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Gene expression signatures differentiate uterine endometrial stromal sarcoma from leiomyosarcoma

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Abstract

Objective—Endometrial stromal sarcoma (ESS) and leiomyosarcoma (LMS) are the two most common uterine sarcomas, but both are rare tumors. The aim of the present study was to compare the global gene expression patterns of ESS and LMS.

Methods—Gene expression profiles of 7 ESS and 13 LMS were analyzed using the HumanRef-8 BeadChip from Illumina. Differentially expressed candidate genes were validated using quantitative real-time PCR and immunohistochemistry.

Results—Unsupervised hierarchical clustering using all 54,675 genes in the array separated ESS from LMS samples. We identified 549 unique probes that were significantly differentially expressed in the two malignancies by greater than 2-fold with 1% FDR cutoff using one-way ANOVA with Benjamini–Hochberg correction, of which 336 and 213 were overexpressed in ESS and LMS, respectively. Genes overexpressed in ESS included *SLC7A10*, *EFNB3*, *CCND2*, *ECEL1*, *ITM2A*, *NPW*, *PLAG1* and *GCGR*. Genes overexpressed in LMS included *CDKN2A*, *FABP3*, *TAGLN*, *JPH2*, *GEM*, *NAV2* and *RAB23*. The top 100 genes overexpressed in LMS included those coding for myosin light chain and caldesmon, but not the genes coding for desmin

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Conflict of interest statement

We declare that we have no conflict of interest.

or actin. *CD10* was not overexpressed in ESS. Results for selected genes were validated by quantitative real-time PCR and immunohistochemistry.

Conclusions—We present the first study in which gene expression profiling was shown to distinguish between ESS and LMS. The molecular signatures unique to each of these malignancies may aid in expanding the diagnostic battery for their differentiation, and may provide a molecular basis for prognostic studies and therapeutic target discovery.

Keywords

Diagnosis; Gene expression array; Uterine sarcoma

Introduction

Uterine sarcomas are rare tumors, comprising 7% of all soft tissue sarcomas and 3% of uterine malignancies [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) [2,3]. In a recent series of all sarcomas in Norway in the period 1970–2000 from our institution, uterine sarcomas comprised 419 of 12,431 (3.4%) uterine malignancies [4]. ESS have traditionally been divided into low-grade and high-grade tumors. However, as high-grade tumors often lack evidence of endometrial stromal cell differentiation and are clinically more aggressive, it has been proposed that they should be classified as undifferentiated endometrial or uterine sarcoma (UUS) [3,5,6].

While the diagnosis of ESS vs. LMS based on morphology is straightforward in many cases, some tumors are difficult to classify, e.g. ESS with smooth muscle differentiation [7]. While immunohistochemistry (IHC) may aid in this differential diagnosis, as in the use of desmin, actin, smooth muscle actin and h-caldesmon to identify muscle differentiation, none of the currently used markers is entirely specific [8,9]. *CD10*, postulated to be ESS-specific, was expressed in most adenosarcomas and LMS and in 33% of UUS by IHC in a recent series. Moreover, 26% of ESS were negative for *CD10*, leaving the marker with little diagnostic value [9].

Genetic studies identified multiple chromosomal aberrations in ESS (reviewed in [10]), the most common characteristic of which is t(7;17)(p15;q21) leading to fusion of the *JAZF1/JJAZ1* zinc finger genes [11]. More recently described molecular changes in ESS include fusion of the PHD finger protein-1 gene *PHF1* at 6p21 with *JAZF1* and the enhancer of polycomb gene *EPC1* [12], as well as with MYST/Esa1-associated factor 6 (*MEAF6*) [13], and fusion of the 14-3-3ε gene *YWHAE* with *FAM22*, involving t(10;17)(q22;p13) [14,15], the latter characterizing an ESS sub-type with more aggressive clinical behavior. Nevertheless, none of these molecular changes is currently widely-used in the diagnostic setting.

