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Chromosome Aberrations in Mesoblastic Nephroma

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Mesoblastic nephroma (MN) is the most common renal tumor diagnosed in infancy. Histologically, MNs are designated as classic, cellular, or mixed type based upon variations in cellularity. Recent karyotypic reports have suggested that extra copies of chromosome 11 are a nonrandom occurrence in MNs. We analyzed nuclear suspensions prepared from a group of 17 formalin-fixed, paraffin-embedded tumors to determine the possible role of chromosome 11 copy number in the genesis of MN. Extra copies of D11Z1 (a probe for the centromeric region of chromosome 11) were detected in seven out of 10 MNs with cellular or mixed histology, whereas each of six classic histology MNs were disomic for D11Z1 (P < 0.05). Additional fluorescence in situ hybridization studies utilizing the probes for the α satellite, centromeric regions of chromosomes 7, 8, 9, 12, 17, and 20 were then carried out on all cases with cellular or mixed histology. Five out of 10 cellular or mixed MNs had extra copies of D8Z1, and four out of 10 had extra copies of D17Z1, suggesting that gains of chromosomes 8 and 17 may be additional nonrandom cytogenetic events associated with the evolution of MNs. DNA aneuploidy, as determined by image analysis, was detected in three tumors: all had greater than four chromosomal aberrations documented by fluorescence in situ hybridization. (Am J Pathol 1993, 143:714–724)

Mesoblastic nephroma (MN) is the most common renal neoplasm occurring in the neonatal period. Recognized as a distinct entity in 1967, it was described as a mesenchymal tumor characterized histologically by a fascicular proliferation of bland, spindle-shaped cells. The tumor was thought to be histogenetically related to, albeit distinct from, Wilms' tumor, and its diagnosis was associated with cure following surgical resection.[1]

After the original histopathological description of MNs was published, it was recognized that the microscopic appearance ranged from the bland, fibromatosis pattern initially described to a much more cellular, less-differentiated lesion with mitoses and necrosis.[2–4] These latter tumors were histologically worrisome and prompted use of additional descriptors such as cellular, atypical, and sarcomatous. Concomitantly, occasional cases of more aggressive MNs characterized by local recurrence or rarely pulmonary metastases were reported.[5–10] These latter observations generated attempts to predict biological behavior based upon microscopic appearance and clinical data. Unlike most tumors however, it became apparent that the presence (or absence) of standard microscopic indicators of malignancy (mitoses, necrosis, and even vascular invasion) were of little prognostic importance in MNs. Currently, it is believed that complete surgical resection is the best predictor of cure. Age of the patient is also important, in that aggressive behavior tends to occur in older infants with MN.[11–16]

These original clinicopathological investigations were followed by studies centered around the cell of origin and the relationship of MN to other renal tumors occurring in infancy and childhood. Ultrastructural and immunohistochemical studies established the fibroblastic/myofibroblastic origin of MN.[17–20] Although the specific mesenchymal progenitor cell in MNs in unknown, postulated candidates include: 1) secondary mesenchyme (that which is no longer ca-

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pable of differentiating into nephronic epithelium), 2) early nephrogenic mesenchyme adjacent to the ureteric bud (central, periureteric bud mesenchyme has been shown to be more stromogenic than epitheliogenic), and 3) nephrogenic mesenchyme that has not been exposed to the epithelial inductive factors associated with the ureteric bud.[1,4,17,20–24] The average older age of occurrence documented in the cellular variant of MNs and the not uncommon observation of cellular areas admixed with classic areas within a given tumor have led to the additional postulate that the cellular regions are the result of genetic progression of tumor originally characterized by purely classic histology.

Efforts to understand the biology of MNs have more recently expanded to include analysis of overall DNA content.[25–27] In addition, occasional reports of karyotypic findings, particularly nonrandom gains of chromosome 11, have been published.[28–31] We performed fluorescence *in situ* hybridization (FISH) and image analysis on disaggregated nuclear suspensions prepared from paraffin-embedded material to evaluate the interrelationships of histology, chromosome copy number, and ploidy in MNs.

Materials and Methods

Mesoblastic nephromas diagnosed at the Children's Hospital of Pittsburgh between 1968 and 1992 comprised the study group. An additional infant with congenital mesoblastic nephroma diagnosed in 1992 at the Children's Hospital of Boston was included in the study as a methodological control incorporating cytogenetic analysis.

Histology

All hematoxylin and eosin- (H&E) stained sections of the resected tumor specimens were reviewed. Tumors were classified as either classic, cellular, or mixed histological variants of mesoblastic nephroma. Classic MNs were characterized by relatively hypocellular interlacing bundles of bland spindle-shaped cells that infiltrated adjacent renal parenchyma as individual fascicles (Figure 1a). Neither pleomorphism nor significant numbers of mitoses were observed in these tumors. Cellular MNs, in addition to being more cellular than those with classic histology, frequently had both mitoses and necrosis (Figure 1c). Nuclei in cellular MNs ranged from round and vesicular to small and spindle-shaped with minimal pleomorphism. Areas of storiform and herringbone patterns were observed, occasionally giving the lesion a sarcomatous appearance. The interface of cellular MN with normal kidney had more of a pushing than an infiltrative appearance. When both classic and cellular patterns were present, the tumor was considered mixed (Figure 1b). One tumor was classified as a maturing MN. This mesenchymal neoplasm was of intermediate cellular density, was associated with a prominent

