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CORRESPONDENCE

Toward a unifying entity that encompasses most, but perhaps not all, inflammatory leiomyosarcomas and histiocyte-rich rhabdomyoblastic tumors

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To the Editor,

We read with great interest the recent article by Cloutier and colleagues where they elucidated the significant overlap of clinicopathologic and genomic features between inflammatory leiomyosarcoma (ILMS) and histiocyte-rich rhabdomyoblastic tumor (HRRMT) and proposed the terminology "inflammatory rhabdomyoblastic tumor" to encompass both entities [1]. To provide compelling evidence, they employed the OncoScan SNP assay and identified in 4 of the 7 HRRMTs distinct genomic patterns of near-haploidization that were essentially identical to those observed in 10 of 11 ILMSs subjected to karyotyping or genomic arrays [2–5]. Specifically, the near-haploidization patterns feature the loss of one set of almost all chromosomes while retaining the heterodisomy of chromosomes 5 and 22, and sometimes also 18, 20, and/or 21, with or without subsequent genome doubling [2–5]. As these tumors are extremely rare, and the concept evolving, we hope to share our own data to contribute to the process toward the formation of consensus.

Prompted by the awareness of highly similar clinicopathologic features and recurrent *NF1* mutations shared by ILMS and HRRMT, as well as the overexpression of multiple skeletal muscle-associated genes in ILMS [5, 6], we hypothesized that the two entities might be closely related. Given the characteristic near-haploid karyotype in ILMS, we examined whether our cases of HRRMT also had similar genomic profiles. Four HRRMTs were collected from the authors' diagnosis services and consultation files, including one reported in the first series of HRRMT [6]. The pertinent clinicopathologic features are summarized in Table 1. Briefly, there were three men and one woman, all in their young adulthood. One patient had neurofibromatosis type 1. Another patient reported trauma at the site of tumor formation 1 year earlier. All tumors arose in the skeletal muscle of the thigh or trunk. All were well-circumscribed with fibrous pseudocapsules; minimal foci of infiltrative growth into the adjacent tissue were noted in 2. Tumor cells were spindly with eosinophilic cytoplasm and mild nuclear atypia, arranged in fascicles or haphazardly (Fig. 1A). Larger ovoid cells containing brightly eosinophilic cytoplasm were also occasionally observed and particularly prominent in Case 1 (Fig. 1B). There were numerous tumor-infiltrating lymphocytes and histiocytes, including frequently clustered foamy cells and scattered Touton giant cells. Mitotic activity was low, and small areas of necrosis were noticed in two cases. All tumors showed diffuse desmin immunostaining and focal immunoreactivity to MyoD1 and myogenin; CD68 highlighted numerous histiocytes (Fig. 1C) while variable staining for smooth muscle actin was also found in three cases. H-caldesmon immunostaining (h-CD clone) was performed in two cases

