Histol Histopathol (2002) 17: 1269-1281

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

The role of CD44 in the development and prognosis of head and neck squamous cell carcinomas

D. Assimakopoulos[1], E. Kolettas[2], G. Patrikakos[1] and A. Evangelou[2]

[1]Department of Otorhinolaryngology and [2]Department of Physiology, University of Ioannina Medical School, Ioannina, Greece

Summary. CD44, the product of a single gene, exists as several isoforms generated by alternative exon splicing and posttranslational modifications, and is widely distributed in different cells and tissues including those of squamocellular origin. CD44 is a cell surface glycoprotein involved in many cellular processes acting as a receptor for cell to cell or cell to matrix adhesion, as a signal transmitter and as a growth factor-presenting molecule. Numerous studies based on immunohistochemical analyses of paraffin-embedded or frozen tissue sections using different monoclonal antibodies to CD44 isoforms and molecular biological techniques have provided evidence that in many types of tumours there is overexpression of CD44 isoforms and aberrant processing of immature CD44 transcripts relative to non-neoplastic control tissues, suggesting a role of CD44 in tumour development and progression. In contrast to these malignancies, one or more of the CD44 splice-variant isoforms are down-regulated in squamous cell carcinomas of the head and neck. CD44-deficient mice develop normally without giving rise to spontaneous tumours, but CD44-negative cells appear to be more susceptible to oncogenic transformation. Reduction in the expression of CD44 may confer growth advantage and malignant properties to tumour cells. The clinical significance of CD44 in squamous cell carcinomas of the head and neck as a tumour marker for cancer diagnosis and prognosis is discussed.

Key words: CD44, Splice variants, Squamous cell carcinoma, Head and neck, Marker

Introduction

CD44 (Cluster of Differentiation 44) comprises a family of transmembrane glycoproteins expressed on a wide range of cells and tissues including hemopoietic, endothelial, mesenchymal and epithelial lineages. The CD44 protein molecule consists of an extracellular, a transmembrane and a cytoplasmic domain, the diversity of which is determined by variable exon usage, glycosaminoglycan substitution, and cell type-specific N- and O-glycosylation. Thus, the CD44 isoforms differ in size, ranging from 90 kDa to 250 kDa, and in their tissue distribution. These isofoms such as CD44s (the standard form), CD44E (the epithelial form) and CD44v (splice variants), arise from differential splicing of one to ten variable exons that encode ortions of the membrane proximal extracellular domain (Lesley and Hyman, 1998; Ponta et al., 1998).

CD44, a cell adhesion molecule, acts as the major hyaluronan receptor and as a receptor for many other extracellular matrix components such as type I and IV collagens, fibronectin and laminin, interacts with signaling molecules such oncogene products possessing protein tyrosine kinase activity and binds soluble growth factors and cytokines, thereby acting not only as a cell adhesion molecule but also as a signal transmitter and as a growth-presenting molecule (Lesley et al., 1993; Bourguignon et al., 1998; Hamada et al., 1998; Lesley and Hyman, 1998; Ponta et al., 1998; Goodison et al., 1999). CD44 has been implicated in a variety of cellular processes, including cell-cell and cell-matrix interactions, cell migration, normal development, immune cell function, tumour progression and metastasis, and these multiple functions, however, appear to be restricted to the various isoforms (Sherman et al., 1996; Naot et al., 1997; Sy et al., 1997; Goodison and Tarin, 1998a,b; Herrlich et al., 1998; Knudson, 1998; Koukoulis et al., 1998).

The neoplastic transformation of normal epithelial cells to metastatic tumour cells is a complex process involving a number of alterations in the expression of genes implicated in cell proliferation, cell adhesion and cell migration. Tumour progression is the process by which tumour cells acquire malignant properties, such as progressive growth, invasion and metastasis (Nowell, 1986). One of the genes involved in these processes is CD44 which appears to be one of the most promising candidates as a cancer diagnosis marker (Herrlich et al.,

Offprint requests to: Professor Dimitrios A. Assimakopoulos, MD, Department of Otorhinolaryngology, University of Ioannina Medical School, 45 110 Ioannina, Greece. Fax: +30651097097. e-mail: dassimak@cc.uoi.gr

1993; Sherman et al., 1996; Naot et al., 1997; Rudzki and Jothy, 1997; Sy et al., 1997; Goodison and Tarin, 1998a,b; Herrlich et al., 1998; Goodison et al., 1999; Caballero et al., 2001). Several studies have provided evidence that the expression of CD44 is specifically altered in many types of tumours. Many types of human tumours show aberrant expression and processing of CD44 transcripts and cell surface expression of CD44 appears to change profoundly during tumour metastasis, particularly during the progression of various carcinomas, as detected by molecular biological techniques such as reverse transcriptase-polymerase chain reaction (RT-PCR), Southern and Northern blotting and immunohistochemical analyses using CD44-specific monoclonal antibodies to epitopes encoded by several different individual exons (Goodison and Tarin, 1998a,b). These studies have confirmed that in many types of common cancers including breast, lung, gastrointestinal, urological, and lymphoid tissue tumours, there is overexpression of CD44 transcript or protein isoforms and abnormal assembly of CD44 transcripts in tumour tissues relative to non-neoplastic control tissues or to normal tissues. These observations indicate that the CD44 adhesion molecule could have an important role in cell transformation, cancer diagnosis and prognosis. In contrast to these malignancies, most of the evidence obtained by molecular biological and immunodetection techniques through literature search and summarized in this review show that some CD44 splice-variant isoforms are down-regulated in squamous cell carcinomas of the head and neck. However, detailed study of the results obtained from numerous tumours of the head and neck leads to the conclusion that there is no specific down-regulation of the expression of any individual splice-variant isoform during the development and progression of head and neck neoplasia. Further, we discuss the plausible mechanisms by which CD44 contributes to the development and progression of squamous cell carcinoma of the head and neck and the clinical importance of such altered CD44 expression in these processes as a biological marker for cancer diagnosis and prognosis.

Structure of CD44

CD44 denotes a family of cell-surface glycoproteins, arising by alternative splicing, that are expressed by a wide variety of cells and tissues and consist of an extracellular, a transmembrane and a cytoplasmic domain. The human single-copy CD44 gene, spanning approximately 60 kb, has been mapped to the chromosomal locus 11p13 (Goodfellow et al., 1982) and it is composed of at least 20 exons (Screaton et al., 1992) (Fig. 1). Exons 1-17 encode the extracellular domain of

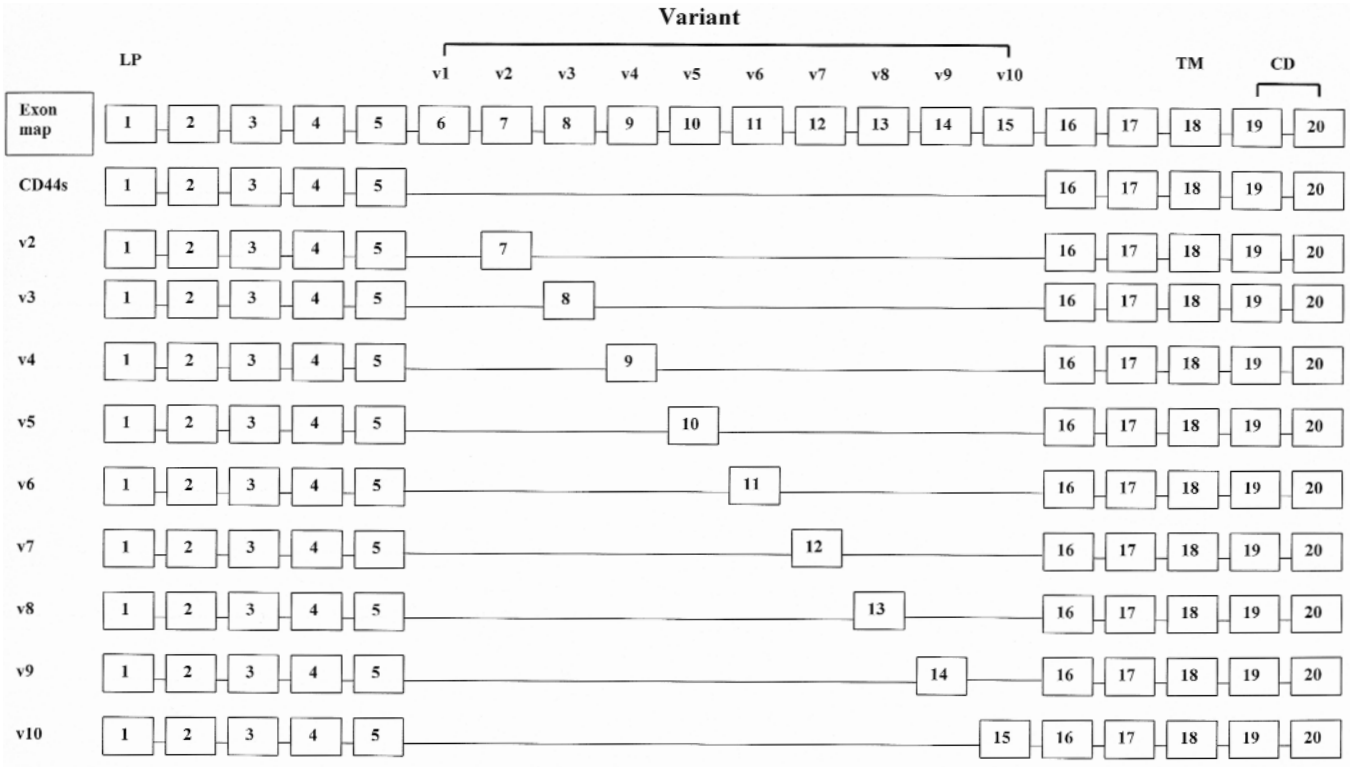

Fig. 1. Gene structure of CD44 and some of its splice variants. The leader peptide (LP) (exon 1), the transmembrane domain (TM) (exon 18) and the cytoplasmic domain (CD) (exons 19 and 20) are shown. The variant exons v1 to v10 (exons 5 to 15) encode the extrracellular domain of the CD44 molecule. Some but no all of the CD44 splice variants implicated in ehad and neck carcinogenesis are also shown.

