

Expression of CD44 by rhabdomyosarcoma: a new prognostic marker?

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Summary The expression pattern of CD44 standard and variant isoforms are prognostically significant in a number of malignancies. The aim of this study was to evaluate the role of the standard isoform of CD44 in predicting the clinical behaviour of rhabdomyosarcoma. Immunohistochemical analysis of CD44 was undertaken using a panel of antibodies recognizing the three core domains of the CD44 molecule. Labelling was repeated in triplicate and reported blind with respect to histological type and outcome. Tumours were characterized as positive in more than 60% of tumour cells labelled and negative if less than 40% of tumour cells labelled. Tumours with 40–60% of tumour cells labelling were considered indeterminate. Eleven of 20 favourable histology tumours were positive for CD44 compared with one of seven unfavourable tumours ($P = 0.07$). Eleven of 12 patients with CD44-positive tumours are alive in first remission compared with five of 15 CD44-negative tumours ($P = 0.001$). Expression of CD44 correlates directly with prognosis; however, larger studies are required so that multivariate analysis can be undertaken.

Keywords: CD44; rhabdomyosarcoma; prognosis

CD44 is an integral membrane glycoprotein which plays an important role in cell–substrate and cell–cell interactions including lymphocyte homing; lymphocyte, endothelial and mucosal interactions; cytokine release, T-cell activation; homotypic and heterotypic cell–cell adhesion and cytoskeletal interactions with the extracellular matrix (Lesley et al, 1993). Altered expression of CD44 has been observed in a large number of tumours of adult life (Matsumura and Tarin, 1992; Abbasu et al, 1993; Heider et al, 1993a; 1993b; Joensuu et al, 1993; Tanabe et al, 1993; Matsumura et al, 1994; Penno et al, 1994; Southgate et al, 1995; Harwood et al, 1996; Nagabhushan et al, 1996) and in the paediatric malignancy neuroblastoma (Favrot et al, 1993; Gross et al, 1994, 1995; Shtivelmann and Bishop, 1991). The aim of this study was to document the pattern of expression of CD44 by rhabdomyosarcomas and to determine the relationship of CD44 expression to prognosis.

MATERIALS AND METHODS

Tumour samples collected prospectively at the time of diagnostic biopsy or definitive tumour excision in Leeds were used for this study. Tumour samples from 28 patients (age range 22 months to 15 years) diagnosed as having rhabdomyosarcoma between 1977 and 1994 were studied. Eleven patients were male and 17 female. Seventeen patients are alive in first remission, two are alive in second or subsequent remission, seven children are dead of disease and two children have died from treatment related complications. Distribution by site, stage and histological type are summarized in Table 1. Tumours had been characterized using an antibody panel including desmin, vimentin and MyoD1.

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Immunohistochemical analysis of CD44 was carried out using a standard streptavidin–biotin–horseradish peroxidase immunohistochemistry protocol [Streptavidin/ABC/HRP detection kit (DAKO)]. Previously characterized primary antibodies recognizing core epitope one (BRIC 222 at 1/60), epitope two (BRIC235 at 1/40) and epitope three (KZ-1 at 1/40) were obtained from International Blood Group Reference Laboratory, Bristol (Anstee et al, 1991). The secondary antibody was a biotinylated F(ab)₂ rabbit anti-mouse immunoglobulin (DAKO, Buckinghamshire, UK). Labelling was detected using diaminobenzidine chromagen and sections counterstained with haematoxylin. Fresh-frozen and paraffin sections were labelled in a similar fashion although formalin-fixed tissue was pretreated by dewaxing and microwave antigen retrieval (Gown et al, 1993). Control samples throughout included omission of primary antibody and the presence of non-tumour tissue within each section examined. Slides were assessed blind by an experienced tumour pathologist (KM) and labelling scored. Tumours were considered negative when tumour cell labelling was less than 40%, indeterminate when 40–60% of tumour cells labelled and positive when greater than 60% of tumour cells labelled.

