



Original contribution

Lysine-specific demethylase 1 is highly expressed in solitary fibrous tumors, synovial sarcomas, rhabdomyosarcomas, desmoplastic small round cell tumors, and malignant peripheral nerve sheath tumors^{☆,☆☆}

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Summary Epigenetic changes including histone methylation, histone acetylation, and DNA methylation are thought to play important roles in the onset and progression of cancer in numerous tumor types. Recent evidence shows that dysregulated epigenetic modifications are as significant as genetic mutations and can act as oncogenic driver lesions causing autonomous growth of cancer cells. Here, we investigated the role of lysine-specific demethylase 1 in mesenchymal tumors. Lysine-specific demethylase 1 is the first discovered histone lysine demethylase and can demethylate both H3K4me2/1 and H3K9me2/1. By analyzing a total of 468 tumors, we describe for the first time high lysine-specific demethylase 1 expression in several highly malignant sarcomas, including synovial sarcomas, rhabdomyosarcomas, desmoplastic small round cell tumors and malignant peripheral nerve sheath tumors. Among the intermediate tumors only solitary fibrous tumors were found to be highly lysine-specific demethylase 1 positive, whereas lysine-specific demethylase 1 expression was low or absent in benign tumors. Lysine-specific demethylase 1 inhibition with small molecule inhibitors resulted in growth inhibition of synovial sarcoma cells in vitro and an increase in global H3K4me2 methylation. Sarcomas continue to remain a clinical challenge and therefore the identification of both diagnostic markers and novel drug targets for the development of new therapeutic options are needed. Our results suggest that dysregulation of lysine-

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specific demethylase 1 is associated with highly malignant sarcomas proposing them as molecular tumor markers as well as targets for the treatment of these tumor types.

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1. Introduction

Mutations in genes controlling the molecular pathways that regulate cell proliferation, differentiation, and cell death all contribute to cancer formation. For a long time, cancer research has focused on the identification of genetic alterations that promote oncogenesis. In many cases, such mutations or chromosomal alterations affect patterns of gene expression in cancer cells, which in turn affect cell identity, cell growth or apoptosis [1]. Recently, epigenetic factors that contribute to the regulation of these processes have been shown to be also implicated in oncogenesis. Enzymes that covalently modify DNA and histones affect these pathways by controlling the dynamic remodeling of the chromatin structure.

The flexible histone tails are modified by a plethora of posttranslational modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. In contrast to other histone modifications, the importance of histone methylations is highlighted due to their enormously specific dynamics with respect to gene regulation. Histone lysine residues on histone H3 and H4 can become mono-, di-, or trimethylated. These modifications are regulated by two classes of enzymes with opposing activities: histone methyltransferases and histone lysine demethylases.

Among the demethylases, lysine specific demethylase 1 (LSD1) was the first identified histone demethylase, which removes one or two methyl group from H3K4 and H3K9 in a flavin adenine dinucleotide (FAD)-dependent manner [2]. Subsequently, another family of histone lysine demethylases structurally different from LSD1 was described, both sharing the conserved jumonji C domain [3].

LSD1 was originally identified as a component of transcriptional repressor complexes comprising transcriptional corepressor protein (CoREST) and histone deacetylase 1/2 (HDAC 1/2) [2,4,5]. The LSD1-CoREST-HDAC core is functionally conserved and associated with various tissue specific factors, involving LSD1 in diverse cellular processes including cellular growth, proliferation, apoptosis and fate specification.

Furthermore, LSD1 has been implicated as an essential player in nuclear receptor signaling. Interestingly, the interaction of LSD1 with nuclear receptors appears to change its substrate specificity from H3K4me_{2/1} to H3K9me_{2/1}, and LSD1 functions as a transcriptional co-activator of androgen receptors (AR) [6]. Recently, Metzger et al showed that phosphorylation of histone H3 at threonine 6 is the key event that prevents LSD1 from demethylating H3K4 during AR-dependent gene activation [7]. In response to AR signaling, protein kinase C beta 1 (PKC β_1) is recruited to AR target gene promoters and leads to histone H3 at threonine 6

phosphorylation which blocks the LSD1-mediated H3K4 demethylation. LSD1 was also shown to interact with the estrogen receptor α in a ligand dependent fashion and was important for activation of a subset of estrogen receptor α -dependent target genes [8]. Gene targeting studies have also supported an activation function for LSD1 in vivo [9].

