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Gene expression signatures of primary and metastatic uterine leiomyosarcoma

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Abstract

Leiomyosarcoma (LMS) is the most common uterine sarcoma. Although the disease is relatively rare, it is responsible for considerable mortality due to frequent metastasis and chemoresistance. The molecular events related to LMS metastasis are unknown to date. The present study compared the global gene expression patterns of primary uterine LMS and LMS metastases. Gene expression profiles of 13 primary and 15 metastatic uterine LMS were analyzed using the HumanRef-8 BeadChip from Illumina. Differentially expressed candidate genes were validated using quantitative real-time PCR and immunohistochemistry. To identify differently expressed genes between primary and metastatic tumors, we performed one-way ANOVA with Benjamini-Hochberg correction. This led to identification of 203 unique probes that were significantly differentially expressed in the two tumor groups by greater than 1.58-fold with p-value <0.01%, of which 94 and 109 were overexpressed in primary and metastatic LMS, respectively. Genes overexpressed in primary uterine LMS included *OSTN*, *NLGN4X*, *NLGN1*, *SLITRK4*, *MASPI*, *XRN2*, *ASS1*, *RORB*, *HRASLS* and *TSPAN7*. Genes overexpressed in LMS metastases included *TNNT1*, *FOLR3*, *TDO2*, *CRYM*, *GJAI*, *TSPAN10*, *THBS1*, *SGK1*, *SHMT1*, *EGR2* and *AGT*. Quantitative real-time PCR confirmed significant anatomic site-related differences in *FOLR3*,

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OSTN and *NLGN4X* levels, and immunohistochemistry showed significant differences in TDO2 expression. Gene expression profiling differentiates primary uterine LMS from LMS metastases. The molecular signatures unique to primary and metastatic LMS may aid in understanding tumor progression in this cancer and in providing a molecular basis for prognostic studies and therapeutic target discovery.

Keywords

Gene expression array; uterine leiomyosarcoma; metastasis

Introduction

Uterine sarcomas are rare tumors, comprising 7% of all soft tissue sarcomas and 3% of uterine cancers [1,2]. With the exclusion of carcinosarcomas, now regarded as metaplastic carcinomas, from this category, the most common uterine sarcomas are endometrial stromal sarcoma (ESS) and leiomyosarcoma (LMS) [2]. In a recent series of all sarcomas in Norway in the period 1970–2000, uterine sarcomas comprised 419 of 12,431 (3.4%) uterine malignancies [3]. LMS are clinically aggressive tumors [3,4].

Several studies have analyzed the molecular profile of LMS. However, the majority of these series consisted predominantly or exclusively of soft tissue LMS, and included other types of soft tissue sarcomas [5–9]. Analyses focusing exclusively or predominantly on uterine LMS are few to date. Quade et al. compared the gene expression profiles of 9 uterine LMS, 7 leiomyomas and 4 normal myometria, as well as 4 non-uterine LMS [10]. A set of 153 probes representing 146 genes differentiated between uterine LMS, leiomyomas and normal myometria. Non-uterine LMS resembled their uterine counterparts.

Skubitz compared the gene expression profiles of 4 uterine and 4 non-uterine LMS, 19 leiomyomas, 46 normal myometria and 250 other tissues [11]. A gene panel differentiating LMS from normal myometrium was defined, with less distinct differences between LMS and the other tissues sampled.

Comparative analysis of 2 uterine LMS and 3 normal myometria identified several overexpressed genes in LMS, of which *GRN*, encoding for acrogranin, was shown to have a biological role *in vitro* and in nude mice model [12]

Two comparative genomic hybridization array analyses of 7 and 15 uterine LMS from one group identified frequently gained and lost genes, although lists differed in these 2 studies [13,14].

Comparative analysis of primary and metastatic soft tissue LMS identified 335 differentially expressed genes [9]. However, data for uterine LMS are unavailable to date. In the present study, we compared the gene expression profiles of 28 primary and metastatic uterine LMS. We identified a set of genes that were differentially expressed in primary and metastatic disease, which may improve our understanding of disease progression in this cancer, as well as provide new potential candidates for targeted therapy.

Material and methods

Patients and material

The clinical material consisted of 28 uterine LMS, submitted for routine diagnostic purposes to the Department of Pathology at the Norwegian Radium Hospital during the period 2002–

2009. Tumors consisted of 13 primary uterine tumors and 15 metastases, the latter consisting of 11 intra-abdominal and 4 distant metastases (1 bone and 3 lung metastases). Metastases were from 10 patients, of whom 1 had 3 lesions, 3 had 2 lesions and 6 had a single metastasis. For patients with >1 metastasis, tumors were metachronous. Primary and metastatic lesions were not patient-matched, with the exception of one patient with primary LMS and lung metastasis.

Tumors were snap-frozen and kept at -70°C . Frozen sections from all tumors were evaluated for the presence of a >80% tumor component and absence of necrosis. Diagnoses were established by experienced gynecologic pathologists based on morphology and immunohistochemistry (IHC) [3,15].

The material analyzed using quantitative real-time PCR (qRT-PCR) consisted of 29 LMS (11 primary, 18 metastatic), including 10 of the 13 primary LMS and all 15 metastatic LMS analyzed in gene expression arrays. The remaining 4 specimens were snap-frozen at the Norwegian Radium Hospital during the same period.

The material analyzed using IHC consisted of 21 patient-matched primary and metastatic LMS operated at the Norwegian Radium Hospital during the period 1998–2011. Cases were chosen based on the availability of patient-matched primary and metastatic tumor. Metastases were predominantly (n=16) intra-abdominal, the remaining 5 consisting of 1 bone and 4 lung metastases. Tumors from 5 of these patients were analyzed by gene expression arrays.

