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ORIGINAL ARTICLE

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Integrins and extracellular matrix-proteins in the different components of the Wilms' tumour

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Abstract The Wilms' tumour (WT) is composed of blastema, epithelium and mesenchyme; the epithelium and possibly also the mesenchyme develop from the blastema, parallel to embryonal development. Since interactions between cell adhesion receptors and extracellular matrix (ECM) proteins play an important role in tissue maturation, we examined the expression of the integrin subunits α1-α6, β1 and β4, and of the ECM proteins fibronectin, laminin and collagen I and IV, in 20 frozen WT samples and in 5 fetal and 2 adult kidneys. The integrin and ECM protein distribution in tumour epithelium and mesenchyme showed strong similarities to that in their fetal counterparts, whereas the tumour blastema differed strongly from the fetal blastema. In the WT blastema different components were recognized. Undifferentiated blastema, characterized by expression of α3 and α6 and the virtual absence of ECM proteins. Blastema with epithelial commitment, showing increased expression of α3 and α6 and the appearance of α2 and, as a very early phenomenon, production of laminin. Blastema with mesenchymal commitment, with loss of α3 and α6 and expression of α1, α4 and α5 and presence of ECM proteins. It is speculated that the inability of the (undifferentiated) blastema to produce ECM proteins is related to its relatively high metastatic potential when compared with epithelium and mesenchyme.

Key words Adhesion molecules · Differentiation Blastema · Renal development

Introduction

Wilms' tumour (WT) or nephroblastoma is the most frequent renal malignancy in children [8]. The classical WT consists of three components: blastema, epithelium and mesenchyme, although biphasic and even monophasic tumours are also seen [2, 4, 7]. It has been suggested that WT recapitulate normal developmental events in the kidney, and that blastema differentiates into epithelium and mesenchyme [6]. It is known that cell-matrix interactions play an important role in normal development as well as in tumour invasion and metastasis [18, 25, 34, 40, 41] and these interactions are mediated by adhesion receptors, to a large extent by the family of integrins. Integrins are formed by various combinations of α- and β-subunits, which in turn are able to interact with various ligands (Table 1). The intracytoplasmic part of the integrin molecules is connected to the cytoskeleton, forming a bridge between extracellular and intracellular compartments [42]. In this way the function of integrins extends beyond adhesion, as it has been demonstrated that they transduce signals from the ECM, to regulate cellular activities such as differentiation and migration [10, 18, 33, 42].

In view of the parallels between WTs and normal development it is of interest to compare the distribution of integrins and their ligands in the WT with that in normal fetal and adult renal tissue. This prompted us to perform an immunohistochemical study of the integrin subunits α1–α6 and β1 and β4 in 20 WTs, with emphasis on their expression in the different tumour components. The dis-

Table 1 Integrins and their ligands (*Coll*, collagen; *LM* laminin; *FN*, fibronectin; *FN alt.*, alternatively spliced CS-1 region in fibronectin; *VCAM*, vascular cell adhesion molecule; *BM*, basement membrane)

Integrins	Ligands
$\alpha1\beta1$	Coll.I, Coll.IV, LM
$\alpha2\beta1$	Coll.I, Coll.IV, LM, FN
$\alpha3\beta1$	Coll.I, LM, FN
$\alpha4\beta1$	FN alt., VCAM-1
$\alpha4\beta7$	FN alt., VCAM-1[a]
$\alpha5\beta1$	FN
$\alpha6\beta1$	LM
$\alpha6\beta4$	BM

[a] The specificity of this receptor is not yet clear. References used: [18, 34]

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tribution of integrin subunits within fetal and adult kidney has been studied previously [20, 32, 41], but the results in these studies were not consistent. We therefore included 5 fetal and 2 adult kidneys, using the same antibodies and titres as used on WT tissue. The distribution of the ECM-proteins fibronectin (FN), laminin (LM), collagen (Coll.) I and IV was studied in the same samples. From our initial results we suspected that LM participated early in the process of epithelial differentiation in WT, as in normal epithelial differentiation [17, 23, 39]. To verify this notion we performed a double-staining using antibodies to LM and an early epithelial marker (Moc-31) [2].

Materials and methods

Surgically resected WT specimens were received from 20 patients ranging in age from 7 to 141 months (median 56 months). Seventeen specimens were taken from primary, untreated WTs and one from a synchronous metastasis in Douglas' pouch. Two samples, a metachronous lung metastasis and a recurrent WT were from patients previously treated chemotherapeutically. Five specimens from normal fetal kidney (17, 28, 33, 35 and 40 weeks post-menstrual age, respectively) and 2 specimens from normal adult kidney (56 and 74 years of age) were obtained from autopsies performed in cases without kidney disease. The collected tissue samples were all snap frozen in either freon or isopentane and stored at −80° C until further use.