the protein, exon 18 encodes a short transmembrane domain and exons 19 and 20 encode the cytoplasmic domain (Knudson, 1998). Ten exons, namely 1-5 and 16-20, are spliced together on all cell types to produce the standard isoform or hematopoietic isoform (CD44s or CD44H). The remaining 10 variable exons (exons 6-15 or v1-v10) that encode portions of the membrane proximal extracellular domain, can be alternatively spliced between exons 5 and 16 leading to an insertion in tandem of one or more variant exons within the standard isoform to generate a number of variant transcripts or proteins (CD44v) (Screaton et al., 1992; Tolg et al., 1993). One of these isoforms is the epithelial isoform (CD44E) which results from the alternative splicing of three additional exons, exons 12-14 (v8-v10) and is preferentially expressed on epithelial cells (Screaton et al., 1992). Several CD44 isoforms have been implicated in tumour progression and metastasis including head and neck tumours (Screaton et al., 1992; Naot et al., 1997; Rudzki and Jothy, 1997; Sy et al., 1997; Bourguignon et al., 1998; Goodison and Tarin, 1998a,b; Knudson, 1998) (Fig. 1).

The smallest CD44s, which lacks the variant exons, codes for a type I transmembrane protein consisting of a 72 amino acid (aa) intracellular domain, a 21 aa transmembrane domain and a 268 aa extracellular domain (including 20 aa of leader sequence) with a size of 37 kDa (Naot et al., 1997; Lesley and Hyman, 1998; Ponta et al., 1998). The CD44 variant transcripts encode proteins similar to CD44s but with additional sequences selected from exons v1-v10 inserted within the membrane proximal region of the extracellular domain (Lesley et al., 1993; Bourguignon et al., 1998; Lesley and Hyman, 1998; Ponta et al., 1998). The molecular and functional diversity of these isoforms can be further modified by post-translational modifications, including N- and O-glycosylation or glycosaminoglycan (GAG) addition to producing a large polymorphic family of transmembrane glycoproteins with diverse functions (Lesley and Hyman, 1998). Thus, CD44 proteins range from 80-90 kDa for the mature CD44s to 250 kDa for CD44 isoforms containing all of the variant exon products and glycosaminoglycans. Glycosylation of CD44 markedly influences its function (Kincade et al., 1997; Lesley and Hyman, 1998). Moreover, CD44 is phosphorylated on serine residues (Lesley and Hyman, 1998). Specifically Ser323 and Ser325 are the only residues of the cytoplasmic domain that are phosphorylated *in vivo*, as determined by mutational analysis, with Ser325 accounting for approximately 90% of the phosphorylation on CD44 (Neame and Isacke, 1994; Pure et al., 1995; Peck and Isacke, 1998). Experimental evidence obtained *in vitro*, using mutant CD44 constructs transfected into mammalian cells suggest that phosphorylation of the CD44 cytoplasmic domain may be involved in mediating events downstream of ligand binding that are necessary for cell migration (Neame and Isacke, 1994; Pure et al., 1995; Lesley and Hyman, 1998; Peck and Isacke, 1998).

Functions of CD44 related to malignancy

CD44 has several different functions related to tumour development, progression and metastasis, due to its interaction with numerous different ligands (Lesley and Hyman, 1998). CD44 is a major hyaluronan receptor (Knudson, 1998), but it also interacts with other extracellular matrix proteins such as fibronectin, collagen types I and IV, serglycin and osteopontin (Lesley et al., 1993; Naot et al., 1997; Ponta et al., 1998) through the oligosaccharide chains of its cytoplasmic domain, hence acting as a matrix receptor (Kincade et al., 1997; Bourguignon et al., 1998; Lesley and Hyman, 1998). The binding to hyaluronan, the major glycosaminoglycan found in the extracellular matrix of mammalian tissues, is known to cause cell adhesion to extracellular matrix components and is also implicated in the stimulation of cell aggregation, cell proliferation, cell migration and angiogenesis (Bourguignon et al., 1998; Knudson, 1998). CD44 associates with cytoskeletal proteins such as ankyrin, and actin microfilaments through its interaction with the ERM protein family members (ezrin, radixin and moesin) (Bourguignon et al., 1998; Lesley and Hyman, 1998), interacts with signaling molecules involved in oncogenesis (Bourguignon et al., 1998; Lesley and Hyman, 1998) such as $p56^{lck}$ (Taher et al., 1996) and $p185^{HER2}$ (Bourguignon et al., 1997) and is induced by overexpression of oncogene products (Hofmann et al., 1993; Jamal et al., 1994; Zhu and Bourguignon, 1996; Bourguignon et al., 1997; Lamb et al., 1997; Ladeda et al., 2001). CD44 also acts as a growth factor-presenting molecule (Hamada et al., 1998). Many of the growth factors and cytokines are heparin-binding factors including bFGF, HB-FGF, HGF, PDGF and IL-8, and bind to heparan sulphate chains of CD44 (Bourguignon, 2001). Further, CD44 expression is induced by several growth factors and cytokines (Hamada et al., 1998). These studies together with the fact that the intracellular domain of CD44 is phosphorylated in some serine residues would suggest that CD44 may affect signal transduction pathways, extracellular matrix and cytoskeletal organization and confer a growth advantage to tumour cells, leading to tumour progression and metastasis.

Experiments with transgenic mice have provided evidence for a role of CD44 in tumourigenesis. Heterozygous and homozygous transgenic mice generated by disrupting exons 2 and 3 of the CD44 gene were born in Mendelian ratio without any obvious developmental or neurological defects, but showed a defect in the distribution of granulocyte-macrophage progenitor cells between bone marrow and spleen (Schmits et al., 1997). However, it was shown that CD44 regulates tumourigenicity in fibroblasts. Primary fibroblasts isolated from CD44-deficient mice were transfected with the SV40 large T antigen and compared with the same fibroblasts carrying the full length mouse CD44s cDNA. While no obvious differences in

morphology or in the in vitro growth properties between CD44-negative and CD44-positive SV40 large T antigen-expressing cells were observed, the former cell type gave rise to large subcutaneous tumours. In contrast, CD44-positive cells gave rise to very small tumours over the same period of time. Histologically both tumours were similar, although CD44-negative tumour cells showed more cellular pleomorphism and had a higher mitotic index (Schmits et al., 1997). Similarly, CD44-deficient mice generated by an in-frame insertion of the lacZ/neo reporter cassette into the CD44 leader peptide to abolish CD44 expression, exhibited normal development and differentiation without developing spontaneous tumours. Although the development of the lymphoid system was not affected in the absence of CD44, mutant lymphoid cells exhibited an impaired homing to the thymus and lymph nodes in the adult animals (Protin et al., 1999). Transgenic mice expressing rat CD44v4-v7 exhibited an accelerated immune response (Moll et al., 1996), thus confirming the role of CD44 on lymphocyte activation.

With regard to squamous tissues, normal epidermis and other stratified squamous epithelia reacted strongly with antibodies specific for CD44s and CD44 isoforms containing exons v4, v6 and v9. Malignant cells in basal carcinoma tissues were found to have low reactivity with antibodies specific for CD44s or variant CD44 molecules (Hale et al., 1995). Similar studies showed that while normal skin expressed CD44s and splice-variants v5, v6, v7, v7-8 and v10, CD44v expression was reduced in basal cell carcinoma and squamous cell carcinoma of the skin, particularly CD44v10 (Seiter et al., 1996). RT-PCR for variant exons v1-v10 confirmed the expression of CD44 including exons v2-v10 in normal keratinocytes and in squamous cell carcinoma cell lines, but no protein expression was detected in the tumour cells (Bloor et al., 2001). Furthermore, expression of CD44s and variant isoforms in human epidermal skin tumours was not correlated with tumour aggressiveness but was down-regulated during proliferation and tumour de-differentiation (Seelentag et al., 1996).

Selective suppression of CD44 in keratinocytes of mice bearing an antisense CD44 transgene driven by the keratin-5 promoter disrupted hyaluronate metabolism and impaired keratinocyte proliferation (Kaya et al., 1997). Mice lacking detectable CD44 expression in skin keratinocytes and corneal epithelium displayed abnormal hyaluronate accumulation in the superficial dermis and corneal stroma, distinct morphological alterations of basal keratinocytes and cornea, defective keratinocyte proliferation in response to mitogens and growth factors and failure of the epidermis to undergo hyperplasia in response to carcinogen. These studies indicated that two major functions of CD44 in skin are the regulation of keratinocyte proliferation in response to external stimuli and the maintenance of local hyaluronate homeostasis (Kaya et al., 1997). It should be emphasised, however, that this phenotype was not observed in CD44 null mice (Schmits et al., 1997; Protin et al., 1999).