To elucidate molecular differences between uterine ESS and LMS, we performed gene expression analysis of 20 tumors, which, to our best knowledge, constitutes the first comparative study of gene expression profiles in these two malignancies. We identified a set

of genes that are overexpressed in each tumor, which may improve our understanding of the biology of these rare entities and provide new diagnostic markers.

Material and methods

Patients and material

Specimens consisted of 20 uterine tumors, including 7 ESS and 13 LMS, submitted for routine diagnostic purposes to the Department of Pathology at the Norwegian Radium Hospital during the period 1993–2009. Tumors were snap-frozen and kept at -70°C . Frozen sections 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 IHC [4,9]. IHC results are shown in Table 1.

All 20 samples that were analyzed using gene expression arrays were also analyzed by quantitative real-time PCR (qRT-PCR), with the addition of 1 ESS and 3 LMS samples. The material analyzed with IHC consisted of 20 ESS and 20 LMS from another case series.

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 tumor types. 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 Micro-array Core. Cluster, developed by the Eisen group (<http://rana.lbl.gov/eisen/>) was used to perform hierarchical clustering analysis using the selected differentially expressed genes.

qRT-PCR

From all differentially expressed genes, 16 were selected for validation using qRT-PCR. These consisted of 7 genes that were over-expressed in ESS (*EFNB3*, *CCND2*, *ECEL1*, *ITM2A*, *NPW*, *PLAG1* and *GCGR*) and 9 genes that were overexpressed in LMS (*FABP3*, *TAGLN*, *JPH2*, *GEM*, *PDLIM5*, *FBXO32*, *MTA21*, *NAV2* and *RAB23*). Genes were chosen based on their potential biological and clinical relevance, as judged by the two senior authors of this manuscript (BD and TLW). 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 2).

Approximately 16–100 ng 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 5 of the 16 genes validated by qRT-PCR 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. Protein expression of cyclin D2 (CCND2), fatty acid binding protein-3 (FABP3), transgelin (TAGLN), neuron navigator 2 (NAV2) and integral membrane protein 2A (ITM2A) was analyzed. Antibody details and staining conditions are provided in Table 3. IHC was performed using the EnVision FLEX + system (Dako, Glostrup, Denmark). Appropriate positive and negative controls were used.

Staining extent and intensity were scored by an experienced gynecopathologist (BD). Tumors were scored as negative (0% stained cells), focally positive (1–20% stained cells) or diffusely positive (staining in >20% cells), corresponding to a score of 0–2, and as negative, weakly stained or strongly stained, similarly corresponding to a score of 0–2. Multiplying the 2 values generated a combined score of 0–4, which was used in the statistical analysis. Differences in protein expression between ESS and LMS were analyzed by Mann Whitney *U* test using the SPSS program (version 18.0, Chicago, IL).

Results

Unsupervised hierarchical clustering was performed to determine the similarity in gene expression patterns among all the samples (Fig. 1A). The results demonstrated that there are two major clusters, with all 7 ESS and 3 of the 13 LMS under one major cluster and the remaining 10 LMS under another cluster. The 3 LMS who clustered under a different hierarchical arm did not exhibit pathological or clinical features different than the other LMS samples. Supervised analysis was performed in order to identify genes with the highest power to separate ESS and LMS. We identified 549 unique probes that were significantly differentially expressed in the two malignancies by greater than 2-fold with 1% FDR cutoff using one-way ANOVA with Benjamini–Hochberg correction, of which 336 and 213 were overexpressed in ESS and LMS, respectively (Fig. 1B). Genes overexpressed in ESS included *SLC7A10*, *EFNB3*, *CCND2*, *ECEL1*, *ITM2A*, *NPW*, *PLAG1* and *GCGR*. Genes overexpressed in LMS included *CDKN2A*, *FABP3*, *TAGLN*, *JPH2*, *GEM*, *NAV2* and *RAB23*. The top 100 genes overexpressed in LMS included several muscle-related genes, such as the *MYLK* and *MYL9* genes, coding for myosin light chain, *CALD1*, coding for caldesmon, and *ACTN1*, coding for actinin A, but not the genes coding for desmin or actin. The *CD10* gene was not one of the genes differentiating these 2 entities. The full gene list is provided in Table S1.