Fisher's exact test with one-tail analysis of CD44 expression by histological subtype was undertaken. For analysis of disease progression the log-rank test was used for testing the difference between the groups. The level of confidence was set at $P = 0.05$. Statistical analysis was undertaken using SPSS for Windows (version 6.1) (Chicago, IL, USA).

RESULTS

Identical labelling patterns were seen when matched pairs of fresh-frozen and formalin-fixed paraffin-embedded tumour samples were analysed. There was good correlation in the expression patterns obtained with labelling using BRIC222 and 235. The antibody KZ-1 labelled more cells in each sample than BRIC222 and 235.

Table 1 Numbers of patients by site and stage of tumour

	I		II		III		IV		Total
	F	U	F	U	F	U	F	U	
Orbit	1								1
PMHN							1		1
non-PMHN				1	1	2	1	1	6
GU-BP					6		1		7
GU-non-BP			1		1				2
Extremity	1	1			4				6
Other					2	2		1	5
Total	2	1	1	1	14	4	3	2	28

F = botryoid, embryonal, mixed embryonal undifferentiated and spindle cell tumours; U = alveolar and undifferentiated tumours. Stage I, II, III and IV refer to IRS clinical groups. PMHN = tumours involving parameningeal sites; non-PMHN = tumours of head and neck not involving parameningeal areas or orbit; GU-BP = tumours arising in bladder or prostate; GU-non-BP = tumours arising in genitourinary tract other than bladder or prostate; Extremity = tumour arising in a limb; Other = tumours arising in all other sites.

Table 2 Table for testing probability of CD44 expression correlating with histological subtype

	CD44-positive	CD44-negative	Total
Favourable	11	9	20
Unfavourable	1	6	7
Total	12	15	27

Favourable = tumours of botryoid, embryonal or spindle cell type; unfavourable = alveolar and undifferentiated. *P* = 0.07.

Table 3 Table for testing probability of association of CD44 expression with disease progression

	CD44-positive	CD44-negative	Total
Disease-free in 1st remission	11	5	16
Disease progression	1	10	11
Total	12	15	27

P = 0.001.

One tumour had approximately equal numbers of labelled and unlabelled cells and has therefore not been included in statistical analysis by predominant cell phenotype. Eleven of 20 favourable histology tumours expressed CD44 (Figure 1) compared with one of seven unfavourable histology tumours (Figure 2) *P* = 0.07 (Table 2). Eleven of 12 patients with CD44 positive tumours are disease-free in first remission compared with five of 15 patients with CD44-negative tumours *P* < 0.001 (Table 3). The Kaplan–Meier survival curve is shown in Figure 3. Nineteen of 27 patients are alive more than 3 years from diagnosis. Seven of the remaining patients died from disease within 3 years of diagnosis and one patient died disease-free from treatment-related toxicity.

DISCUSSION

CD44 is a transmembrane glycoprotein expressed on virtually all cell types where it acts as a receptor for hyaluronate (Picker et al, 1989; Culty et al, 1990; Lesley et al, 1993). It is encoded by a gene occupying 60–80 kb located at chromosome 11p13 and consists of

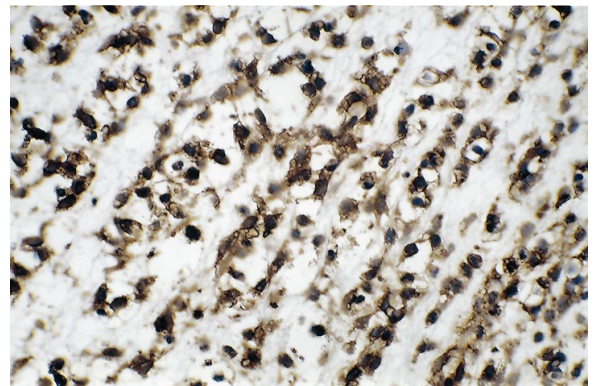


Figure 1 Rhabdomyosarcoma with focal labelling of the majority of tumour cells for CD44, x120