Several studies have implicated the CD44 transmembrane protein family in tumour development and metastasis (Gunthert et al., 1991; Hoffmann et al., 1993; Ponta et al., 1994; Kogerman et al., 1997; Yu et al., 1997; Reeder et al., 1998; Harada et al., 2001). Others, however, showed that loss or reduced expression of CD44 molecules may contribute to cell transformation (Takahashi et al., 1995; Gao et al., 1997; Schmits et al., 1997) suggesting that CD44 acts both as a growth- and invasiveness-promoting molecule and as a tumour-suppressing co-factor (Herrlich et al., 2000). For example, introduction of antisense CD44v6 (Reeder et al., 1998) or antisense CD44s cDNA (Harada et al., 2001) into metastatic colorectal tumour cells inhibited tumour growth and metastasis. In contrast, re-introduction of CD44 in colorectal tumour cells that exhibited reduced CD44s expression compared with normal colonic cells, was found to reduce tumourigenicity (Takahashi et al., 1995). The tumour-promoting and the tumour-suppressing actions of CD44 appear to depend on the cell type, on the growth state of the cells, on the isoform pattern expressed, on the binding of growth factors and their presentation to their authentic high affinity receptors, on the recruitment of ERM proteins (ezrin and moesin), on the nature of extracellular matrix proteins and on the binding of CD44 to the tumour suppressor protein, merlin (Herrlich et al., 2000).

Expression of CD44 in normal and neoplastic head and neck squamous tissues

The CD44 protein family is ubiquitously expressed, but the expression of variant isoforms appears to be cell- and tissue-specific (Sherman et al., 1996; Goodison and Tarin, 1998a). Immunohistochemical, immuno-fluorescence and immunoblotting studies using CD44-specific antibodies and molecular biological techniques such as Northern blotting and reverse transcriptase-polymerase chain reaction (RT-PCR) utilising variant-isoform-specific probes or primers, respectively, have been used to detect the expression of CD44s and CD44v in normal and tumour head and neck samples. The monoclonal antibodies used to detect CD44 expression can be categorised into two classes: antibodies that recognise epitopes located in the cytoplasmic domain, encoded by non-variant sequences and therefore detecting all forms of CD44 with no distinction between CD44s and CD44v proteins (pan-CD44 antibodies), and those that are specific for particular CD44v proteins and allow a distinction both between different CD44v proteins and between CD44v and CD44s (Ponta et al., 1998).

Expression of CD44 in normal head and neck tissues

Immunohistochemical staining using monoclonal antibodies for CD44s and the product of variant exons,

Table 1. Expression of CD44 and its splice variants in neoplastic head and neck tissues.

CD44	TISSUE	METHOD	EXPRESSION AND COMMENTS	REFERENCES
CD44	13 oral SCC (tongue, tonsil, antrum)	Im, FS, PE pan-CD44	Strong to moderate in 12/13	
v3		(3G5)	Reduced in 13/13 with total loss in all poorly-differentiated tumours and in 50% of moderate and well-differentiated	Hudson et al., 1996
v4-5		3D2	Absent or very weak in 7/13	
v6		2F10	Absent or very weak in 5/13	
v8		1E8	Absent or very weak in 9/13	
CD44s	38 primary tongue cancers (T1/T2N0)	Im, PE pan-CD44	Decreased, particularly in the group with late nodal metastases	Masuda et al., 2000
v3 v4-5 v6	56 SCC of the border of the tongue	Im BBA11 BBA25 BBA13	Downregulation of v3 in 37.5% of cases, of v4-5 in 67.9% of cases and of v6 in 33.9% of cases which correlated with cell differentiation, tumour grade and invasion	Fonseca et al., 2001
v4-5	11 primary oral SCCs without metastases 9 primary carcinomas with 19 metastases	Im, FS, PE 3D2	Loss of CD44v4-5 in all but more marked in metastases. No correlation with behaviour or grade.	Oliveira et al., 1998
v5 v6 v7 v8 v10	55 oral SCCs and 29 lymph metastases from tongue (12), oral cavity (5), pharynx (35), larynx (31)	Im, FS VFF8 VFF7 VFF9 VFF17 VFF16	Reduction of CD44v7, v8 and v10 but not of v5 and v6. Total loss of v7, v8 and v10 in lymph node metastases	Herold-Mende et al., 1996
v5 v6 v7-8	62 oral SCCs	Im Anti-splice variant	Reduction of CD44v7-8 in 22/62 (35.5%) which correlated negatively with shorter survival time	Kuo et al., 1998
v6	100 oral SCCs	Im, FS 2F10 or Var3.1	Reduction or loss of CD44v6 which correlated with tumour cell differentiation and increased frequency of regional lymph node metastases	Bahar et al., 1997 Kunishi et al., 1997 Soukka et al., 1997
v4 v5 v6 v7 v9	99 primary oral or oropharyngeal SCCs	Im, PE Anti-splice variant mAbs	Reduction of one or more isoforms in 39/99 (39.4%). Reduction of v7 and v9 correlated negatively with shorter survival and recurrence-free interval	Stoll et al., 1998 Stoll et al., 1999
v9	40 primary oral SCC	Im Anti-v9	Reduction of v9 in 19/40 (47.5%) cases which correlated with tumour cell differentiation, primary and secondary metastasis to lymph node	Ue et al., 1998
CD44s v2 v6	89 head and neck SCCs	Im, PE	No change in CD44s and v6. Down-regulation of v2 correlated with poor tumour cell differentiation and shorter survival time	Kanke et al., 2000
v6 CD44 With intron9	9 oral SCCs	RT-PCR/ Southern blot	Increase of v6 and CD44/intron 9 but no correlation with tumour stage or metastasis	Higashikawa et al., 1996
v4 v5 v6 v9	100 oral SCCs	Im, PE	No change in v5 and v6 but decrease of v4 and v9	Piffko et al., 1996 Piffko et al., 1999
v6	277 HNSCC	Im U36, U39 VFF18	No, or marginal downregulation of CD44v6 in 268/277 tumours	Van Hal et al., 1999
CD44s	Sinonasal inverted papillomas (SIPs) and associated SCCs	Im, PE A3D8	76 SIPs expressed CD44s, 2SIPs with SCC in situ showed strong expression No expression in 6/10 (60%) SIPs With SCC and weak expression in 4/10 (40%) SIPs with SCC	Ingle et al., 1998
CD44s v6	70 LSCCs	Im 2C5 2F10	Decrease of CD44s and v6 correlated with an increase in metastasis and a decrease in survival	Spafford et al., 1996
v3	12 LSCCs	Im	Reduction of v3 irrespective of TNM stage	Repassy et al., 1998
v5 v6	28 LSCCs without metastases and 26 LSCCs with metastases	Im, PE VFF8 VFF7	Reduction of v5 and v6 in the inner proliferative tumour area, but no correlation to metastatic ability	Ostwald et al., 1997
CD44	27 dysplastic laryngeal epithelium 172 LSCCs	Im, PE Hermes 3	No change in dysplastic epithelium compared to normal. Focal reduction in LSCCs. Loss of CD44 was associated with poor differentiation, increased mitotic index and nodal or metastatic spread	Hirvikoski et al., 1999
CD44	66 LSCCs 67 keratosis 97 carcinomas	Im, PE pan-CD44	Progressive overexpression of CD44 in laryngeal carcinogenesis	Sugar et al., 1997
CD44	34 LSCCs 13 in situ carcinomas 35 dysplastic tissue 10 papillomas 17 keratosis	Im, PE pan-CD44	Progressive overexpression of CD44 in laryngeal carcinogenesis	Ioachim et al., 1999

Im, immunohistochemistry; RT-PCR, reverse transcription-polymerase chain reaction;
SCCs, squamous cell carcinoma; LSCCs, laryngeal SCCs; FS, frozen tissue sections; PE, paraffin-embedded tissues

of frozen and formalin-fixed paraffin-embedded sections has demonstrated their expression in normal oral epithelium. Expression of CD44v4, v6 and v9 has been reported in tonsillar epithelium (Salmi et al., 1993; MacKay et al., 1994) and of CD44v5, v6, v7, v7-8 and v10 in oral and pharyngeal epithelia using frozen tissue (Herold-Mende et al., 1996). Similarly, immunohistochemical staining of CD44s and its splice variants v3, v4-5, v6 and v9 of frozen and formalin-fixed paraffin-embedded sections of lip vermilion border, buccal mucosa, dorsum and ventrum of tongue, floor of mouth, gingiva and hard palate showed that all epithelia stained strongly for all variants in basal, suprabasal and pickle cells, but cornified and surface layers and the basal surface of basal cells were all negative. Despite the structural heterogeneity within oral epithelium, no regional variation was detected (Oliveira and Odell, 1997). The physiological expression of various CD44 isoforms such as CD44v4, v5, v6, v7, v7-8, v8 and v9 in oral squamous cell epithelium has also been confirmed by others by immmunohistochemical staining and immunofluorescence using variant isoform-specific

Table 2. Summary of the expression of CD44 and its splice variants in neoplastic head and neck tissues.