Ingenuity pathway analysis identified genes participating in canonical pathways related to the actin cytoskeleton, RhoA signaling and germ cell–Sertoli cell junction signaling in LMS. Pathways highlighted in ESS were those related to taurine biosynthesis, the G1/S checkpoint of the cell cycle and noradrenaline and adrenaline degradation (Table 4).

Validation experiments

Expression levels of the 16 selected transcripts were analyzed in 8 ESS and 16 LMS using qRT-PCR. As shown in Fig. 2, genes found to be overexpressed in ESS using gene expression arrays were significantly overexpressed in this tumor compared to LMS samples by qRT-PCR, all except for *EFNB3* and *GCGR* with $p < 0.01$. Similarly, qRT-PCR validated the array data for all LMS-specific genes ($p < 0.01$ for all).

IHC was applied to analyze 5 proteins, including 3 protein products of genes overexpressed in LMS (FABP3, TAGLN, NAV2), and 2 of genes overexpressed in ESS (CCND2, ITM2A). IHC confirmed the array findings for all 5 genes (Fig. 3), with statistically significant differences for all proteins ($p < 0.001$ for CCND2 and TAGLN, $p = 0.002$ for NAV2, $p = 0.004$ for ITM2A, $p = 0.003$ for FABP3). Staining for NAV and TAGLN was cytoplasmic, whereas ITM2A and CCND2 staining had nuclear localization, although concomitant cytoplasmic staining for the latter was seen in some cases. Staining for FABP3 was both nuclear and cytoplasmic.

Discussion

ESS and LMS are both rare diseases, and knowledge regarding their molecular biology, especially in the case of LMS, is limited. ESS is a relatively indolent disease which responds to hormonal treatment, as the majority of tumors express estrogen and progesterone receptors. However, adnexal and lymph node metastases, as well as metastases outside the pelvis, are found in some patients at diagnosis, and recurrences, especially within the abdominal cavity or in the lungs, are frequent [4,17–20]. In the present cohort, 1 of the 7 tumors recurred to date, a figure which may increase in view of the short follow-up period for some of the patients. LMS is a clinically aggressive disease with high mortality [4,20]. As the role of chemotherapy is limited in both diseases, targeted therapy is increasingly regarded as an important modality in these malignancies [20].

In the present study, ESS and LMS specimens clustered separately and differentially expressed a large number of genes, many of which were present in 2 or more copies in the list of significantly over- and underexpressed markers (see Table S1). Several muscle-related genes were overexpressed in LMS, including *CALDI*, coding for caldesmon, which is used in the diagnostic setting. However, the genes coding for CD10, desmin or actin were not strong differentiators between these tumors.

Among genes that were overexpressed in ESS were several genes which have not been studied in this tumor, although they have been shown to have a role in the biology of other tumors. *EFNB3* codes for Ephrin-B3, part of the Eph (erythropoietin-producing hepatoma)/Ephrin family of receptor tyrosine kinases, which consists of A-type and B-type receptors and ligands that are both membrane-bound. Family members may have both tumor-promoting and suppressing roles and are involved in several aspects of tumor biology, including adhesion, migration, invasion, metastasis, survival and proliferation, as well as in angiogenesis [21,22]. Ephrin-B3 levels were reported to be increased in ovarian carcinoma, neuroblastoma and glioblastoma compared to corresponding normal tissue [22]. Studies of

sarcoma specimens are to date limited to a small series of osteosarcomas, in which *EFNB3* mRNA was not found [23].