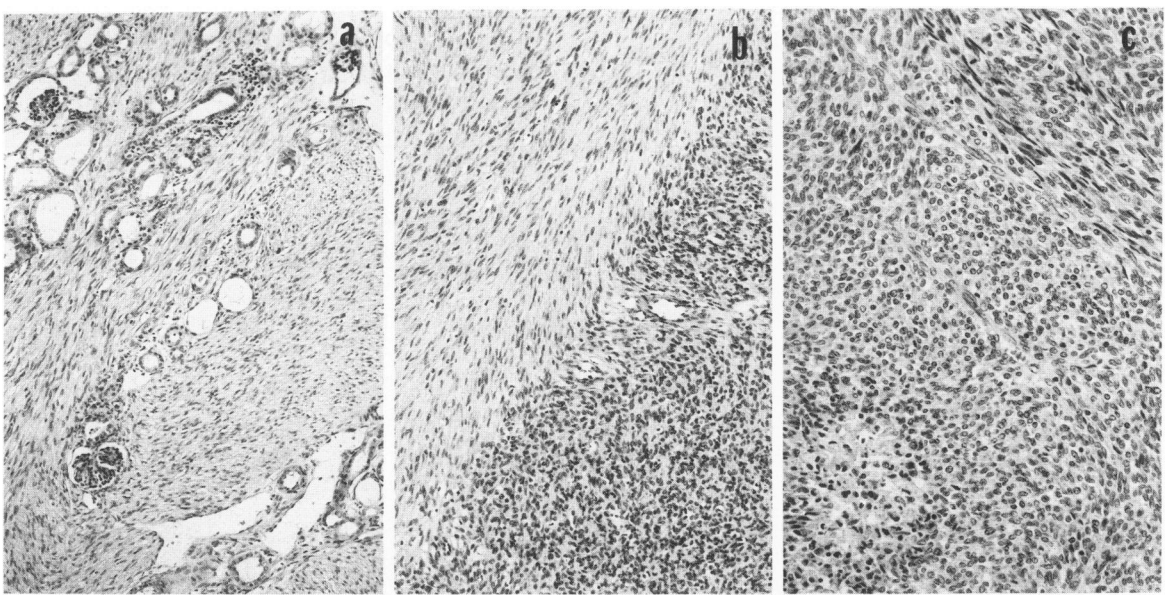

Figure 1. *H&E-stained sections of classic* (a), *cellular* (c), *and mixed* (b) *histology MNs are illustrated. The classic MN is characterized by fascicles of bland, spindle-shaped tumor cells infiltrating normal renal parenchyma. In the mixed MN, cellular and classic areas are histologically distinct. In the cellular MN, mitoses and foci of necrosis are occasionally seen.*

connective tissue background and lacked a fascicular pattern. Neither epithelial nor blastematous elements were present.

Preparation of Nuclear Preparations for DNA Content and FISH Analyses

After review of all H&E stained slides from each case, two tissue blocks were selected for FISH and image analysis. If the tumor had a homogeneous microscopic appearance, the two blocks were selected randomly. If the tumor was heterogeneous in appearance (as in the mixed MNs), one block was selected from the more cellular areas and one from the less cellular areas. In addition, one block was comprised primarily of tumor tissue, whereas the other block was comprised of a mixture of tumor and normal kidney.

Two 60-μ sections were cut from each block. They were disaggregated according to the method described by Hedley et al.[32] Briefly, deparaffinization in xylene was followed by rehydration to distilled water in graded alcohols (100, 90, 70, and 50%). The sections were then digested with pepsin (Sigma Chemical Co., St. Louis, MO, catalog #P-6887) for 30 to 60 minutes in a 37 C water bath. The tissue was then vigorously drawn up and down in a 5-ml syringe with 18 and 21 gauge needles and filtered through nylon mesh (Small Parts, Inc, Miami, FL, #CMN-105). The samples were rinsed with 4 ml of phosphate-buffered saline and centrifuged at 1,000 rpm for 10 minutes. The resulting nuclear pellet was resuspended in a small amount of phosphate-buffered saline and pipetted onto a glass slide. The slides were air-dried 1 to 30 days at room temperature before either FISH or determination of DNA content by image analysis.

Fluorescence in Situ Hybridization

Slides of nuclear suspensions prepared from two tissue blocks of all MNs were analyzed for chromosome 11 copy number. No effort was made to separate tumor from normal kidney before proteolytic digestion. Reagents used for FISH were obtained from Oncor (Gaithersburg, MD). The modified hybridization procedure was carried out according to a previously published method.[33] D11Z1 α satellite probe (1.5 ng/15 ul) was hybridized to slides overnight at 37 C. Probe detection and amplification were as described,[33] and signals were visualized with a Zeiss Axiophot fluorescent microscope (Carl Zeiss, Oberkochen, Germany) using a BP 450–490 excitation filter, a FT 510 beam splitter, and a LP 520 barrier filter.

D11Z1 primary hybridization signals were clear, distinct, and easily counted. Hybridization efficiency was greater than 95%. Secondary hybridization signals were occasionally present, although of markedly diminished intensity when compared with the primary signals, posing no problem in interpretation. There was no significant variation in hybridization efficiency when different areas of the slides were examined. The number of signals visualized in 200 nuclei were tabulated from random areas of the slides in which nuclear overlap was minimal.