Haematoxylin-eosin stained frozen tumour sections were analysed for the presence of blastemal, epithelial and mesenchymal components as defined by Bennington and Beckwith [7] and Beckwith and Palmer [5]. Only clearcut tubular formations with a central lumen were considered to be epithelial. Areas composed of loosely arranged spindle cells, of which the ratio of the length to diameter of the nucleus exceeded 3:1, were considered to be mesenchymal [5].

The antibodies used in the current study are listed in Table 2.

A two- and a three-step immunoperoxidase technique was performed on 4 μ frozen sections of all collected material. The two-step technique was used when the specific antibodies were raised in rabbit (polyclonal), the three-step when raised in mouse, rat (both monoclonal) or goat (polyclonal). In the first step the sections were incubated with 50 μl of a specific antibody for 60 min. The subsequent steps are represented schematically in Table 3. In these steps each section was incubated with 50 μl of horseradish-peroxidase-conjugated immunoglobulin antibody solution (Dakopatts, Copenhagen, Denmark), supplemented with 1% human AB-serum, for 30 min. 3-Amino-9-ethylcarbazole (Sigma, St. Louis, Mo., USA), was used as a substrate for the demonstration of peroxidase activity. All sections were counterstained with haematoxylin.

A double-staining technique was employed on sections of specimens from three WTs, using anti-LM and Moc-31. Here, four separate incubation steps had to be performed, i.e. anti-LM (50 μl, 1:500, 60 min), swine-anti-rabbit peroxidase immunoglobin Abs (50 μl, 1:20, 30 min; Dakopatts), Moc-31 (50 μl, undiluted, 30 min), rabbit-anti-mouse alkaline phosphatase immunoglobin Abs (50 μl, 1:20, 15 min; Dakopatts). Alkaline phosphatase activity, visualizing Moc-31, was demonstrated as described previously [16].

Table 2 Characteristics of antibodies (*Tsc*, T-cell science, Cambridge; *CLB*, Central Laboratory of the Netherlands red cross blood transfusion service, Amsterdam; *ATTC*, American Type Culture Collection; *T*, Telios, San Diego; *UBI*, Upstate Biotechnology, Inc., Lake Placid; *M*, Monosan, Uden, The Netherlands; *L*, de Ley, Groningen [12]; *SBA*, Southern Biotechnology Associates, Birmingham, Alabama; *C*, Cedarlane, Hornby, Ontario; *Int*, Integrin

	Source	Reactivity	Generated	Dilution
Monoclonal				
TS 2/7	Tcs	α1-subunit	Mouse	1:5000
CLB-tromb/4	CLB	α2-subunit	Mouse	1:500
P1B5	T	α3-subunit	Mouse	1:2000
B5G10	UBI	α4-subunit	Mouse	1:5000
VLA-5 (CD49e)	M	α5-subunit	Mouse	1:500
GoH3	CLB	α6-subunit	Rat	undiluted
TS 2/16	ATTC	β1-subunit	Mouse	1:5000
3E1	T	β4-subunit	Mouse	1:5000
anti-FN[a]	T	Fibronectin	Mouse	1:5000
Moc-31	L	epithelia	Mouse	undiluted
Polyclonal				
anti-LM	T	Laminin	Rabbit	1:500
anti-Coll.I	SBA	Collagen I	Goat	1:100
anti-Coll.IV	SBA	Collagen IV	Goat	1:1000

[a] [30]

Table 3 Schematic representation of subsequent steps in a two- and three-step immunoperoxidase technique. (*ab*, Antibody; *RAM*, rabbit-anti-mouse; *RAR*, rabbit-anti-rat; *RAG*, rabbit-anti-goat; *SAR*, swine-anti-rabbit; *PO*, peroxidase. All peroxidase-conjugated antibodies were obtained from Dakopatts, Copenhagen, Denmark. Dilutions used, are given in parentheses)

Ab raised in	Abs used in second incubation step	Abs used in third incubation step
Mouse	RAM-PO (1:50)	SAR-PO (1:50)
Rat	RAR-PO (1:50)	SAR-PO (1:50)
Goat	RAG-PO (1:50)	SAR-PO (1:50)
Rabbit	SAR-PO (1:20)	–

Results

In the haematoxylin-eosin stained sections, we were able to identify blastemal components in 19 tumours, mesenchymal components in 10 and epithelial components in 8. We further observed areas in the blastema that had lost the typical appearance of this component. In these areas, we identified either primitive epithelial structures, characterized by cells with larger nuclei forming solid nests or rosettes, but without clearcut tubules, or loosely organized blastemal cells, that appeared somewhat elongated though spindle shaped cells could not be identified. As these areas could not be characterized unequivocally as epithelium or mesenchyme, we defined them as blastema with an epithelioid, or mesenchymal aspect, respectively (Fig. 1, 3d; cf. also [2].