Cyclins are major positive regulators of the cell cycle. D-type cyclins consist of the closely-related cyclin D1, D2 and D3, which have a great degree of homology, particularly in their cyclin box, where they bind their activators cyclin-dependent kinase (CDK) 4 and 6, as well as their inhibitors p21, p27 and p57. Binding of CDK4 and CDK6 to cyclin D results in phosphorylation of several targets, including the retinoblastoma protein, and cell cycle progression, as well as other biological effects, such as differentiation and migration. Cyclins additionally have non-catalytic roles, regulating transcription of genes related to proliferation and differentiation. Cyclin D1 is an established oncogene which has been shown to be deregulated in multiple cancers, and drugs targeting cyclin D are currently evaluated in clinical trials [24]. Cyclin D1 was recently shown to be expressed in only 1/17 ESS [25], suggesting, together with our data, that cyclin D2 is the more frequently expressed member of this family in ESS. The identification of genes related to the G1/S checkpoint of the cell cycle in Ingenuity pathway analysis suggests the possibility of therapeutic intervention at this point of the cell cycle in ESS.

ECEL1, coding for endothelin-converting enzyme-like 1 protein, is part of the M13 family of endopeptidases, which regulates neuropeptide and peptide activities and are localized to the plasma membrane and the endoplasmic reticulum [26]. High *ECEL1* expression was associated with clinical and biological parameters of a less aggressive disease and with a favorable outcome in neuroblastoma [27], and the *ECEL1* gene was recently reported to be frequently hypermethylated in bladder cancer [28]. The methylation status of *ECEL1* in uterine sarcomas remains to be investigated.

ITM2A, coding for integral membrane protein 2A, is regulated by PAX3, a transcription factor involved in neural, muscle and facial development during embryogenesis that is mutated in alveolar rhabdomyosarcoma, and its fusion product PAX3-FKHR in the latter tumor [29]. It was additionally shown to inhibit chondrogenic differentiation in mesenchymal stem cells [30]. Both reports, as well as the frequent nuclear localization of this protein in our ESS series, suggest a biological role for this molecule in sarcomas.

PLAG1 (pleomorphic adenoma gene 1) is a gene coding for a zinc finger protein which resides on chromosome 8q12 and is involved in t(3;8)(p21;q12) translocation, with reciprocal swapping of promoters with the β -catenin gene CTNNB1 [31]. Involvement of *PLAG1* in soft tissue tumors was reported in lipoblastoma, where the hyaluronic acid synthase 2 (*HAS2*) and collagen Ia2 (*COL1A2*) gene promoter regions are fused to the *PLAG1* coding sequence [32].

In agreement with our observation that *PLAG1* is overexpressed in ESS compared to LMS, a recent comprehensive analysis of 243 mesenchymal tumors showed no expression of the *PLAG1* protein in 8 LMS, of which 4 were of uterine origin [33]. ESS was not analyzed in the latter study.

The molecules playing a role in the biology of uterine LMS are largely unknown to date. We identified several genes that were overexpressed in LMS compared to ESS, of which some,

including the aforementioned *MYLK*, *MYL9*, *CALD1* and *ACTN1*, are related to muscle differentiation and the actin cytoskeleton, the latter identified as a central pathway in Ingenuity pathway analysis. An additional gene related to this group is *JPH2*, which codes for junctophilin-2, a member of the JPH family. JPH2 regulates calcium release in the heart by keeping the plasma membrane and sarcoplasmic reticulum at a fixed distance during the excitation–contraction process and mutations in the JPH2 gene result in hypertrophic cardiomyopathy [34]. The potential role of JPH2 in cancer is at present unknown.

FBXO32 (*Homo sapiens* F-box protein 32), a.k.a. muscle atrophy F-box (MAFbx) and Atrogin-1, is a skeletal muscle ubiquitin ligase which is highly expressed during muscle atrophy, including in cancer cachexia [35,36]. It is upregulated in gastrointestinal stromal tumor (GIST) cells following treatment with Imatinib mesylate (Gleevec®) [37], but was not shown to be amplified in analysis of genes residing at chromosome 5p, an area shown to be frequently amplified in analysis of 34 soft tissue sarcomas [38]. *FBXO32* was more recently reported to be a target gene of transforming growth factor- β (TGF β)/SMAD4 that undergoes promoter hypermethylation in ovarian carcinoma, and *FBXO32* methylation was associated with poor progression-free survival [39]. In contrast, a higher *FBXO32* level by gene expression arrays was reported to be predictive of lymph node metastasis in oral cancer as part of a 4-gene panel, and was related to poor overall and cancer-specific survival [40].

