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COMMENTARY

Molecular subtypes of leiomyosarcoma: Moving toward a consensus

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Leiomyosarcoma (LMS) is one of the most common soft tissue sarcoma (STS) subtypes, accounting for up to 20% of STS diagnoses.[1] LMS are tumours of smooth muscle origin, most often developing in the extremities, retroperitoneum and uterus. The 5-year recurrence rate of LMS varies from 10% to 43% dependent on anatomical site, and long-term patient follow-up shows that late recurrences (>10 years) can occur in extremity, abdominal and retroperitoneal patients.[2] In addition, only a subset of LMS patients respond to conventional chemotherapy and radiotherapy. Due to advancements in gene expression profiling technologies, our understanding of the molecular basis of LMS has improved over the past decade and supports the concept of LMS molecular subtypes to explain some of the extensive clinical heterogeneity observed across patients.

In 2009, molecular subtypes of LMS were first documented by Beck et al., through gene expression microarray profiling in a cohort of 51 samples.[3] Following on from this pivotal report, several additional studies utilising transcriptomics have consistently identified 3 different molecular subtypes.[4–8] The different features of the molecular subtypes reported in each of these studies are summarised in Table 1. Whilst the relationship between the molecular subtypes identified in different studies has not been formally assessed, these studies are broadly considered to have identified highly similar subtypes. Across the studies, some common molecular subtype-specific features have been reported, such as anatomical site distribution, expression of myogenic markers, immune activity and association with clinical outcomes.

1 | ANATOMICAL SITE DISTRIBUTION

LMS molecular subtypes are reported to show differential anatomical site distribution. This includes the consistent identification of a uterine LMS (uLMS)-enriched subtype (Beck group III, Guo subtype III, Chudasama SG1, Hemming uLMS, Anderson subtype 3).[3,4,6–8] However, the level of uterine representation varies greatly between these studies, with uLMS accounting for between 34% and 92% of all samples within the putative uLMS-enriched subtype. Moreover, uLMS are found to also be present in other molecular subtypes, comprising between 19% and 59% of samples in the other non-uLMS enriched groups. In support of anatomically driven subtyping, Hemming et al. reported preserved expression of uterine-specific

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TABLE 1 Overview of leiomyosarcoma (LMS) molecular subtypes identified from transcriptomic studies

Subtype	Proportion (%)	Clinical features	Biological features	Survival analysis	Comments
Beck group I[3]	25%	92% stLMS, 77% conventional histology	Enriched in muscle-related genes, phosphoproteins and kinases. Lower genomic stability	Improved DSS in multivariable analysis	Survival analysis performed on separate cohorts using expression measure of group I IHC markers
Beck group II[3]	24%	75% stLMS, 50% conventional histology	Enriched in metabolic, cell proliferation and organ development genes	–	
Beck group III[3]	51%	42% uLMS, 79% pleomorphic/mixed histology, mostly non-primary	Enriched in organ development, ribosomal, ECM and wound response genes	–	
Guo subtype I[7]	35%	72% stLMS, similar proportions of low, intermediate and high-grade tumours	Enriched in muscle-related genes	Improved DSS in univariable analysis	Survival analysis performed on separate cohorts classified based on IHC markers
Guo subtype II[7]	22%	59% uLMS, 68% high grade	Enriched in translation & protein localization genes	Poorer DSS in univariable analysis	
Guo subtype III[7]	13%	92% uLMS, 77% high grade	Enriched in metabolic and transcription genes	–	
Guo ungrouped[7]	29%	–	–	–	
TCGA uLMS[5]	34%	100% uLMS	High DNA damage response, hypomethylation of ESR1 targets, altered AKT pathway	–	Supervised separation of uLMS from stLMS
TCGA stLMS C1[5]	31%	100% stLMS	High HIF1α signalling compared to uLMS, altered AKT pathway, generally hypermethylated, 40% *MYOCD* amplification,	Poorer RFS and DSS in the univariable analysis compared to stLMS C2	

(Continues)

TABLE 1 (Continued)

Subtype	Proportion (%)	Clinical features	Biological features	Survival analysis	Comments
TCGA stLMS C2[5]	35%	100% stLMS	High HIF1α signalling compared to uLMS, generally hypomethylated, high inflammatory signatures (NK and mast cells)	–	
Chudasama SG1[6]	16%	34% uLMS	Enriched in platelet degranulation, complement activation and metabolic genes	–	
Chudasama SG2[6]	14%	19% uLMS	Enriched in muscle-related genes	–	
Chudasama SG3[6]	70%	19% uLMS	Intermediate expression of muscle-related genes, and cell-cell signalling genes	–	
Hemming cLMS[8]	49%	10% metastasis	High expression of muscle-associated transcripts and *IGF1R*	Improved DSS compared to iLMS in univariable analysis	
Hemming iLMS[8]	28%	10% metastasis	Enriched in immune-related genes. Estimated high M2 macrophage and CD8+ T cell infiltration	–	
Hemming uLMS[8]	23%	88% uLMS, 40% metastasis	Expression of uterogenic transcripts	–	
Anderson Subtype 1[4]	18%	43% gLMS	High occurrence of *DMD* deletions (evidence of dedifferentiation), high in immune activity (M2 macrophages)	–	Subtype 2 split into 2a (31%; mostly abdominal) and 2b (69%; mixed abdominal and extremity)
Anderson Subtype 2[4]	65%	81% abdominal or extremity LMS	*MYOCD* amplifications	Improved OS & DSS compared to combined subtypes 1 and 3 in univariable analysis	
Anderson Subtype 3[4]	17%	91% gLMS	*DMD* deletions and *MYOCD* amplifications	–	