In the fetal specimens we were able to identify a blastemal component in three cases, with menstrual ages of 17, 28 and 33 weeks, respectively.

The most prominent integrin subunit expressed in the blastemal component (Fig. 2) was α6 (Fig. 3a); the α3 subunit was expressed throughout the blastema as well (Fig. 3b). The staining intensity of α6 and α3 tended to be less in blastemal areas with a mesenchymal aspect, whereas especially α6 was stronger in areas with an epithelioid aspect.

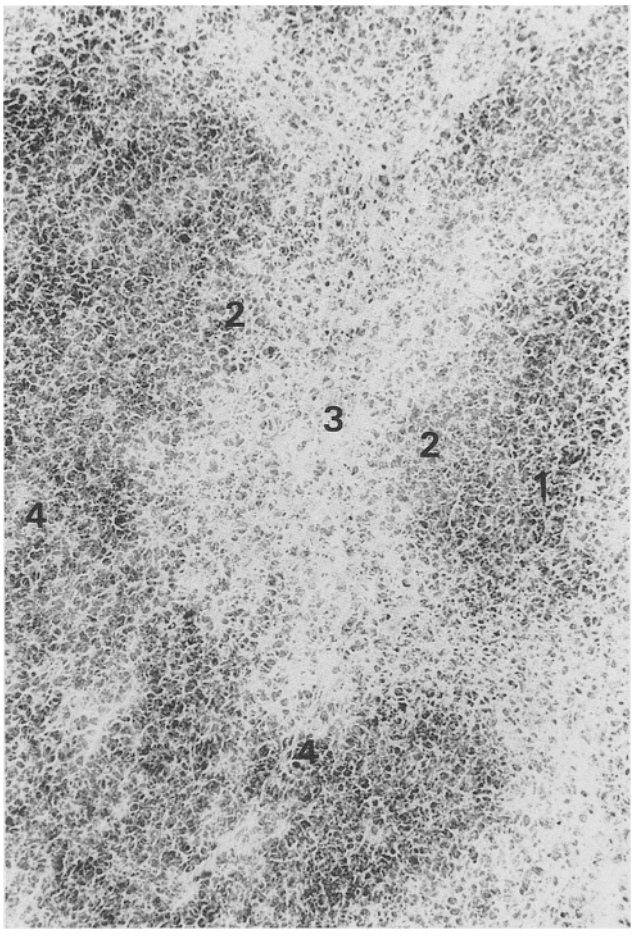
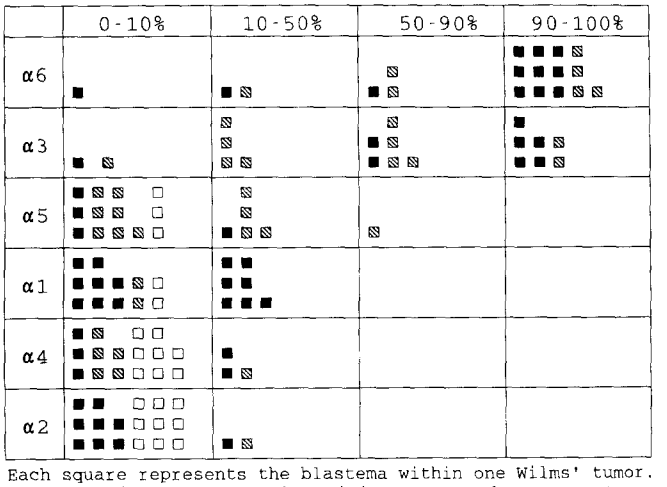


Fig. 1 Undifferentiated blastema (*1*), blastema with a mesenchymal aspect (*2*), mesenchyme (*3*) and blastema with an epithelioid aspect (*4*). H & E stained frozen section (×140)



Each square represents the blastema within one Wilms' tumor. □ : no staining, ▨ : weak staining, ■ : moderate to strong staining.

Fig. 2 Summary of the findings of the α subunits in WT blastema, indicating the intensity and the approximate percentage of immunoreactive cells

In contrast to the $\alpha 3$ and $\alpha 6$ subunits, the $\alpha 1$, $\alpha 2$, $\alpha 4$ and $\alpha 5$ subunits were expressed only to a limited extent. The integrin subunits $\alpha 1$, $\alpha 4$ and $\alpha 5$, were primarily demonstrated in blastemal areas with a mesenchymal aspect, whereas $\alpha 2$ was primarily expressed in blastema with an epithelioid aspect.