Another muscle-related gene overexpressed in LMS was *TAGLN*, encoding for transgelin, a 22-kDa actin-binding protein of the calponin family that may be involved in calcium-independent smooth muscle contraction [41]. Transgelin was reported to be a tumor suppressor that reduces matrix metalloproteinase-9 (MMP9) levels and is downregulated in breast and colon carcinoma [42,43]. However, a more recent study showed association between transgelin expression and lymph node metastasis in colon carcinoma, as well as promotion of invasion, resistance to anoikis and survival *in vitro* in colon carcinoma cell lines [44]. In agreement with this, transgelin expression was higher in tumorigenic CD133+ Huh-7 hepatocellular compared to non-tumorigenic CD133– cells, and was related to invasion and expression of the pro-metastatic chemokine receptor CXCR4 [45]. Stromal transgelin expression in non-small cell lung carcinoma was unrelated to clinicopathologic parameters or survival [46].

PDLIM5, also termed enigma homolog 1 (ENH1), is member of the PDZ-LIM family whose members provide a scaffold for protein–protein interactions involving transcription factors, cytoskeletal proteins and protein kinases. It is highly expressed in skeletal muscle and the heart, and was recently reported to regulate protein kinase C activity [47]. As with transgelin, stromal PDLIM5 expression was unrelated to clinicopathologic parameters or survival in non-small cell lung carcinoma [46]. It was additionally shown to be deleted in oral squamous cell carcinoma by comparative genomic hybridization [48].

Four additionally validated genes with less specificity for muscle tissue in the present study were *FABP3*, *NAV2*, *RAB23* and *MTA2*. The fatty acid binding protein family consists of 9 members, which have a high affinity for long-chain fatty acids and are differentially expressed in normal tissues [49]. The *FABP3* promoter is hypermethylated in lung cancer [50], but no members of this family have been studied in uterine sarcomas.

Voltage-gated Na⁺-permeable channels, termed NAV1 and NAV2 are highly conserved proteins which mediate electrical excitability in animals [51]. NAV2 is involved in cerebellar and cranial nerve development, as well as in blood pressure regulation [52,53]. Recently, the NAV2 gene was found to be frequently fused to the WNT pathway gene *TCF7L1* in colorectal carcinoma in a study by the Cancer Genome Atlas Network [54].

Rab GTPases, coordinators of vesicle traffic, are small GTP-binding proteins that form the largest family within the Ras superfamily of small GTPases, with over 70 human genes characterized [55]. Rab23 was studied in different carcinomas [56,57], but its role in uterine sarcoma is yet to be defined.

Metallothionein 2A is member of the metallothionein family, small cysteine-rich proteins involved in proliferation, apoptosis and differentiation, which was reported to be transcriptionally repressed in hepatocellular carcinoma, but shown to be related to chemoresistance in breast carcinoma [58,59].

The expression and biological roles of the 16 genes chosen for validation in the normal endometrium or myometrium is largely unknown to date, with some data available for *TAGLN* and *CCND2*. Transgelin protein expression by two-dimensional (2D) gel electrophoresis was reported to be downregulated following exposure of myometrial tissue to oxytocin [60]. Higher *TAGLN* mRNA expression by qRT-PCR was found in endometriosis compared to patient-matched eutropic endometrium [61] and higher protein expression was observed in these lesions compared to normal peritoneum [62]. Cyclin D2 mRNA and protein expression was higher in proliferative compared to secretory endometrium [63], and cyclin D2 protein levels were higher in leiomyomas compared to normal myometrium [64]. Understanding of the role of the genes differentiating LMS from ESS in tumorigenesis requires further research.

