Virchows Archiv (1995) 426:19–25

ORIGINAL ARTICLE

Thomas Barth · Peter Möller · Gunhild Mechtersheimer

Differential expression of β1, β3 and β4 integrins in sarcomas of the small, round, blue cell category

Received: 20 May 1994 / Accepted: 18 October 1994

Abstract Integrins are a large and complex family of membrane spanning αβ heterodimeric cell surface glycoproteins mediating cell/cell and cell/matrix interactions. Small, round, blue cell sarcomas (SRBCS) are a group of poorly differentiated tumours of various and in part uncertain histogenesis displaying similar cytomorphology. Among them are rhabdomyosarcomas (RMS), ganglioneuroblastomas [(G)NB], primitive peripheral neuroectodermal tumours (pPNET) and Ewing's sarcomas (ES). Thirty-two SRBCS were studied immunohistochemically for the distribution of β1, β3 and β4 integrins in situ. We found complex and to some extent differential patterns of β1, β3 and β4 integrin subunit expression in different types of SRBCS: all of the sarcomas studied were consistently $\beta1^+$, $\beta4^-$, $\alpha2^-$. Four of nine RMS were completely negative for all other integrin subunits studied while one RMS was $\alpha5^+$ throughout and three RMS were focally $\alpha5^+$. Three RMS expressed the α6 and αv chains. In contrast to RMS, pPNET and ES, all of which were $\alpha1^-$, $\alpha3^-$, (G)NB were $\alpha3^+$ and frequently co-expressed α1. The eight pPNET and seven ES studied showed a similarily restricted integrin profile that was limited to the expression of β1 and α5 in nearly all cases. In summary, RMS were $\beta1^+$, $\alpha1^-$, $\alpha3^-$ and heterogeneously expressed α5 and α6. (G)NB were generally $\beta1^+$, $\alpha1^+$, $\alpha3^+$, $\alpha5^-$, $\alpha6^-$. pPNET and ES were $\beta1^+$, $\alpha1^-$, $\alpha3^-$, $\alpha5^+$, $\alpha6^-$. The data illustrate a complex expression pattern of various integrins in SRBCS, a differential expression pattern of some of the integrin subunits among different types of SRBCS and almost identical integrin profiles in pPNET and ES.

Key words Small round blue cell sarcomas · Integrins · Immunohistochemistry

This paper is dedicated to Prof. Dr. Dres. h.c. Wilhelm Doerr on the occasion of his 80th birthday

T. Barth · P. Möller (✉) · G. Mechtersheimer
Institute of Pathology, University of Heidelberg,
Im Neuenheimer Feld 220,
D-69120 Heidelberg, Germany

Introduction

Interactions between cells and extracellular matrix components are mediated, in part, via a large family of cell surface adhesion molecules known as integrins (recently reviewed in [23, 30]). Integrins are non-covalently linked membrane spanning αβ heterodimeric glycoproteins. Through an extracellular receptor domain which is built up by both subunits and the cytoplasmic portion of the β subunit which interacts with talin and α-actinin, integrins connect the extracellular milieu with the cytoskeleton [8, 27]. Currently, at least 15 different α chains and eight different β chains are known [22, 28, 30]. The integrin family is actually subdivided into different subgroups based on types of β chains which, in turn, can dimerize with different α chains. The largest subgroup are the β1 integrins, formerly designated very late antigens since they were first described on T cells during late phases of activation in vitro [18]. The β1 integrins consist of the common β1 chain [CD29 according to the cluster of differentiation (CD) nomenclature of cell surface molecules] that combines with at least six α chains (CD49a–f) to form six different αβ heterodimeric cell surface receptors. Furthermore, the β1 chain has been shown to associate alternatively with the αv chain (CD51) of the vitronectin receptor αvβ3 (CD51/CD61) [39]. Another example of the promiscuity within the different integrin subunits is the α6 chain which can also dimerize with the β4 chain (CD104) [20]. Receptors of the β1, β3 and β4 integrin type were shown to bind to a variety of extracellular matrix proteins and to some cell adhesion molecules (Table 1). As cell adhesion molecules they are involved in different biological events such as embryogenesis, wound healing and immune response. Integrins are differentially expressed on a broad spectrum of normal cell types and their neoplastic equivalents [1, 2, 7, 24, 29, 45]. The descriptive term ‚small, round, blue cell sarcoma' (SRBCS) encompasses different tumours of neuroectodermal and mesenchymal derivation that, although similar in cytomorphology, have different biological properties [10]. Among SRBCS are

Table 1 Integrin subunits detected and monoclonal antibodies used in this study (*CD* cluster of differentiation, *VCAM* vascular cell adhesion molecule, *ICAM* intercellular adhesion molecule)

Antigen	Molecular weight (kDa)	CD number[a]	Clone	Isotype	Receptor for *function*	Reference
β1	130	CD29	K20	IgG2a	*Common β chain of α1 to α6*	[4]
β3	110	CD61	SZ.21	IgG1	*Common β chain of αv and αIIb* (CD41)	[31]
β4	205	CD104	3E1	IgG1	Alternative *β chain of α6*/laminin	[21]
α1	210	CD49a	TS2/7	IgG1	Collagen/laminin	[19]
α2	170	CD49b	CLB-thromb/4	IgG1	Collagen/(laminin)/(fibronectin)	[16]
α3	130	CD49c	J143	?	Collagen/laminin/fibronectin/epiligrin/entactin	[14]
α4	150	CD49d	HP2/1	IgG1	Fibronectin/VCAM-1(CD106)/ICAM-2(CD102)	[32]
α5	135	CD49e	SAM1	IgG2b	Fibronectin	[37]
α6	120	CD49f	GOH3	IgG2a	Laminin	[35]
αv	120	CD51	AMF7	IgG1	Vitronectin/fibronectin/fibrinogen/von Willebrand's factor/collagen/osteopontin/thrombospondin	[38]

