expressed by epidermal cells but not by MM cells (arrowheads); (h) MMM (same as in f): CD44v5 expressed by epidermal cells but not by MM cells (arrowheads) (MAb VFF8, 100×); (i) MMM (same as in f): CD44v6 is not expressed by MM cells (arrowheads) (MAb VFF7, 100×); (j) MMM (same as in f): CD44v7/v8 is expressed by MM cells (arrowheads) (MAb VFF17, 100×); (k) MMM (same as in f): CD44v10 is expressed by MM cells (arrowheads) (MAb VFF16, 100×); (l) BMN (compound type): Leu 44 staining on epidermal cells, dermal stromal cells and on naevus cells (arrowheads) (400×); (m) BMN (same as in l): CD44v5 is not expressed by BMN cells (arrowheads) (MAb VFF8, 400×); (n) BMN (same as in l): CD44v6 is not expressed by BMN cells (arrowheads) (MAb VFF7, 400×); (o) BMN (same as in l): CD44v10 is expressed by naevus cells (arrowheads) (MAb VFF16, 400×).**

melanocytes displayed the same pattern of CD44v expression as did primary and metastatic MM, i.e. lack of v5 and v6 (Figure 2m, n, Table 2) but expression of v7/8 and v10 (Figure 2o, Table 2). Protein sequences encoded by exons v3 and v4 were detected in the minority of PMM, MMM and BMN (Table 2). We conclude that there is no change in CD44 epitope expression during melanocyte carcinogenesis. Presumably, one isoform occurs throughout, comprising the N-terminal exons of CD44s, exons v7 to v10 and the C-terminal region.

*CD44 variant-specific RNA expression in human skin cancers*

To compare epitope expression with RNA splice patterns in NS, BCC, SCC and primary tumours or metastases of MM, RNA was isolated from NS and tumours that had also been examined by immunohistochemistry. RNA was reverse transcribed, amplified by PCR, and hybridised to v6-specific probes. Using primers located 5′ and 3′ of the insertion site of the variant exons, we obtained one predominant PCR product in all samples, its size of 440 bp (base pairs) being indicative of CD44s (Figure 3a). PCR products of minor abundance containing v6-specific RNAs were detected in almost all samples after blotting on to Nylon membranes and hybridisation with a v6-specific probe (Figure 3b). RNA derived from NS and SCC yielded remarkably similar patterns of v6-containing splice variants up to sizes of approximately 1400 bp. This size is generated if variant exon sequences from v3 to v10 are included in CD44 RNA. By contrast, with RNA derived from BCC, PCR products of approximately 750 bp and 1050 bp were hybridised (Figure 3b). The smaller band could represent v6 plus one or two additional variant exons, while the larger band may contain v6 plus 4 to 5 additional variant exons, both of which still need to be verified. Finally, RNAs isolated from 1/1 PMM and 4/5 MMM also resulted in v6-specific RT–PCR signals. In both PMM and MMM, a major fragment of approximately 560 bp was detected (Figure 3b), that could originate from a CD44 splice variant including v6 exon sequences only (theoretical size of 564: 440 bp for CD44s plus 124 bp for v6). In addition, a larger band of approximately 750 bp was found in some MMM which may result from RNA containing v6 plus one additional variant exon, as discussed above (Figure 3b). However, the expression of v6-specific mRNAs in the melanomas was minor compared to SCC or to normal skin.