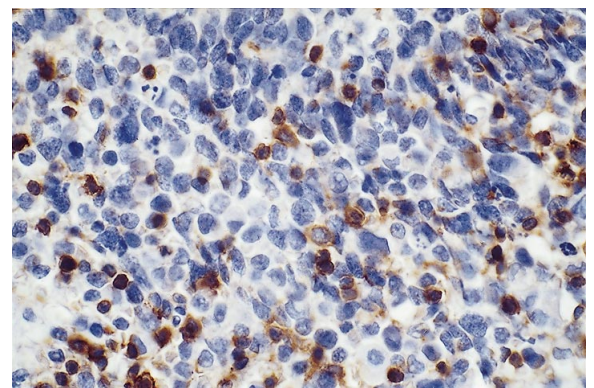


Figure 2 Labelling of inflammatory cells for CD44 within an alveolar RMS negative for CD44, x120

at least 21 exons (Forsberg et al, 1989; Jackson et al, 1992; Sreaton et al, 1992). The CD44 molecule has three core epitopes encoded by ten exons with alternative mRNA splicing of the remaining exons generating multiple isoforms (CD44v). The standard form of CD44 (CD44s) is expressed on almost all cell types and is heavily glycosylated. Variant isoforms are expressed in a cell- and tissue-specific manner (Arch et al, 1992; Herrlich et al, 1993; Lesley et al, 1993; Mackay et al, 1994).

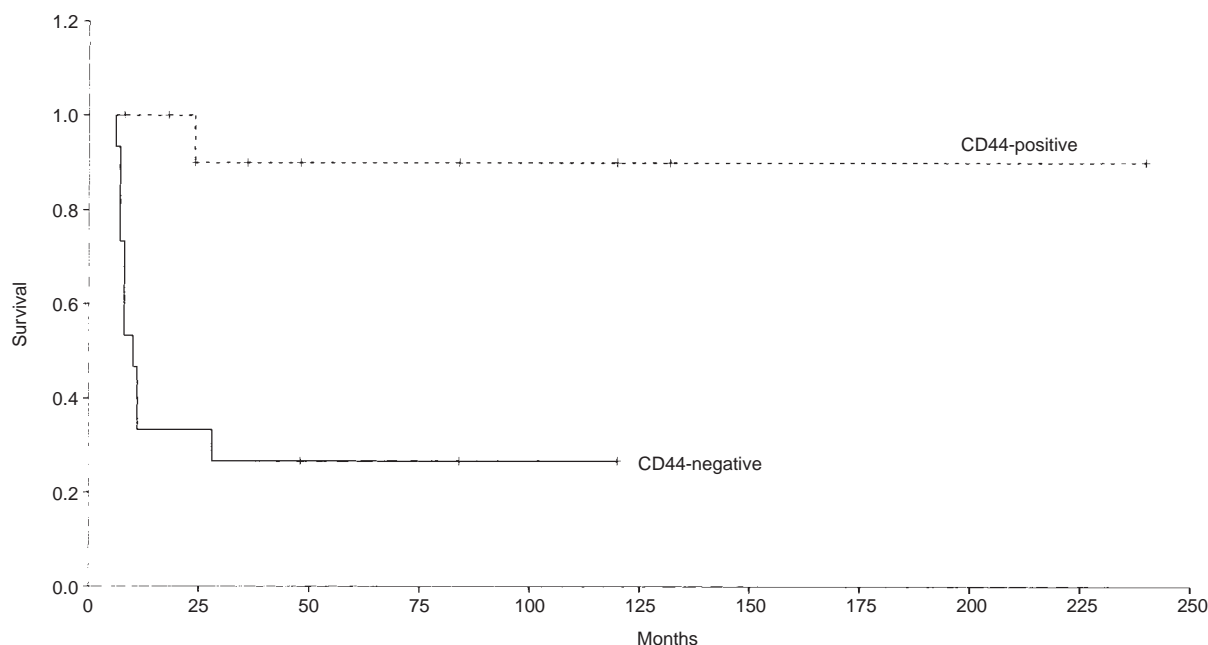


Figure 3 Kaplan-Meier survival curve demonstrating improved survival in patients with CD44-positive tumours, $P = 0.001$