[a] According to the fixing of the Nomenclature Committee of the 5th International Workshop and Conference on Leukocyte Differentiation Antigens, Boston, November 1993 [33]

rhabdomyosarcomas (RMS), ganglioneuroblastomas (GNB), neuroblastomas (NB), primitive peripheral neuroectodermal tumours (pPNET) and Ewing's sarcomas (ES). Histogenetic assignment of pPNET and ES is a matter of ongoing controversy [3, 5, 36, 41].

Most data on integrin expression in SRBCS are based on in vitro studies of various cell lines, such as NB, ES and RMS cell lines [6, 9, 11, 43]. The analysis in situ is still very incomplete. We therefore analysed the distribution pattern of β1, β3 and β4 integrin subunits immunohistochemically in a series of 32 unselected SRBCS.

Materials and methods

Fresh frozen tumour tissue from 32 SRBCS was collected at our institute in a period of 5 years. The tumour series comprised nine RMS, five GNB, three NB, eight pPNET and seven ES. The diagnosis of each tumour case was based on routine paraffin sections (haematoxylin and eosin, periodic acid-Schiff, Masson-Goldner and Gomori silver stains) according to standard histopathological criteria as described by Enzinger and Weiss [12], combined with immunohistochemical analysis using monoclonal antibodies and antisera against structural antigens of mesenchymal cells (for review see [12, 26, 42]). All tissue samples were taken from incisional biopsies or surgical specimens. The tissue was snap-frozen in liquid nitrogen within 1 h after removal and stored at −70° C. Serial frozen sections of about 1 cm^2 and 4–6 μm thickness were air-dried, acetone-fixed at room temperature for 10 min and immunostained immediately or stored at −20° C for a short period.

The primary monoclonal antibodies (mAb) used in this study are listed in Table 1. The mAb K20 (anti-β1), Gi9 (anti-α2), HP2/1 (anti-α4), SAM1 (anti-α5), GOH3 (anti-α6), SZ.21 (anti-β3) and AMF7 (anti-αv) were obtained from Dianova (Hamburg, Germany); mAb TS2/7 (anti-α1) was purchased from T Cell Sciences (Cambridge, Mass., USA); mAb P1B5 (anti-α3) and 3E1 (anti-β4) were obtained from Telios Pharmaceuticals (San Diego, Calif., USA). A polyclonal biotinylated sheep antibody to mouse immunoglobulin (Ig; reactive with all mouse isotypes), a polyclonal biotinylated sheep antibody to rat Ig for detection of rat-derived mAb GOH3, and a streptavidin-biotinylated peroxidase complex, all obtained from Amersham (Buckinghamshire, UK), served as a detection system for the primary antibodies. 3-Amino-9-ethylcarbazole (AEC) and N′N-dimethylformamide were obtained from Sigma Chemical Company (St. Louis, Mo., USA).

After rehydration with phosphate-buffered saline solution (PBS; pH 7.4), the frozen sections were incubated for 1 h with primary mAb. The mAb as culture supernatants were applied undiluted, ascites preparations were diluted 1:2000 in PBS, purified reagents were used in a protein concentration of about 10 μg/ml PBS. All incubation steps were carried out in a humid chamber at room temperature and were followed by double rinsing with PBS. AEC was used as chromogen [0.4 mg/ml in 0.1 mol/l (molar) acetate buffer, pH 5.0, with 5% N′N-dimethylformamide and 0.01% hydrogen peroxide for about 20 min]. The peroxidase reaction caused an intense red precipitate at the binding site of the primary antibody. The sections were then rinsed in tap water, counterstained with Harris' haematoxylin and mounted with glycerol gelatin.

Negative controls were performed in each case by substituting the primary antibody with PBS. No staining was observed except for scattered granulocytes due to endogenous peroxidase. This reaction was not blocked for the benefit of optimal antigenicity. Strongly stained stromal cells and/or endothelial cells and/or lymphocytes and histiocytes, always present in combinations characteristic of the respective antigen under study, served as positive intrinsic controls. The staining intensity corresponding to the integrin antigenic density in neoplastic cells was evaluated against the background of these cells and was assessed in a semiqualitative fashion as follows: +, strong staining; (+), weak staining; − no staining. In the presence of positive and negative neoplastic cells a semiquantitative evaluation was carried out: +/− indicates positive and negative tumour cells in about equal amounts, +>− indicates a clear prevalence of positive tumour cells; −>+ indicates a clear prevalence of negative tumour cells.

Results

The distribution patterns of β1, β3, β4, α1-α6 and αv integrin subunits within the tumour cells of 32 SRBCS studied are shown in detail in Table 2 and are summarized in Table 3. Generally, the cells displayed a granular staining that was located on both the cell membrane and in the cytoplasm.