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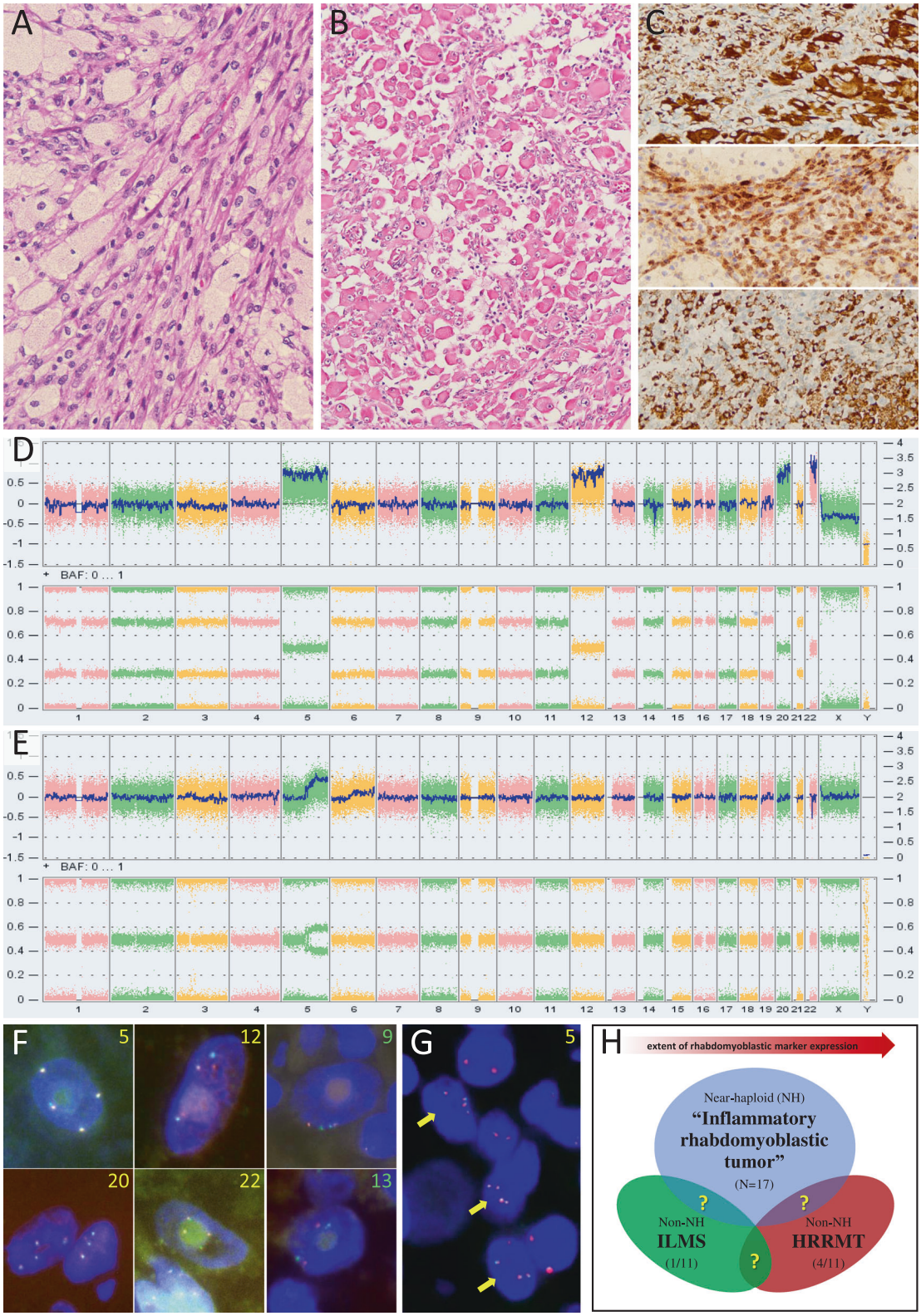
Table 1 Clinicopathologic and molecular information of the cases.

Case#	Age	Sex	Location	Max. dimension	Tumor border	Mitosis	Necrosis	Resection Margin	Positive immunostaining[a]	Follow-up (weeks)	OncoScan-inferred karyotype[b]	FISH-derived average copy number: chr# (copy number)	Note
1	38	M	Thigh	6.8 cm	Encapsulated	2/50 HPF	Present	Close (<1 mm)	Desmin, SMA, MyoD1, myogenin	NED (9)	54, X, +X, −Y, +**5**, +**5**, +**12**, +**12**, +**20**, +**20**, +**22**, +**22**	Chr5q (3.225), Chr9 (1.75), Chr12 (2.825), Chr13 (1.675), Chr17 (1.625), Chr18q (1.5), Chr20q (3.025), Chr22q (3.325)	Trauma 1 year before
2	35	F	Hip	11 cm	Encapsulated, focally infiltrative	1/50 HPF	Present	Positive	Desmin, MyoD1, myogenin	NED (6)	46, XY (with a gain of 5q14.3-q35.3)	Chr5q (3.3), Chr9 (1.95), Chr12 (1.9), Chr17 (1.9), Chr18q (1.875), Chr20q (1.775), Chr22q (1.85)	Adjuvant radiotherapy
3	23	M	Thigh	7.2 cm	Encapsulated	5/50 HPF	Absent	Close (<1 mm)	Desmin, SMA, MyoD1, myogenin	NED (146)	52, XY, +X, +Y, +**5**, +**5**, +**22**, +**22**	Not performed	Adjuvant radiotherapy. Previously reported (ref. [6])
4	18	M	Back	5.3 cm	Encapsulated, focally infiltrative	2/50 HPF	Absent	Close (<1 mm)	Desmin, SMA, MyoD1, myogenin	NED (6)	58, XY, +X, +Y, +**5**, +**5**, +**18**, +**18**, +**20**, +**20**, +**21**, +**21**, +**22**, +**22**	Not performed	Neurofibromatosis type 1