CD44	TISSUE	METHOD	EXPRESSION AND COMMENTS	REFERENCES
CD44	Oral SCC	Im	Strong to moderate in 12/13	Hudson et al., 1996
CD44s		Im	Decrease in 38/38 cases No change in 89/89 cases	Masuda et al., 2000 Kanke et al., 2000
v2		Im	Reduction in 89/89 cases	Kanke et al., 2000
v3		Im	Reduced in 34/69 cases	Hudson et al., 1996; Fonseca et al., 2001
v4		Im	Reduction in most of 199 cases	Piffko et al., 1996; Stoll et al., 1998, 1999
v4-5		Im	Reduction in 65/89 cases and in 19/19 metastases	Hudson et al., 1996; Fonseca et al., 2001; Kanke et al., 2000
v5		Im	No change in 200 oral SCCs and in 29 lymph metastases	Herold-Mende et al., 1996; Piffko et al., 1996, 1999; Kuo et al., 1998
v6		Im	Reduction in 163/268 cases	Hudson et al., 1996; Bahar et al., 1997; Kunichi et al., 1997; Stoll et al., 1998, 1999
		Im	No change in 306 cases and 29 metastases	Herold-Mende et al., 1996; Piffko et al., 1996, 1999; Kuo et al., 1998; Kanke et al., 2000
		RT-PCR	Increase in 9/9 cases	Higashikawa et al., 1996
v7		Im	Reduction or loss in 94/154 cases and in 29 metastases	Herold-Mende et al.,1996; Stoll et al., 1999
v7-8		Im	Reduction in 22/62 cases	Kuo et al., 1998
v8		Im	Reduction in 64/68 cases, loss in 29 metastases	Herold-Mende et al., 1996; Hudson et al., 1996
v9		Im	Reduction in 152/239 cases	Piffko et al., 1996, 1999; Ue et al., 1998, 1998, 1999
v10		Im	Reduction in 55/55 cases, loss in 29 metastases	Herold-Mende et al., 1996
CD44	LSCC	Im	Progressive overexpression of CD44 (100 LSCCs, 84 keratosis, 10 papillomas, 35 dysplastic tissue and 110 carcinomas using pan-CD44)	Ioachim et al., 1999; Sugar et al., 1997
			No change in 27 dysplastic tissues. Focal reduction in 172 LSCCs associated with poor differentiation	Hirvikoski et al., 1999
CD44s		Im	Reduction in 70 LSCCs correlated with increased metastases and decreased survival	Spafford et al., 1996
v3		Im	Reduction in 12/12	Repassy et al., 1998
v5		Im	Reduction in 54 cases. No correlation with metastases	Ostwald et al., 1997
v6		Im	Reduction in 124 cases	Spafford et al., 1996; Ostwald et al., 1997

Im, immunohistochemistry; RT-PCR, reverse transcription-polymerase chain reaction; SCCs, squamous cell carcinoma; LSCCs, laryngeal SCCs.

monoclonal antibodies or molecular biological techniques such as RT-PCR (Salmi et al., 1993; Hudson et al., 1996; Piffko et al., 1996; Bahar et al., 1997; Kunishi et al., 1997; Soukka et al., 1997; Kuo et al., 1998; Ue et al., 1998; Stoll et al., 1998, 1999; Piffko et al., 1999). Expression of CD44s and CD44v6 has also been detected in normal laryngeal epithelium by immunohistochemical staining using monoclonal antibodies (Spafford et al., 1996; Hirvikoski et al., 1999; Ioachim et al., 1999; Stoll et al., 1999). RT-PCR/Southern blot techniques utilising DNA probes for CD44 variable region exons 11 (v6) to 14 (v9) and also for intronic sequences examined the expression of aberrant CD44 transcripts in several normal head and neck tissues (Higashikawa et al., 1996). In these studies strong expression of CD44s and CD44-variant isoforms such as CD44v6, v7, v8, v7-8, v8-9 and v7-8-9 was detected in gingiva, tongue and larynx and moderate expression in parotid and submandibular glands (Higashikawa et al., 1996). Using exon 9-10 (v4-5) containing the intron 9 and an intron 9-specific probe showed that expression of CD44 variants was restricted to squamous epithelia and in salivary gland. In summary, these studies show that normal oral, pharyngeal and laryngeal epithelium express high levels of CD44s, and multiple CD44-variant isoforms.

Expression of CD44 in head and neck tumours

Numerous studies have provided evidence for a role of CD44 in the progression and metastasis of several tumour tissues of mesenchymal and epithelial origin. Changes in the abundance or isoform composition of CD44 appear to be involved in neoplastic conversion or metastasis in a number of different tissues and cell culture models (Naot et al., 1997; Sy et al., 1997; Bourguignon et al., 1998; Goodison and Tarin, 1998a,b; Goodison et al., 1998, 1999; Herrlich et al., 1998; Knudson, 1998; Koukoulis et al., 1998; Kahara et al., 2000; Ylagan et al., 2000) including head and neck tumours (Korabiowska et al., 1995; Stoll et al., 1998, 1999). These studies employing immonohistochemical staining, RT-PCR and Northern blotting, have confirmed that in many types of common cancers, including breast, lung, gastrointestinal, bladder, cervical and lymphoid tissue tumours, there is overexpression of CD44s and of certain variant isoforms and aberrant CD44 mRNA processing in tumour tissues relative to non-neoplastic control tissues (Goodison and Tarin 1998a,b). Such expression is not seen in every cancer cell but is limited to tumour cells and is heterogeneous throughout the tumour. In contrast to these malignancies, neuroblastomas exhibit reduced or loss of CD44 variant isoform expression (Shtivelman and Bishop, 1991; Gross et al., 1994).

Early studies showed that in contrast to many other malignancies, expression of CD44v6 was down-regulated in squamous cell carcinomas, as detected by immunohistochemistry using mAb Var 3.1, but it was not possible to correlate it to any clinical parameters in the small and heterogeneous group containing both recurrent and primary carcinomas (Salmi et al., 1993). Since then, several studies have analyzed the expression of CD44-variant isoforms in squamous cell carcinomas of head and neck in an attempt to correlate it with clinicopathological parameters and prognosis (Tables 1, 2).

Immunohistochemical staining employing monoclonal antibodies reacting with an epitope present on all forms of CD44 (mAb E1/2) or recognising specific splice variants was used to detect the expression of CD44 in 13 oral squamous cell carcinomas derived from the tongue, tonsil and antrum (Hudson et al., 1996). Staining with E1/2 showed a strong or moderate expression of CD44 in 12/13 oral SCCs, consistent with the findings by employing pan-CD44 antibodies. However, staining with an antibody (mAb 3G5) to v3 domain showed reduced intensity in all 13 samples, with total loss of expression in all poorly differentiated tumours and in 50% of the moderate and well-differentiated ones. Using several other monoclonal antibodies to specific splice variants including v4-5 (mAb 3D2), v6 (mAb 2F10) and v8 (mAb 1E8) domains, showed that all the tumour samples showed reduced expression of at least one variant exon, with v3 loss being more frequent; staining was absent or very weak in 7/13 samples for v4-5, 5/13 samples for v6 and 9/13 samples for v8, with loss being more common in poorly differentiated tumours (Hudson et al., 1996). Immunohistochemical evaluation of the expression of CD44s in formalin-fixed, paraffin-embedded specimens of 38 cases of primary T1/T2N0 tongue cancers showed a decreased expression of CD44s, particularly in the group that had late nodal metastases (Masuda et al., 2000). Assessment of the level of expression of CD44 isoforms v3 (BBA11), v4-5 (BBA25) and v6 (BBA13) by immunohistochemical staining of 56 consecutive case of squamous cell carcinomas of the border of the tongue showed that CD44v3 was downregulated in 37.5% of the cases, v4-5 in 67.9% and v6 in 33.9% and down-regulation of these variant isoforms correlated with cell differentiation, tumour grade and the pattern of neoplastic invasion (Fonseca et al., 2001). Loss of expression of CD44v4-5 as determined using mAb 3D2, was also observed in most of the 11 primary oral SCCs without metastases and of the 9 primary carcinomas with 19 matched metastases examined. Loss of expression was more marked in metastases but there was no correlation between expression and behaviour or grade (Oliveira et al., 1998). In a similar study using frozen tissue sections, it was shown that expression of CD44v7 (mAb VFF9), v8 (mAb VFF17) and v10 (mAb VFF16), but not CD44v5 (mAb VFF8) and v6 (mAb VFF7) was significantly reduced in 55 oral SCCs and lost in 29 lymph node metastases, derived from tumours of the tongue (12), the oral cavity (5), the pharynx (35) and the larynx (31) (Herold-Mende et al., 1996). Expression of CD44s and CD44 variant isoforms (CD44v5, v6, v7-8)

