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ANATOMICAL PATHOLOGY

Congenital mesoblastic nephroma: a study of 19 cases using immunohistochemistry and *ETV6-NTRK3* fusion gene rearrangement

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Summary

Mesoblastic nephroma (MN) is the most common renal tumour in the first 3 months of life and accounts for 3–5% of all paediatric renal neoplasms. To further understand the morphological variants of MN, we identified 19 cases of MN (five classic, eight cellular and six mixed) and examined each case for markers known to be important in urogenital embryological development (PAX8, WT1 and RCC), stem cell associated markers (Oct 4, CD34 and c-kit), muscle/myofibroblastic markers (muscle specific actin, calponin and h-caldesmon), aberrant transcription factors, cell cycle regulation and other oncogenic proteins (p16, cyclin D1 and beta-catenin). Fluorescence *in situ* hybridisation (FISH) testing for *ETV6-NTRK3* gene fusion/rearrangement revealed further differentiation between the subtypes with *ETV6-NTRK3* gene fusion detected in 0/5 of the classic MN, 8/8 of the cellular MN and 5/6 of the mixed MN cohorts, respectively. Our results conclude that cyclin D1 and beta-catenin may be useful markers for differentiating between cellular MN and classic MN when the histology is not conclusive. The absence of expression of stem cell markers and markers involved in urogenital development suggests that MN is not a nephroma and most likely represents a soft tissue tumour, with congenital infantile fibrosarcoma representing cellular MN with a predilection to arise in the kidney. In addition, the immunophenotype and genetic fingerprint of mixed MN most likely represents a heterogenous group of tumours that are mostly cellular type, with areas that are phenotypically less cellular.

Key words: Mesoblastic nephroma; fusion gene; cyclin D1.

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INTRODUCTION

Mesoblastic nephroma (MN) is one of the most common paediatric neoplasms. There are three morphological variants: classic, cellular, and mixed (which shows a combination of morphological features of classic and cellular variants).[1,2] Nearly all cases of MN involve the renal sinus and share the gross characteristic appearance of being centred on the hilum of the kidney. Mesoblastic nephroma, regardless of its morphological subtype, typically occurs in very young children.

Histopathologically, classic and cellular variants are predominantly monomorphic neoplasms composed of uniform, elongated spindle cells arranged in bundles, and interspersed areas of entrapped glomeruli and tubules. 'Herring bone' pattern is often observed, particularly in cellular MN.[3] The mesenchymal, fibroblastic, or myofibroblastic lineage is evident by the expression of vimentin, smooth muscle actin, and muscle specific actin. There are higher rates of recurrence and metastatic disease for the cellular variant compared to the classic variant.[4] Mitoses and necrosis are more commonly seen in cellular MN, and classic MN is a relatively hypocellular tumour compared to cellular MN. While the histomorphological characteristics of classic MN are similar to infantile fibromatosis/myofibromatosis, cellular MN shares morphological and genetic characteristics with congenital infantile fibrosarcoma (CIFS).

Classic and cellular MN also differ with respect to *ETV6-NTRK3* fusion transcripts and/or *ETV6*-region rearrangement, which is associated with all cases of cellular MN but not with classic MN.[5] In cases of mixed MN, the *ETV6-NTRK3* fusion gene rearrangement has not been consistently demonstrated. Argani *et al.*[6] and Anderson *et al.*[7] found all five and six of their tested cases, respectively, to be negative for the presence of the fusion gene, but Knezevich *et al.*[5] and Rubin *et al.*[8] found all cases (five in total) of mixed MN to be positive for the fusion gene.

The aim of this study was to determine if the morphological variants of MN, especially mixed MN, can readily be distinguished from each other using a panel of immunohistochemical (IHC) markers.

METHODS

The study was approved by Children's Hospital of Eastern Ontario Ethics Research Board and Children's Healthcare of Atlanta IRB. Archives of both hospitals were searched for MN during the period 2000–2012. Clinical data, histology, and ancillary studies were reviewed by two pathologists independently. We performed a panel of IHC stains for markers (Table 1) known to be important in urogenital embryological development (WT1 and RCC), stem

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Table 1 Panel of IHC stains performed

Antibody	Clone	Dilution	Supplier
OCT3/4	NINK	RTU	Leica
Cyclin D	SP4	1 IN 10	Biocare
DOG-1	K9	RTU	Leica
CD117	C-KIT	1 IN 100	Dako
PAX8	BC12	1 IN 25	Biocare
p16	JC8	1 IN 500	Leica
Calponin	26A11	RTU	Leica
RCC	SPM314	1:50	Dako
Beta-catenin	17C2	1:50	Leica
WT-1	WT49	RTU	
MSA	HHF35	1:50	Leica
CD34	QB-end/10	RTU	Leica
Caldesmon	HCD	1:50	Dako
Vimentin	V9	RTU	Leica

RTU, ready to use.

cell associated markers (Oct 4, CD34, and c-kit), and muscle/myofibroblastic markers (muscle specific actin, calponin, and h-caldesmon). We also stained for PAX8, a gene that is involved in embryogenesis of the thyroid, müllerian, and renal/upper urinary tracts.[2] Additional analysis to identify aberrant transcription factors, cell cycle regulation and other oncogenic proteins (p16, cyclin D1, and beta-catenin), and other oncogenic markers shown to be involved in gastrointestinal stromal tumours and mesenchymal tumours (DOG-1 and vimentin)[9,10] were performed. We also used our IHC panel on our control group, consisting of five cases each of infantile fibromatosis/myofibromatosis (IMF), desmoid fibromatosis (DF), and congenital infantile fibrosarcoma (CIFS).