NED no evidence of disease.

[a]Only desmin was diffusely and strongly positive, while the others were focal.

[b]Heterozygous chromosomes shown in bold numbers.



and was negative in both (data not shown). No tumor recurrence or metastasis occurred within the admittedly short follow-up durations.

To determine the global copy number profile, DNA from paraffin-embedded tissues was subjected to the same OncoScan SNP assay used by Cloutier et al. [1, 7] Three

◀ **Fig. 1 Representative histology, immunostaining, OncoScan plots, FISH, and the proposed schematic. A**, **B** Histology of the tumors revealing spindle cells with eosinophilic cytoplasm and generally low-grade nuclear atypia (**A**; original magnification: ×400). There were increased mononuclear inflammatory cells, usually including small lymphocytes and foamy histiocytes. Markedly enlarged cells with abundant brightly eosinophilic cytoplasm, large vesicular nuclei, and prominent nucleoli were present to varying extents (**B**; ×200). **C** Immunohistochemistry showed multifocal to diffuse desmin (upper) and usually focal MyoD1 (middle) staining; CD68 staining (lower) highlighted the stromal histiocytes that seemingly outnumbered the tumor cells. **D**, **E** OncoScan SNP array results. Case 1 (**D**) exhibited inferred copy numbers of around 3 (indicated in the upper panel) for chromosomes 5, 12, 20, and 22 with retained heterozygosity (disclosed with BAF, or B-allele frequency, in the lower panel) while the other somatic chromosomes seemed to be disomic despite the loss of heterozygosity. This genotype indicated near-haploidization with subsequent genome doubling. Of note, the results represented the average data of all cells and were therefore diluted by the numerous diploid inflammatory cells; the copy numbers of the heterozygous chromosomes were roughly 4 after adjustment based on the tumor cell percentage inferred by the sex chromosome copy numbers and BAF. In contrast, Case 2 (**E**) was diploid and largely heterozygous except for gains of 5q. **F** FISH showed 3 or 4 copies of *FGF1* (chr.5q), *MDM2*/CEP12 (chr.12), *NCOA3* (chr.20q), and *EWSR1* (chr.22q) in contrast with two copies of *CDKN2A*/CEP9 (chr.9) and *RB1*/CEP13 (chr.13) in representative cells in Case 1. **G** Many tumor cells in Case 2 had four copies of *FGF1* (chr.5q). **H** The schematic diagram summarizing the current knowledge of karyotypes of ILMS/HRRMT. While 10 ILMS and 7 HRRMT harbored characteristic near-haploid (NH) genomes with or without doubling, 1 ILMS and 4 HRRMT instead showed diploidy. Whether these non-NH cases are interrelated (despite being differently named ILMS vs. HRRMT) and whether they should be regarded as being within the spectrum of the newly proposed "inflammatory rhabdomyoblastic tumor" remains to be determined. Considering the inherent variations in rhabdomyoblastic marker expression, the term ILMS might remain justified for those showing unconvincing rhabdomyoblastic immunophenotype, such as the sole diploid ILMS.