Aberrant signal expression was defined as the observation of extra signals in at least 20% of nuclei. The exception to this cutoff is those tumors with extra signals (usually three) in greater than 20% of

Table 1. *Histology, DNA Content, and D11Z1 Correlation*

					D11Z1 Signals/Nucleus*			
Case	Age	Sex	Histology	DNA Content	1	2	3	4
1	3 d	M	Classic	Diploid	1	98	1	0
2	13 d	F	Classic	Diploid	1	99	0	0
3	3 d	F	Classic	Diploid	1	98	1	0
4	24 d	M	Classic	Diploid	3	95	1	1
5	2 d	F	Classic	Diploid	4	92	3	1
6	7 d	M	Classic	Diploid	4	94	2	0
7	8 m	F	Maturing	Diploid	2	93	4	1
8	1 d	M	Cellular	Diploid	0	97	2	1
9	1 y	M	Cellular	Diploid	3	94	2	1
10	2 y	F	Cellular	Diploid	2	96	1	1
11	1 d	M	Cellular	Diploid	2	10	***87***	1
12	1 d	M	Mixed	Diploid	3	62	***34***	1
13	10 w	M	Mixed	Diploid	1	27	***71***	1
14	5 d	F	Mixed	Diploid	1	66	***31***	2
15	8 d	F	Mixed	Aneuploid	1	20	***77***	2
16	7 m	F	Cellular	Aneuploid	1	11	***88***	0
17	2 d	F	Mixed	Aneuploid	3	37	***35***	***25***

* Reported as percentage of hybridizing nuclei/ n = 400.
Underlined/italicized: aberrant D11Z1 copy number.

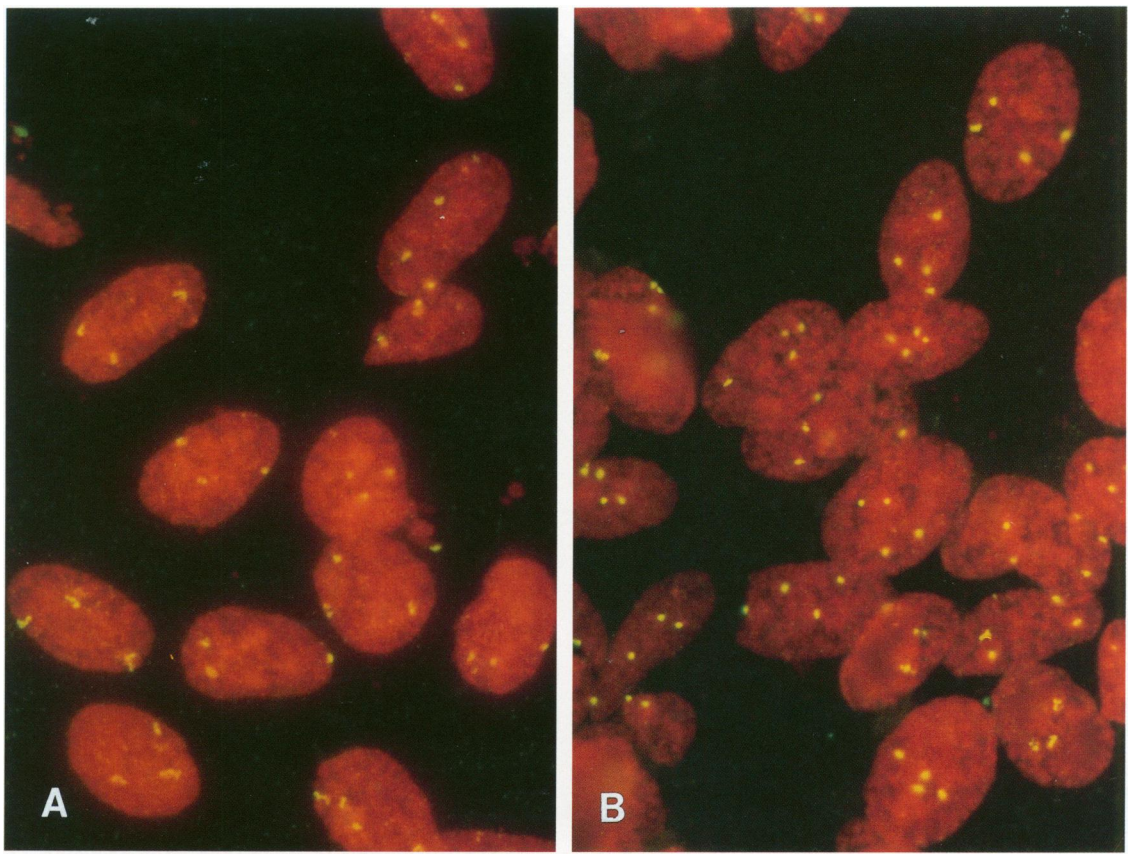

Figure 2. *Case 16 FISH results utilizing the probes D11Z1* (a) *and D17Z1* (b) *are illustrated. Nuclei prepared from tissue processed and embedded in 1979 show one extra copy of D11Z1 and two extra copies of D17Z1 (500×).*

nuclei in whom an additional population of nuclei also characterized by extra signals (usually four) was observed. The cutoff for this second population of nuclei was dropped to 5% as this number is still above values observed in tumors classified as disomic (see Table 1).