examined in 62 samples of oral SCCs by immunohistochemical analysis showed a significant reduction in the expression of CD44v7-8 (22/62, 35.5%) which correlated negatively with survival time of the patients (Kuo et al., 1998). The expression of CD44v6 using mAb 2F10 or Var3.1 has also been examined by several research groups in over 100 tissue samples of oral squamous cell carcinomas by immunohistochemical staining and shown to be dramatically reduced or lost in tumour cells (Bahar et al., 1997; Kunishi et al., 1997; Soukka et al., 1997) and down-regulation of CD44v6 correlated with the degree of tumour cell differentiation (Bahar et al., 1997; Kunishi et al., 1997; Soukka et al., 1997) and an increased frequency of regional lymph node metastasis (Piffko et al., 1996). In 39 out of 99 cases of primary oral or oropharyngeal SCC (39.4%), the expression of one or more CD44 isoforms examined (CD44v4, v5, v6, v7 and v9) was decreased in the tumour cells as determined by immunohistochemical staining of paraffin-embedded tumour samples using variant isoform-specific monoclonal antibodies (Stoll et al., 1999). A decreased expression of the isoforms v4, v5 and v6 was found only in a few cases so that a significant correlation to any other parameter was not expected when considered separately. More often, a diminution for the isoforms CD44v7 and v9 was observed which correlated negatively with a shorter survival time as well as a shorter recurrence-free interval (Stoll et al., 1998, 1999). The expression of CD44v9 was found to be reduced in 19/40 (47.5%) primary oral SCCs and this correlated with tumour cell differentiation and primary and secondary metastasis to lymph nodes, suggesting a role of CD44v9 in lymphatic metastasis (Ue et al., 1998). A recent study evaluated the expression of CD44s and splice variants CD44v2 and v6 by immunohistochemistry in paraffin-embedded tissues of 89 head and neck squamous cell carcinomas (Kanke et al., 2000). No change in CD44s or CD44v6 expression was observed in cancer tissues, but a significant correlation was found between the down-regulation of CD44v2 and poorer differentiation of the tumour cells as well as with shorter overall survival (Kanke et al., 2000). Collectively, these studies showed that reduced or loss of expression of one or more of the variant exons of CD44 may be used as biological markers during malignant transformation of oral squamous cell epithelium. Investigation of the expression of CD44v5 and v6 isoforms during development and progression of oral squamous cell carcinomas in formalin-fixed and paraffin-embedded tissues of 100 oral carcinomas by immunohistochemical staining using splice-variant isoform-specific monoclonal antibodies, showed that both variant isoforms were strongly expressed in normal and dysplastic mucosa and all primary and metastatic SCCs suggesting that CD44v5 and v6 expression is not altered during development and progression of oral carcinomas (Piffko et al., 1996). However, the expression of CD44v4- and CD449-variant isoforms was significantly decreased compared to normal oral epithelium (Piffko et al., 1999).

In contrast to these studies, investigation of the expression of CD44 in only 9 oral squamous cell carcinoma cases using RT-PCR/Southern blotting, showed that all the tumour tissues overexpressed high molecular weight CD44 variants, compared to their corresponding normal stratified squamous epithelium, and in particular CD44v6 and CD44 variants containing the intron 9 sequence of CD44, but no correlation with tumour stage or metastasis was found (Higashikawa et al., 1996). Mab U36, which recognizes CD44v6, stained more than 50% of the cells intensely in 188 of 196 (96%) head and neck SCC biopsies (Van Hal et al., 1996). The expression of CD44v6 was examined qualitatively and quantitatively in normal oral mucosa and compared to that in a large panel of head and neck squamous cell carcinomas (277 tumours) and cell lines by immunohistochemistry using three different anti-CD44v6 antibodies (U36, U39 and VFF18) and by RT-PCR using CD44v6 splice-variant-specific primers on head and neck SCC cell lines, microdissected normal mucosa and primary as well as metastatic head and neck SCC tissues (Van Hal et al., 1999). The results showed that there was no, or only marginal, down-regulation of CD44v6 in head and neck SCCs, as detected by immunohistochemistry and the CD44v6 splice variants present in the head and neck primary tumours and metastases were identical to those expressed in normal mucosa, indicating that CD44v6 does not play a role in malignant progression of head and neck SCCs (Van Hal et al., 1999).

CD44 expression has also been studied in sinonasal inverted papillomas (SIP) and associated squamous cell carcinoma by automated immunohistochemistry on paraffin-embedded tissue sections using mAb A3D8 which reacts with human CD44s (Ingle et al., 1998). All 76 SIPs expressed CD44, 2 SIPs with SCC in situ maintained strong expression in benign and severely dysplastic foci and 6 of 10 SIPs (60%) with SCC showed complete loss of CD44s expression, while the remaining 4 cases of SIPs with SCC (40%) showed weak expression. Thus, in SIPs developing an invasive SCC, CD44s expression is lost or dramatically reduced (Ingle et al., 1998). Examination of 20 cases of non-cancerous nasopharyngeal mucosa and 80 cases of nasopharyngeal carcinoma (NPC) by immunohistochemistry showed no significant difference in the rate of positive expression of CD44s, v3 and v6 proteins between non-cancerous nasopharyngeal mucosa and NPC or in NPC with and without lymph node metastasis, indicating that these gene products did not correlate with lymph node metastasis in NPC (Huang et al., 2000).

CD44 expression has also been implicated in laryngeal carcinogenesis. Early studies examining the expression of CD44s and CD44 splice-variant v6 by immunohistochemistry using mAb 2C5 and mAb 2F10, respectively, in paraffin-embedded archival tissue of 70 patients with laryngeal squamous cell carcinoma (LSCC) revealed a decreased expression of both CD44s and

CD44v6 which correlated with an increase in metastasis and a decreased survival (Spafford et al., 1996). Analysis of 12 laryngeal SCCs (7 laryngeal and 5 hypopharyngeal cancers) by immunohistochemistry for the expression of CD44v6/v3 exhibited a reduced CD44v3 expression, irrespective of the TNM stages (Repassy et al., 1998). Immunohistochemical staining of paraffin-embedded sections of 28 laryngeal carcinomas without metastases, 26 with metastases and 20 lymph node metastases from larynx carcinomas with anti-CD44v5 (VFF8) and v6 (VFF7) monoclonal antibodies showed that in all cases the same staining intensity was observed, suggesting that CD44v5 and v6 may be valuable markers of proliferation but not of metastatic behaviour (Ostwald et al., 1997). Further, there was no difference between carcinomas with and without metastases or the lymph node metastases but a strong difference in staining was detected between the carcinoma cells of the outer and the inner proliferative tumour areas. Whereas the former cells stained very intensely, the latter showed a dramatic reduction in intensity or no staining at all. These results indicated a role of CD44v5 and v6 in laryngeal carcinogenesis but no connection to metastatic ability could be established (Ostwald et al., 1997). Irregular expression of hyaluronan and its CD44 receptor was associated with metastatic phenotype in laryngeal SCC (Hirvikoski et al., 1999). Immunohistochemical analysis for the expression of CD44 on paraffin-embedded tissue sections of 27 dysplastic samples of laryngeal epithelium and 172 LSCCs showed that hyaluronan (HA) and CD44 (Hermes 3) were expressed in 90% of the dysplastic and neoplastic samples as compared to normal laryngeal epithelium. However, while in the normal epithelium their distributions were homogeneous, malignant transformation was associated with focal reductions of both HA and its receptor. Moreover, the loss of HA and CD44 in LSCC was associated with poor differentiation and increased mitotic index and nodal or metastatic spread. The focal decrease in HA and CD44 staining detected in more that a quarter of the LSCC, most prevalent in poorly differentiated tumours, also occurred in dysplasias, suggesting that it may be a common and often early event in the set properties that LSCC adopts during its development (Hirvikoski et al., 1999).

CD44 functions as a 'part time' proteoglycan expressing a chondroitin sulfate or heparan sulfate glycosaminoglycan side chain (Jackson et al., 1995; Piepkorn et al., 1997; Sleeman et al., 1997, 1998; Chiu et al., 1999). Further support for reduction or loss of CD44 during laryngeal carcinogenesis has been provided by immunohistochemical analyses of Chondroitin-6-sulfate chains of normal and tumour samples of laryngeal epithelium. Immunohistochemical staining for chondroitin-6-sulfate on paraffin-embedded sections showed positive immunoreactivity in the cell/basement membrane of basal and suprabasal cells of the laryngeal epithelium. Focal immunostaining was observed in 67 out of 71 (94%) biopsies with no dysplasia, in 39/45 (87%) biopsies with mild/moderate dysplasia, and in 16/16 (100%) biopsies with severe dysplasia or carcinoma in situ. However, it was seen in only 2 out of 18 biopsies with invasive squamous cell carcinoma of the larynx, although in neither of these was chondroitin-6-sulfate immunostaining seen at the actual site of invasion (Uhlman and Niehans, 1999). The loss of chondroitin-6-sulfate immunostaining concurrent with squamous cell carcinoma invasion in the larynx suggests that loss of a chondroitin-6-sulfate-containing proteoglycan or a change in proteoglycan side-chain composition, is a critical step in laryngeal epithelial tumour invasion.

In contrast, immunohistochemical staining of 66 cases of paraffin-embedded adult-onset squamous cell papilloma, 67 cases of keratosis and 97 carcinomas employing monoclonal pan-CD44 antibodies showed a progressive overexpression of CD44 at successive stages of human laryngeal carcinogenesis (Sugar et al., 1997). Similarly, examination of CD44 expression using pan-CD44 antibodies in 34 formalin-fixed, paraffin-embedded squamous cell carcinomas, 13 in situ carcinomas, 35 cases with various degrees of epithelial dysplasia, 10 papillomas and 17 cases of keratosis confirmed the progressive CD44 overexpression in laryngeal carcinogenesis (Ioachim et al., 1999). However, as mentioned above, pan-CD44 antibodies cannot differentiate between CD44s and the CD44 variant isoforms, hence these studies investigated the expression of all forms of CD44 during laryngeal tumour progression. Similarly, it was shown that expression of CD44s and CD44v6 in 32 samples of pharynx/larynx squamous cell carcinomas and their lymph node metastases, studied by immunohistochemistry, it was essentially unaltered in relation to their primary sites (Hernandez Gaspar et al., 1999).