Patient consent was obtained according to national guidelines and the study was approved by the Regional Committee for Medical Research Ethics in Norway.

Microarray Expression and GeneChip analysis

RNA was prepared from tumor samples using a Qiagen RNeasy kit (Qiagen, Valencia, CA). Illumina HumanRef-8 BeadChip arrays were used to analyze gene expression in both tumors. The BeadChip includes ~24,500 well-annotated transcripts with up-to-date content derived from the National Center for Biotechnology Information Reference Sequence (NCBI RefSeq) database (Build 36.2, Release 22). RNA labeling, hybridization and scanning of the arrays were performed using the standard protocols in the Johns Hopkins Medical Institutions Microarray Core.

qRT-PCR

Among the above-detailed differentially expressed genes, we selected 14 for validation using qRT-PCR. These consisted of 8 genes that were overexpressed in primary LMS (*OSTN*, *NLGN4X*, *NLGN1*, *EGR2*, *SLITRK4*, *MASP1*, *XRN2*, and *HRASLS*) and 6 genes that were overexpressed in metastatic LMS (*TNNT1*, *FOLR3*, *TDO2*, *SHMT1*, *CRYM* and *THBS1*). Genes were chosen based on their potential biological and clinical relevance, as judged by the two senior authors of this manuscript (BD and TLW). Specifically, genes known to have a role in cancer and/or expressed in soft tissue, particularly in muscle, were prioritized. qRT-PCR was performed using a SyBr Green-based detection system as previously described [16]. Primers were designed to test the performance in qRT-PCR and those generating robust and specific PCR products in melting curve analysis with minimal primer dimers were selected for analysis (Table 1).

Approximately 16–100ng of cDNA was used in the qRT-PCR analysis, performed on an iCycler. Threshold cycle numbers (Ct) were obtained using the iCycler Optical system interface software (Bio-Rad Lab, Hercules, CA). Averages in the Ct of duplicate measurements were obtained. The results were expressed as the difference between the Ct of

the gene of interest and the Ct of a control gene (APP) for which expression is relatively constant among previously analyzed SAGE libraries. In cases where no gene expression was observed, a cutoff Ct value of 45 cycles was used.

IHC

Protein expression of 10 gene products, including 6 overexpressed in primary LMS (*ASS1*, *NLGN4X*, *NLGN1*, *SLITRK4*, *XRN2*, and *HRASLS*) and 4 overexpressed in metastatic LMS (*TDO2*, *SHMT1*, *CRYM* and *GJAI*) products was analyzed using IHC. The choice of proteins for validation was based on the availability of commercial antibodies with adequate performance in formalin-fixed paraffin-embedded material. Antibody details and staining conditions are provided in Table 2. IHC was performed using the EnVision FLEX+ system (Dako, Glostrup, Denmark). Appropriate positive and negative controls were used and were satisfactory in each staining round.

Immunostained slides were scored by a gynecopathologist (BD). A subset of the slides (25%) was additionally scored by a second gynecopathologist (VMA). Staining extent was scored on a 0–3 scale, corresponding to 0%, 1–10%, 11–50% and 51–100% stained cells, whereas staining intensity was scored as negative (score=0), weak (score=1) or strong (score=2). Multiplying these 2 values provided a staining score scale of 0–6, which was used in the statistical analysis. Differences in protein expression between primary and metastatic LMS were analyzed by the Mann Whitney U test using the SPSS program (version 18.0, Chicago IL).

Results

Unsupervised hierarchical clustering, performed on primary tumors to determine the similarity in gene expression patterns among these 13 samples, demonstrated two major clusters (Figure 1-A). Review of the morphology of tumors in these two clusters did not show differences with respect to the degree of atypia or mitotic counts. The 15 metastatic samples were derived from 10 patients, of whom 1 had 3 lesions (S3–S4–S14), 3 had 2 lesions (S5–S6, S17–S18, S24–S30), and 6 had a single metastasis. To test if metastatic samples from the same patient shared similar transcriptome profiles, unsupervised hierarchical clustering analysis was performed. This analysis demonstrated that metastatic samples derived from the same patient all clustered under the same branch (Figure 1-B). This indicates that although tumor cells from an individual patient have metastasized to different organs, their transcriptome profiles are by-and-large very similar. Of note, the 5 metastases in the right branch originated from primary LMS with very high mitotic count (>20/10 high-power fields).

To identify genes differentially expressed in primary and metastatic LMS, we performed one-way ANOVA with Benjamini-Hochberg correction and found 203 unique probes that were significantly differentially expressed by greater-than-1.58-fold with $p < 0.01\%$ (Figure 2). Among these genes, 94 and 109 were overexpressed in primary and metastatic LMS, respectively. Genes overexpressed in primary uterine LMS included *OSTN*, *NLGN4X*, *NLGN1*, *SLITRK4*, *MASPI*, *XRN2*, *ASS1*, *RORB*, *HRASLS* and *TSPAN7*. Genes overexpressed in LMS metastases included *TNNT1*, *FOLR3*, *TDO2*, *CRYM*, *GJAI*, *TSPAN10*, *THBS1*, *SGK1*, *SHMT1*, *EGR2* and *AGT*. Differences among the groups were in general less pronounced than in our previous comparative analysis of ESS and LMS using the same platform [17]. The full gene list is provided in Supplementary Table 1.