DNA Content

Slides from two tissue blocks of all MNs were analyzed separately for DNA content. One block contained normal kidney and tumor, providing an internal measure of diploid DNA mass. As 60-μ tissue sections were cut from this block, they were divided into normal and tumor tissue. Deparaffinization and digestion were performed in parallel according to the method outlined above. Tumor and normal nuclei from this same tissue block were then spread onto two separate halves of a slide for staining and analysis. Tissue sections from the second block (generally pure tumor) were processed intact.

All slides used for determination of DNA content were postfixed in 10% formalin and stained for DNA content with a purified Feulgen stain obtained from Cell Analysis System (CAS, Elmhurst, IL). Quantitation of nuclear DNA content was performed using the Cell Analysis System (200 Image Analysis System) equipped with the Quantitative DNA Analysis software package (CAS, Elmhurst, IL). The slides were calibrated using the predeposited calibration cells, and a minimum of 200 nuclei were analyzed. Tumor DNA index was calculated as the DNA mass of the tumor nuclei divided by the DNA mass of the normal nuclei. On the basis of the DNA index, tumors were categorized as diploid (index: 0.85 to 1.1), tetraploid (index: 1.8 to 2.2) or aneuploid (index: > 1.1 and <1.8).

Karyotyping

Fresh, sterile tumor tissue from case 11 was received after 15 hours refrigeration in Hanks' balanced salt solution. It was cross-minced with opposing scalpel blades and disaggregated overnight in a 200 unit/ml collagenase (Sigma) solution overnight.[34] After short-term culture, including a single passage, metaphases were harvested at 1 week using a 14-hour incubation with 0.002 g/ml of colce-

mid. Slides were made according to conventional techniques, stained by the trypsin-giemsa method[35] and analyzed and photographed on a Zeiss Axiophot microscope. Ten metaphases were analyzed.

Results

Clinical information, along with the histological classification, DNA content, and D11Z1 copy number for 17 MNs are summarized in Table 1.

Clinical Information

Patient age at the time of diagnosis ranged from 1 day to 2 years. Sixteen out of 17 tumors were completely excised. The exception was case 10, in which a microscopic focus of tumor was identified within the adventitia of the renal vein resection margin. Five patients (cases 9, 10, 12, 13, and 16) received postoperative actinomycin D and vincristine. Fifteen out of 17 patients are alive without evidence of disease (followup of 8 months to 22 years). In case 8, the neonate died 5 days postoperatively due to uncontrollable hemorrhage. In case 10, the child developed pulmonary metastases 8 months after resection (confirmed by needle biopsy). It should be noted that cases 1 to 3, 7, 8, and 12 to 14 correspond to cases 1 to 4, 6, 7, 9, and 10 of a previous study examining cellularity and ultrastructure of mesoblastic nephromas.[19]

Histology

Six MNs were characterized by classic histology, five by variable combinations of cellular and classic histology, and five by cellular histology in all sections examined. There were subtle microscopic variations within the group of cellular histology MNs, but definite histological differences could not be defined. Neither could specific differences between the histology of these purely cellular tumors and the cellular areas of mixed MNs be defined. In case 10, a cellular MN, the histology of the renal primary and pulmonary metastases were similar. Finally, case 7 was classified as a maturing MN. The average age of patients with cellular or mixed tumors, cases 8 to 17, was significantly older (140 days) than patients with classic MNs, cases 1 to 6 (9 days), $P < 0.001$. All classic histology MNs were diagnosed in infants less than 1 month of age.

D11Z1 FISH Results

D11Z1 FISH results are illustrated in Figure 2a and the percentage of nuclei with 1, 2, 3, or 4 signals (tabulated from 200 nuclei analyzed on each of two blocks or 400 nuclei total) is summarized in Table 1. Six cases were characterized by three D11Z1 hybridization signals (indicating trisomy 11) and one case by a combination of three and four D11Z1 hybridization signals (indicating trisomy and tetrasomy 11) in greater than 30% of nuclei. The remaining nine cases were characterized by two D11Z1 hybridization signals in 97 to 99% of hybridizing cells. D11Z1 polysomy was found only in MNs with cellular components ($P < 0.05$, Fisher's exact test), an observation to be addressed later.

The percentage of nuclei with extra D11Z1 hybridization signals varied from case to case as illustrated in Table 1. FISH results were similar in the two regions sampled from all classic histology MNs. There was some variation of the percentage of abnormal nuclei in different regions of the pure cellular

Table 2. *Fish Results for Cellular/Mixed Mesoblastic Nephromas*

Case	Probe*								DNA index†
	D7Z1	D8Z1	D9Z1	D11Z1	D12Z3	D17Z1	D18Z1	D20Z1	
7	Di	Di	Di	Di	Di	Di	Di	Di	1‡
8	Di	Di	Di	Di	Di	Di	Di	Di	1‡
9	*Tri*	*Tri/Tet*	Di	Di	Di	Di	Di	Di	1‡
10	Di	Di	Di	Di	Di	Di	Di	Di	1‡
11	Di	Di	Di	*Tri*	Di	Di	Di	Di	1‡
12	Di	Di	Di	*Tri*	Di	Di	Di	Di	1‡
13	Di	Di	Di	*Tri*	Di	Di	*Tri*	Di	1‡
14	Di	*Tri*	Di	*Tri*	Di	*Tri*	Di	Di	1.10
15	*Tet*	*Tri/Tet*	Di	*Tri*	Di	*Tri*	Di	Di	1.21
16	Di	*Tri*	*Tri*	*Tri*	Di	*Tet*	*Tri*	Di	1.28
17	Di	*Tri/Tet*	Di	*Tri/Tet*	*Tri*	*Tri/Tet*	Di	Di	1.48

* Di: two hybridization signals/nucleus; *Tri: three hybridization signals/nucleus; Tet:* four hybridization signals/nucleus.
† Expressed as tumor DNA mass/DNA mass of normal kidney in same tissue block.
‡ Tumor DNA mass indistinguishable from normal DNA mass.