The epithelial cells expressed $\alpha 6$ in an even stronger fashion than the blastemal cells. The immunoreactivity was localized at the cell surface and was generally more prominent at the basal side. The epithelial structures expressed $\alpha 2$ and $\alpha 3$ integrin subunits as well, but no $\alpha 1$, $\alpha 4$ or $\alpha 5$ subunits. The $\alpha 2$ integrin subunit was expressed by only a minority of the tubules, which appeared small as compared to the $\alpha 2$-negative tubules. Expression of the $\alpha 3$ subunit was seen in both smaller and larger tubules.

The pattern of expression of integrin subunits in the mesenchyme was largely complementary to that in the epithelial structures. Thus, $\alpha 1$, $\alpha 4$ and $\alpha 5$ were expressed by almost all mesenchymal cells. The number of positive cells for $\alpha 2$, $\alpha 3$ and $\alpha 6$ ranged from less than 10% to nearly a 100%. The staining intensity was usually weak, though moderate and sometimes even strong expression was seen.

The $\beta 1$-integrin subunit was observed throughout all three tumour components. It was especially prominent at the basal side of epithelial structures. The $\beta 4$-integrin subunit was only found at the basal side of some epithelial structures.

FN and Coll.I were abundantly present in the mesenchymal component. In some cases they were also present at the basal side of tubules. In the undifferentiated blastemal areas FN distribution was limited and displayed a very irregular and fragmented pattern (Fig. 3c), whereas in those blastemal areas with a mesenchymal aspect a more diffuse staining pattern was observed. Coll.I was only seen in blastemal areas with a mesenchymal aspect, though not as prominently as FN.

The basal membrane protein LM was strongly expressed around tubules. In 3 cases up to approximately 60% of the blastemal component was also immunoreactive. In 3 other cases LM was distributed in blastema in a circular configuration, similar to that seen around epithelial structures, though morphologically no epithelial differentiation could be recognized. The double-staining in these three cases showed that Moc-31 and LM were largely present in the same areas, but not all cells enclosed by LM were Moc-31 positive (Fig. 3d). In the mesenchyme, LM expression varied in intensity and was strongest in the most differentiated areas.

Like LM, Coll. IV was seen in the basal membrane of tubules and to some extent also in the mesenchyme. In the blastema only some irregular or fragmented Coll. IV expression was seen.

The results of integrin expression in fetal and adult kidneys are represented in Table 4 and illustrated in Fig. 3e, f.