RMS

The RMS studied comprised primary and metastatic lesions in about equal numbers and included six embryonal and three alveolar RMS. All RMS were consistently $\beta1^+$, $\beta3^-$, $\beta4^-$ (Fig. 1). The expression of the α chains was heterogeneous. Two embryonal and two alveolar RMS lacked any detectable α1–6 and αv chains. The un-

Table 2 Detailed expression patterns of β1, β3 and β4 integrin subunits in neoplastic cells of small round blue cell sarcomas (*RMS a* alveolar rhabdomyosarcoma, *RMS e* embryonal rhabdomyosarcoma, *UC* undifferentiated tumour cells, *RB* rhabdomyoblasts, *GNB* ganglioneuroblastoma, *Nbl* neuroblasts, *GC* immature ganglion cells, *NB* neuroblastoma, *pPNET* primitive peripheral neuroectodermal tumour, *ES* Ewing's sarcoma, + strong positivity for all tumour cells, *(+)* weak positivity for all tumour cells, – negative for all tumour cells, +/– positive and negative tumour cells in about equal amounts, +>– positive tumour cells clearly dominate negative tumour cells, –>+ negative tumour cells clearly dominate positive tumour cells

Type		β1	β3	β4	α1	α2	α3	α4	α5	α6	αv
RMS a		+	–	–	–	–	–	–	–	–	–
RMS a		+	–	–	–	–	–	–	–	–	–
RMS a		+	–	–	–	–	–	+	–	+	–
RMS e		+	–	–	–	–	–	–	–	–	–
RMS e		+	–	–	–	–	–	–	–>(+)	–	–
RMS e		+	–	–	–	–	–	–	+	–	(+)
RMS e		(+)	–	–	–	–	–	–	–>+	+>–	(+)
RMS e		+	–	–	–	–	–	–	–	–	–
RMS e	UC	+	–	–	–	–	–	+/–	+>–	+/–	–
	RB	+	–	–	+	–	+/–	+	+	+	+
GNB	Nbl	+	–	–	–	–	+	–	–	(+)/–	–
	GC	+	–	–	–	–	+	–	–	–	–
GNB	Nbl	+	+/–	–	(+)>–	–	+>–	–	–	(+)/–	+
	GC	+	–	–	(+)>–	–	+/–	–	–	(+)/–	–
GNB	Nbl	+	–	–	–	–	+	–	–	–	–
	GC	+	–	–	–	–	+	–	–	–	–
GNB	Nbl	+	–	–	+	–	+	–	–	–	–
	GC	+	–	–	–	–	+	–	–	–	–
GNB	Nbl	(+)	–	–	–	–	+	–	–	–	–
	GC	(+)	–	–	–	–	+	–	–	–	–
NB		+	–>(+)	–	+	–	+	–	–	(+)>–	(+)
NB		+	–	–	+	–	+	–	–	–	–
NB		+	–	–	+	–	+	–	–	–	–
pPNET		(+)	–	–	–	–	–	–	(+)	–	–
pPNET		+	–	–	–	–	–	–	+	–	–
pPNET		+	–	–	–	–	–	–	(+)	–	+
pPNET		(+)	–	–	–	–	–	–	(+)>–	–	–
pPNET		+	–	–	–	–	–	–	(+)>–	–	–
pPNET		+	–	–	–	–	–	–	+	–	–
pPNET		(+)	–	–	–	–	–	–	–	–	–
pPNET		(+)	–	–	–	–	–	–	–>(+)	–	–
ES		(+)	–	–	–	–	+/–	–	+	–	–
ES		+	–	–	–	–	–	–	(+)	–	–
ES		+	–	–	–	–	–	–	(+)	–	–
ES		+	–	–	–	–	–	–	(+)	–	–
ES		+	–	–	–	–	–	–	+	–	–>+
ES		(+)	–	–	–	–	–	(+)	(+)	–	(+)
ES		(+)>–	–	–	–	–	–	–	+/(+)	–	(+)

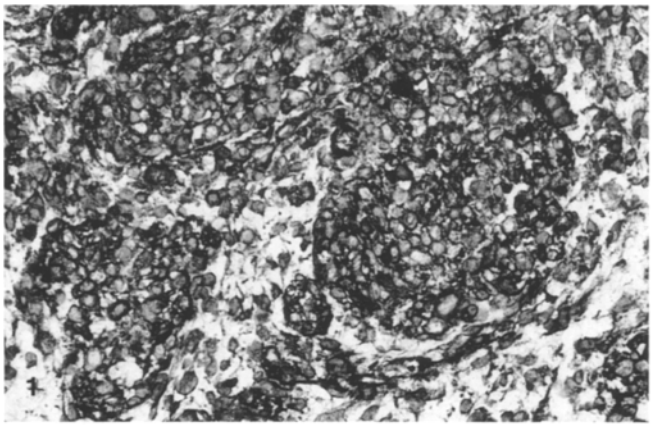
Table 3 Summarized expression of β1, β3 and β4 integrin subunits in neoplastic cells of small, round, blue cell sarcomas (Figures without parentheses indicate the number of cases in which all tumour cells expressed the corresponding integrin subunit, those with parentheses include the number of cases in which the respective antigen was detectable in at least a minor neoplastic population)