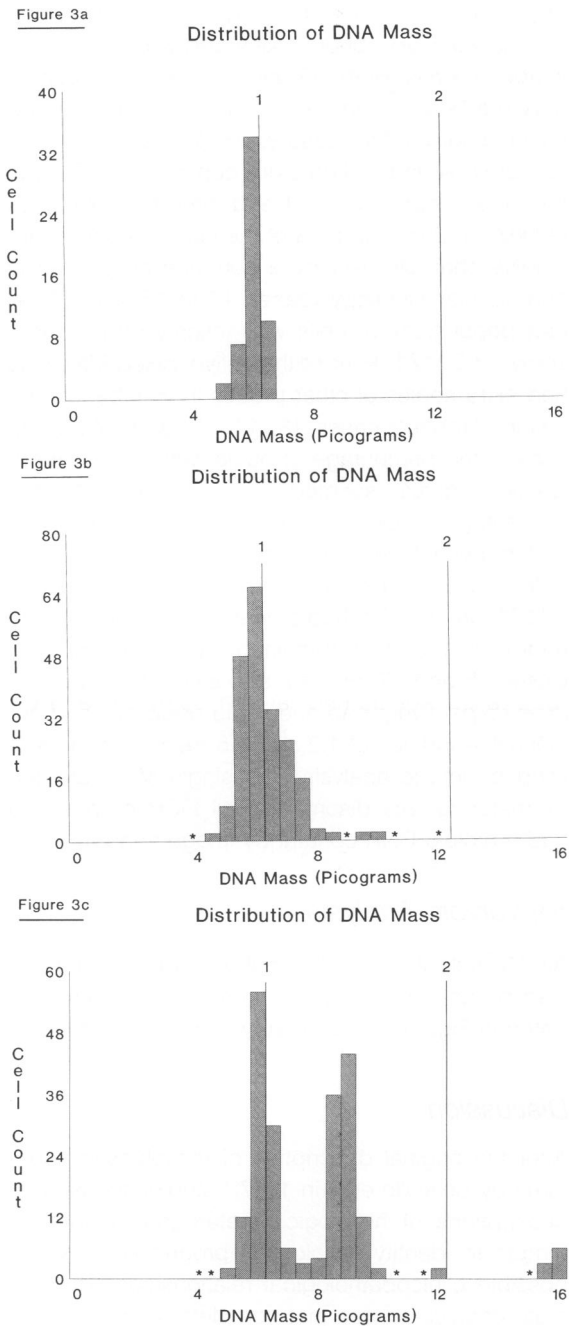

Figure 3. *DNA histograms from a mixed histology MN (case 17).* **a:** *DNA mass of normal kidney nuclei.* **b:** *DNA mass of nuclei obtained from classic histology regions of this tumor—the DNA mass is identical to that in the normal kidney cells. Although there is a shoulder present on the histogram in* **b**, *an aneuploid peak is not present.* **c:** *DNA mass of nuclei from mixed cellular and classic regions—one DNA peak is identical to that in normal kidney and another has a DNA index of 1.48.*

MNs, but this variation correlated with estimated percentage of tumor nuclei (vs normal) in the tissue sections. However, in tumors with mixed cellular and classic histology, the percentage of nuclei with aberrant D11Z1 expression was heterogeneous and correlated with both the percentage of tumor (vs normal) nuclei and the percentage of cellular (vs classic) histology: those sections/blocks with primarily cellular histology had a greater percentage of nuclei with extra D11Z1 signals than sections/blocks with primarily classic histology. Thus, FISH distributions varied between tumors, (eg, cases 14 and 15 with D11Z1 trisomy in 31 and 77% of nuclei, respectively) and also between different areas within certain tumors.

Classification as disomic, trisomic, trisomic/tetrasomic, or tetrasomic was based upon the criteria outlined in the methods section. In the purely classic or cellular histology tumors, percentages were calculated based upon a summation of results obtained from the two different areas sampled as there was minimal variation other than that accounted for by normal tissue. In the mixed MNs, classification as disomic, trisomic, trisomic/tetrasomic, or tetrasomic was based upon results from the block containing the greater amounts of cellular histology.

Overall DNA Content

Fourteen of 17 tumors were characterized by DNA content similar to that of the corresponding normal kidney tissue (DNA index: 0.85 to 1.1). There were no cases of classic histology MN that had a population of aneuploid cells that could be detected by image analysis. All three cases of DNA aneuploidy were observed in cellular MNs (case 16) or mixed MNs (cases 15 and 17). In case 17, one of the tissue blocks was comprised of approximately 30% normal kidney and 70% tumor of predominantly classic histology. The other was comprised of pure tumor of approximately 80% cellular and 20% classic histology. Although a shoulder was present on the diploid peak of the first block, an aneuploid population of nuclei was not detected (Figure 3, a and b). A large aneuploid peak (DNA index = 1.48) was identified in the second block containing tumor with predominantly cellular histology (Figure 3c).