Next we performed GeneGo MetaCore analysis to determine functional ontologies that are enriched in the 203 differentially expressed gene probes. The most enriched GeneGo biological processes are related to angiotensin maturation as well as PKA signaling (Figure

3). Closer look at the angiotensin-related pathway showed that angiotensin I-IV and the angiotensin precursor angiotensinogen were up-regulated in metastatic LMS (Supplementary Figure 1).

Validation experiments

Expression levels of 14 selected transcripts were analyzed in 11 primary and 18 metastatic LMS using qRT-PCR. Statistically significant differences were observed for *OSTN* ($p=0.044$) and *NLGN4X* ($p=0.002$), overexpressed in primary LMS, and for *FOLR3* ($p=0.044$), overexpressed in metastatic LMS (Figure 4). A trend for higher expression in primary LMS was seen for *SLITRK4* ($p=0.07$), and p -values <0.3 were found for several additional genes, including *TDO2* ($p=0.13$), *MASPI* ($p=0.14$), *EGR2* ($p=0.15$) and *SHMT1* ($p=0.23$) (data not shown). Marked differences in expression level were seen within each group for the majority of genes.

IHC was applied to analysis of 10 proteins, of which 6 and 4 were overexpressed in primary and metastatic LMS, respectively, in 21 patient-matched paired tumors. Eight of the proteins (*NLGN4X*, *NLGN1*, *SLITRK4*, *XRN2*, *HRASLS*, *TDO2*, *CRYM* and *Cx43/GJA1*) had variable degree of expression across tumors, with evident expression differences observed in some primary-metastasis pairs (Figure 5). *SHMT1* was diffusely expressed in all tumors irrespective of anatomic site, whereas *ASS1* protein was not expressed in any of the tumors. Statistical analysis showed significantly higher *TDO2* expression in LMS metastases ($p=0.01$), with no significant differences for the remaining proteins. Inter-observer agreement for staining score was 75%. Differences were predominantly of one staining extent or intensity level, and were resolved in a consensus session.

Discussion

Uterine LMS is a rare aggressive cancer, and there is currently little knowledge regarding the molecular events undergone by LMS cells in the process of metastasis. In the present study, we applied gene expression profiling to address this biological question. We identified 203 unique probes that significantly differentiated between primary and metastatic LMS. These showed little overlap with the genes identified by Lee et al. as differentiators between primary and metastatic soft tissue LMS [9]. One possible reason for this, apart from technical considerations, may be the fact that uterine LMS, while sharing many morphological and biological characteristics with soft tissue LMS, is a distinct entity, and arises in an organ which differs considerably from soft tissue. That would imply different microenvironment, in turn modifying the genotype of tumor cells.

The majority of genes found to be overexpressed in primary uterine LMS have not been previously studied in this tumor, although some have been localized to muscle tissue. Osteocrin, encoded by *OSTN*, was the most overexpressed gene in primary LMS. Osteocrin was originally identified as a secreted protein in osteoblasts, but was later found in other mesenchymal cells, including muscle. It has structural homology to natriuretic peptides and is considered to be a hormone-like peptide, with possible role in metabolic regulation in muscle [18].

Two Neuroligin family members, Neuroligins 1 and 4X, were overexpressed in primary LMS. Neuroligins are encoded by 5 genes and undergo alternative splicing. Together with Neurexins, they are expressed in the nervous systems, where they form trans-synaptic complexes and are thought to regulate synaptic development and function. Genetic alterations have been described in both gene families in autism. Though predominantly expressed within the nervous system, Neuroligin 3 was detected in pancreas, skeletal and cardiac muscle and glia, and Neuroligin mRNA was found in endothelial cells. In

endothelium, Neuroligins may regulate crosstalk between nerves and vessels or angiogenesis, the latter suggesting a role in tumor biology [19]. Both Neuroligins were widely expressed at the protein level in our series and their clinical role may merit further study in a larger series.

As for Neuroligins, Slitrk family members, encoded by *SLITRK1-6*, are nervous system proteins. Slitrk proteins are integral membrane proteins resembling Trk tyrosine kinase receptors, which are involved in neural development. They are expressed in different brain tumors, including both gliomas and small blue round cell tumors such as medulloblastoma and primitive neuroectodermal tumor (PNET) [20]. The current report is the first to describe expression of a Slitrk family member in sarcomas.

XRN2 is member of the 5'→3' exoribonuclease (XRN) family, conserved enzymes in eukaryotes which regulate RNA metabolism. *XRN2* is hypermethylated in salivary adenoid cystic carcinoma [21]. It has not been studied in sarcomas to date.

Tetraspanins are a large family of 33 transmembrane adhesion molecules which interact with both membrane and cytoplasmic partners, including integrins, claudin-1 and EGFR. Tetraspanins are deregulated in multiple cancers and regulate angiogenesis, invasion and metastasis, promoting or inhibiting tumor progression depending on the tumor and family member involved, and are considered as candidates for targeted therapy [22]. Uterine sarcomas were reported to have low expression of the tetraspanin member KAI-1. However, 5 of the 15 analyzed tumors in this group were carcinosarcomas, which are currently not considered as sarcomas, the remaining 10 consisting of 8 ESS and 2 LMS [23]. In the present study, *TSPAN7 (CD231)* was overexpressed in primary LMS whereas *TSPAN10* was overexpressed in metastatic LMS. The clinical significance of this alteration, if any, remains to be established.