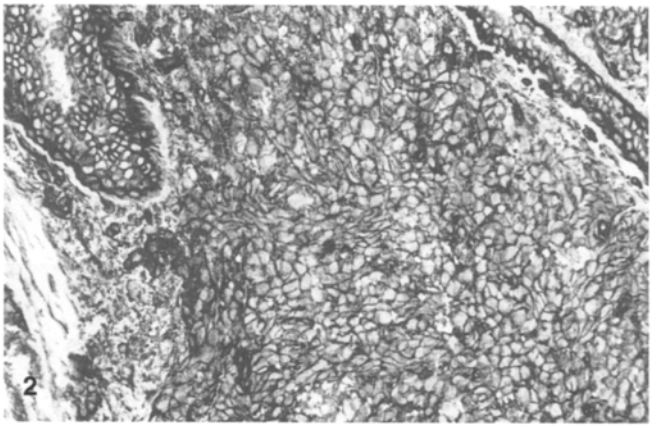
	RMS (*n*=9)	GNB (*n*=5)	NB (*n*=3)	pPNET (*n*=8)	ES (*n*=7)
β1	9	5	3	8	7
β3	–	(1)	(1)	–	–
β4	–	–	–	–	–
α1	(1)	(2)	3	–	–
α2	–	–	–	–	–
α3	(1)	4(1)	3	–	(1)
α4	1(1)	–	–	–	1
α5	1(3)	–	–	4(3)	7
α6	1(2)	(2)	(1)	–	–
αv	2(1)	(1)	1	1	2(1)

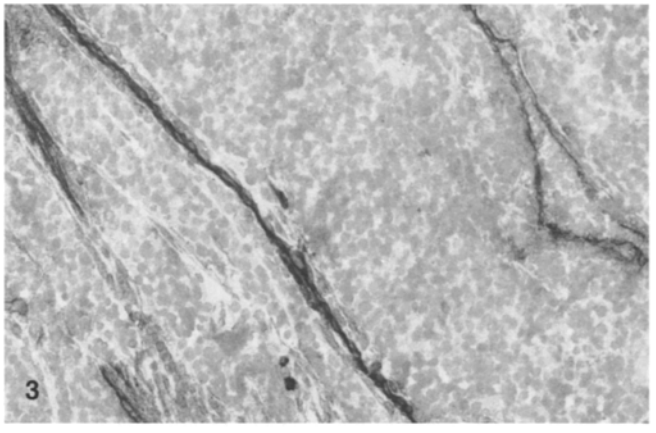
differentiated tumour cell component of all RMS was $\alpha1^-$, $\alpha3^-$. One embryonal RMS contained a rhabdomyoblastic subpopulation which was $\alpha1^+$, $\alpha3^+$. One alveolar RMS was completely $\alpha4^+$. In one embryonal RMS the rhabdomyoblastic tumour cells and about half of the undifferentiated tumour cells were $\alpha4^+$. The α5 subunit was expressed in the entire tumour cell population of one embryonal RMS. Three further embryonal RMS expressed α5 in a neoplastic subset. The α6 chain was consistently present in one alveolar RMS (Fig. 2) and heterogeneously expressed in two embryonal RMS. Two embryonal RMS were entirely αv^+ while in one embryonal RMS expression of αv was limited to a tumour cell subset.

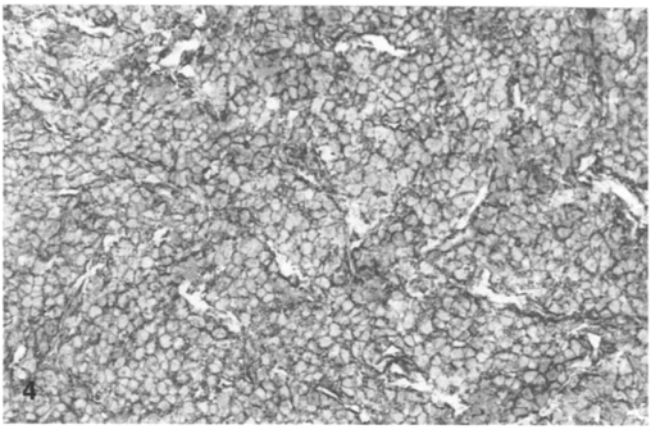
(G)NB

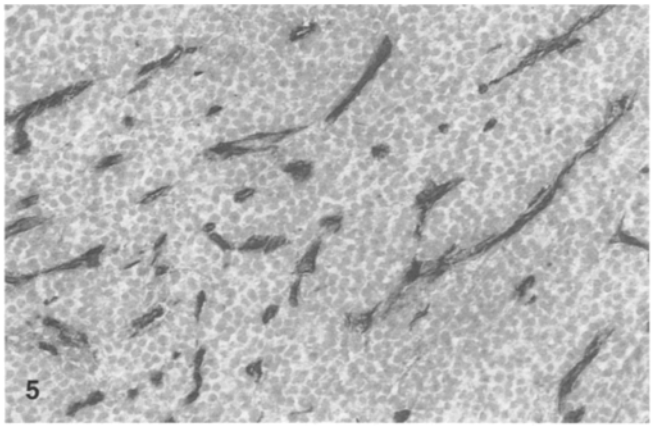
The GNB and NB studied showed a strong expression of the β1 subunit. β3 was present in about half of the neuroblastic tumour cells of one GNB. One GNB and one NB expressed β3 in a neoplastic subset. All (G)NB were $\beta4^-$. Again, expression of the α chains was heterogeneous. All (G)NB were $\alpha2^-$, $\alpha4^-$ and $\alpha5^-$ (Fig. 3). α1 was present in the neuroblastic cells of one GNB. Another GNB expressed α1 in subsets of both the neuroblasts and ganglion cells. All NB were evenly $\alpha1^+$ (Fig. 4). Four of five GNB were entirely $\alpha3^+$, one case comprised $\alpha3^+$ and $\alpha3^-$ neoplastic cells in about equal amounts. All NB