In conclusion, the first gene expression array analysis performed with the aim of comparing ESS and LMS identified sets of genes that are differentially expressed in these 2 malignancies. LMS-related genes are in part muscle-related molecules that may improve current diagnostic panels, but may be of significance with respect to tumor biology as well. Other genes overexpressed in each sarcoma are known to be involved in central cellular processes, such as intracellular signaling and transcriptional regulation of proliferation, invasion and metastasis, suggesting they may be of biological, prognostic and therapeutic relevance, the latter potentially directed against pathways that are critical for tumor cells, such as the cell cycle and RhoA signaling. This may be of benefit for patients with uterine sarcoma, who critically depend on targeted therapy for prolonging survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- ▶ Gene expression profiling differentiates uterine endometrial stromal sarcoma from leiomyosarcoma.
- ▶ The tumor-specific molecular signatures identified may aid in diagnosis and clinical stratification and generate candidate markers for therapeutic intervention.

Fig. 1.

A: Unsupervised hierarchical clustering of uterine endometrial stromal sarcomas (ESS; 7 tumors) and leiomyosarcomas (LMS; 13 tumors). Two major clusters are seen, one with all 7 ESS and 3 of the 13 LMS, the other with the 10 remaining LMS. B. Volcano plot of two-fold differentially expressed genes identified using modified *t* test with FDR at 1%.

Fig. 2.

Quantitative real-time PCR was performed to validate 16 differentially-expressed genes in 24 tumors (8 ESS, left; 16 LMS, right). The expression level of each gene in the individual specimen is shown as a pseudo-color gradient based on the relative expression level of a given gene to the average value derived from the control gene, *APP*. The first 7 genes are more highly expressed in ESS, while the remaining 9 genes are overexpressed in LMS.

Fig. 3.

Immunohistochemistry analysis for the protein products of *FABP3*, *TAGLN*, *NAV2*, *CCND2* and *ITM2A*, evaluated as markers for the differentiation of ESS from LMS. LMS express *FABP3* (A), *TAGLN* (C) and *NAV2* (E), which are absent in ESS (staining of small vessels for *TAGLN* seen in D). ESS show nuclear immunostaining for *ITM2A* (H) and *CCND2* (J), whereas LMS (G and I, respectively) are negative. Cytoplasmic staining for *ITM2A* is shown in few leukocytes, whereas LMS stain weakly positive at the cytoplasm for *CCND2*.

Table 1

Diagnostic immunohistochemistry results in the studied cases.

Case	Diagnosis	CD10	Actin	SMA	Desmin
1	LMS	Negative	Negative	Positive	Positive
2	LMS	Negative	Positive	Positive	NP ^a
3	LMS	Negative	Positive	Positive	Positive
4	LMS	Focal ^b	Negative	Negative	Positive
5	LMS	Positive	Focal	Focal	Positive
6	LMS	Negative	Positive	Positive	Positive
7	LMS	Focal	NP	Positive	Negative
8	LMS	Negative	Negative	Positive	Negative
9	LMS	Positive	Positive	Positive	Positive
10	LMS	Negative	Positive	Positive	Positive
11	LMS	Focal	Positive	Positive	Positive
12	LMS	Negative	Positive	Positive	Positive
13	LMS	Negative	Focal	Positive	Focal
14	ESS	Positive	Focal	Positive	Positive
15	ESS	Positive	Focal	Positive	Positive
16	ESS	Positive	Negative	Focal	Negative
17	ESS	Positive	NP	Focal	Focal
18	ESS	Positive	Negative	Positive	Positive
19	ESS	Positive	Negative	Negative	Focal
20	ESS	Positive	Negative	Positive	Negative

^aNP = not performed.

^bFocal 20% of cells.

Table 2

Primers used in quantitative PCR analysis.