Genes overexpressed in LMS metastases consisted mainly of molecules which have been characterized in sarcomas, non-malignant muscle cells or other cancers. The latter category included 2 molecules involved in folate metabolism, *FOLR3* and *SHMT1*. *FOLR3*, the secreted member of the folate receptor family, is overexpressed in ovarian carcinoma [24]. *SHMT1* encodes serine hydroxymethyltransferase 1, vitamin B6-dependent enzyme catalyzing the conversion of tetrahydrofolate to 5,10-methylene tetrahydrofolate, which provides one-carbon units for the synthesis of purine, thymidilate and methionine [25]. *SHMT1* polymorphisms are associated with higher risk for different cancers, e.g. childhood acute lymphoblastic leukemia [25], and *SHMT1* was found to be one of the oncogenic genes at chromosome 17p11.2–p12 in osteosarcoma [26].

TDO2 encodes tryptophan 2,3-dioxygenase, one of three heme-containing enzymes which convert tryptophan to *N*-formyl kynurenine, and is highly expressed in normal liver [27]. While *TDO2* has not been well characterized in cancer to date, indoleamine 2,3-dioxygenase 2 (*IDO2*), one of the other enzymes mediating this reaction, was associated with poor metastasis-free and overall survival in osteosarcoma [28].

TNNT1 is part of a 7-gene group encoding for thin filament skeletal proteins, in which >140 different mutations have been identified, forming the molecular basis for congenital skeletal myopathies [29]. The role of this gene in sarcomas has not been studied to date.

EGR2 (early growth response 2) is involved in myelination by Schwann cells and is mutated in peripheral neuropathies. A risk locus for Ewing sarcoma was recently identified 5kb upstream to the *EGR2* gene [30].

Another gene found to be overexpressed in metastatic LMS was *SGK1*, encoding the serum/glucocorticoid-induced kinase SGK-1, which is widely expressed in normal tissue and regulates ion transport. SGK-1 is a serine-threonine kinase belonging to the AGC kinase family, which includes protein kinases A-C and G (PKA, PKB/Akt, PKC, PKG), and is induced by mTOR, a downstream target of Akt. SGK-1 mediates cell survival and tumor growth in colon carcinoma [31].

Finally, we observed overexpression of *CRYM* and *GJA1* in metastatic LMS. Crystallins are a family of structural lens proteins, and mutations in Crystallin genes are associated with cataract. Crystallin μ , encoded by *CRYM*, is expressed in various tissues, including muscle, and *CRYM* mutations are associated with deafness [32]. *CRYM* expression has not been studied in sarcomas to date. However, *CRYAB*, another member of this family, was reported to be overexpressed in osteosarcoma compared to normal bone [33].

Connexins (Cx) are a family of gap junction proteins which are deregulated in different cancers and are associated with tumor progression and metastasis, and thus considered as potential therapeutic targets [34]. Expression of Cx43, the most widely-studied member of this family, is associated with poor survival in Ewing sarcoma [35].

In conclusion, the first gene expression array analysis comparing primary and metastatic LMS identified sets of genes that are differentially expressed at different anatomic sites in this cancer. Differences in gene expression levels were, however, more subtle than in our previous comparison of ESS and LMS [17], which is likely to have impacted our validation analyses. Metastatic samples from the same patient have similar overall transcriptome. Ideally, to identify genes differentially expressed in primary versus metastatic samples, patient-matched lesions should be studied. However, obtaining frozen material from the primary tumor and metastasis for rare tumors is difficult. Such analysis was nevertheless performed for protein expression by IHC. Validation by qPCR and IHC did confirm anatomic site-related differences in the expression of several molecules which may be relevant for tumor biology and disease progression in LMS, and analysis of the genomic landscape may help further delineate the pathogenesis in developing recurrence of LMS. Further studies of larger series are likely to elucidate the prognostic and potential therapeutic relevance of these molecules, hopefully benefiting a patient population that critically depends on targeted therapy for prolonging survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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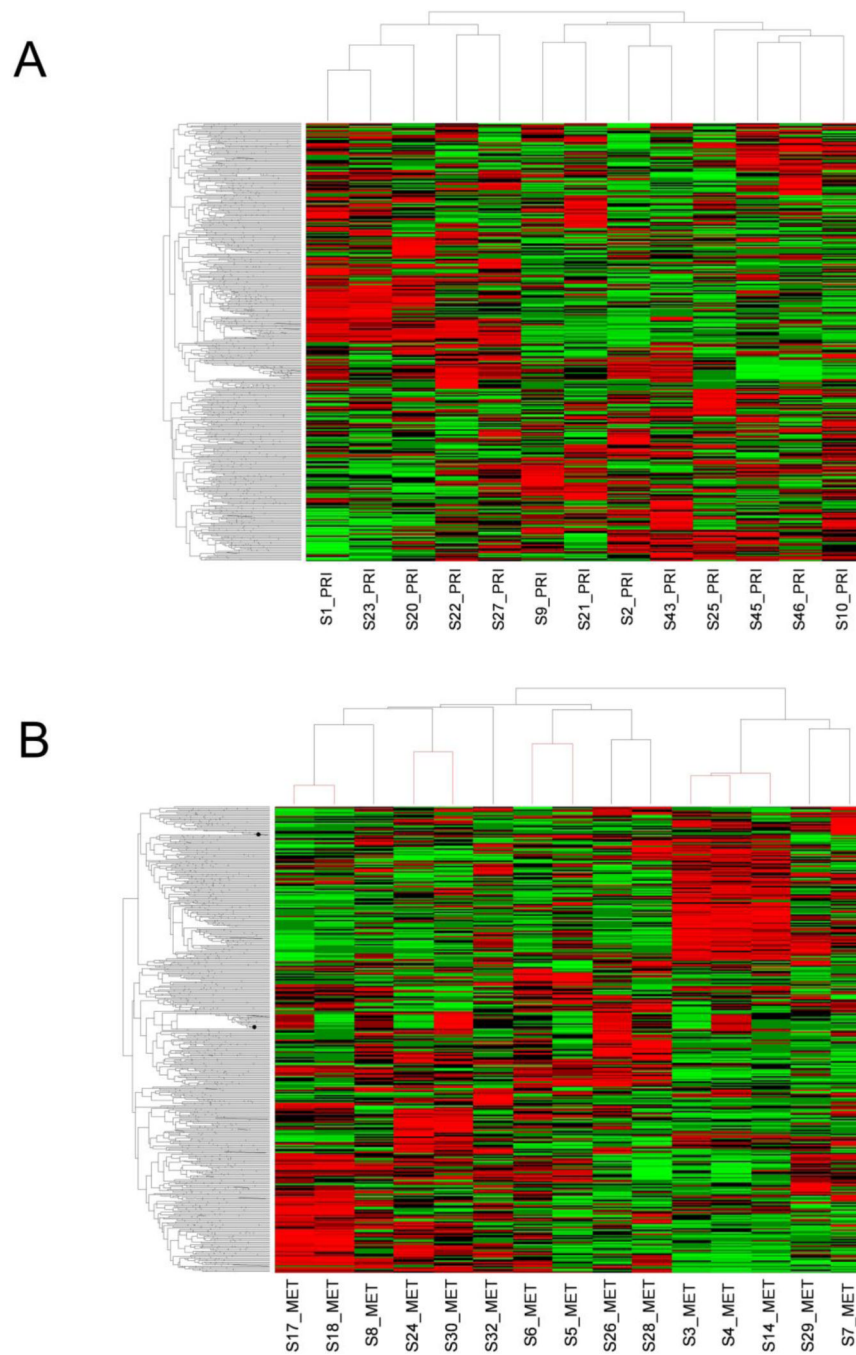