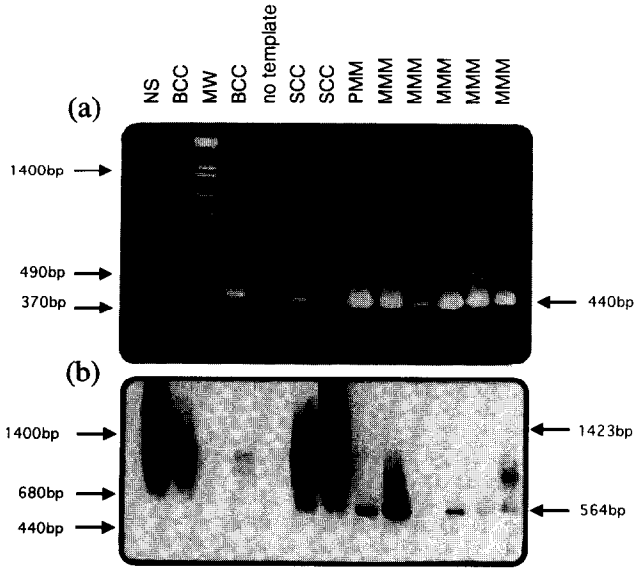


**Figure 3. CD44v6 messenger RNA analysis in individual samples of skin cancers and normal skin. Total cellular RNA was extracted from two NS, two BCC, two SCC, one PMM and five MMM. In each case, 3 μg of RNA was used for the RT-PCR reaction using primers located 5′ and 3′ of the insertion site of the variant exons. (a) PCR products were analysed by 1.2% agarose gel electrophoresis. The arrows on the left mark sizes of 370, 490 and 1400 bp. The arrow on the right marks a size of 440 bp, the predicted size of CD44s. (b) PCR products were transferred to a Nylon membrane and hybridised with a $^{32}$P-labelled v6-specific probe, followed by autoradiography (30 min exposure). The arrows on the left mark sizes of 440, 680 and 1400 bp. The arrows on the right mark sizes of 564 and 1423 bp, the predicted sizes of v6 or v3–v10, plus the amplified flanking region of CD44s, respectively.**

## DISCUSSION

In this paper, we describe the expression of CD44 isoforms in normal skin and in skin cancers of different metastatic potential. In NS, we found the epidermis to express isoforms containing sequences encoded by exons v3–v10. By immunohistochemistry, the respective epitopes were detected throughout the epidermis as reported previously [6, 22, 23], suggesting that keratinocytes in all layers of the epidermis have the capacity to synthesise presumably one specific large isoform, CD44v3–v10. According to RT–PCR analysis, NS contains alternatively spliced CD44 transcripts ranging in length from approximately 560 to 1400 bp. These results indicate that cells in the NS samples can generate different CD44 mRNAs, a major form of which (1400 bp) codes for CD44v3–v10. The normal function of CD44v in the epidermis is presently unknown, although a role in the homotypic adhesion of keratinocytes has been suggested [22].

During malignant transformation, the distribution of CD44 isoforms in epidermal cells changes. Interestingly, the two cancers of keratinocyte origin, BCC and SCC, differ markedly with respect to their intratumoral localisation of CD44 molecules. Immunohistochemistry revealed that CD44 epitopes were expressed exclusively in the centre of BCC, whereas in SCC, the same epitopes were found in the invasive periphery. PCR analysis of RNA derived from BCC, SCC and NS revealed a complex pattern of different v6-containing splice variants. However, the RNA sample may have been contaminated by RNA derived from non-tumour cells and thus the data do not necessarily need to match those from immunocytochemistry. According to these PCR data, NS and SCC displayed a similar expression pattern, including the predominant transcript that may represent CD44v3–v10. This differs from observations in other epithelial tumours, for example, gastric and colorectal cancers which express v6-splice patterns distinct from those of the normal mucosa [11, 17]. Interestingly, the CD44 RNA splice pattern of BCC differed from that of SCC and NS. BCC synthesise two major RNA species whose PCR amplification products hybridise to v6. Both PCR products were smaller than the 1400 bp form representing CD44v3–v10. This indicates that in BCC, v6 is associated with smaller transcripts composed of only a few, rather than all the variant exons (CD44v3–v10) that are present in keratinocytes and SCC. Taking into account the immunohistochemical data, the larger RNA species in BCC should encode CD44v5–v10.

In 16/16 SCC CD44v6 protein as well as v6-specific RNA were found expressed at similar levels as in NS. This result

appears to differ from work published by Salmi and colleagues [23], who observed by immunohistochemistry that the v6 epitope was downregulated or absent in SCC, when compared to normal epithelia. It should be noted that different anti-v6 antibodies were used in both studies, raising the possibility that the epitope recognised by MAb Var3.1 [23] is masked on SCC, e.g. by glycosylation, while those detected by our reagents (VFF-4, VFF-7, VFF-18) are not. The notion that their MAb Var3.1 and our reagents identify distinct v6 epitopes is supported by their different staining patterns in normal epidermis. Another explanation for the disparate results could be differences in the tumours studied, i.e. Salmi and associates [23] examined a heterogeneous group of head and neck SCC originating mostly from the larynx, while we studied SCC arising exclusively from skin.