cases revealed characteristic near-haploidization with subsequent genome doubling, where at least chromosomes 5 and 22 retained heterozygosity (with an inferred copy number of 4 in the neoplastic cells) in all (Fig. 1D). The other heterozygous chromosomes were all highly recurrent in ILMS, including chromosomes 18, 20, and 21, with the exception of chromosome 12, which was found to retain heterozygosity in Case 1. In contrast, Case 2 harbored a diploid genome with gains of a major part of chromosome 5q (5q14.3–5q35.3) (Fig. 1E). These results are summarized in Table 1.

Meanwhile, FISH analyses were performed in two cases for cross-validation. Break-apart probes for *FGF1* (chr.5q31), *SS18* (chr.18q11), *NCOA3* (chr.20q13), and *EWSR1* (chr.22q12), along with centromeric probes contained in the copy number probes for *CDKN2A* (chr.9), *MDM2* (chr.12), *RB1* (chr.13), and *ERBB2* (chr.17), were used to represent respective chromosomes. A pathologist blinded to OncoScan results interpreted each of the FISH slides by counting 40 nuclei. Consistent with the SNP array results, the average copy numbers of chromosomes 5, 12, 20, and 22 were roughly twice those of the other somatic chromosomes in Case 1, whereas Case 2 showed a gain of chromosome 5q and was otherwise disomic (Fig. 1F, G and Table 1).

In summary, our current findings strengthened those reported by Cloutier et al., together showing 7 of 11 HRRMT harbored near-haploid genomes that were characteristic of ILMS. We also added a second case with type 1 neurofibromatosis, suggesting *NF1* aberrations may play a role in a subset of tumors. Collectively, the significantly overlapped clinical features (sex, age, location, and indolent behavior), histopathology (myogenic cells and mononuclear inflammatory cells), and molecular characteristics (near-haploidization, *NF1* mutations, and expression of rhabdomyoblastic genes) strongly suggest ILMS and HRRMT should belong to one entity. Exceptions exist, however, as some HRRMTs (4/11) and ILMS (1/11) showed no near-haploidization [1, 3]. Moreover, the sole diploid ILMS, identified by Chang and colleagues, was negative for myogenin immunostaining; [3] therefore, its inclusion under the rubric of inflammatory "rhabdomyoblastic" tumor could incur some controversy despite the limited coverage of rhabdomyoblastic immunophenotype. Instead, the term ILMS might be preserved for such cases. The relationship between the near-haploid "inflammatory rhabdomyoblastic tumor" and those diploid exceptions clearly requires further investigation (Fig. 1H).

The current findings also suggest that FISH could be explored as a surrogate of the less accessible SNP arrays, except for some caveats. The cases with larger tumor nuclei (e.g., Case 1) could be more subject to nuclear truncation in sectioning (hence underestimated copy numbers), while in cases with smaller nuclear sizes (e.g., Case 2) the distinction between neoplastic cells and histiocytes could be challenging. Furthermore, we confirmed the retained heterozygosity of chromosome 12 in Case 1 to be a recurrent phenomenon despite a low frequency [1]. Meanwhile, Case 2 served as an unprecedented example of ILMS/HRRMT with 5q gain and otherwise diploidy. Therefore, the use of chromosomes 5q and 12 FISH as representatives for disomic and monosomic chromosomes, respectively, entails cautious interpretation. For this purpose, we suggest the FISH should cover at least chromosomes 5 and 22 as markers of retained heterodisomy, plus 2 or more chromosomes expected to be monosomic in ILMS/HRRMT.

Data availability

The data supporting the findings of this study are available from the corresponding authors, JCL and HYH, upon reasonable request.

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Author contributions JCL conceived, designed, and implemented the study and wrote the paper; WSL and YCK provided critical opinions on the experiment design and the content of the paper; YCC implemented the experiments and assisted in data analysis; HYH provided cases and supervised the process of study implementation and paper writting.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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