Taken into consideration that LSD1 controls broad expression programs, is involved in malignant progression of cancer [6,10-12] and represents a potential therapeutic target, we here analyzed the role of LSD1 in mesenchymal tumors.

These tumors, especially soft tissue sarcomas and some types of benign and intermediate tumors, constitute a major health care issue. Owing to the low frequency of single tumor entities and the heterogeneity of tumor subgroups, no specific targeted therapies are available for most soft tissue tumors. Therefore, we aimed at screening a larger cohort of well-characterized benign, intermediate, and malignant soft tissue tumors for the expression of LSD1.

An awareness of the dynamic nature of histone modification has stimulated interest in the concept that drugs targeting histone methylation/demethylation might provide treatment options for cancer [13,14]. Because LSD1 belongs to the family of FAD-dependent amine oxidases, certain inhibitors of monoamine oxidases (MAOs), including the clinically used antidepressant trans-2-phenylcyclopropylamine (tranylcypromine) and reversible MAO inhibitors chloxylin, are also capable of inhibiting LSD1 [5]. Therefore, we investigated if LSD1 might serve as a potential therapeutic target for mesenchymal tumors.

2. Materials and methods

2.1. Tissue microarrays

Tissue microarrays were prepared as described previously [10] from formalin-fixed, paraffin-embedded tissue specimens of 468 mesenchymal tumors selected from the archival files of the Institute of Pathology, University of Bonn Medical School. Three different tissue cores within a single tumor were arrayed from formalin-fixed, paraffin-embedded tissue blocks using a manual device (Beecher Instruments, Sun Prairie, WI). Two-micrometer paraffin sections were cut from every tissue microarray and used for subsequent immunohistochemical analyses.

We investigated different mesenchymal tumors in this study, including entities from several histotypes as well as lesions from the soft tissues and the viscera. Therefore, not only sarcomas were investigated but also benign mesenchymal tumors and neoplasias with an intermediate

Table 1 TMA composition with the numbers of cases included (total n = 468)

Adipocytic tumors (72)	
Benign (19)	2 Hibernomas 2 Angiolipomas 3 Spindle cell lipomas 12 Lipomas, NOS
Malignant (53)	20 Well differentiated liposarcomas/ atypical lipomatous tumors 22 Dedifferentiated liposarcomas 5 Myxoid liposarcomas 6 Pleomorphic liposarcomas
Fibroblastic/myofibroblastic tumors (110)	
Benign (48)	10 Superficial fibromatoses 2 Nodular fasciites 1 Myositis ossificans 4 Calcifying fibrous tumors 1 Angiomyofibroblastoma 11 Inflammatory fibrous polyps 4 Fibromas of the tendon sheath 1 Elastofibroma 14 Other types
Intermediate (42)	25 Desmoid-type fibromatoses 17 Solitary fibrous tumors
Malignant (20)	3 Dermatofibrosarcoma protuberans 6 Fibrosarcomas, adult type 7 Myxofibrosarcomas 2 Low-grade fibromyxoid sarcomas 2 Sclerosing epithelioid fibrosarcomas
Fibrohistiocytic tumors (36)	
Benign (13)	7 Giant cell tumors 1 Pigmented villonodular synovitis 5 Xanthogranulomas and dermatofibromas
Malignant (23)	23 Pleomorphic undifferentiated sarcomas (malignant fibrous histiocytomas)
Smooth muscle tumors (83)	
Benign (41)	4 Angioleiomyomas 8 Uterine leiomyomas and retroperitoneal leiomyomas of uterine type 26 Leiomyomas of the gastrointestinal tract 3 Other types
Intermediate (1)	1 Retroperitoneal smooth muscle tumor of unknown malignant potential (STUMP)
Malignant (41)	2 Vascular leiomyosarcomas 4 Uterine leiomyosarcomas 8 Leiomyosarcomas of the gastrointestinal tract 27 Leiomyosarcomas of other localisations
Pericytic tumors (3)	
Benign (3)	2 Glomus tumors 1 Myopericytoma
Tumors with skeletal muscle differentiation (12)	
Malignant (12)	2 Alveolar rhabdomyosarcomas 7 Embryonal rhabdomyosarcomas 3 Pleomorphic rhabdomyosarcomas
Vascular tumors (28)	
Benign (11)	5 Capillary hemangiomas 1 Epithelioid hemangioma 5 Other types