Clinical implications of altered CD44 expression in head and neck squamous cell carcinoma

Studies on early premalignant lesions and on early-stage malignancies of several types of common tumours, such as breast, bladder and colon, have reported increased CD44 isoform expression and aberrant CD44 transcript processing, but also a marked heterogeneity in the pattern of expression within the tumour. These specific alterations in CD44 expression become clear and distinct with tumour progression, with higher expression levels achieved in invasive and metastatic tumour cells. However, observations on deeply invading late-stage tumours, there is a dramatic reduction or no expression of any CD44 isoform, as the tumour cells reach the boundaries of the organ or penetrate surrounding tissues. Contradictory reports on whether CD44 expression in a given tumour is elevated or decreased may have resulted from lack of accurate information on the staging of the tumours used and the method and reagents of analyses (Goodison and Tarin, 1998a,b; Martegani et al., 1999). Several mechanisms, based on the properties of CD44 as the major hyaluronan

receptor and as a signal transmitter and growth-presenting molecule, have been proposed to explain the role of elevated CD44 expression during tumour development and progression (Goodison and Tarin , 1998a; Knudson, 1998; Ponta et al., 1998).

Normal squamous epithelium of the head and neck express CD44s and several splice-variant isoforms at high levels. Although the different CD44 isoforms appear to modulate ligand-binding and cell-cell and cell-matrix interactions (Bourguignon et al., 1998; Hamada et al., 1998; Knudson, 1998; Lesley and Hyman, 1998) their functions in normal head and neck squamous epithelium is poorly understood. However, the concentration of CD44 along cellular boundaries *in vivo* suggests that at least certain isoforms mediate homotypic cell interactions within the epithelium. Reduction in the expression of CD44 in squamous cell carcinomas of the head and neck may have important functional consequences to tumour cell proliferation and migration. Since CD44 acts as cell adhesion and growth-factor binding molecule, down-regulation of its expression would decrease the homotypic cell interactions and allow enhanced binding of the growth factor with its specific receptor, thereby increasing the malignant properties of tumour cells such as progressive growth, invasion and metastasis.

Several monoclonal antibodies have been developed and characterised as candidates for radioimmunotherapy (RIT) of squamous cell carcinomas and these include VFF18, specific for an epitope encoded by the human CD44v6 (Heider et al., 1996), BIWA1 (Heider et al., 1995) and U36 (Schrijvers et al., 1993; De Bree et al., 1995; Van Hal et al., 1996) which are both CD44v6-specific but bind to different epitopes. U36 binds to an epitope consisting of amino acids 365-376 and BIWA1 to an epitope consisting of amino acids 360-370 and with a 35-fold higher affinity (Van Hal et al., 1996; Stroomer et al., 2000). A phase I RIT study of Rhenium-186 (^{186}Re)-labeled chimeric monoclonal antibody U36 was conducted in 13 patients with recurrent or metastatic head and neck SCCs (Colnot et al., 2000). In this carefully designed trial, some antitumour effects were seen. Two patients with dose-limiting myelotoxicity showed a marked reduction in tumour size and one patient showed stable disease for 6 months after treatment. Although these results provide encouragenment for the approach, the use of RIT using anti-CD44 monoclonal antibodies as adjuvant therapy in patients with head and neck SCCs remains questionable for several reasons (reviewed in Breitz, 1999), among which is the antigen expression by the tumour cells. If antigen expression is not evident or greatly reduced, then the use of RIT will not be beneficial to the patient. Although more experimental evidence is required, particularly on laryngeal carcinogenesis, collectively, the few above studies described in this review, using several different high affinity monoclonal antibodies to CD44 isoforms on paraffin-embedded or frozen tissue sections show that CD44s and one or more of its splice variants are downregulated during head and neck carcinogenesis (Tables 1 and 2). The focal reduction of these cell adhesion molecules, particularly in the inner proliferative mass of the tumour as detected, mainly, by immunohistochemical studies, and the fact that knock-out experiments do not give rise to spontaneous tumours in the transgenic mice (Schmits et al., 1997; Protin et al., 1999), but that CD44-negative cells isolated from these transgenic animals are more susceptible to tumourigenesis by activated oncogenes (Schmits et al., 1997), points towards the use of CD44s and one or more of its isoforms as a prognosis marker in head and neck carcinogenesis.

References

Bahar R., Kunishi M., Kayada Y. and Yoshiga K. (1997). CD44 variant 6 (CD44v6) expression as a progression marker in benign, premalignant and malignant oral epithelial tissues. Int. J. Oral Maxillofac. Surg. 26, 443-446.

Bloor B.K., Jelvagharan M., White K.N. and Odel E.W. (2001). Characterization of CD44 splicing patterns in normal keratinocytes, dysplastic and squamous carcinoma cell lines. Int. J. Oncol. 18, 1053-1058.

Bourguignon L.Y.W. (2001). CD44-mediated oncogenic signaling and cytoskeleton activation during mammary tumour progression. J. Mammary Gland Biol. Neoplasia 6, 287-297.

Bourguignon L.Y.W., Zhu H., Chu A., Iida N., Zhang L. and Hung M.C. (1997). Interaction between the adhesion receptor CD44 and the oncogene product, p185H^{HER2}, promotes human ovarian tumour cell activation. J. Biol. Chem. 272, 27913-27918.

Bourguignon L.Y.W., Zhu D. and Zhu H. (1998). CD44 isoform-cytoskeleton interaction in oncogenic signalling and tumour progression. Front. Biosci. 3, d637-d649.

Breitz H.B. (2000). How far have we come with solid (nonhematologic) tumor radioimmunotherapy? J. Nucl. Med. 41, 2011-2013.

Caballero O.L., de Souza S.J., Bretani R.R. and Simpson A.J. (2001). Alternative spliced transcripts as cancer markers. Dis. Markers 17, 67-75.

Chiu R.K., Droll A., Dougherty S.T., Carpenito S., Cooper D.L. and Dougherty G.J. (1999). Alternatively spliced CD44 isoforms containing exon v10 promote cellular adhesionthrough the recognition of chondroitin sulfate-modified CD44. Exp. Cell. Res. 248, 314-321.

Colnot D.R., Quak J.J., Roos J.C., van Lingen A., Wilhelm A.J., van Kamp G.J., Huijgens P.C., Snow G.B. and van Dongen G.A.M.S. (2000). Phase I therapy study of ^{186}Re-labelled chimeric monoclonal antibody U36 in patients with squamous cell carcinoma of the head and neck. J. Nucl. Med 41, 1999-2010.

De Bree R., Roos J.C., Quak J.J., Den Hollander W., Snow G.B. and van Dongen G.A.M.S. (1995). Radioimmunoscintigraphy and biodistribution of technicium-99m-labelled monoclonal antibody U36 in patients with head and neck cancer. Clin. Cancer Res. 1, 591-598.

Fonseca I., Pereira T., Rosa-Santos J. and Soares J. (2001). Expression of CD44 isoforms in squamous cell carcinoma of the border of the tongue: A correlation with histological grade, pattern of stromal invasion and cell differentiation. J. Surg. Oncol. 76, 115-120.

Gao A.C., Lou W., Dong J.T. and Isaacs J.T. (1997). CD44 is a

metastasis suppressor gene for prostatic cancer located on human chromosome 11p13. Cancer Res. 57, 846-849.

Goodfellow P.N., Banting G., Wiles M.V., Tunnacliffe A., Parkar M., Solomon E., Dalchau R. and Fabre J.W. (1982). The gene, MIC4, which controls expression of the antigen defined by monoclonal antibody F10.44.2, is on human chromosome 11. Eur. J. Immunol. 12, 659-663.

Goodison S. and Tarin D. (1998a). Clinical implications of anomalous CD44 gene expression in neoplasia. Front. Biosci. 3, e89-e109.

Goodison S. and Tarin D. (1998b). Current status of CD44 variant isoforms as cancer diagnostic markers. Histopathology 32, 1-6.

Goodison S., Yoshida K., Churchman M. and Tarin D. (1998). Multiple intron retention occurs in tumour cell CD44 mRNA processing. Am. J. Pathol. 153, 1221-1228.

Goodison S., Urquidi V. and Tarin D. (1999). CD44 cell adhesion molecules. Mol. Pathol. 42, 189-196.

Gross N., Beretta C., Peruisseau G., Jackson D., Simmons D. and Beck D. (1994). CD44H expression by human neuroblastoma cells: relation to MYCN amplification and lineage differentiation. Cancer Res. 54, 4238-4242.

Guenthert U., Hofmann M., Rudy W., Reber S., Zoller M., Haussmann I.M., Matzku S., Wenzel A., Ponta H. and Herrlich P. (1991). A new variant of glycoprotein C44 confers metastatic potential to rat carcinoma cells. Cell 65, 13-24.

Hale L.P., Patel D.D., Clark R.E. and Haynes B.F. (1995). Distribution od CD44 variant isoforms in human skin: differential expression in components of benign and malignant epithelia. J. Cutan. Pathol. 22, 536-554.

Hamada J.I., Sawamura Y. and Van Meir E.G. (1998). CD44 expression and growth factors. Front. Biosci. 3, d657-d664.

Harada N., Mizoi T., Kinouchi M., Ishii S., Shiiba K. and Matsuno S. (2001). Introduction of antisense CD44s cDNA down-regulates expression of overall CD44 isoforms and inhibits tumour growth and metastasis in highly metastatic colon carcinoma cells. Int. J. Cancer 91, 67-75.

Heider K-H., Mulder J-W.R., Ostermann E., Susani S., Patzelt E., Pals S.T. and Adolf G.R. (1995). Splice variants of the cell surface glycoprotein CD44 associated with metastatic tumour cells are expressed in normal tissues of humans and cynomolgus monkeys. Eur. J. Cancer 31A, 2385-2391.

Heider K-H., Sproll M., Susani S., Patzelt E., Beaumier P., Ostermann E., Ahorn H. and Adolf G.R. (1996). Characterization of a high-affinity monoclonal antibody specific for CD44v6 as candidate for immunotherapy of squamous cell carcinomas. Cancer Immunol. Immunother. 43, 245-253.