IHC was performed on the cellular MN, classic MN, and mixed MN cases. The IHC stains were interpreted as positive when staining of the tumour phenotype accounted for more than 5% of the tumour, provided that the staining was noted throughout the tumour and not only seen focally. Negative interpretation was restricted to those tumours showing focal staining accounting for less than 5% compared to controls. In addition, fluorescence *in situ* hybridisation (FISH) testing for *ETV6-NTRK3* gene was performed using *ETV6* break apart (Vysis) in all MN and CIFS cases.

RESULTS

We identified 19 cases of MN (five classic, eight cellular, and six mixed) (Table 2) and we included a total of 15 cases of other soft tissue tumours (IMF, DF, and CIFS). Overall, the age at MN presentation ranged from one day to 12 months, with a mean age of 93 days ± 134 days. A slight male predominance was observed, with a male to female ratio of 10:9. Tumours were localised to the right kidney slightly more often than the left (10 versus 9, respectively).

FISH testing for *ETV6* break apart showed *ETV6* gene rearrangement in 0/5 of the classic MN, 8/8 of the cellular MN, and 5/6 of the mixed MN cohorts, respectively. The case of mixed MN with absent *ETV6* break apart was positive for cyclin D1 and beta-catenin.

Table 2 Summary of immunohistochemistry and karyotyping of mesoblastic nephroma and select fibroblastic/myofibroblastic tumours in infants

	Classic MN $n = 5$	Mixed MN $n = 6$	Cellular MN $n = 8$
Cyclin D1	5+	5+	0+
Beta-catenin (cytoplasmic only)	5+	6+	0+
DOG-1	3+	1+	0+
p16/PAX8	5+	5+	5+
Caldesmon/Calponin	0+	0+	0+
WT-1	0+	0+	0+
ETV6-NTRK3 gene rearrangement	0+	5+	8+

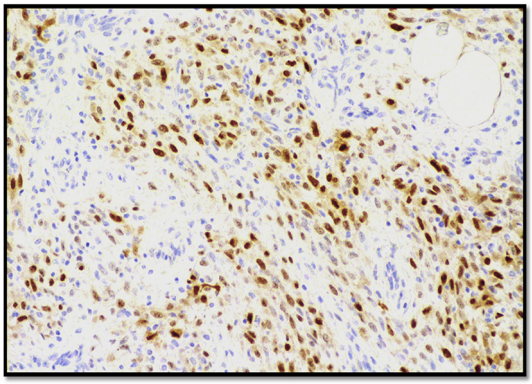

Fig. 1 Classic mesoblastic nephroma, positive for cyclin D1 expression.

All cases of the classic MN demonstrated strong and diffuse (>75%) nuclear expression for cyclin D1 (Fig. 1) and cytoplasmic (but not nuclear) expression for beta-catenin (Fig. 2), while these markers were negative (<5% staining and limited to rare foci) in cellular MN (Fig. 3 and 4) (Table 2). Similar to classic MN, the diffuse (>95%) positive expression beta-catenin (cytoplasmic) is seen in all of the mixed MN cases, and five of six cases of mixed MN showed staining for cyclin D1 (Table 2). IMF showed diffuse positive (cytoplasmic) expression for cyclin D1, while cyclin D1 expression for DF was negative. Beta-catenin expression was positive for DF (nuclear and cytoplasmic), but negative for IMF cases. All cases of MN, CIFS, and IMF in contrast to DF showed no expression of either caldesmon or calponin.

Negative expression for markers of urogenital development (with the exception of PAX8) and stem cell markers was noted in all of the cases of MN and the 15 cases of fibroblastic/myofibroblastic tumours (CIFS, IMF, and DF). Specifically, there was no WT-1 cytoplasmic or nuclear staining in any of the cases. PAX8 and p16 were strongly expressed in all cases of MN and fibroblastic/myofibroblastic tumours studied, with all of the 15 cases of fibroblastic/myofibroblastic tumours [CIFS (5/5), IMF (5/5) and DF (5/5)] staining diffusely and strongly positive for PAX8 and p16 (Fig. 5 and 6). The DOG-1 stain was positive in 3/5 and 1/6 classic MN and mixed MN, respectively, but was negative in all cases of fibroblastic/myofibroblastic tumours studied. There was no correlation between c-kit and DOG-1, since all cases included in the study were negative for C-kit.

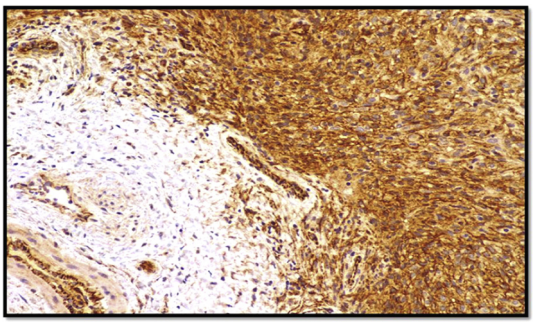

Fig. 2 Classic mesoblastic nephroma, showing beta-catenin cytoplasmic expression.