Table 1 (continued)

Adipocytic tumors (72)	
Intermediate (1)	1 Retiform hemangioendothelioma
Malignant (16)	16 Angiosarcomas of various types and localisations (3 post-radiation AS)
Neurogenic tumors (49)	
Benign (29)	9 Neurofibromas 14 Schwannomas 6 Other types
Intermediate (2)	2 Gangliocytic paraganglioma
Malignant (18)	15 MPNST 3 PNET
Tumors with uncertain differentiation (48)	
Benign (13)	3 Angiomyxomas 2 Myxomas 5 PEComas ^a (3 angiomyolipomas, 2 lymphangiioleiomyomatoses) 1 Cellular angiofibroma 2 Other types
Intermediate (1)	1 Abdominopelvic PEComa ^a
Malignant (34)	5 Chordomas 5 DSRCT 10 Endometrial stromal sarcomas 1 Epithelioid sarcoma 1 Extraskelatal myxoid chondrosarcoma 5 Synovial sarcomas 7 Unclassifiable sarcomas
Gastrointestinal stromal tumors (27)	
	25 Stomach, 1 small bowel, 1 esophagus 11 Low, 4 intermediate, 12 high risk (according to [16]) 15 Spindled, 5 epithelioid, 7 mixed type

Abbreviation: MPNST, malignant peripheral nerve sheath tumor; DSRCT, desmoplastic small round cell tumors; PNET, Peripheral primitive neuroectodermal tumors

^a Classification according to [29].

biological potential. The latter group consists of tumors that grow locally aggressive and recur often, but do not metastasize or metastasize very rarely.

We included 217 malignant, 177 benign and 74 intermediate tumors into this study (Table 1). The classification of cases was basically done according to the World Health Organization (WHO) classification system [15] which rests mainly on morphological similarities of tumors to known types of soft tissues (ie, adipocytic or fibroblastic/myofibroblastic tumors). Additional tumor types not included into the WHO system were categorized as appeared appropriate (eg, endometrial stromal sarcomas as “malignant tumors of uncertain differentiation”). In order to simplify the classification system and strengthen further statistical analysis, some tumor categories were unified (eg, the WHO categories “intermediate—locally aggressive” and “intermediate—rarely metastasizing” were merged to “intermediate”, and well differentiated liposarcomas and atypical lipomatous tumors were fused to one group). The whole group of gastrointestinal stromal tumors was grouped into the “intermediate” category

(the full risk classification—according to Fletcher et al [15,16] - indicated in Table 1). The tumor diagnoses were made by three experienced pathologists on the basis of clinical information, tumor morphology and suitable immunohistochemistry. Comprehensive analyses by fluorescence in situ hybridisation, including translocation tests for *EWS*, *FUS*, *SS18*, *FOXO1A*, *CHOP* and amplification of *MDM2* were applied to all appropriate cases.

2.2. Immunohistochemistry

Immunohistochemical staining was done as described previously [11] using an α -LSD1 antibody (Novus Biologicals) at a 1:250 dilution. Only nuclear immunostaining results for LSD1 were considered positive. For semiquantitative analysis of LSD1 immunostaining a 3-tiered scoring system was used, ranging from score 0: <10% to score 1: \geq 10% to score 2: \geq 80% immunopositive tumor cell nuclei.