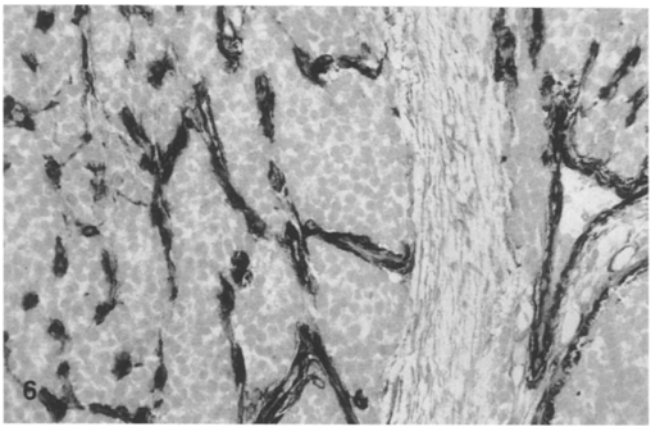

1

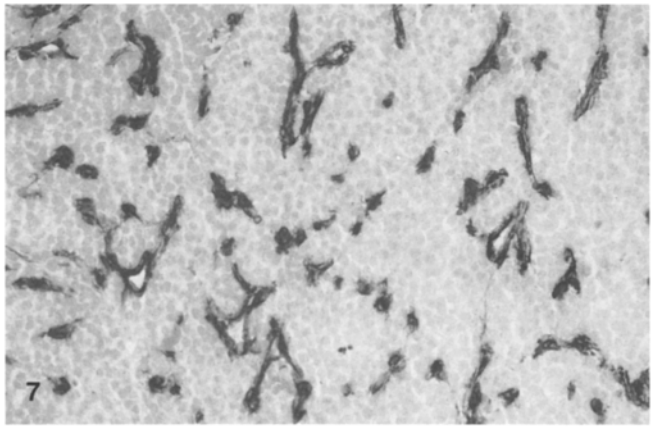

2

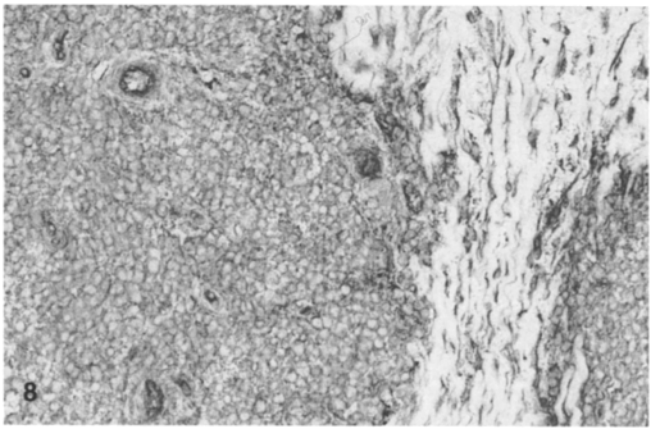

3


4


5


6


7


8

were $\alpha3^+$ throughout. The $\alpha6$ subunit was focally expressed in ganglion cells and neuroblasts of one GNB and in the neuroblastic tumour cell population of one further GNB. One NB expressed $\alpha6$ in the majority of tumour cells. Expression of the αv subunit was restricted to the neoplastic population of one NB and to neuroblastic tumour cells of a single GNB.

pPNET and ES

pPNET and ES showed a similar pattern of integrin subunit expression: all pPNET and ES were essentially $\beta1^+$, $\beta3^-$, $\beta4^-$, $\alpha1^-$ to $\alpha4^-$, $\alpha6^-$ (Figs. 5, 6, 7). As exceptions, one ES contained a $\beta1^-$ neoplastic subset, another case was focally $\alpha3^+$ and one further ES was evenly $\alpha4^+$. Some degree of heterogeneity emerged concerning the expression of the $\alpha5$ subunit. Four pPNET were entirely $\alpha5^+$ and three further pPNET were partly $\alpha5^+$. All seven ES were evenly $\alpha5^+$ (Fig. 8). The αv subunit was expressed in one of eight pPNET and in two of seven ES; one further ES showed an αv^+ neoplastic subset.

Against the background of tumour type three integrin subunit phenotypes emerged: RMS were essentially $\beta1^+$, $\alpha1^-$, $\alpha3^-$ and expressed $\alpha5$ and $\alpha6$ heterogeneously, (G)NB were generally $\beta1^+$, $\alpha1^+$, $\alpha3^+$, $\alpha5^-$, $\alpha6^-$ and pPNET and ES had similar integrin profiles being $\beta1^+$, $\alpha1^-$, $\alpha3^-$, $\alpha5^+$, $\alpha6^-$. Thus, the different types of SRBCS exhibited characteristic differences in their integrin subunit equipment.