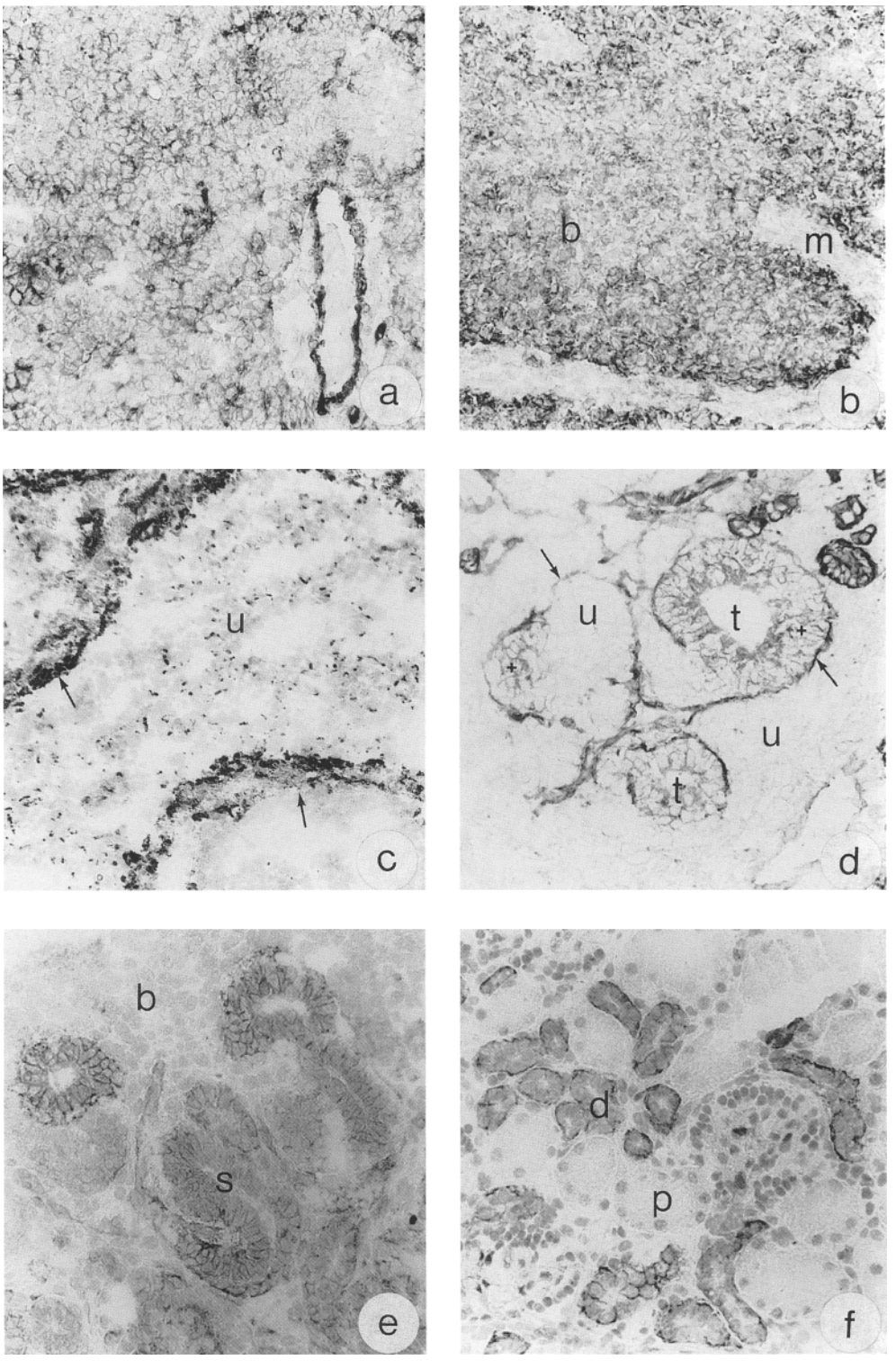


Fig. 3a–d Wilms' tumour; (**a**) immunoperoxidase staining for $\alpha 6$ in the blastemal component (×224). (**b**) Immunoperoxidase staining for $\alpha 3$ in the blastemal component (*b*), with negative mesenchyme (*m*) (×224). (**c**) Immunoperoxidase staining for fibronectin showing a fragmented reaction pattern in the undifferentiated blastema (*u*) and a stronger reaction pattern in the mesenchyme (*arrows*; ×140). (**d**) Double-staining for laminin (peroxidase) and Moc-31 (alkaline phosphatase) showing two tubules (*t*) with cellular immunoreactivity for Moc-31 (+), surrounded by laminin positive matrix (*arrows*). An adjacent undifferentiated blastemal area (*u*) shows a circular pattern of laminin immunoreactivity, whereas only a part of the enclosed cells is immunoreactive for Moc-31 (×224). (**e**) and (**f**) Fetal tissue; (**e**) immunoperoxidase staining for $\alpha 6$ in primitive tubules and an S-shaped body (*s*), with negative blastema (*b*) (×224); (**f**) $\alpha 2$ immunoperoxidase staining detected at distal (*d*), but not proximal tubules (*p*)

LM and Coll.IV were detected at the basal side of the tubular structures in fetal and adult kidney. FN and Coll. I were present in the uninduced blastema and in the interstitium. FN was located at the basal side of immature epithelial structures, whereas in the more mature structures, expression could no longer be detected.

Discussion

The current study compared the distribution of integrin subunits and ECM proteins in the different components of the WT to that in normal fetal and adult kidney. In a study like this it is not feasible to take into account all

Table 4 The distribution of integrin subunits within fetal and adult kidney tissue (fetal/adult kidney), (*unind.bl.*, uninduced blastema; *pr*, primary; *s-shap.*, s-shaped; *coll.*, collecting; *interst.*, interstitium; *w*, weak; * not applicable; +, positive; –, negative; ±, some cells positive, some negative)

	β1	β4	α1	α2	α3	α4	α5	α6
unind.bl.	+/*	–/*	w/*	–/*	–/*	+/*	±/*	–/*
pr.vesicle	+/*	–/*	–/*	+/*	w/*	–/*	–/*	+/*
s-sh.body	+/*	–/*	–/*	+/*	w/*	–/*	–/*	w/*
glomerulus								
visceral	–/+	–/–	–/–	–/–	–/w	–/–	–/–	–/–
parietal	+/+	–/–	–/–	–/–	+/+	–/–	–/–	±/±
mesangial	+/+	–/–	w/+	+/w	±/±	–/–	+/+	±/±
tubules[a]								
proximal	+/+	–/–	–/–	–/–	–/–	–/–	–/–	+/+
distal	+/+	–/–	–/–	+/+	±/+	–/–	–/–	+/+
coll.ducts	+/+	+/+	–/–	+/+	±/+	–/–	–/–	+/+
interst.	+/+	–/–	–/–	–/–	–/–	–/–	+/+	–/–