According to the staining results, several tumor entities with more than 50% strongly positive tumor cells were detected. In other entities only few samples were strongly positive. These subgroups were included in a further validation study.

2.3. Validation study

To validate the frequency of strong LSD1 expression, we prepared tissue microarrays including a total of 110 additional tumor samples, that is, 54 dedifferentiated liposarcomas, 3 myxofibrosarcomas, 26 unclassifiable sarcomas, 26 angiosarcomas and 1 extraskeletal myxoid chondrosarcoma. The tissue microarrays were processed and evaluated as described above.

2.4. Western blot analysis

Proteins of snap-frozen sarcoma tissue samples with a tumor cell content of >50% and proteins of sarcoma cells were extracted in 150 mmol/L NaCl, 10 mmol/L Tris (pH 7.2), 0.1% sodium dodecyl sulfate, 1% Triton X-100, 1% deoxycholate, and 5 mmol/L EDTA, and centrifuged at 13,000 \times g for 20 minutes at 4°C. 10 μ g protein lysates were denatured in Laemmli buffer (Roth) at 95°C for 10 minutes, loaded on a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel, and subjected to electrophoresis under reducing conditions. Proteins were transferred onto a polyvinylidene difluoride membrane (Roti-PVDF; Roth, Karlsruhe, Germany) using standard protocols. After blocking in 5% nonfat dry milk/phosphate buffered saline Tween-20 (PBST) for 1 to 2 hours, the membranes were incubated for 1 to 2 hours using the following antibodies and dilutions: α -LSD1 antibody (Novus Biologicals) 1:1.000; α -K4H3me2 (Abcam) 1:1.000; β -actin (Sigma) 1:5.000. Then, the membranes were washed, incubated with horseradish peroxidase–conjugated secondary antibody

(dilution 1:1,000; Dako, Glostrup, Denmark), and developed using enhanced chemiluminescence (Amersham).

2.5. Cell culture

The synovial sarcoma cell lines Fuji and CME-1 were cultured in RPMI supplemented with 10% FBS (Invitrogen), HS-SY-II and SYO-1 were cultivated in Delbucco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

2.6. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide cell proliferation assay

Cells were seeded into 96 well plates at a density of 2500 cells per well and cultured as described above. Medium was replaced daily. Treatment with clorgyline (Sigma-Aldrich) or tranlycypromine (Biomol) was done as indicated. A colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (Roche) was performed to assess cell viability. A multiwell scanner was used to measure the absorbance at 570 nm.

2.7. Detection of SS18 translocations

The synovial sarcoma cell lines CME-1, 1273/99, SYO-1, Fuji and HS-SY-II have been described before [17–21]. Fluorescence in situ hybridisation was carried out by using the SYT LSI break apart probe (Abbott Molecular, Wiesbaden, Germany) according to the manufacturer's recommendations. The presence of the specific SS18/SSX1 or SS18/SSX2 translocation subtype was confirmed by polymerase chain reaction (PCR). Briefly, total RNA was extracted using Trizol (Invitrogen, Karlsruhe, Germany) as indicated by the manufacturer and cDNA was synthesized using the Superscript III reverse transcriptase kit (Invitrogen). SS18, SSX1, and SSX2 primers employed for PCR amplification were described before [6]. PCR was performed in a Uno Thermocycler (Biometra, Göttingen, Germany) using FastStart Taq polymerase (Roche) as indicated by the manufacturer.

2.8. Statistical analysis

Statistical analyses were performed by using PASW Statistics 18.0, version 18.0.0 (SPSS; SPSS, Chicago, IL). Fisher exact test was calculated, and $P < .05$ was considered statistically significant. PASW Statistics 18.0 was also used to create the histogram.