Discussion

This is the first comparative immunohistochemical analysis of $\beta1$, $\beta3$, $\beta4$ integrin subunit expression in different types of SRBCS in situ. Apart from homologies in integrin subunit expression like the consistent expression of the $\beta1$ chain and the absence of the $\alpha2$ and $\beta4$ chains in all SRBCS studied, we found three immunophenotypic patterns associated with the different types of SRBCS: RMS were $\alpha1^-$, $\alpha3^-$ and showed a heterogeneous expression of $\alpha5$ and $\alpha6$, (G)NB were $\alpha1^+$, $\alpha3^+$, $\alpha5^-$, $\alpha6^-$ and pPNET and ES were close to identical in their integrin subunit profile in being $\alpha1^-$, $\alpha3^-$, $\alpha5^+$, $\alpha6^-$. Thus, the three different modes of $\alpha1$, $\alpha3$, $\alpha5$, $\alpha6$ expression can be regarded as novel characteristics of RMS, (G)NB and pPNET/ES.

Our results are in most aspects in accordance with the rare data on integrin subunit expression in SRBCS in situ but contrast in some instances to findings observed in SRBCS cell lines in vitro.

Concerning RMS, current knowledge on the expression of integrins is limited to a few studies in vitro. Thus, the RMS cell line RD was shown to express the $\beta1$, $\alpha1$, $\alpha4$, $\alpha5$ and $\alpha6$ subunits while lacking the $\alpha2$ and $\alpha3$ chains [9, 40]. Expression of the $\alpha4$ and $\alpha5$ subunits was confirmed in various other RMS cell lines [6, 43]. Comparable to these data in vitro, some of our RMS expressed the $\alpha4$, $\alpha5$ and $\alpha6$ subunits in parallel to the $\beta1$ chain. However, in some cases the β chain was expressed in the absence of any detectable $\beta1$-associated α subunit. It is conceivable that in RMS cells the $\beta1$ chain associated with another, as yet unknown α subunit to form a functionally active $\alpha\beta$ heterodimer. One such candidate, called α' or H36-$\alpha7$, has been described in chicken and murine myoblasts [25, 34]. Experimental evidence suggests that the presence of $\alpha2$ in a RMS context might augment the metastatic potential of this tumour. Thus, Chan et al. [9] showed that the constitutively $\alpha2^-$ and poorly tumourigenic RMS cell line RD had an enhanced growth rate and a higher capacity to form metastases in nude mice once successfully transfected with $\alpha2$ cDNA. In our study, although limited to a small number of specimens, all RMS were $\alpha2^-$, both at the primary site and within the metastatic lesions. This does not exclude, however, that the disseminating RMS cells might have been $\alpha2^+$ during the metastatic cascade.

NB and GNB were characterized by the presence of $\beta1$, $\alpha1$ and $\alpha3$ chains while the other integrin subunits studied were absent in the clear majority of (G)NB studied. These data are in agreement with in situ studies performed by other investigators [13, 17]. In vitro, however, Yoshihara et al. [43] described differences in integrin subunit expression between the three different morphological types [neuroblastic (N-type), intermediate and substrate adherent (S-type)] NB cells. In line with our findings in situ N-type NB cells were characterized by very low levels of $\alpha5$ expression when compared to S-type NB. Unexpectedly, however, expression of $\alpha3$ was more prominent in S-type NB cell lines. Moreover, in contrast with our data in situ and those presented by others [13, 17], expression of $\alpha4$ was found in a variety of NB cell lines, irrespective of the morphological type [43]. These discrepancies might be due either to a clonal selection during the establishment of the cell lines or to culture conditions. Such effects have been proposed as

Fig. 1 Embryonal rhabdomyosarcoma. All rhabdomyoblastic and undifferentiated tumour cells are $\beta1^+$ (×178)

Fig. 2 Alveolar rhabdomyosarcoma. All tumour cells are $\alpha6^+$. Duct remnants of infiltrated mammary gland are also $\alpha6^+$ (×142)

Fig. 3 Neuroblastoma. The tumour cell population is $\alpha5^-$ contrasting with the $\alpha5^+$ endothelial cells (×142)

Fig. 4 Neuroblastoma. The entire neoplastic population is $\alpha1^+$ (×214)

Fig. 5 Primitive peripheral neuroectodermal tumour. The tumour cell population is evenly $\beta4^-$. $\beta4^+$ endothelial cells serve as a positive intrinsic control of the immune reaction (×129)

Fig. 6 Primitive peripheral neuroectodermal tumour. All tumour cells are $\alpha1^-$. Endothelial cells are strongly $\alpha1^+$ (×129)

Fig. 7 Primitive peripheral neuroectodermal tumour. Expression of $\alpha3$ is restricted to endothelial cells while being absent in the tumour cell population (×129)

Fig. 8 Ewing's sarcoma. The entire neoplastic population is $\alpha5^+$. Endothelial cells and stromal fibrocytes/-blasts are $\alpha5^+$ as well (×142)

an explanation for conflicting in situ/in vitro data in the melanoma system [2].

The pPNET and ES studied showed close to identical integrin profiles. In addition to the β1 subunit, pPNET and ES expressed the α5 subunit in nearly all cases while most other β1-associated integrin subunits, especially the α1 and α3 chains, were undetectable or only extremely rarely found in these tumours. This is in accordance with the phenotype of a PNE cell line that was $\alpha1^-$ but $\alpha5^+$ in vitro [11]. Furthermore, expression of α5 was a consistent feature in three ES cell lines examined in vitro [43].

