Uterine leiomyosarcomas exhibit distinct drug resistance molecular profiles compared to extrauterine leiomyosarcomas: A comprehensive analysis of 1,023 leiomyosarcomas

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ABSTRACT

Objectives: Controversy exists as to whether uterine leiomyosarcomas (ULMS) and extrauterine leiomyosarcomas (ELMS) represent distinct pathological and molecular entities. We aim to evaluate molecular, genomic, and protein expression patterns in a large cohort of leiomyosarcomas (LMS) in hopes of identifying novel treatment strategies.

Methods: A total of 1,023 cases of LMS were submitted for molecular analysis from 2009 to 2015, including 635 ULMS and 388 ELMS. Testing included a combination of sequencing (Sanger or next-generation sequencing [NGS]), protein expression (immunohistochemistry), and gene amplification (fluorescence in situ hybridization [ISH]/chromogenic ISH).

Results: The mean age in the LMS cohort was 56.8 years, with 34% of ELMS occurring in men. Figure 1 summarizes molecular and sequencing alterations in ULMS and ELMS. Of the LMS samples evaluated using NGS, TP53 was most commonly altered (41%), followed by BRCA2 (6.3%) and RB1 (4.5%). Evaluating markers of drug resistance, RRM1 expression, associated with gemcitabine resistance, was seen in 36% of ULMS and higher than in ELMS (P < .0001). On subanalysis, ERCC1-expressing LMS had higher expression of TOP2A (P < .0001) and TOP1 (P = .0309), suggesting a potential role for anthracyclines and topotecan in these lines. Significantly more ULMS expressed TUBB3, a marker correlated with taxane resistance (33% ULMS vs 17% ELMS, P < .0001). Lower ERCC1 expression was seen in ULMS. Hormone receptor expression was frequent in LMS overall (45.2% ER, 34.2% PR and 24% AR), but much more common in ULMS than ELMS: AR (P = .0014); ER (P < .0001); PR (P < .0001). In ULMS, ER/PR negative LMS, epidermal growth factor receptor overexpression, via immunohistochemistry and ISH, were significantly elevated (P = .04, P = .0001, respectively). Of interest, 28.6% of LMS expressed PDL1 on tumor cells, and 46% expressed PDL1 protein on tumor-infiltrating lymphocytes. Table 1 summarizes statistically significant differences in biomarker expression profiles between ULMS and ELMS.

Conclusions: Our findings highlight the molecular heterogeneity in LMS, and distinct differences between ULMS and ELMS. Uterine LMS display significantly more biomarkers implicating drug resistance than extrauterine LMS. Of interest, one-third of ULMS expressed biomarkers associated with gemcitabine and docetaxel resistance. Alternative strategies such as anthracyclines, hormonal therapy, PD-1 inhibitors, and tyrosine kinase inhibitors may be considered as adjuvant therapy.

1,023 cases of LMS were submitted for molecular analysis from 2009 to 2015:
- 635 Uterine LMS
- 388 Extrauterine LMS

Patient Demographics:
- Mean age of LMS cohort 56.8
- 34% of ELMS occurred in men

Sequence Alterations:
- No statistical difference between sequencing alterations of ULMS and ELMS
- Most common sequencing alterations among entire cohort:
  - TP53 alterations in 41%
  - BRCA2 alterations in 6.3%
  - RB1 alterations in 4.5%

Table 1: Molecular profile distinctions between Leiomyosarcoma of uterine and extra-uterine origin

Table 1 summarizes statistically significant differences in biomarker expression profiles between ULMS and ELMS.

<table>
<thead>
<tr>
<th>Molecular Profile</th>
<th>Uterine LMS (n=635)</th>
<th>Extra-Uterine LMS (n=388)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone Receptors</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ER</td>
<td>60.1%</td>
<td>48.4%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PR</td>
<td>41.8%</td>
<td>29.1%</td>
<td>0.0014</td>
</tr>
<tr>
<td>AR</td>
<td>29.1%</td>
<td>19.6%</td>
<td>0.0014</td>
</tr>
<tr>
<td>DNA Repair</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERCC1</td>
<td>40.0%</td>
<td>50.0%</td>
<td>0.0352</td>
</tr>
<tr>
<td>DNA Replication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOP2A</td>
<td>33.3%</td>
<td>16.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TOP1</td>
<td>32.5%</td>
<td>39.7%</td>
<td>0.0412</td>
</tr>
<tr>
<td>Drug Resistance AssOCIated Proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRM1</td>
<td>43.0%</td>
<td>18.5%</td>
<td>0.0032</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ImmunoOncologIcalCheckpoints</td>
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<tr>
<td>PDL1</td>
<td>27.5%</td>
<td>31.2%</td>
<td>0.5692</td>
</tr>
<tr>
<td>PDL1</td>
<td>47.0%</td>
<td>51.0%</td>
<td>0.6923</td>
</tr>
</tbody>
</table>