3. Results

3.1. LSD1 expression

Immunohistochemical LSD1-staining was informative in 458 of 468 tumors (98%). The frequencies of high labeling

Table 2 Numbers of tumors with strong LSD1 expression (LSD1 labeling index = 2); total n = 82

Adipocytic tumors (n = 7)	0/19 Benign adipocytic tumors 0/20 Well differentiated liposarcomas 4/22 Dedifferentiated liposarcomas ^a 1/5 Myxoid liposarcomas 2/6 Pleomorphic liposarcomas
Fibroblastic/myofibroblastic tumors (n = 20)	0/47 Benign fibroblastic/myofibroblastic tumors 1/1 Angiomyofibroblastoma 0/25 Desmoid-type fibromatoses 14/17 Solitary fibrous tumors 1/3 Dermatofibrosarcoma protuberans 2/6 Fibrosarcomas, adult type 1/7 Myxofibrosarcomas 0/2 Low-grade fibromyxoid sarcomas 1/2 Sclerosing epithelioid fibrosarcomas
Fibrohistiocytic tumors (n = 1)	0/13 Benign fibrohistiocytic tumors 1/23 Pleomorphic undifferentiated sarcomas (malignant fibrous histiocytomas)
Smooth muscle tumors (n = 0)	0/41 Benign smooth muscle tumors 0/42 Intermediate and malignant tumors
Pericytic tumors (n = 1)	1/2 Glomus tumors 0/1 Myopericytoma
Tumors with skeletal muscle differentiation (n = 10)	2/2 Alveolar rhabdomyosarcomas 6/7 Embryonal rhabdomyosarcomas 2/3 Pleomorphic rhabdomyosarcomas
Vascular tumors (n = 4)	0/11 Benign vascular tumors 0/1 Retiform hemangioendothelioma 4/16 Angiosarcomas (3/3 post-radiation AS) ^a
Neurogenic tumors (n = 12)	0/31 Benign and intermediate neurogenic tumors 11/15 MPNST 1/3 PNET 1/3 Angiomyxomas 1/2 Myxomas 1/5 PEComas 1/1 Cellular angiofibroma 0/3 Other benign and intermediate tumors 2/5 Chordomas
Tumors with uncertain differentiation (n = 27)	5/5 DSRCT 5/10 Endometrial stromal sarcomas 1/1 Epithelioid sarcoma 1/1 Extraskelletal myxoid chondrosarcoma ^a 4/5 Synovial sarcomas 5/7 Unclassifiable sarcomas ^a
Gastrointestinal stromal tumors (n = 0)	

Entities with more than one tumor and strong LSD1 expression in more than 50% of the investigated tumors are indicated in bold.

^a These entities were further analyzed in a validation study: frequencies of strongly LSD1 positive tumors in the additional cohort were: 6 of 54 dedifferentiated liposarcomas (total 10/76), 4 of 26 angiosarcomas (8/42), 13 of 26 unclassifiable sarcomas (18/33), 2 of 3 myxofibrosarcomas (3/10), 1 of 1 extraskelletal myxoid chondrosarcoma (2 of 2).

scores among tumor groups are displayed in [Table 2](#) and [Fig. 1](#). 213 tumors (46.5%) did not express LSD1 significantly (score 0), 163 tumors (35.6%) showed a moderate (score 1), and 82 tumors (17.9%) a strong LSD1 staining (score 2). LSD1 expression did not occur accidentally; rather, malignant mesenchymal tumors demonstrated a strong expression more frequently. We observed a significantly higher rate of LSD1-Score 2 cases in the group of malignant tumors ([Table 2](#); Fisher exact test: $P < .001$). Among 172

benign tumors 111 showed LSD1-Score 0 (<10% immunopositive tumor cells), 55 LSD1-score 2 ($\geq 10\%$), and only 6 LSD1-score 2 ($\geq 80\%$). In contrast, 62 of 212 malignant tumors demonstrated LSD1-score 2 (76 tumors score 0, 74 tumors score 1). A moderate LSD1 expression (score 1) was found in all tumor groups without significant differences.