[a] Integrin expression was primarily located at the basal side, especially in the more mature tubules

different isoforms of the various ECM proteins [29, 37] and therefore we used polyclonal antibodies, except for FN. In general, the epithelial and mesenchymal components of the WT resembled their fetal and adult counterparts in respect to their expression of integrins as well as their ECM protein production. The differentiated epithelial structures of the WT expressed α6, α3 and to some extent α2, similar to the fetal and adult kidney. However, when compared with normal fetal and adult renal tissue, integrin expression by the tubular structures of the WT showed less polarisation (see [41]), parallel to previous observations in normal and neoplastic epithelium of the breast [24]. The expression of LM, Coll. IV and FN at the basal side of tubular structures of the WT is also similar to that seen in normal fetal and adult renal tissue [1, 9, 13, 14] and indicates a high level of differentiation in this tumour component. This may explain why WTs with a large epithelial component are associated with good survival [3, 15, 19, 22]. In the mesenchyme of the WT, the α1, α4 and α5 integrin subunits were markedly expressed, which, with the exception of α4, were expressed in the interstitium of the fetal and adult kidney as well. In both tumour and normal mesenchyme FN and Coll. I were abundant.

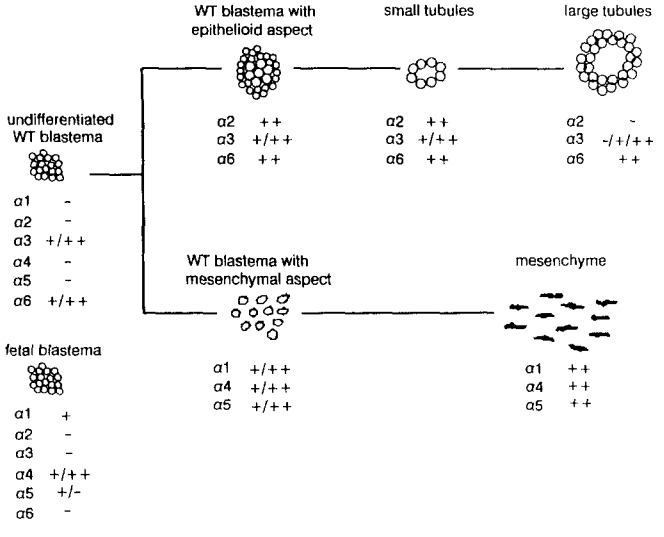


Fig. 4 Diagram summarizing the major findings of α subunits of integrins in the different components of the Wilms' tumour, indicating the differentiation into epithelial and mesenchymal structures. For comparison the fetal blastema is represented as well

The tumour and fetal blastema differed in their integrin expression as well as in their distribution of ECM-proteins. The undifferentiated neoplastic blastema expressed α3 and α6 integrin subunits and as reported earlier [36] virtually no ECM proteins. The uninduced fetal blastema expressed α1, α4 and to some extent α5 integrin subunits, but in contrast to the findings by Terpe et al. [41], no α6. As in earlier reports [13, 28], the uninduced blastema also showed FN and Coll.I. During normal development the blastema is assumed to be the germinal matrix with gradually gives rise to epithelial and mesenchymal components. In parallel, the WT blastema may have the potential to differentiate into epithelial and mesenchymal structures. A morphological transition between undifferentiated blastema and epithelium and, less clearly, mesenchyme can be observed and previous immunohistochemical findings have indicated that the expression of early epithelial markers in WT blastema precedes morphological epithelial differentiation [2]. The current findings extend this notion and indicate a gradual change in integrin expression and ECM production with progressive epithelial and mesenchymal differentiation (Fig. 4). Progressive epithelial differentiation was associated with increased expression of α3 and α6 and the appearance of α2. The focal, circular distribution of LM in the blastema suggests that in the WT this ECM protein is an early manifestation of epithelial differentiation (see also [21b]). The double stainings, using an early epithelial marker (Moc-31) together with a polyclonal antibody to LM, suggest that the production of LM is in fact the first step in epithelial differentiation and precedes morphological differentiation and even expression of Moc-31. During epithelial differentiation in normal renal development the integrins are