Figure 1. A: Unsupervised hierarchical clustering of primary leiomyosarcomas (n=13). B: Unsupervised hierarchical clustering of metastatic leiomyosarcomas (n=15). The 4 red branches correspond to metastatic samples from 4 different patients.

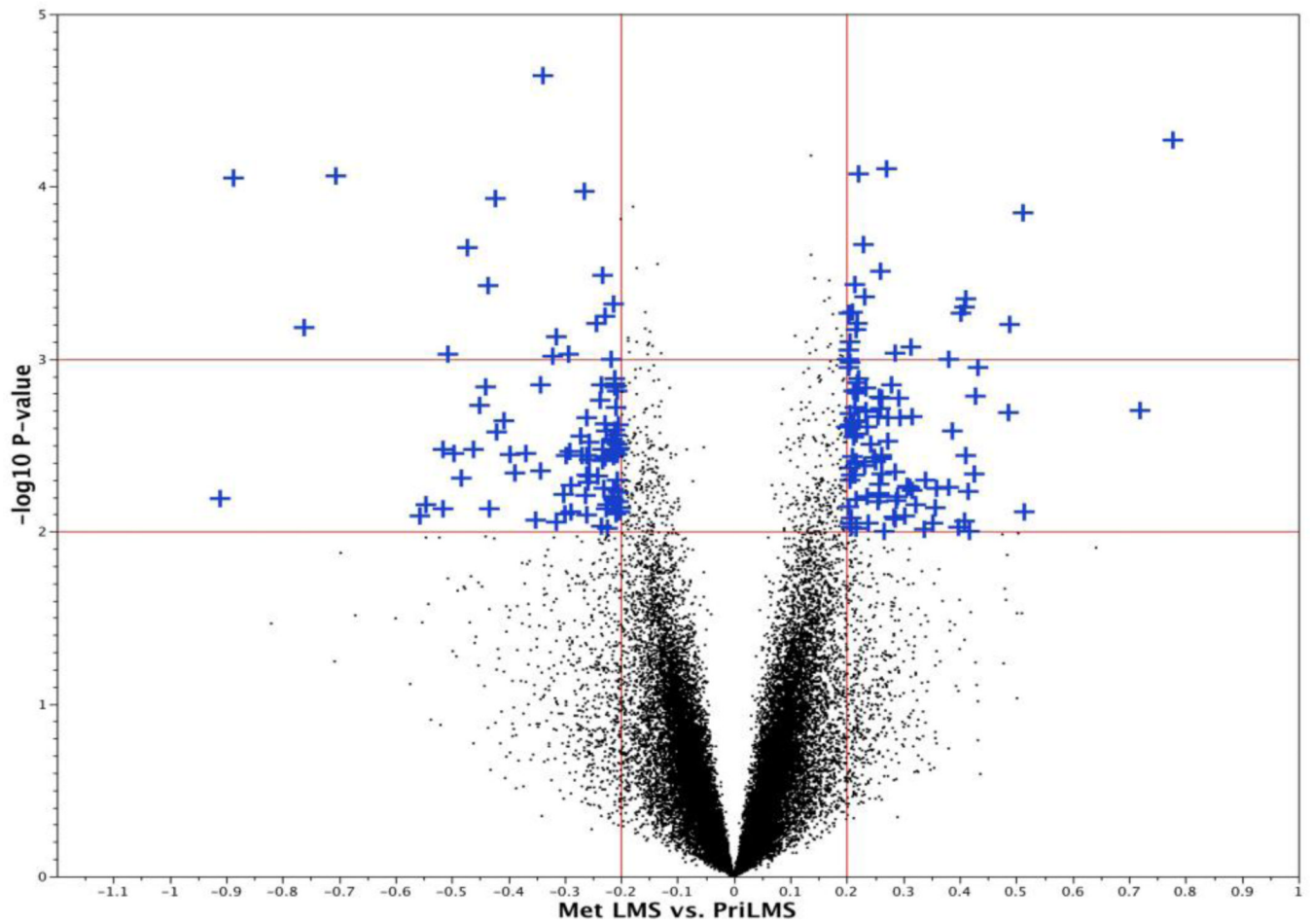


Figure 2. Volcano Plot depicting the results of the t-test for differential expression between primary and metastatic LMS. 203 gene probes were significantly differentially expressed in these two groups by >1.58-fold change ($p < 0.01$).

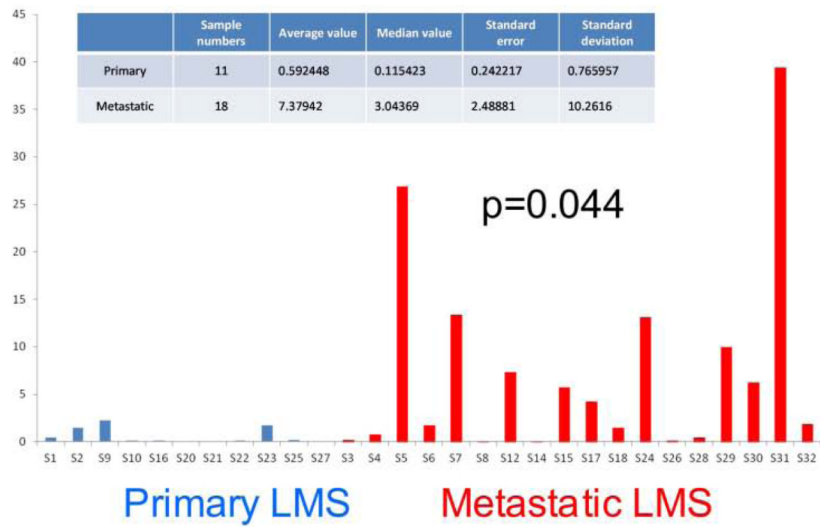
#	Maps	0	2	4	6	8	-log(pValue)	pValue †	Ratio
1	Protein folding and maturation_Angiotensin system maturation \ Human version							1.895e-10	8/43
2	Protein folding and maturation_Angiotensin system maturation \ Rodent version							4.813e-10	8/48
3	Signal transduction_PKA signaling							2.917e-4	4/51
4	Development_Slit-Robo signaling							8.722e-4	3/30
5	Transport_Aldosterone-mediated regulation of ENaC sodium transport							8.722e-4	3/30

Figure 3.

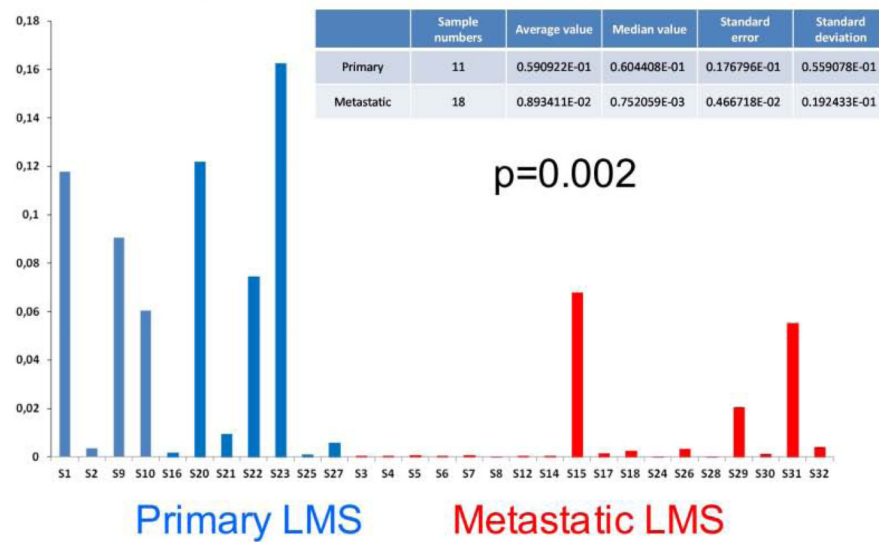
The five most significantly enriched GeneGo biological processes in the 203 gene probes. The bars represent significance as $-\log(p\text{-value})$. All ontology enrichments were filtered to allow no more than 10% false discovery rate.