We could demonstrate high LSD1 expression in specific highly malignant tumor groups ([Table 2](#), [Fig. 1](#)): synovial sarcomas, rhabdomyosarcomas of different types,

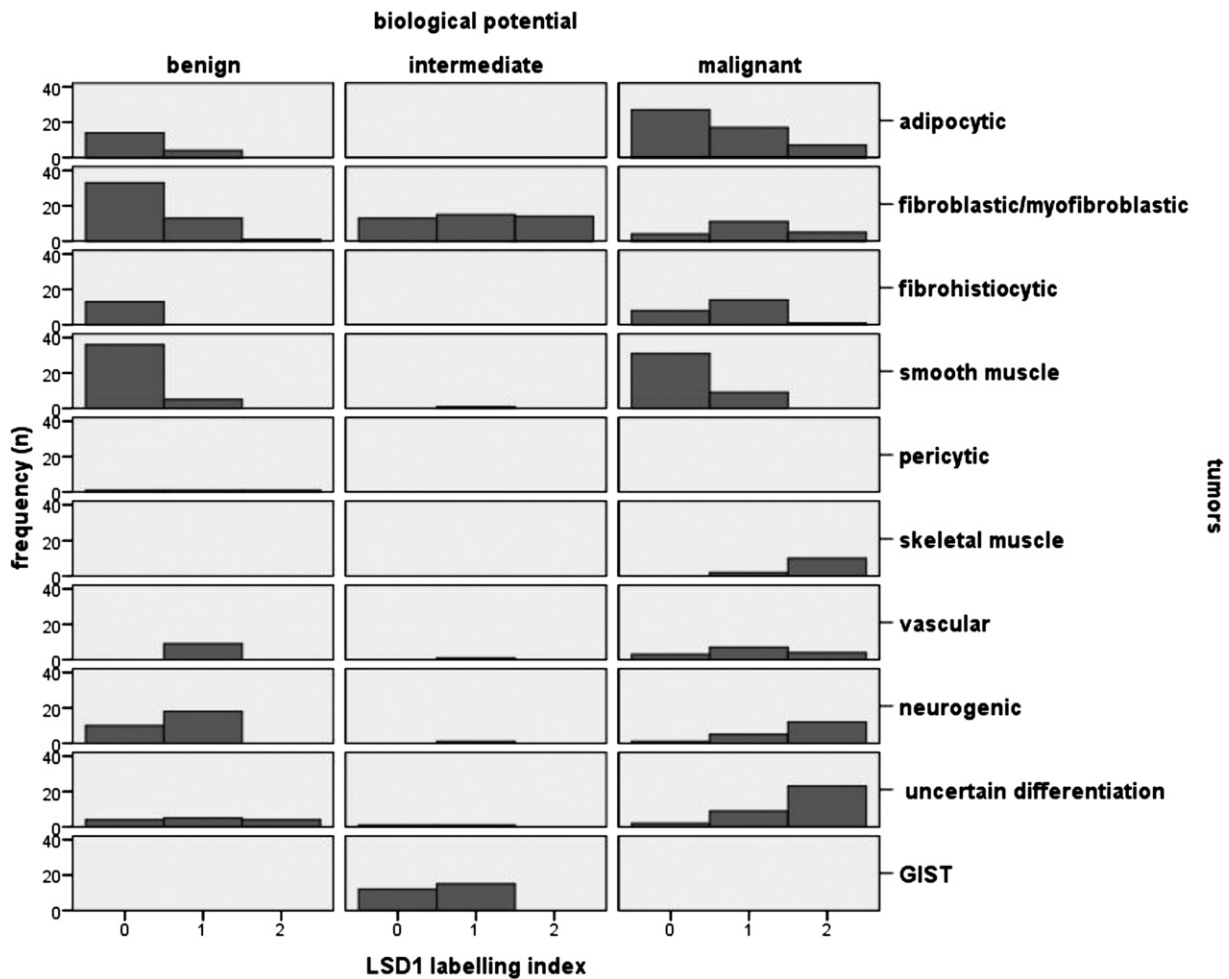


Fig. 1 Histograms of tumors according to their biological potential and LSD1 labeling index.

desmoplastic small round cell tumors and malignant peripheral nerve sheath tumors (MPNST) were highly immunopositive in the majority of cases. In the group of intermediate tumors, only solitary fibrous tumors turned out as strongly positive: 14 out of 74 evaluable intermediate tumors (14 of 17 solitary fibrous tumors [SFTs] included in this study) showed LSD1-score 2. Only 6 benign tumors showed LSD1-Score 2, among them myxomas, angiomyxomas, cellular angiofibromas, and angiomyofibroblastomas, which have some overlap in terms of morphology and clinical behaviour. Interestingly, high LSD1 expression did not occur in smooth muscle tumors (neither leiomyomas nor leiomyosarcomas), in gastrointestinal stromal tumors, and in undifferentiated pleomorphic sarcomas.