Gene symbol	Forward primer	Reverse primer
<i>FABP3</i>	GTGGTAGGCTTGGTCATGCT	GCACCTGGAAGCTAGTGGAC
<i>TAGLN</i>	CTCATGCCATAGGAAGGACC	GTCCGAACCCAGACACAAGT
<i>JPH2</i>	TGCCTCAGCTTTGGCCT	GCCAGAAAGTGGAGCACAGT
<i>GEM</i>	ACTTTCCCCATCAACCATCA	CCACTCTGGCCAACATCTTT
<i>PDLIM5</i>	CCATCAATGCTGAGAACCAC	AGGGCGGTAAGGATTTC AAC
<i>FBXO32</i>	TCAGGGATGTGAGCTGTGAC	GGGGGAAGCTTTCAACAGAC
<i>MT2A</i>	TCTTTGCATTTCAGGAACC	CAACCTGTCCCGACTCTAGC
<i>NAV2</i>	TGATTGGCCAGTCTGTGTA	CCTGAAATCGCAGGTGCT
<i>RAB23</i>	GAAACTGCTTCAAAAGATTCCCT	GCAGGTCAGGAGGAATTGTA
<i>EFNB3</i>	AGAGGAGTGAGGGCCAGG	GCCTGTCTACTGGAAC TCGG
<i>CCND2</i>	ACGTTGGTCCTGACGGTACT	TGAGCTGCTGGCTAAGATCA
<i>ECEL1</i>	GCCAGGACCTTCTCACTGTC	ACAGGAACTCCTCGCGCTAC
<i>ITM2A</i>	GTCCTGCCAAGATGAATGAAA	AACTCAGATACTGACCGGCAA
<i>NPW</i>	GGAGCTGGCCAGAGACTTC	CTATGAGCGGCTCCTGGAA
<i>PLAG1</i>	GCTCCAAACTCTAGCAAGGC	GAGGGAGGATGTTAAAGCCC
<i>GCCR</i>	GGTGTCTCCCTGAAGC	CGAAGACGAGCGGTCAC
<i>APP</i>	CCACAGAACATGGCAATCTG	TTTGGCACTGCTCCTGCT

Table 3

Antibodies used for validation by immunohistochemistry.

Antibody ^a	Source	Manufacturer	Catalog nr.	Antigen retrieval	Dilution	Control
ITM2A	Rabbit	Sigma-Aldrich (St. Louis, MO) ^b	AV33823-100UG	Citrate	1:100	ESS
CCND2	Rabbit	Proteintech (Chicago, IL)	10934-1-AP	Citrate	1:500	ESS
FABP3	Rabbit	Sigma-Aldrich	HPA002918	Citrate	1:200	LMS
TAGLN	Rabbit	Sigma-Aldrich ^b	HPA019467	Citrate	1:100	LMS
NAV2	Rabbit	Sigma-Aldrich ^b	HPA011891	Citrate	1:200	LMS

^a ITM2A=integral membrane protein 2A; CCND2=cyclin D2; FABP3=fatty acid binding protein-3; TAGLN=Transgelin; NAV2=neuron navigator 2.

^b Prestige Antibodies® affinity-isolated, powered by Atlas Antibodies (Stockholm, Sweden).

Table 4

Ingenuity pathway analysis showing canonical pathways enriched in the ESS and LMS transcriptomes.

Tumor type	Ingenuity canonical pathways	−log (p-value)	Ratio	Molecules
LMS	Actin cytoskeleton signaling	8.73E+00	8.73E+00	ITGB1, PFN1, MYL6, ACTB, IQGAP1, MYLK, MYL9, ACTR3, FLNA, MYH9, PIK3CB, ACTN4, ACTN1, MSN
	RhoA signaling	6.76E+00	6.76E+00	MYLK, MYL9, ACTR3, PFN1, MYL6, ACTB, CDC42EP3, ANLN, MSN
	Germ cell– Sertoli cell junction signaling	6.15E+00	6.15E+00	ITGB1, TUBA1A, TUBB6, ACTB, TUBG1, PIK3CB, TUBA1C, ACTN4, IQGAP1, ACTN1
ESS	Taurine biosynthesis	3.81E+00	8.73E+00	CSAD, CDO1
	Cell cycle: G1/S checkpoint regulation	2.21E+00	6.76E+00	NRG1, CCND2, E2F5, BTRC
	Noradrenaline and adrenaline degradation	2.14E+00	6.15E+00	ALDH1A1, ALDH1A3, PNMT