FOLR3

Normalized fold expression

**NLGN4X**

Normalized fold expression



OSTN

Normalized fold expression

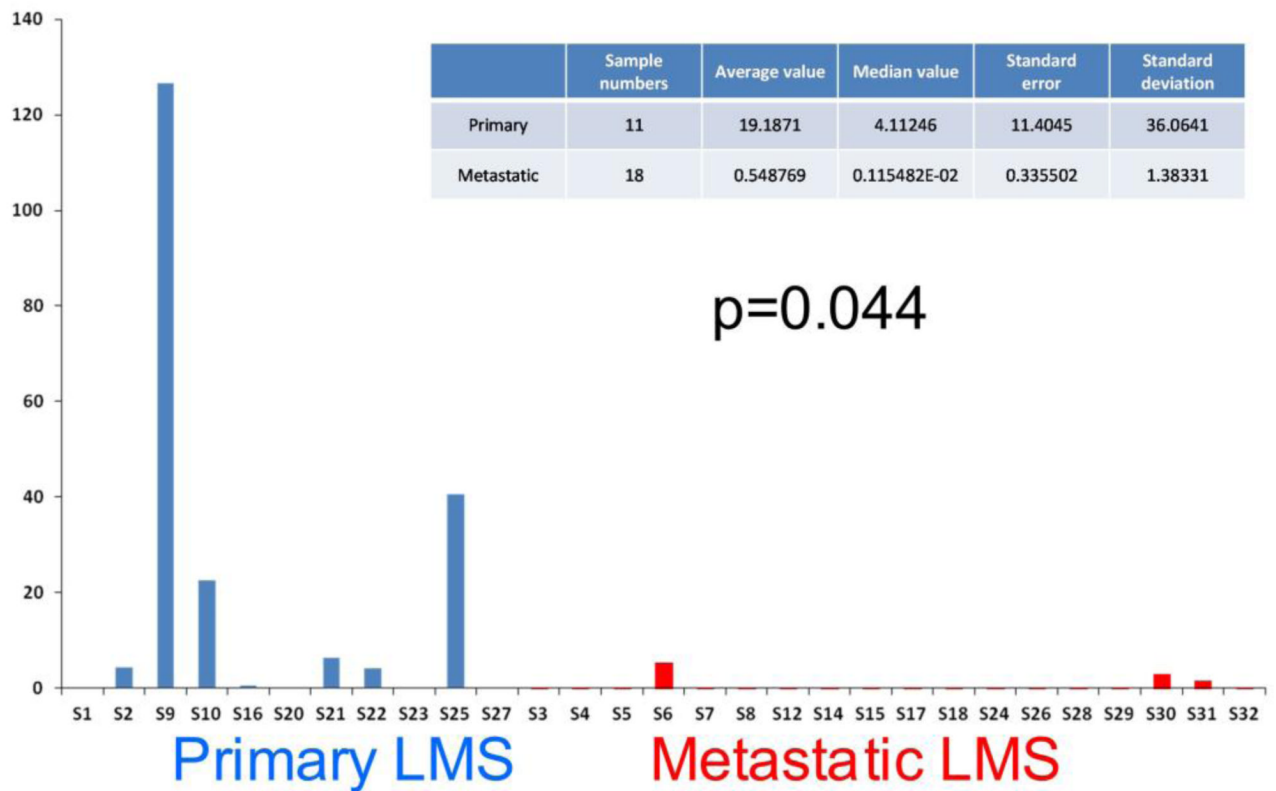


Figure 4. qRT-PCR results for the 3 genes with greatest degree of differential expression among 14 analyzed genes. *OSTN* and *NLGN4X* are overexpressed in primary LMS, whereas *FOLR3* is overexpressed in metastatic LMS.