We included a larger number of cases to determine the incidence and the significance of strong expression of LSD1 in some mesenchymal tumor types in a second validation study (Table 2). Although these tumors are rare, we prepared additional tissue microarrays with additional 110 tumor samples (54 dedifferentiated liposarcomas, 3 myxofibrosarcomas, 26 unclassifiable sarcomas, 26 angiosarcomas and 1 extraskeletal myxoid chondrosarcoma). This validation

study confirmed the same ratio of tumors with high LSD1 expression as described in Table 2.

To confirm these data, we performed western blot analyses in a subset of tumor specimens. Results shown in Fig. 2 clearly indicated that LSD1 protein was more strongly expressed in tumor extracts which correspond to specimens with high LSD1 expression detected by immunohistochemistry (LSD1-score 2), whereas the amount of protein was lower or negative in LSD1-score 1 and LSD-score 0 cases, respectively. Overall, immunohistochemistry of LSD1 was highly concordant with protein expression evaluated by western blot (Fig. 2A and B).

3.2. Inhibition of LSD1 impairs cell growth in vitro

LSD1 belongs to a large family of FAD-utilizing amine oxidases. These include polyamine oxidases and MAOs. Monoamine oxidase inhibitors (MAOIs) are a class of small-molecule inhibitors of the MAO family, which reversibly and irreversibly inhibit various MAOs and are in clinical use for treatment of depression and Parkinson's disease.

We investigated whether inhibition of LSD1 leads to cancer cell growth inhibition. Therefore, we tested several

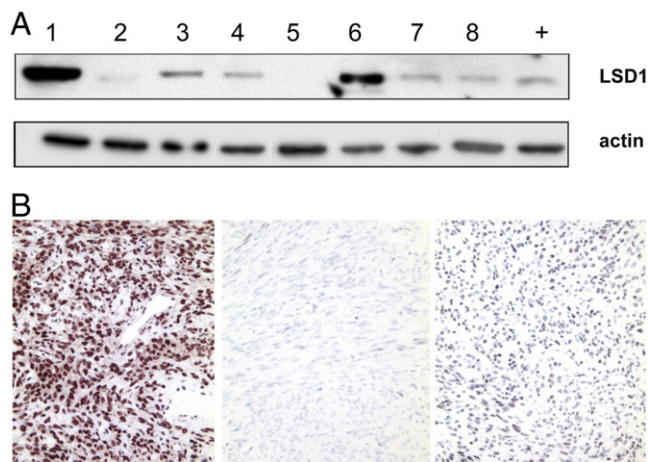


Fig. 2 Expression of LSD1 in different mesenchymal tumors. A, LSD1 expression in tumor tissue extracts of different mesenchymal tumors selected from the archival files was determined by western blot. β -actin served as the loading control. B, Strong immunohistochemical staining of LSD1 in was observed in solitary fibrous tumor (left panel, LSD1-score +2) and weaker staining was observed in MPNST (right panel LSD1-score +1), whereas in leiomyosarcoma (middle panel) LSD1 staining was absent. 1, Solitary fibrous tumor; 2, leiomyosarcoma; 3, myxoid liposarcoma; 4, leiomyosarcoma; 5, Leiomyosarcoma; 6, Angiosarcoma; 7, MPNST; 8, desmoid fibromatosis. + indicates in vitro translated human LSD1.

synovial sarcoma cells lines. Treatment of the synovial sarcoma cells Fuji, CME-1, HS-SY-II and SYO-1 with the irreversible MAOI tranlylcypromine, and the reversible MAOI chlorgyline impaired cell growth in a dose-dependent manner (Fig. 3A). Reduced viability was accompanied by increased global dimethylation of lysine 4 in histone 3 (H3K4me2, Fig. 3B) indicating that the enzymatic function of LSD1 is indeed inhibited.