Abbreviations: DSS, disease-specific survival; ECM, extracellular matrix; gLMS, gynecological LMS; IHC, immunohistochemistry; NK, natural killer; OS, overall survival.; RFS, recurrence free survival; stLMS, soft-tissue LMS (non-uterine); uLMS, uterine LMS.

transcripts in the uLMS-enriched subtype (Hemming uLMS) and absent or minimal expression of these transcripts in other molecular subtypes.[8] In a separate study, Anderson et al., illustrated that most samples of the putative uLMS subtype (Anderson subtype 3) co-localised with normal gynaecological smooth muscle tissue in unsupervised clustering of the transcriptomic data.[4]

Anderson et al. also showed that a putative non-uLMS subtype (Anderson subtype 2) could be further stratified into two clusters, which appear to be driven by anatomical site.[4] Anderson subtype 2a comprised mostly abdominal lesions, and clustered with normal digestive smooth muscle, whereas Anderson subtype 2b comprised a mix of abdominal and extremity lesions, and clustered with normal vascular smooth muscle. Retroperitoneal and extremity LMS frequently arise in association with the vasculature, and therefore the stratification between 2a and 2b may illustrate distinct LMS tissue lineages. Collectively, the evidence suggests that there is some association between molecular subtypes and anatomical site but these are not definitive. Rather, it appears that anatomical site may contribute to disease heterogeneity but it does not fully explain the molecular differences observed across LMS.

2 | MYOGENIC MARKERS AND IMMUNE ACTIVITY

LMS is derived from a smooth muscle lineage and diagnosis entails immunohistochemical assessment of a number of smooth muscle markers. Notably, differential myogenic gene expression levels have been observed across the three LMS molecular subtypes. In particular, a 'high-myogenic' subtype has been reported (Beck group I, Guo subtype I, TCGA soft tissue LMS (stLMS) C1, Chudasama SG2, Hemming cLMS, Anderson subtype 2).[3–8] This subtype is characterised by overexpression of several muscle-specific genes and is thought to be a molecular subtype of low/intermediate grade and majority non-uterine tumours of mostly conventional histology. Genomically, the 'high-myogenic' group has been characterised by hypermethylation, lower genomic stability compared to other LMS, and myocardin (*MYOCD*) amplifications (Table 1).

Anderson et al. also reported a 'low myogenic' or 'dedifferentiated' molecular subgroup (Anderson subtype 1).[4] Clustering analysis with other STS histologies showed that the majority of Anderson subtype 1 LMS tumours tend to co-cluster with non-LMS tumours, including undifferentiated pleomorphic sarcomas. Histologically observed dedifferentiation within LMS tumours has previously been reported.[9,10] These reports describe tumours with regions of classical LMS tissue, co-occurring alongside de-differentiated non-myogenic components. Dedifferentiation is a well-studied phenomenon across oncology and often confers a higher grade and more aggressive tumour type,[11] which may identify a high-risk patient population.

The different myogenic subtypes also differ in their immune composition. In particular, the 'low-myogenic' or 'dedifferentiated' subtype (TCGA stLMS C2, Chudasama SG1, Hemming iLMS, Anderson subtype 1) has been shown to possess higher immune cell infiltrates.[4–6,8] Through in silico deconvolution estimation of transcriptomic data, these studies have reported enrichment of different immune cell types including high M2 macrophage, NK cells, CD8+ T cells, or mast cells infiltration in the 'low myogenic' molecular subtype (Table 1).

3 | CLINICAL SIGNIFICANCE

Some studies report improved outcomes in the 'high-myogenic' subtype compared to the 'low-myogenic' subtype (Table 1). Although these findings are not consistent across the different studies. Beck et al. and Guo et al. utilised immunohistochemical analysis of selected protein markers representing the relevant molecular subtypes and assessed their association with patient survival.[3,7] Both studies indicated significantly improved disease specific survival (DSS) in the 'high-myogenic' group. Hemming et al., found improved DSS for the cLMS subtype (high-myogenic) compared to the iLMS subtype (low-myogenic) while Anderson et al. reported improved DSS for subtype 2 (high-myogenic) compared to the other two molecular subtypes combined.[4,8] However, neither subtype variables remained independent prognosticators following adjustment for key clinicopathological variables in multivariable analyses. In contrast, the study by The Cancer Genome Atlas (TCGA) consortium revealed a significantly poorer relapsed free survival (RFS) and DSS in stLMS C1 (high-myogenic) versus stLMS C2 (low-myogenic).[5] However, significance was again lost in multivariable analyses. The inconsistent survival observations made by TCGA compared to other studies may be explained in part by the semi-supervised approach TCGA took to LMS subtyping, where uLMS and stLMS were separated prior to analysis. In summary, given the lack of consistency between the different studies and absence of independent prognostic value, the clinical utility of LMS molecular subtypes for prognostication remains to be determined.

4 | FUTURE PERSPECTIVES

Robust transcriptomic-based molecular subtyping has been deployed in precision oncology applications in

routine clinical management and clinical trials of other cancer types such as breast and colorectal cancer.[12,13] To advance molecular subtyping in the clinical management of LMS, there is a need to first establish a robust and consistent molecular classification. Due to differences in gene expression profiling platforms and patient cohorts, there are inconsistencies between the different LMS molecular subtype studies that have been reported thus far. In order to resolve some of the discrepant results, we suggest the need for a community-driven multi-national collaborative effort to undertake a cross-comparison analysis develop a robust consensus molecular classification system. This will facilitate the clinical translation of molecular subtyping for the benefit of patients affected by LMS.

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