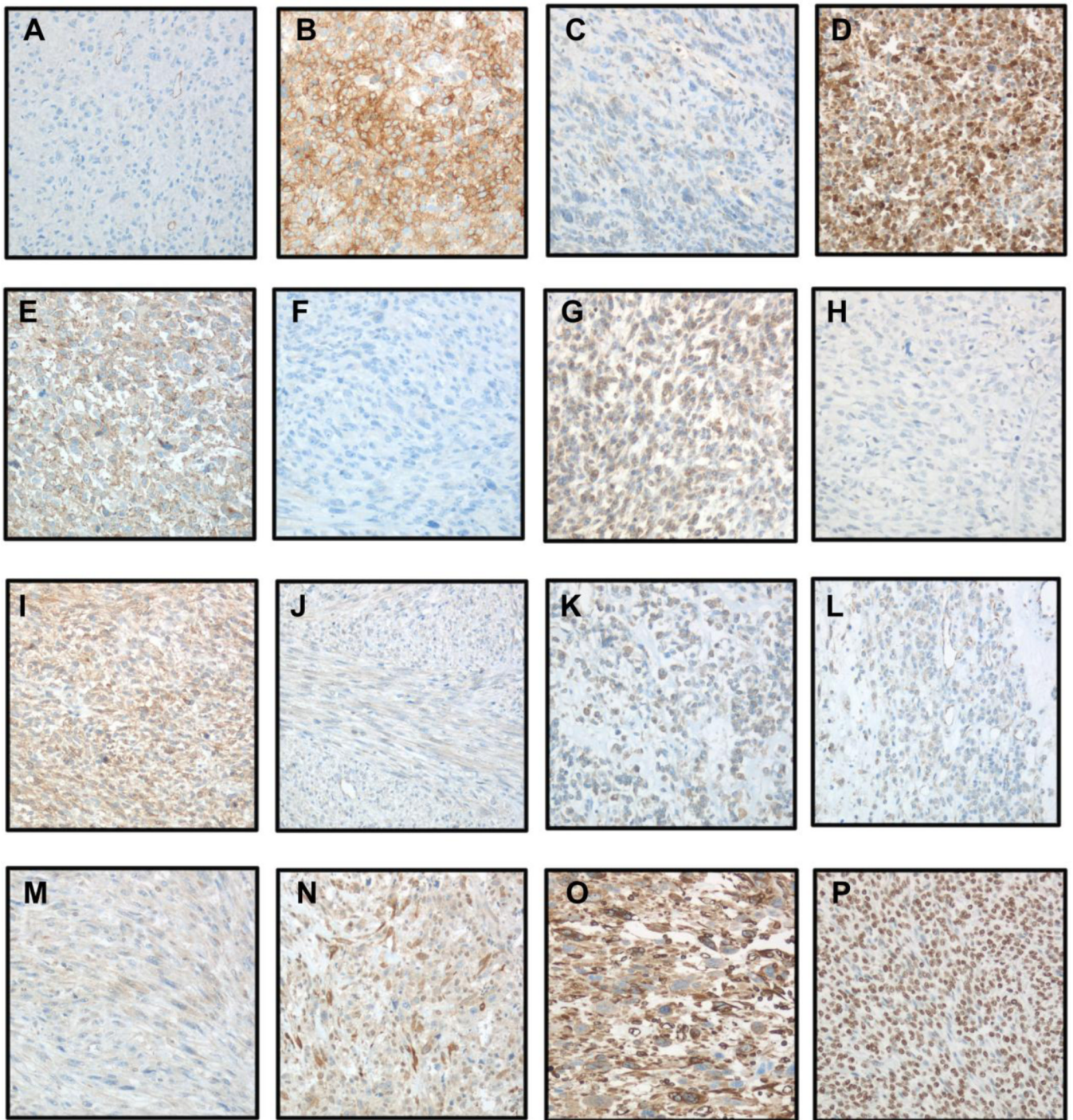


Figure 5.
 IHC analysis of NLGN4X, NLGN1, SLITRK4, XRN2, HRASLS, TDO2, CRYM, Cx43/
 GJA1 and SHMT1 protein expression in LMS (A–N: primary LMS in left column,
 metastatic LMS in right column; $\times 200$ magnification for all).
 A–B: Higher Cx43/GJA1 expression in metastatic LMS
 C–D: Higher CRYM expression in metastatic LMS
 D–F: Higher HRASLS expression in primary LMS

G–H: Higher NLGN1 expression in primary LMS
I–J: Higher NLGN4X expression in primary LMS
K–L: Higher SLITRK4 expression in primary LMS
M–N: Higher TDO2 expression in metastatic LMS
O: SHMT1 in primary LMS
P: XRN2 in primary LMS

Table 1

Primers used in quantitative PCR analysis

Gene Symbol	Forward Primer	Reverse Primer
<i>OSTN</i>	AATCCGATCCATGGGGATA	CCCCTTGACAGACTCTCAGC
<i>NLGN4X</i>	GTGCCCTCCATGTAAGATCC	CTACGTGCCACGGAAGA
<i>NLGN1</i>	ATAGTCCCTTTGCAGCCT	TGTCTTGGCAAGTTATGGCA
<i>EGR2</i>	AGCAAAGCTGCTGGGATATG	TTGACCAGATGAACGGAGTG
<i>SLITRK4</i>	GGGCTGACAAAATCAGAAACA	ACCAGACGCTGGGCTCTA
<i>MASPI</i>	GCACCTGGTCCTCAGTTTCT	CACTGTCCCAGATGGGTTC
<i>XRN2</i>	TGGATTAGGTTTACTGGCATCA	GCAAGTACCCGTCATCATAG
<i>HRASLS</i>	GGTTGCCAGGGTAGTTCAAA	AAGAGAGACCCCAGGACACA
<i>TNNT1</i>	CAGCTCCAGCAGGTCTTTCT	AGAGCCAGAAGAGGAACGC
<i>FOLR3</i>	AGCAGGCATTCTTCTCCAG	CTCAATGTCTGCATGAACGC