thought to interact with ECM proteins [21a, 39] and it seems that a similar phenomenon occurs in the neoplastic blastema. In this respect, the current findings suggest that the LM production characterizes the switch from undifferentiated blastema to "epithelially committed" blastema. The mesenchyme differed from blastema by the absence of $\alpha 3$ and $\alpha 6$ integrin subunits and increased expression of $\alpha 1$, $\alpha 4$ and $\alpha 5$ as well as ECM production. Despite the above described epithelial and mesenchymal commitment of blastema components it is unlikely that all neoplastic blastema has the potential for further differentiation. It may be assumed that an undifferentiated, non-committed component remains at a stem cell level, which according to the present findings, is characterized by expression of $\alpha 3$ and $\alpha 6$ integrin subunits and the virtual absence of ECM proteins. Although we cannot state with certainty that no isoform of FN was expressed by WT blastema, the absence of all ECM proteins in the fetal blastema contrasts sharply with normal blastema, which showed both FN and Coll. I. This is in keeping with previous studies which have documented the incomplete production of ECM by malignant tumours compared with their normal counterparts [11, 34]. Some authors have speculated that the lack of ECM gives the tumour cells an added degree of freedom [31, 34] resulting in the potential to invade and metastasize. However, it has also been suggested that the altered expression of adhesion proteins by malignant tumour cells may be more important than the loss of ECM, as migrating tumour cells still have to penetrate the normal matrix secreted by neighbouring cells [31]. In this respect, the presence of $\alpha 3$ and $\alpha 6$ in the blastema is of special interest, as it enables cells to interact with interstitial and basal membrane proteins (FN and LM) a factor thought to be important in the process of haematogenous metastasis [11, 24, 35]. Either way, the absence of ECM production supports the malignant character of the undifferentiated blastema.

The current findings suggest that the WT blastema is composed of undifferentiated and epithelially or mesenchymally committed components, a composition reminiscent of that of other pediatric neoplasms [26, 27]. The switch from undifferentiated to epithelially committed WT blastema appears to be associated with the initiation of production of LM. Among the WT components the blastema is more likely to metastasize than the epithelium and mesenchyme [3, 19, 38, 43]. It is tempting to assume that it is especially the undifferentiated component which displays this relatively aggressive biological behaviour, which in turn may be related to the inability of these cells to produce ECM proteins.

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References

1. Abrahamson DR, Leardkamolkarn V (1991) Development of kidney tubular basement membranes. Kidney Int 39:382–393
2. Albeda FW, Molenaar WM, de Leij L, Thijs-Ipema AH (1989) Heterogeneity of Wilms' tumour blastema. An immunohistological study. Virchows Arch [A] 414:263–271
3. Bannayan GA, Huvos AG, D'Angio GJ (1971) Effect on irradiation on the maturation of Wilms' tumor. Cancer 27:812–818
4. Beckwith JB (1983) Wilms' tumor and other renal tumors of childhood: a selective review from the national Wilms' tumor study pathology center. Hum Pathol 14:481–492
5. Beckwith JB, Palmer NF (1978) Histopathology and prognosis of Wilms' tumor. Results from the first national Wilms' tumor study. Cancer 41:1937–1948
6. Beckwith JB, Kiviat NB, Bonadio JF (1990) Nephrogenic rests, nephroblastomatosis, and the pathogenesis of Wilms' tumor. Pediatr Pathol 10:1–36
7. Bennington JL, Beckwith JB (1975) Tumors of the kidney, renal pelvis, and ureter. 2nd edn, Armed Forces Institute of Pathology, Washington, D.C.
8. Breslow NE, Langholz B (1983) Childhood cancer incidence: geographical and temporal variations. Int J Cancer 32:703–716
9. Cooper M, Tamura RN, Quaranta V (1991) The major laminin receptor of mouse embryonic stem cells is a novel isoform of the $\alpha 6\beta 1$ integrin. J Cell Biol 115:843–850
10. Damsky H, Werb Z (1992) Signal transduction by integrin receptors for extracellular matrix: cooperative processing of extracellular information. Curr Opinion Cell Biol 4:772–781
11. Dedhar S, Saulnier R (1990) Alterations in integrin receptor expression on chemically transformed human cells: specific enhancement of laminin and collagen complexes. J Cell Biol 110:481–489
12. deLeij L, Broers J, Ramaekers F, Berendsen H, Wagenaar SjSc (1986) Application of monoclonal antibodies in tumor pathology. Martinus Nijhof, Amsterdam, pp 191–210
13. Ekblom P, Vestweber D, Kemler R (1986) Cell-matrix interactions and cell adhesion during development. Ann Rev Cell Biol 2:27–47
14. Fleming S (1991) Cell adhesion and epithelial differentiation. J Pathol 164:95–100
15. Green DM (1985) The diagnosis and management of Wilms' tumor. Pediatr Clin N Am 32 (3):735
16. Harms G, Dijkstra C, Hardonk M (1990) Glycosyl receptors in macrophage subpopulations of rat spleen and lymph node. Cell Tissue Res 262:35–40
17. Hay ED (1991) Collagen and other matrix glycoproteins in embryogenesis. In: Hay ED (ed) Cell biology of extracellular matrix. 2nd edn, Plenum Press, New York, pp 419–462
18. Hynes RO (1992) Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69:11–23
19. Jereb B, Sandstedt B (1973) Structure and size versus prognosis in nephroblastoma. Cancer 31:1473–1481
20. Korhonen M, Ylänne J, Laitinen L, Virtanen I (1990) The $\alpha 1$–$\alpha 6$ subunits of integrins are characteristically expressed in distinct segments of developing and adult human nephron. J Cell Biol 111:1245–1254
21a. Korhonen M, Laitinen L, Ylänne J, Gould VE, Virtanen I (1992) Integrins in developing, normal and malignant human kidney. Kidney Int 41:641–644
21b. Kumar S, Carr T, Marsden HB, Morris-Jones PH (1986) Study of childhood renal tumours using peroxidase conjugated lectins. J Clin Pathol 39:736–741
22. Lawler W, Marsden HB, Palmer MK (1977) Histopathological study of the first medical research council nephroblastoma trial. Cancer 40:1519–1525
23. Lin CQ, Biskell MJ (1993) Multifaceted regulation of cell differentiation by extracellular matrix. FASEB J 7:737–743
24. Liotta LA, Rao CN, Wewer UM (1986) Biochemical interactions of tumor cells with the basement membrane. Ann Rev Biochem 55:1037–1057