Correlation of Histology, DNA Content, and Expanded FISH Data

In this study, all MNs with pure classic histology (cases 1 to 6) were diploid and had two copies of the chromosome 11 α satellite. Aberrant copy number of D11Z1 and DNA aneuploidy were restricted to cellular or mixed histology MNs. Because the

aneuploid DNA indices suggested polysomy for additional chromosomes, we performed further FISH studies on the group of cellular or mixed histology tumors. We included the maturing MN (case 7) in these studies as this case was unique histologically.

Probes for chromosome 7 (α satellite, D7Z1-1 ng/15 ul), chromosome 8 (α satellite, D8Z1-1 ng/15 ul), chromosome 9 (classic satellite, D9Z1-1 ng/15 ul), chromosome 17 (α satellite, D17Z1-1 ng/15 ul), chromosome 18 (α satellite, D18Z1-1 ng/15 ul), and chromosome 20 (α satellite, D20Z1-2.5 ng/15 ul) were chosen for the additional FISH studies because polysomies for these chromosomes have been described in MNs.[20–23] In addition, we assessed copy number of D12Z3 (1 ng/15 ul) because trisomy 12 is the most frequent numerical chromosomal aberration in Wilms' tumor.[36,37]

Hybridization efficiency and signal intensity with D7Z1, D8Z1, D9Z1, D12Z3, D17Z1, and D18Z1 were comparable to those obtained with D11Z1. Hybridization efficiency with the chromosome 20 α satellite probe, D20Z1, ranged from 25 to 95%, and signals were weak, making interpretation more difficult. In all cases, the percentages of non-disomic nuclei with D7Z1, D8Z1, D9Z1, D12Z3, D17Z1, and D18Z1 were approximately equal to, or less than, those observed with the D11Z1 probe.

The results of additional FISH studies performed on cases 7 to 17 are summarized in Table 2. Polysomies were observed for D8Z1 (five cases), D17Z1 (four cases, Figure 2b), D7Z1 and D18Z1 (two cases each), and D9Z1 and D12Z3 (one case each). Polysomy for D20Z1 was not detected in any of the cases. Polysomies for D7Z1, D8Z1, D9Z1, D12Z1, D17Z1, and D18Z1 were generally associated with extra copies of D11Z1. In case 9, however, D7Z1 trisomy and D8Z1 trisomy/tetrasomy were the only FISH abnormalities detected.

Of five MNs with pure cellular histology, two (cases 11 and 16) were characterized by D11Z1 trisomy in greater than 90% of nuclei. In case 11, this was an isolated finding, whereas in case 16, it was accompanied by additional copies of D8Z1, D9Z1, D17Z1, and D18Z1 (Table 2). Of the three remaining MNs with pure cellular histology, two (cases 8 and 10) lacked FISH abnormalities and were diploid by image analysis. Both the primary tumor and pulmonary metastasis were analyzed in case 10. The remaining cellular MN, case 9, also had a diploid content of DNA and lacked extra copies of D11Z1, but had three copies of D7Z1 and three to four copies of D8Z1 in 25% and 67% of the nuclei, respectively.

MNs characterized by a combination of classic and cellular histology (cases 12 to 15 and 17) all had populations of cells with trisomy and/or tetrasomy for D11Z1. Four of these five mixed MNs also had extra copies of other probes in variable combinations (Table 2 cases 13, 14, 15, and 17). Variations in the percentage of nuclei with extra hybridization signals seemed to correlate with the percentage of cellular histology. This is illustrated by the raw data in case 17 (Table 3). Case 14, characterized by aberrant copy number of D8Z1, D11Z1, and D17Z1, had a near diploid DNA content (index of 1.1) as determined by image analysis. Cases 15 and 17, with six and seven FISH polysomies respectively, had aneuploid populations of nuclei (DNA indices of 1.2 and 1.5 respectively) identified by image analysis. The single MN classified as maturing was disomic for all FISH probes and had a diploid DNA content by image analysis.

Karyotypic Analysis

All 10 metaphases fully analyzed were characterized by an extra copy of chromosome 11 as illustrated in Figure 4. The karyotype was 46,X,-Y,+11.

Discussion

After the original description of mesoblastic nephroma by Bolande et al in 1967,[1] studies focused on descriptions of histological heterogeneity with attempts to identify histological prognosticators and possible clinicopathological relationships. The ultimate conclusion was that complete surgical resection seems to be the most important prognostic factor,[15] whereas patient age and histology are of

Table 3. *Intratumor Heterogeneity of Fish Results (Case 17)*

	D8Z1 probe*				D11Z1 probe				D12Z1 probe				D17Z1 probe			
Block	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
A (10†)	02	71	*27*	00	05	65	*28*	02	02	97	01	00	02	72	*21*	*05*
B (80†)	01	14	*25*	*60*	02	10	*42*	*46*	01	68	*28*	03	02	08	***33***	*57*

* Expressed as the percentage of nuclei with 1, 2, 3, or 4 primary hybridization signals (underlined/italicized = aberrant copy number).
† Estimated percentage of cellular histology per tissue block.