<i>TDO2</i>	TCAGCCACCTGTTCTCTTT	TCTGGGGAAAGCTGAAAAA
<i>SHMT1</i>	GTTGACTGGCATCGTCATTG	GTCAGCGGGTCTGGGACT
<i>CRYM</i>	TAATGGCTGTAGGCCTGGAC	GAACAGCTGCAGTTTCTGCC
<i>THBS1</i>	CACAGCTCGTAGAACAGGAGG	CAATGCCACAGTTCTGATG
<i>APP</i> ^a	CCACAGAACATGGCAATCTG	TTTGGCACTGCTCCTGCT

^aReference gene

Table 2

Antibodies used for validation by immunohistochemistry

Antibody ^a	Type ^b	Cat. #	Manufacturer	Dilution	Antigen retrieval
ASS1	Rabbit PC	ab96433	Abcam (Cambridge, UK)	1:100	Citrate
NLGN4X	Rabbit PC	HPA001651	Atlas Antibodies (Stockholm, Sweden)	1:100	Citrate
NLGN1	Rabbit PC	HPA006680	Atlas Antibodies	1:100	Citrate
SLITRK4	Rabbit PC	HPA001039	Atlas Antibodies	1:300	Citrate
XRN2	Rabbit PC	ab74408	Abcam	1:1000	Citrate
HRASLS	Goat PC	ab134828	Abcam	1:150	Citrate
TDO2	Mouse MC	ab128532	Abcam	1:150	Citrate
SHMT1	Mouse MC	ab115665	Abcam	1:100	Citrate
CRYM	Mouse MC	ab54669	Abcam	1:50	Citrate
Cx43/GJA1	Rabbit PC	ab11370	Abcam	1:1000	Citrate

^a ASS1=Argininosuccinate synthetase 1; NLGN4X=Neurologin 4X; NLGN1=Neurologin 1 protein-3; SLITRK4=SLIT and NTRK-like family, member 4; HRASLS=HRAS like suppressor; TDO2=Tryptophan 2,3-dioxygenase; SHMT1=Serine hydroxymethyltransferase-1; CRYM=mu-Crystallin; Cx43=Connexin 43

^b PC=polyclonal; MC=monoclonal