Hormone receptor expression more common in ULMS than ELMS:
- ER/PR negative LMS, epidermal growth factor receptor overexpression noted
- ERCC1 expression in ULMS was significantly different compared to ELMS
- In ULMS, ERCC1 expression was significantly higher than in ELMS (P < .0001)
- ERCC1 expression was significantly higher in extrauterine LMS compared to uterine LMS (P < .0001)
- In ULMS, ER/PR negative LMS, epidermal growth factor receptor overexpression, via immunohistochemistry and ISH, were significantly elevated (P = .04, P = .0001, respectively)
- Of interest, 28.6% of LMS expressed PDL1 on tumor cells, and 46% expressed PDL1 protein on tumor-infiltrating lymphocytes

Higher expression of TUBB3 in ULMS compared to ELMS:
- Class III beta-tubulin: hypothesize TUBB3 may increase dynamic instability of microtubules
- Correlated with taxane resistance
- Higher RRM1 expression in ULMS compared to ELMS
- Gemcitabine diphosphate functions by inhibiting ribonucleotide reductase, enzyme required for synthesis of dNTPs
- Increased expression associated with gemcitabine resistance
- On subanalysis, RRM1 expressing LMS had higher expression of TOP2A and TOP1
- Suggesting a potential role for anthracyclines and topotecan in these patients

Among entire LMS cohort, 28.6% expressed PDL1 on tumor cells, and 46.8% PDL1 protein on tumor-infiltrating lymphocytes
- Potential role for PD-1 inhibitors

INTRODUCTION

Leiomyosarcoma (LMS) represent 25% of all soft tissue sarcomas with the uterus, retroperitoneum, and extremities comprising the most common primary anatomic sites.

Only recently ULMS has been studied uniquely in clinical trials separate from other gynecological sarcomas or soft tissue sarcomas of other sites.

Continuously exists as to whether uterine LMS and extrauterine LMS represent distinct biological and clinical entities.

Observational studies have demonstrated that ULMS responds differently to adjuvant treatment compared to ELMS.

METHODS

Retrospective data was done on uterine leiomyosarcoma (ULMS) and extrauterine leiomyosarcoma (ELMS) cases that were submitted to a commercial referral laboratory (Columbia University Life Sciences, Phoenix, AZ) for molecular profiling aimed to provide therapeutic information based on tumor biology.

Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS), protein expression (IF/Cand gene amplification (CISH or FISH).

ICP analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark [H00048880-01], Ventana, and Autostainer LS 4800, Dako), and commercially available antibodies.

Fluorescence in situ hybridization (FISH) was used for evaluation of the HER2/neu (HER2/CEP777 probe), EGFR (EGFR/CEP777 probe), and MET (MET/CEP7 probe, Abbott Molecular/Vysis). HER2/neu and ATM status were also analyzed using chromogenic in situ hybridization (INHER2 Dual IISH DNA Probe Cocktail, commercially available and chromosome 7 DIG probe, Ventana).

Direct sequencing analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using Illumina MiSeq platform. Mutation analysis of 47 genes of the genome were amplified using the Illumina TruSeq exome research kit (Middlesex Medical Sciences, Cambridge, MA). Mutation analysis was performed by Sanger sequencing included selected regions of BRAF, KRAS, NRAS, V600E, EGFR, and PD1CA genes and was performed by using M13-linked PCR primers.

CONCLUSIONS

LMS demonstrate considerable molecular heterogeneity.

Although there are similarities in the molecular, genomic, and protein expression patterns of ULMS and ELMS, there are important and potentially clinically different between the two.

Uterine LMS display more biomarkers implicating drug resistance compared to extrauterine LMS.

Of interest, one-third of ULMS expressed proteins associated with gemcitabine and docetaxel resistance.

Alternate strategies such as anthracyclines, hormonal therapy, PD-1 inhibitors, and tyrosine kinase inhibitors may be considered as adjuvant therapy.