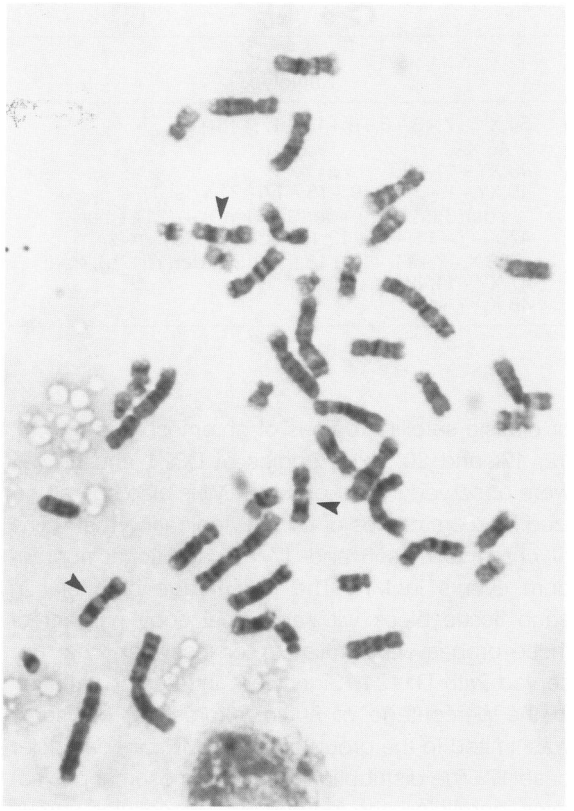

Figure 4. *This GTG banded metaphase spread consists of 46 chromosomes—normal male karyotype other than the three copies of chromosome 11 (arrow heads) and absence of the Y chromosome.*

secondary importance. The single case of metastatic MN in the present series occurred in a 2-year-old child, was documented at the renal vein resection margin, and was a cellular tumor.

Based on reports of DNA aneuploidy (summarized in Table 4) and nonrandom gains of chromosome 11 (summarized in Table 5) in MNs, we performed a retrospective study of both DNA content and chromosome 11 copy number. Overall DNA content was determined by image analysis, and chromosome 11 copy number was assessed by FISH with a probe for the α satellite region of chromosome 11 (D11Z1). Because of case reports of intratumor heterogeneity with respect to DNA content and karyotypic analysis, two separate areas from all tumors were analyzed. Both image analysis and FISH studies were performed on disaggregated nuclear suspensions prepared from paraffin-embedded material. So far, most FISH studies performed on paraffin-embedded tissue have utilized microscopic sections placed on glass slides. Though preserving the relationship of individual cells with respect to their neighbors, this method can be difficult to interpret as only portions of nuclei are examined (varying with the thickness of the tissue section). The use of nuclear suspensions circumvents this limitation, and interpretation of results is facilitated by correlation with the corresponding H&E-stained section.

We observed extra D11Z1 hybridization signals in seven of 17 mesoblastic nephromas. Aberrant copy number was restricted to tumors with either mixed or cellular histology; there were no tumors with pure classic histology that had extra copies of D11Z1. Variable results were observed in tumors with pure cellular histology in that two of five were trisomic for D11Z1, whereas three were disomic. The group of MNs with mixed histology was extremely interesting in that all had trisomy or tetrasomy for D11Z1. Moreover, the percentage of trisomic and tetrasomic nuclei varied within different

Table 4. *Ploidy Studies*

DNA Content		Histology	Outcome
Diploid (n = 39)			
$n = 13^{(25)}$		NA	ANED
$n = 9^{(27)}$		Classic (8)	ANED(7)/DOPD(1)
		Cellular(1)	ANED
$n = 1^{(29)}$		Cellular	ANED
$n = 2^{(26)}$		Classic(1)	ANED
		Cellular(1)	ANED
n = 13 (current series)		Classic(6)	ANED
		Mixed(6)	ANED
		Cellular(4)	ANED(2)/AWMD(1)/POD(1)
Aneuploid (n = 6)			
$n = 2^{(27)}$	(DI 1.4)	Cellular	ANED
	(DI 1.3)	Mixed	ANED
$n = 1^{(26)}$		Mixed	ANED
n = 3(current series)	(DI 1.5)	Mixed	ANED
	(DI 1.2)	Mixed	ANED
	(DI 1.3)	Cellular	ANED

ANED = alive, no evidence of disease; AWMD = alive with metastatic disease; DOPD = died of progressive disease; POD = post-operative death.

Table 5. *Karyotypic Findings*

Case	Patient age	Histology	Karyotype
1[28]	5 days	Mixed	54,XX,+7,+8,+8,+9,+11,+17,+18,+20 46,XX
2[30]	NS*	NS	48,XY,+11,+20
3[29]	8 months	Cellular	45,XY,−1,−3,−9,−9,−15,−17,−21,+del(1), +der(3)t(3;9;15),+der(9),+der(9),+r(21),+mar
4[31]	1 month	NS	47,X,−Y,+11,−17,t(12;15),+der(Yq17q),+mar/ 48,X,−Y,+11,−17,+18,t(12;15),+der(Yq17q),+mar
5[31]	1 month	NS	47,XY,+11/48,XY,+11,+11
6(case 10)	1 day	Cellular	46,X,−Y,+11

* Not specified.

areas of individual tumors and correlated with the percentage of cellular histology present in the corresponding H&E-stained section. Similar intratumor heterogeneity was not detected in either the classic or cellular variants of MN.