25. McCarthy JB, Skubitz APN, Iida J, Mooradian DL, Wilke MS, Furcht LT (1991) Tumor cell adhesive mechanisms and their relationship to metastasis. Cancer Biol 2:155–167
26. Molenaar WM, Oosterhuis JW, Oosterhuis AM, Ramaekers FCS (1985) Mesenchymal and muscle-specific intermediate filaments (vimentin and desmin) in relation to differentiation in childhood rhabdomyosarcomas. Hum Pathol 16:838–843
27. Molenaar WM, Jansson D, Gould VE, Rorke LB, Franke WW, Lee VMY, Trojanowski JQ (1989) Molecular markers of primitive neuroectodermal tumors (PNETs) and other pediatric central nervous system tumors. Lab Invest 61:635–643
28. Mounier F, Foidrat J, Gubler M (1986) Distribution of extracellular matrix glycoproteins during normal kidney development of human kidney. Lab Invest 54:394–401
29. Paulsson M (1992) Basement membrane proteins: structure, assembly, and cellular interactions. Crit Rev Biochem Mol Biol 27:93–127
30. Pierschbacher MD, Hayman HG, Ruoslahti E (1981) Location of the cell-attachment site in fibronectin with monoclonal antibodies and proteolytic fragments of the molecule. Cell 26:259–267
31. Plantefaber LC, Hynes RO (1989) Changes in integrin receptors on oncogenically transformed cells. Cell 56:281–290
32. Rahilly AM, Fleming S (1992) Differential expression of integrin alpha chains by renal epithelial cells. J Pathol 167:327–334
33. Ruoslahti E (1991) Integrins. J Clin Invest 87:1–5
34. Ruoslahti E (1992) Control of cell motility and tumour invasion by extracellular matrix interactions. Br J Cancer 66:239–242
35. Ruoslahti E, Giancotti FG (1989) Integrins and tumor cell dissemination. Cancer Cells 1:119–126
36. Sariola H, Ekblom P, Rapola J, Vaheri A, Timpl R (1985) Extracellular matrix and epithelial differentiation of Wilms' tumor. Am J Pathol 118:96–107
37. Schwarzbauer JE (1991) Alternative splicing of fibronectin: three variants, three functions. Bioassays 13:527–533
38. Shimmoto K, Ushigome S, Nikaido T, Kikuchi Y, Kobayashi N, Yamazaki Y (1991) Maturation of pulmonary metastases of Wilms' tumor after therapy: a case report. Pediatr Hematol Oncol 8:147–157
39. Sorokin L, Ekblom P (1992) Development of tubular and glomerular cells of the kidney. Kidney Int 41:657–664
40. Terpe HJ, Tajrobekhar K, Günthert U, Altmannsberger M (1993) Expression of cell adhesion molecules alpha-2, alpha-5 and alpha-6 integrin, E-cadherin, N-CAM and CD-44 in renal cell carcinomas. An immunohistochemical study. Virchows Arch [A] 422:219–224
41. Terpe HJ, Stark H, Ruiz P, Imhof BA (1994) Alpha 6 integrin distribution in human embryonic and adult tissues. Histochemistry 101:41–49
42. Teti A (1992) Regulation of cellular functions by extracellular matrix. J Am Soc Nephrol 2:S83–S87
43. Van Leeuwen EH, Postma A, Oosterhuis JW, Meiring A, Cornelisse CJ, Koudstaal J, Molenaar WM (1987) An analysis of histology and DNA-ploidy in primary Wilms' tumors and their metastases and a study on the morphological effects of therapy. Virchows Arch [A] 410:487–494