The almost identical integrin profile of pPNET and ES adds further evidence to the close nosological relationship of these two tumours that up to date is based on the common reciprocal translocation t(11,22)(q24,q12) [41, 44], the common expression of the MIC-2 antigen [3], and other immunophenotypic correspondences such as the consistent expression of HLA-A,B,C and the absence of CD9 and CD56/neural cell adhesion molecule [15, 26].

SRBCS are currently regarded as a morphologically rather uniform group of highly malignant and aggressive sarcomas. The phenotypic differences among the different types of SRBCS we have described herein most likely reflect differences in the mode of tumour growth and spread, since the molecules involved are fundamentally important regulators of the social behaviour of cells in tissues. However, one should refrain from speculating too much on the net effects that these different integrin receptor profiles might have in vivo. At this point we do not know whether the integrins expressed on SRBCS are assembled in a functional fashion. Furthermore, their ligand specificity will have to be re-defined for the cell types we have been dealing with. We cannot exclude the possibility that integrin functions and ligands not yet known might play a significant role. Nevertheless, it may well be that the adhesion receptor profile contributes to the fact that SRBCS typing is of clinical importance.

Acknowledgements This study was supported by a grant from the Deutsche Krebshilfe/Dr. Mildred Scheel-Stiftung (W57/93/Mö3). We are indebted to Simone Westenfelder and Andrea Müller for skillful technical assistance and to John Moyers for photographic assistance.

References

1. Albelda SM (1993) Role of integrins and other cell adhesion molecules in tumor progression and metastasis. Lab Invest 68:1–17
2. Albelda SM, Mette SA, Elder DE, Stewart RM, Damjanovich L, Herlyn M, Buck CA (1990) Integrin distribution in malignant melanoma: association of the β3 subunit with tumor progression. Cancer Res 50:6757–6764
3. Ambros IM, Ambros PF, Strehl S, Kovar H, Gadner H, Salzer-Kuntschik M (1991) MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumors. Cancer 67:1886–1893
4. Amiot M, Bernard A, Tran HC, Leca G, Kanellopoulos JM, Boumsell L (1986) The human cell surface glycoproteien complex (gp120,200) recognized by monoclonal antibody K20 is a component binding to phytohaemagglutinin on T cells. Scand J Immunol 23:109–118
5. Aurias A, Rimbaut C, Buffe D, Dubousset J, Mazabraud A (1983) Chromosomal translocations in Ewing's sarcoma. N Engl J Med 309:496–498
6. Bednarczyk JL, McIntyre BW (1992) Expression and ligand-binding function of the integrin α4β1 (VLA-4) on neural-crest-derived tumor cell lines. Clin Exp Metastasis 10:281–290
7. Bosman FT (1993) Integrins: cell adhesives and modulators of cell function. Histochemical J 25:469–477
8. Burridge K, Fath K, Kelly T, Nuckolls G, Turner C (1988) Focal adhesion: transmembrane junction between the extracellular matrix and the cytoskeleton. Annu Rev Cell Biol 4:487–525
9. Chan BMC, Matsuura N, Takada Y, Zetter BR, Hemler ME (1991) In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells. Science 251:1600–1602
10. Crist WM, Kun LE (1991) Common solid tumors of childhood. N Engl J Med 324:461–471
11. Dedhar S, Haqq CH, Gray V (1989) Specific overproduction of very late antigen 1 integrin in two human neuroblastoma cell lines selected for resistance to detachment by an Arg-Gly-Asp-containing synthetic peptide. J Biol Chem 264:4832–4836
12. Enzinger FM, Weiss SW (1988) Soft tissue tumors. Mosby, St. Louis
13. Favrot MC, Combaret V, Goillot E, Lutz P, Frappaz D, Thiesse P, Thyss A, Dolbeau D, Bouffet E, Tabone E, Philip T (1991) Expression of integrin receptors on 45 clinical neuroblastoma specimens. Int J Cancer 49:347–355
14. Fradet Y, Cordon-Cardo C, Thomson T, Daly ME, Whitmore WF, Lloyd KO, Melamed MR, Old LJ (1984) Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. Proc Natl Acad Sci USA 81:224–228
15. Garin-Chesa P, Fellinger EJ, Huvos AG, Beresford HR, Melamed MR, Triche TJ, Rettig W (1991) Immunohistochemical analysis of neural cell adhesion molecules. Differential expression in small round cell tumors of childhood and adolescence. Am J Pathol 139:275–286
16. Giltay JC, Brinkman HJ, Modderman PW, Borne AEGK von dem, Mourik JA van (1989) Human vascular endothelial cells express a membrane protein complex immunochemically indistinguishable from the platelet VLA-2 (glycoprotein Ia–IIa) complex. Blood 73:1235–1241
17. Gross N, Favre S, Beck D, Meyer M (1992) Differentiation-related expression of adhesion molecules and receptors on human neuroblastoma tissues, cell lines and variants. Int J Cancer 52:85–91
18. Hemler ME, Sanchez-Madrid F, Flotte TJ, Krensky AM, Burakoff SJ, Bahn AK, Springer TA, Strominger IL (1984) Glycoproteins of 210.000 and 130.000 m.w. on activated T cells: cell distribution and antigenic relation to components on resting cells and T-cell lines. J Immunol 132:3011–3018
19. Hemler ME, Jacobson JG, Brenner MB, Mann D, Strominger JL (1985) VLA-1: a T cell surface antigen which defines a novel late stage of human T cell activation. Eur J Immunol 15:502–508
20. Hemler ME, Crouse C, Sonnenberg A (1989) Association of the VLA α6 subunit with a novel protein. J Biol Chem 264:6529–6535
21. Hessle H, Sakai LY, Hollister DW, Burgeson RE, Engvall E (1984) Basement membrane diversity detected by monoclonal antibodies. Differentiation 26:49–54
22. Hynes RO (1992) Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69:11–25
23. Juliano R (1994) Signal transduction by integrins and its role in the regulation of tumor growth. Cancer Metastasis Rev 13:25–30
24. Koretz K, Schlag P, Boumsell L, Möller P (1991) Expression of VLA-α2, VLA-α6, and VLA-β1 chains in normal mucosa and adenomas of the colon, and in colon carcinomas and their liver metastases. Am J Pathol 138:1–10
25. Mark H von der, Dürr J, Sonnenberg A, Mark K von der, Deutzmann R, Goodman SL (1991) Skeletal myoblasts utilize