The biological relevance of polysomy 11 remains to be determined in MNs. Trisomy 11 is an uncommon aberration in most solid tumors, but has been reported in mesenchymal breast tumors,[38] infantile fibrosarcomas,[39–41] and benign chondroid tumors.[30] These observations suggest that trisomy 11 might convey a proliferative advantage to several mesenchymal cell types. Abnormal proliferation in some or all of these tumors might result from increased copy number of a gene (or genes) on chromosome 11. Several growth factor-related genes and oncogenes have been mapped to chromosome 11 (IGF-II, HRAS, INT2, SEA, FL11, HSTLF1, and ETS1), and increased IGF-II expression seems to be characteristic of some MNs.[42] Accordingly, it will be interesting to determine whether IGF-II expression correlates with chromosome 11 polysomy in MNs.

Populations of cells with aneuploid DNA content were identified in three cellular or mixed histology MNs. An additional mixed histology MN, although technically DNA diploid, had a DNA index of 1.10. In the mixed histology MNs, the aneuploid populations were most prominent in sections with greater amounts of the cellular component. In case 17, DNA aneuploidy was not detected in tumor tissue obtained from areas of primarily classic histology, although a large population of cells with a DNA index of 1.48 was detected in an area of the tumor with predominantly cellular histology (Figure 3). This intratumor heterogeneity is similar to that described in a previous report.[26]

Because DNA aneuploidy and aberrant D11Z1 copy number were found only in cellular or mixed histology MNs, we expanded our FISH studies on this group of tumors (Table 2), utilizing probes for α or classic satellite regions of chromosomes 7, 8, 9, 12, 17, and 20. Extra copies of D8Z1 and D17Z1 were observed in four of seven MNs also characterized by extra copies of D11Z1, indicating that gains of chromosomes 8 and 17 are additional nonrandom events in MN. The percentage of nuclei in each tissue block with abnormal copy number of these probes was similar to, or less than, that observed with D11Z1. Significant intratumor variation in the percentage of nuclei expressing polysomy was limited to the group of mixed MNs, in particular case 17. The distribution of all chromosomal polysomies paralleled the percentage of cellular versus classic histology (Table 3). Not surprisingly, DNA aneuploidy was detected in those tumors with the greatest number of FISH polysomies.

Aberrant copy numbers of D7Z1 (chromosome 7) and D8Z1 (chromosome 8) in case 9 were not associated with D11Z1 polysomy. It is possible that this MN had partial trisomy for chromosome 11, excluding the α satellite region detected by D11Z1. Alternatively, this tumor might have resulted from oncogenetic events not involving a gain of chromosome 11 material.

It is interesting that the MN associated with subsequent pulmonary metastases (case 10) lacked demonstrable genetic aberrations in this study. The tumor had a purely cellular histological appearance and was documented at the renal vein resection margin. The histology of the metastasis did not differ from that of the primary tumor. Analysis of DNA content revealed no detectable population of aneuploid cells by image analysis of Feulgen stained nuclei and FISH studies (performed on both the primary tumor and pulmonary metastasis) revealed disomy for D7Z1, D8Z1, D9Z1, D11Z1, D12Z3, D17Z1, D18Z1, and D20Z1.

The present study suggests an oncogenetic basis for histological variability in MNs. Trisomy 11 seems to be the most common cytogenetic abnormality in MN, but this aberration was found in only

MNs with cellular components. Accordingly, gains of chromosome 11 material might be associated with histological progression of some MNs. It seems that most classic histology MNs result from oncogenetic events other than gains of chromosome 11. Trisomy 11 might then be a later mutation in classic histology MNs that is manifest microscopically as conversion to mixed and then possibly cellular histology. Cellular components might then develop additional chromosomal aberrations, as evidenced by the nonrandom polysomies of chromosomes 8, 17, and 18 observed in the present series. This hypothesis does not account for the subset of cellular MNs that apparently lack chromosome 11 gains. These tumors might be characterized by structural chromosomal abnormalities resulting in partial trisomy for chromosome 11 not detected by the D11Z1 α satellite probe. However, it is also possible that some cellular MNs arise by a separate and divergent oncogenetic pathway that does not involve progression from classic histology. In this regard, it is interesting that cellular MNs lacking D11Z1 abnormalities did not have identifiable areas of classic histology, and all mixed histology tumors had extra copies of D11Z1.

The typical chromosome aberrations in MN seem to be unique among pediatric renal tumors. Numerical chromosomal abnormalities are observed frequently in Wilms' tumor and its variants. However, trisomy or tetrasomy for chromosome 12 is characteristic of Wilms' tumors, and trisomy 11 is seen in fewer than 5% of cases.[36,37] In addition, chromosome 11 abnormalities have not been reported in clear cell sarcoma of the kidney,[31,43] a tumor that in the past has been hypothesized to be the malignant variant of MN.[44] Given the histological similarity between MN and the spectrum of fibrous lesions occurring in infancy/childhood, it is interesting that polysomy 11, often accompanied by polysomy 8, 17, and 20 also characterizes infantile fibrosarcoma[39–41] and that high levels of IGF-II have been documented in both lesions.[42] The shared cytogenetic aberrations suggest that MN and infantile fibrosarcoma arise by similar oncogenetic pathways.

In conclusion, we have determined that increased chromosome 11 copy number is a characteristic aberration in MNs with cellular histological components. Additional characteristic polysomies in cellular or mixed histology MNs include extra copies of chromosomes 8 and 17. In contrast, chromosome 11 aberrations were not found in classic histology MNs. Few MNs recur or metastasize, however, the genetic aberrations described in this study do not seem to correlate with prognosis.

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