Qualitative and quantitative changes in expression of CD44 have been demonstrated in vitro in the vascular dissemination of melanoma (Birch et al, 1991) and lymphoma cells (Sy et al, 1991), and in the migration of rat pancreas carcinoma cells on the extracellular matrix (Günthert et al, 1991). In vivo, enhanced or up-regulation of CD44 (core or variant) expression has been found to be related to tumour progression in breast (Joensuu et al, 1993), colorectal (Abbasu et al, 1993a; Tanabe et al, 1993; Wielenga et al, 1993), gastric (Heider et al, 1993a; Mayer et al, 1993), cervical (Dall et al, 1994) and bladder (Matsumura et al, 1994; Southgate et al, 1995) carcinomas, non-Hodgkin's lymphoma (Koopman et al, 1993) and brain tumours (Terpe et al, 1993). Conversely, loss of or reduction in expression of CD44v isoforms is associated with disease progression in squamous cell (Salmi et al, 1993) and endometrial carcinomas (Fujita et al, 1994). In addition, loss of CD44s isoforms has been reported in metastatic prostatic (Nagabhushan et al, 1996) and bladder cancer (Southgate et al, 1995) and melanomas during their vertical growth phase (Harwood et al, 1996).

Several studies have confirmed the potential importance of CD44 as a prognostic indicator for neuroblastoma tumours with stage 1-3 and 4s disease expressing CD44 (Favrot et al, 1993; Gross et al, 1994, 1995; Christiansen et al, 1995; Terpe et al, 1995). Studies of stage 4 neuroblastomas comparing MYCN amplification with CD44 expression have shown that there is a highly significant inverse relationship between MYCN amplification and CD44 expression (Favrot et al, 1993; Gross et al, 1994, 1995; Christiansen et al, 1995; Terpe et al, 1995). In rhabdomyosarcoma, MYCN amplification appears to be a relatively infrequent observation except in those of alveolar subtype (Dias et al, 1990; Maillet et al, 1992; Driman et al, 1994; Tsuda et al, 1998). This study combined with that of Saxon et al (1997) suggests that most alveolar rhabdomyosarcomas are CD44-negative; therefore, studies correlating CD44 expression with MYCN amplification should be carried out on embryonal and alveolar rhabdomyosarcomas to see if MYCN

amplification is directly related to histological type and to establish if the two are causally related.

In 1958, Horn and Enterline divided rhabdomyosarcomas into four subtypes – embryonal, botryoid, alveolar and pleomorphic. In an attempt to improve the prognostic value of basic histology an alternative classification divides tumours into: favourable (botryoid and spindle cell), moderately favourable (all other embryonal tumours), and unfavourable (solid alveolar, alveolar and undifferentiated) (Newton et al, 1995). In 1997, Saxon reported that none of five alveolar rhabdomyosarcomas studied expressed CD44 and that embryonal tumours had a heterogeneous pattern of labelling but did not attempt to correlate this with disease outcome. This study suggests that alveolar tumours are predominantly CD44-negative. This small study appears to indicate that low expression of CD44 correlates with poor outcome, these results must be interpreted with caution because of the small sample size and possible confounding influence of subset analysis. To confirm the hypothesis that low CD44 expression predicts poor outcome and to establish if this is independent of histological subtype further studies should be performed using a large retrospective data set with multivariate analysis.

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