a novel β1-series integrin and not α6β1 for binding to the E8 and T8 fragments of laminin. J Biol Chem 266:23593–23601
26. Mechtersheimer G, Barth T, Ludwig R, Staudter M, Möller P (1993) Differential expression of leukocyte differentiation antigen in small, round, blue cell sarcomas. Cancer 71:239–248
27. Otey CA, Pavalko FM, Burridge K (1990) An interaction between α-actinin and the β1 integrin subunit in vitro. J Cell Biol 111:721–729
28. Palmer EL, Ruegg C, Ferrando R, Pytela R, Sheppard D (1994) Sequence and tissue distribution of the integrin α9 subunit, a novel partner of β1 that is widely distributed in epithelial and muscle. J Cell Biol 123:1289–1297
29. Peltonen J, Larjava H, Jaakola S, Grainick H, Akiyama SK, Yamada KM, Uitto J (1989) Localization of integrin receptors for fibronectin, collagen, and laminin in human skin. J Clin Invest 84:1916–1923
30. Ruoslahti E, Noble NA, Kagami S, Border WA (1994) Integrins. Kidney Int 45:17–22
31. Ruan C, Du X, Wan H, Hu X, Xi X, Li P (1987) Characterization of the fibrinogen binding sites using monoclonal antibodies to human platelet membrane glycoproteins IIb/IIIa (abstract). Thromb Haemost 58:243 (abstr.)
32. Sanchez-Madrid F, Landazuri MO de, Morago G, Cebrian M, Acevedo A, Bernabeu C (1986) VLA-3: a novel polypeptide association within the VLA molecular complex: cell distribution and biochemical characterization. Eur J Immunol 16:1343–1349
33. Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein RL, Springer TA, Tedder TF, Todd RF (1994) CD antigens 1993. Blood 83:879–880
34. Song WK, Wang W, Foster RF, Bielser DA, Kaufman SJ (1992) H36-α7 is a novel integrin alpha chain that is developmentally regulated during skeletal myogenesis. J Cell Biol 117:643–657
35. Sonnenberg A, Jansen H, Hogervorst F, Calafat J, Hilgers J (1987) A complex of platelet glycoprotein Ic and IIa identified by a rat monoclonal antibody. J Biol Chem 21:10376–10383
36. Thiele CJ (1991) Biology of pediatric peripheral neuroectodermal tumors. Cancer Metastasis Rev 10:311–319
37. Velde A te, Klomp JP G, Yard BA, Vries JE de, Figdor CG (1988) Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. J Immunol 140:1548–1554
38. Vries JE de, Keizer GD, Velde AA te, Voordouw A, Ruitter D, Rumke P, Spits H, Figdor CG (1986) Characterization of melanoma-associated surface antigens involved in the adhesion and motility of human melanoma cells. Int J Cancer 38:465–473
39. Vogel BE, Tarone G, Giancotti FG, Gailit J, Ruoslahti E (1990) A novel fibronectin receptor with an unexpected subunit composition (αvβ1). J Biol Chem 11:5934–5937
40. Weitzman JB, Pasqualini R, Takada Y, Hemler ME (1993) The function and distinctive regulation of the integrin VLA-3 in cell adhesion, spreading, and homotypic cell aggregation. J Biol Chem 268:8651–8657
41. Whang-Peng J, Triche TJ, Knutsen T, Miser J, Douglass EC, Israel MA (1984) Chromosomal translocation in peripheral neuroepithelioma. N Engl J Med 311:584–585
42. Wick MR, Swanson PE (1988) Immunohistochemistry of neoplasia. Soft tissue tumors. In: Bhan AK, McCluskey TE (eds) Diagnostic Immunopathology. Raven Press, New York, pp 365–400
43. Yoshihara T, Esumi N, Humphries MJ, Imashuku S (1992) Unique expression of integrin fibronectin receptors in human neuroblastoma cell lines. Int J Cancer 51:620–626
44. Zucman J, Delattre O, Desmaze C, Plogastel B, Joubert I, Melot P, Peter M, Jong P de, Rouleau G, Aurias A, Thomas G (1992) Cloning and characterization of the Ewing's sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. Genes Chrom Cancer 5:271–277
45. Zutter MM, Mazoujian G, Santoro SA (1990) Decreased expression of integrin adhesive protein receptors in adenocarcinoma of the breast. Am J Pathol 137:836–870