PMM and MMM were shown by immunohistochemistry to express CD44s, which is in concordance with previous work [24, 25]. Furthermore, we found the majority of PMM and MMM to express CD44 isoforms containing v7/8 and v10. However, we failed to detect surface expression of CD44 isoforms containing epitopes encoded by exons v5 or v6 in 16/16 primary and 18/18 metastatic MM. This was not due to an inadequate staining technique, since strong epidermal v5 and v6 reactivity was observed in normal skin within the same tumour section. However, v5 and v6 in these tumours may not be accessible to the MAb due to a masking of epitopes, for example by differential glycosylation. We consider this possibility unlikely, since two different anti-v5 and three different anti-v6 reagents all failed to stain PMM or MMM. Furthermore, limited deglycosylation of cryostat sections of MMM had no effect on v5 and v6 staining (data not shown). These findings are in partial concordance with a recent paper by Manten-Horst and coworkers [24], who also failed to detect expression of CD44v6 on human melanomas. However, these authors report expression of CD44v5 in 8/19 PMM and 11/19 MMM and a loss of CD44v10 expression in 17/19 PMM and 13/19 MMM. Differences in the patient populations studied or in the immunohistological scoring system may account for these disparate findings. Specifically, Manten-Horst and coworkers [24] defined a MM as positive if >10% of melanocytic cells stained positive, whereas, based on previous experience [18], we required >25% of melanoma cells to stain with an intensity comparable to that of the epidermis in the same section to term a melanoma positive.

Interestingly, in the majority of neuroblastomas, a highly metastatic childhood neoplasm, no v5 or v6 containing CD44 isoforms have been detected [26–28]. Both neuroblastomas and malignant melanomas arise from neural crest-derived cells [2, 26], raising the possibility that tumours of neuroectodermal origin, unlike certain epithelial tumours, do not require surface expression of CD44 isoforms containing v5 or v6 for metastasis formation. It should be noted that Schwannomas, which are tumours that also originate from the neural crest but rarely metastasise, do express CD44v epitopes encoded by exons v5, v7/8 and v9–v10 in certain circumstances [29].

It has been hypothesised that CD44v might only be relevant for the lymphogenic but not for the haematogenic spread of tumours [5]. To rule out the possibility that this may account for our negative v5/v6 staining in MM, we compared MM metastases originating from lymphogenic tissue (locoregional and distant lymph nodes) with those that had spread systemically (distant cutaneous tissues and distant visceral organs). No differences were observed between the two groups, i.e. 18/18 MMM failed to express v5 and v6 on their surface, irrespective of their localisation. We also found benign melanocytic naevi, the precursor lesions of MM [2] to express the same pattern of CD44v (v7/8 and v10) as PMM or MMM. BMN expressed these epitopes irrespective of their degree of dysplasia, unlike the precursors of gastric or colonic cancer [6, 17]. Taken together, these results suggest that PMM or MMM do not express the putative metastasis-associated exon v6 sequence on CD44 molecules on their surface.

However, by RT–PCR, we detected v6-specific mRNAs in the same PMM and MMM that had proven to be v6-negative by means of immunohistochemistry. It should be noted that the samples used for RNA preparations contain heterogeneous cell populations (i.e. melanoma cells, keratinocytes and possibly lymphocytes and macrophages); we therefore cannot exclude that the PCR signals resulted from a contamination with non-melanoma cells. Further, the v6-specific PCR signals were weak, particularly if normalised to the 440 bp CD44s PCR product. Therefore, an explanation for the discrepancy between immunohistochemical and RT–PCR data could be that MM cells express only minor amounts of v6-specific mRNA, below the detection level of immunohistochemistry.

In conclusion, different CD44 isoforms were detected in BCC, SCC, PMM and MMM. Only SCC, but not BCC, resembled non-transformed keratinocytes in their expression pattern of CD44 isoforms. Non-transformed melanocytes and melanocytes from benign naevi resembled melanoma cells with respect to their expression of CD44 isoforms. Finally, an association of CD44v6 surface expression and metastatic potential, particularly in human malignant melanoma, was not observed.


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