Hernandez Gaspar G., de los Toyos J.R., Alvarez Marcos C., Riera J.R. and Sampedro A. (1999). Quantitative immunohistochemical analyses of the expression of E-cadherin, thrombomodulin, CD44H and CD44v6 in primary tumours of pharynx/larynx squamous cell carcinoma and their lymph node. Anal. Cell. Pathol. 18, 183-190.

Herold-Mende C., Seiter S., Borno A., Patzelt E., Schupp M., Zoller J., Bosch F.X. and Zoller M. (1996). Expression of CD44 splice variants in squamous epithelia and squamous carcinomas of the head and neck. J. Pathol. 179, 66-73.

Herrlich P., Morrison H., Sleeman J., Orian-Rousseau V, Kinig H., Weg-Remers S. and Ponta H. (2000). CD44 both acts as growth- and invasiveness-promoting molecule and as a tumour suppressing co-factor. Ann. NY Acad. Sci. 910, 106-120.

Herrlich P., Zoller M., Pals S.T. and Ponta H. (1993). CD44 splice variants: metastases meet lymphocytes. Immunol. Today 14, 395-399.

Herrlich P., Sleeman J., Wainwright D., Konig H., Sherman L., Hilberg F. and Ponta H. (1998). How tumour cells make use of CD44. Cell. Adhes. Commun. 6, 141-147.

Higashikawa K., Yokozaki H., Ue T., Taniyama K., Ishikawa T., Tarin D. and Tahara E. (1996). Evaluation of CD44 transcription variants in human digestive tract carcinomas and normal tissues. Int. J. Cancer 66, 11-17.

Hirvikoski P., Tammi R., Kumpulainen E., Virtaniemi J., Parkkinen J.J., Tammi. M., Johanson R., Agren U., Karhunen J. and Kosma V.M. (1999). Irregular expression of hyaluronan and its CD44 receptor is associated with metastatic phenotype in laryngeal squamous cell carcinoma. Virchows Arch. 434, 37-44.

Hofmann M., Rudy W., Gunthert U., Zimmer S.G., Zawadzki V., Zoller M., Lichtner R.B., Herrlich P. and Ponta H. (1993). A link between ras and metastatic behaviour of tumour cells: ras induces CD44 promoter activity and leads to low level expression of metastasis-specific variants of CD44 in CREF cell. Cancer Res. 53, 1516-1521.

Huang G.W., Mo W.N., Kuang G.Q., Nong M.Y., Sunagawa M. and Kosugi T. (2001). Expression of p16, nm23-H1, E-cadherin and CD44 gene products and their significance in nasopharyngeal carcinoma. Laryngoscope 111, 1465-1471.

Hudson D.L., Speight P.M. and Watt F.M. (1996). Altered expression of CD44 isoforms in squamous cell carcinomas and cell lines derived from them. Int. J. Cancer 66, 457-463.

Ingle R., Jennings T.A., Goodman M.L., Pilch B.Z., Bergman S. and Ross J.S. (1998). CD44 expression in sinonasal inverted papillomas and associated squamous cell carcinoma. Am. J. Clin. Pathol. 109, 309-314.

Ioachim E., Assimakopoulos D., Goussia A.C., Peschos D., Skevas A. and Agnantis N.J. (1999). Glycoprotein CD44 expression in benign, premalignant and malignant epithelial lesions of the latynx: An immunohistochemical study including correlation with Rb, p53, Ki-67 and PCNA. Histol. Histopathol. 14, 1113-1118.

Jackson D.G., Bell J.I., Dickinson R., Timans J., Shields J. and Whittle N. (1995). Proteoglycan forms of the lymphocyte homing receptor CD44 are alternatively spliced variants containing the v3 exon. J. Cell. Biol. 128, 673-685.

Jamal H.H., Cano-Gauci D.F., Buick R.N., and Filmus J. (1994). Activated ras and src induce CD44 overexpression in rat intestinal epithelial cells. Oncogene 9, 417-423.

Kahara N., Ozaki T., Doi T., Nishida K., Kawai A., Shibahara M. and Inoue H. (2000). CD44 in soft tissue sarcomas. Virchows Arch. 436, 574-578.

Kanke M., Fujii M., Kameyama K., Kanzaki J., Tokumaru Y., Imanishi Y., Tomita T. and Matsumura Y. (2000). Clinicopathological significance of expression of CD44 variants in head and neck squamous cell carcinoma. Jpn. J. Cancer Res. 91, 410-415.

Kaya G., Rodriguez I., Jorcano J.L., Vassalli P. and Stamenkovic I. (1997). Selective suppression of CD44 in keratinocytes of mice bearing an antisense CD44 transgene driven by a tissue-specific promoter disrupts hyaluronate metabolism in the skin and impairs keratinocyte proliferation. Genes Dev. 11, 996-1007.

Kincade P.W., Zheng Z., Katoh S. and Hanson L. (1997). The importance of cellular enviroment to function of the CD44 matrix receptor. Curr. Opin. Cell. Biol. 9, 635-642.

Knudson W. (1998). The role of CD44 as a cell surface hyaluronan receptor during tumour invasion of connective tissue. Front. Biosci.

3, d604-d615.

Kogerman P., Sy M.S. and Culp L.A. (1997). Overexpressed human CD44s promotes lung colonization during micrometastasis of murine fibrosarcoma cells: facilitated retention in the lung vasculature. Proc. Natl. Acad. Sci. USA 94, 13233-13238.

Korabiowska M., Brinck U., Honig J., Mora O., Bartkowski S. and Schauer A. (1995). CD44: a marker of metastases of head and neck melanomas. In Vivo 9, 253-256.

Koukoulis G.K., Patriarca C. and Gould V.E. (1998). Adhesion molecules and tumour metastasis. Hum. Pathol. 29, 889-892.

Kunishi M., Kayada Y. and Yoshiga K. (1997). Down-regulated expression of CD44 variant 6 in oral squamous cell carcinomas and its relationship to regional lymph node metastasis. Int. J. Oral Maxillofac. Surg. 26, 280-283.

Kuo M.Y., Cheng S.J., Chen H.M., Kok S.H., Hahn L.J. and Chiang C.P. (1998). Expression of CD44s, CD44v5, CD44v6 and CD44v7-8 in betel quid chewing-associated oral premalignant lesions and squamous cell carcinomas in Taiwan. J. Oral Pathol. Med. 27, 428-433.

Ladeda V., Frankel P., Feig L.A., Foster D.A., Bal de Kier Joffe E. and Aguirre-Ghiso J.A. (2001). RalA mediates v-src, v-ras and v-raf regulation of CD44 and fibronectin expression in NIH3T3 fibroblasts.Biochem. Biophys. Res. Commun. 283, 854-861.

Lamb R.F., Hennigan R.F., Turnbull K., Katsanakis K.D., MacKenzie E.D., Birnie G.D. and Ozanne B.W. (1997). AP-1-mediated invasion requires increased expression of the hyaluronan receptor CD44. Mol. Cell Biol. 17, 963-976.

Lesley J., Hyman R. and Kincade P.W. (1993). CD44 and its interactions with the extracellular matrix. Adv. Immunol. 54, 271-235.

Lesley J. and Hyman R. (1998). CD44 structure and function. Front. Biosci. 3, d616-d630.

MacKay C.R., Terpe H.J., Stauder R., Marston W.L., Stark H. and Gunthert U. (1994). Expression and modulation of CD44 variant isoforms in humans. J. Cell Biol. 124, 71-82.

Martegani M.P., Del Prete F., Gasbarri A., Natali P.G. and Bartolazzi A. (1999). Structural variability of CD44v molecules and reliability of immunodetection of CD44 isforms using mAbs specific for CD44 variantexon products. Am. J. Pathol. 154, 291-300.

Masuda M., Kuratomi Y., Shiratsuchi H., Nakashima T., Naonobu K. and Komiyama S. (2000). Decreased expression in early-stage tongue carcinoma associates with late nodal metastases following interstitial brachytherapy. Head Neck 22, 662-665.

Moll J., Schmidt A., van der Putten H., Plug R., Ponta H., Herrlich P. and Zoller M. (1996). Accelerated immune response in transgenic mice expressing rat CD44v4-v7 on T cells. J. Immunol. 156, 2085-2094.

Naot D., Sionov R.V. and Ish-Shalom D. (1997). CD44: Structure, function and association with the metastatic process. Adv. Cancer. Res. 71, 241-319.

Neame S. and Isacke C. (1994). Phosphorylation of CD44 in vivo requires both Ser323 and Ser325, but does not regulate membrane localization or cytoskeletal interaction in epithelial cells. EMBO J. 11, 4733-4738.

Nowell P.C. (1986). Mechanisms of tumour progression. Cancer Res. 46, 2203-2207.

Oliveira D.T. and Odell E.W. (1997). Expression of CD44 variant exons by normal oral epithelia. Oral Oncol. 33, 260-262.

Oliveira D.T., Sherriff M. and Odell E.W. (1998). Expression of variant exons by primary and metastatic oral squamous carcinomas. J. Oral Pathol. Med. 27, 303-307.

Ostwald J., Pracht O., Rhode E. and Kramp B. (1997). Are the products of CD44 exons v5 and v6 markers for mestastasis of laryngeal carcinomas. Laryngorhinootologie 76, 295-299.

Peck D. and Isacke C. (1998). Hyaluronan-dependent cell migration can be blocked by a CD44 cytoplasmic domain peptide containing a phosphoserine at position 325. J. Cell. Sci. 111, 1595-1601.

Piepkorn M., Hovingh P., Bennett K.L., Aruffo A. and Linker A. (1997). Chondroitin sulfate composition and structure in alternatively spliced CD44 fusion proteins. Biochem. J. 327, 499-506.

Piffko J., Bankfalvi A., Klauke K., Dreier R., Joos U., Bocker W. and Schmid K.W. (1996). Unaltered strong immunohistochemical expression of CD44v6 and v5 isoforms during development and progression of oral squamous cell carcinomas. J. Oral Pathol. Med. 25, 502-506.

Piffko J., Bankfalvi A., Joos U., Ofner D., Krassort M. and Schmid K.W. (1999). Immunophenotypic analysis of normal mucosa and squamous cell carcinoma of the oral cavity. Cancer Detect. Prev. 23, 45-56.

Ponta H., Sleeman J., Dall P., Mall J., Sherman L. and Herrlich P. (1994-1995). CD44 isoforms in metastasis cancer. Invasion Metastasis 14, 82-86.

Ponta H., Wainwright D. and Herrlich P. (1998). The CD44 protein family. Int. J. Biochem. Cell. Biol. 30, 299-305.

Pure E., Camp R., Peritt D., Panettieri R.A., Lazaar A.L. and Nayak S. (1995). Defective phosphorylation and hyaluronate binding of CD44 with point mutations in the cytoplasmic domain. J. Exp. Med. 181, 55-62.

Reeder J.A., Gotley D.C., Walsh M.D., Fawcett J. and Antalis T.M. (1998). Expression of antisense CD44 variant 6 inhibits colorectal metastasis and tumour growth in a wound enviroment. Cancer Res. 58, 3719-3726.

Protin U., Schweighoffer T., Jochum W. and Hilberg F. (1999). CD44-deficient mice develop normally with changes in subpopulations and recirculation of lymphocyte subsets. J. Immunol. 163, 4917-4923.

Repassy G., Forster-Horvath C., Juhasz A., Adany R., Tamassy R. and Timar J. (1998). Expression of invasion markers CD44v6/v3, NM23 and MMP2 in laryngeal and hypopharyngeal carcinoma. Pathol. Oncol. Res. 4, 14-21.

Rudzki Z. and Jothy S. (1997). CD44 and the adhesion of neoplastic cells. Mol. Pathol. 50, 57-71.

Salmi M., Grön-Virta K., Sointu P., Grenman R., Kalimo H. and Jalkanen S. (1993). Regulated expression of exon v6 containing isoforms of CD44 in man: Downregulation during malignant transformation of tumors of squamocellular origin. J. Cell. Biol. 122, 431-442.

Screaton G.R., Bell M.V., Jackson D.G., Cornelis F.B., Gerth Uu. and Bell J.I. (1992). Genomic structure of DNA encoding the lyphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. Proc. Natl. Acad. Sci. USA 89, 12160-12164.

Schmits R., Filmus J., Gerwin N., Senaldi G., Kiefer F., Kundig T., Wakeham A., Shahinian A., Catzavelos C., Rak J., Furlonger C., Zakarian A., Simard J.J.L., Ohashi P.S., Paige C.J., Gutierrez-Ramos J.C. and Mak T.W. (1997). CD44 regulates hematopoietic progenitor distribution, granuloma formation and tumourigenicity. Blood 90, 2217-2233.

Schrijvers A.H.G.J., Quak J.J., Uyterlinde A.M., van Walsum M., Meijer C.J.L.M., Snow G.B. and van Dongen G.A.M.S. (1993). U36, a novel monoclonal antibody successful in immunotargeting of squamous cell carcinoma of the head and neck. Cancer Res. 53, 4383-4390.

Seelentag W.K., Guenthert U., Saremaslani P., Futo E., Pfaltz M., Heitz P.V. and Roth J. (1996). CD44 standard and variant isoform expression in human epidermal skin tumours is not correlated with tumour aggressiveness but down-regulated during proliferation and tumour de-differentiation. Int. J. Cancer 69, 218-224.

Seiter S., Tilgen W., Errman K., Schadendarg D., Patzelt E., Moller P. and Zoller M. (1996). Expression of CD44 splice variants in human skin and epidermal tumours. Virchows Arch. 428, 141-149.

Sherman L., Sleeman J., Dall P., Hekele A., Moll J., Ponta H. and Herrlich P. (1996). The CD44 proteins in embryonic development and in cancer. Curr. Top. Microbiol. Immunol. 213, 249-269.

Shtivelman E. and Bishop J.M. (1991). Expression of CD44 is repressed in neuroblastoma cells. Mol. Cell. Biol. 11, 5446-5453.

Sleeman J.P., Kondo K., Moll J., Ponta H. and Herrlich P. (1997). Variant exons v6 and v7 together expand the repertoire of glycosaminoglycans bound by CD44. J. Biol. Chem. 272, 31837-31844.

Sleeman J.P., Rahmsdorf U., Steffen A., Ponta H. and Herrlich P. (1998). CD44 variant 5 encodes a tyrosine that is sulphated. Eur. J. Biochem. 255, 74-80.

Soukka T., Salmi M., Joensuu H., Hakkinen L., Sointu P., Koulu L., Kalimo K., Klemi P., Grenman R. and Jalkanen S. (1997). Regulation of CD44v6-containing isoforms during proliferation of normal and malignant epithelial cells. Cancer Res. 57, 2281-2289.

Spafford M.F., Koeppe J., Pan Z., Archer P.J., Meyers A.D. and Franklin A.D. (1996). Correlation of tumour markers p53, bcl-2, CD34, CD44H, CD44v6 and ki-67 with survival and metastasis in laryngeal squamous cell carcinoma. Arch. Otolaryngol. Head Neck Surg. 122, 627-632.

Stoll C., Baretton G., Soost F. and Lohrs U. (1998). The influence of CD44 splice variants to the outcome of patients with oral squamous cell carcinomas. Adv. Exp. Med. Biol. 451, 51-55.

Stoll C., Baretton G., Soost F., Terpe H.J., Domide P. and Lohrs U. (1999). Prognostic importance of the expression of CD44 splice variants in oral squamous cell carcinomas. Oral Oncol. 35, 484-489.

Stroomer J.W.G., Roos J.C., Sproll M., Quak J.J., Heider K-H., Wilhelm B.J., Castelijns J.A., Meyer R., Kwakkelstein M.O., Snow G.B., Adolf G.R. and van Dongen G.A.M.S. (2000). Safety and biodistribution of 99mTechnetium-labelled anti-CD44v6 monoclonal antibody BIWA1 I head and neck cancer patients. Clin. Cancer Res. 6, 3046-3055.

Sugar J., Vereczkey I., Toth J., Peter I. and Banhidy F. (1997). New aspects in the pathology of the preneoplastic lesions of the larynx. Acta Otolaryngol. (Stockh.) 527, 52-56.

Sy M.S., Mori H. and Liu D. (1997). CD44 as a marker in human cancers. Curr. Opin. Oncol. 9, 108-112.

Taher T.E.I., Smit L., Griffioen A.W., Schilder-Tol E.J., Borst J. and Pals S.T. (1996). Signaling through CD44 is mediated by tyrosine kinases. J. Biol. Chem. 271, 2863-2867.

Takahashi K., Stamenkovic I., Cutler M., Saya H. and Tanabe K.K. (1995). CD44 hyaluronate binding influences growth kinetics and tumourigenicity of human colon carcinomas. Oncogene 11, 2223-2232.

Tolg C., Hofmann M., Herrlich P. and Ponta H. (1993). Splicing choice from ten variant exons establishes CD44 variability. Nucleic Acids Res. 21, 1225-1229.

Ue T., Yokozaki H., Kagai K., Higashikawa K., Yasui W., Sugiyama M., Tahara E. and Ishikawa T. (1998). Reduced expression of the CD44 variant exons in oral squamous cell carcinoma and its relationship to metastasis. J. Oral Pathol. Med. 27, 197-201.

Uhlman D.L. and Niehans G.A. (1999). Immunohistochemical study of chondroitin-6-sulfate and tenascin in the larynx: A loss of chondroitin-6-sufate expression accompanies squamous cell carcinoma invasion. J. Pathol. 189, 470-474.

Van Hal N.L., van Dongen G.A., Knippels-Road P.M.C., van de Volk P., Snow G.B. and Brakenhoff R.B. (1996). Monoclonal antibody U36, a suitable candidate for clinical immunotherapy of squamous cell carcinoma, recognises a CD44 isoform. Int. J. Cancer 68, 520-527.

Van Hal N.L., van Dongen G.A., Stigter-van Walsum M., Snow G.B. and Brakenhoff R.B. (1999). Characterization of CD44v6 isoforms in head and neck squamous cell carcinoma. Int. J. Cancer 82, 837-845.

Ylagan L.R., Scholes J. and Demopoulos R. (2000). CD44: a marker of squamous differentiation in adenosquamous neoplasms. Arch. Pathol. Lab. Med. 124, 212-215.

Yu Q., Toole B.P. and Stamenkovic I. (1997). Induction of apoptosis of metastatic mammary carcinoma cells in vitro by disruption of tumour cell surface CD44 function. J. Exp. Med. 186, 1985-1996.

Zhu D. and Bourguinon L.Y.W. (1996). Overexpression of CD44 in p185neu-transfected NIH3T3 cells promotes an up-regulation of hyaluronic acid-mediated membrane-cytoskeleton interaction and cell-adhesion. Oncogene 12, 2309-2314.

Accepted June 12, 2002