4. Discussion

Several epigenetic drugs, such as HDAC-inhibiting trichostatin and suberoylanilide hydroxamic acid (SAHA), are effective in various malignancies, such as breast cancer, multiple myeloma, and cutaneous T-cell lymphoma [22]. On the other hand, DNA methyltransferase inhibitors, such 5-azacytidine, are known to be effective in myelodysplastic syndrome [23]. These inhibitors have proven that epigenetic inhibitors are useful drug candidates. The discovery of a large number of histone demethylases suggests an important role for dynamic regulation of histone methylation in biological processes. The observation that overexpression, amplification or mutations of several histone demethylases have been found in many types of tumors, raises the possibility of using these enzymes as diagnostic tools as well as pave a way for the discovery of novel therapeutic targets and treatment modalities. However, epigenetic therapies that target histone demethylation are at the concept stage.

In this study, we were able to demonstrate that certain distinct groups of mesenchymal tumors strongly express LSD1. A major finding of our study is the consistent and strong LSD1 expression in distinct highly malignant sarcoma subgroups, as in rhabdomyosarcomas, synovial sarcomas, desmoplastic small round cell tumors (DSRCT) and MPNST. These tumors often occur in childhood (eg rhabdomyosarcomas) or in young adults (eg, DSRCT, synovial sarcomas) and are mostly characterised by a dismal prognosis despite a multimodal therapy. The currently used therapy regimens, however, consist basically of surgery, conventional chemotherapy, and radiation, whereas targeted therapies are still not available.

We and others have demonstrated that LSD1 is involved in the regulation of many cancers, such as breast cancer [12], prostate cancer [6,10], colon cancer [24], and neuroblastoma [11]. High expression of LSD1 in prostate cancer was a predictive marker for aggressive tumor biology and tumor recurrence during therapy [10]. Recently, our group could show that LSD1 expression was highly upregulated in poorly differentiated neuroblastoma and estrogen receptor-negative breast cancer, correlating LSD1 with adverse clinical outcome [11,12]. At the cellular level, RNA interference inhibition of LSD1 markedly inhibited proliferation of cancer cells and led to a broad change in proliferation-related gene expression profile. Treatment of neuroblastoma xenografts in nude mice by the non-selective LSD1 inhibitor tranlylcypromine provided proof-of-principle of exploiting LSD1 inhibition as a target for cancer therapy [11]. Here, we demonstrate that the monoamine oxidase inhibitors tranlylcypromine and clorgyline block effectively LSD1 and thereby synovial sarcoma cell growth in vitro. Our previous work [11] has shown that only relatively high doses reduced xenograft tumor growth in vivo in a prevention model. The doses required were higher than those effectively inhibiting neurotransmitter deamination, resulting in excessive side effects in vivo. However, LSD1 inhibitors have still to be improved, in terms of their specificity to LSD1 and their potential side effects with other FAD-dependent enzymes, to be a tool to alter chromatin state with promise of a block of tumor cell growth. Then, we hypothesize, LSD1 inhibitors would be clinically useful for treatment of synovial sarcomas, which are characterized by an aggressive phenotype even under the current therapy regimens.

A second class of inhibitors, polyamine analogues, which inhibit polyamine oxidase in the FAD-dependent enzyme family, reportedly block LSD1 and allow re-expression of aberrantly silenced secreted frizzled-related proteins, Wnt signaling pathway antagonist genes in colon carcinoma cells [24,25]. The combined use of the polyamine analogues with DNA methyltransferase inhibitors represents a promising anti-cancer treatment for colon tumors [24].

On the basis of the report that JMJD2C demethylates trimethylated Lys 9 of histone H3 cooperatively with LSD1 and is involved in the regulation of gene expression [26], a very recent work has shown that inhibition of JMJD2 acts

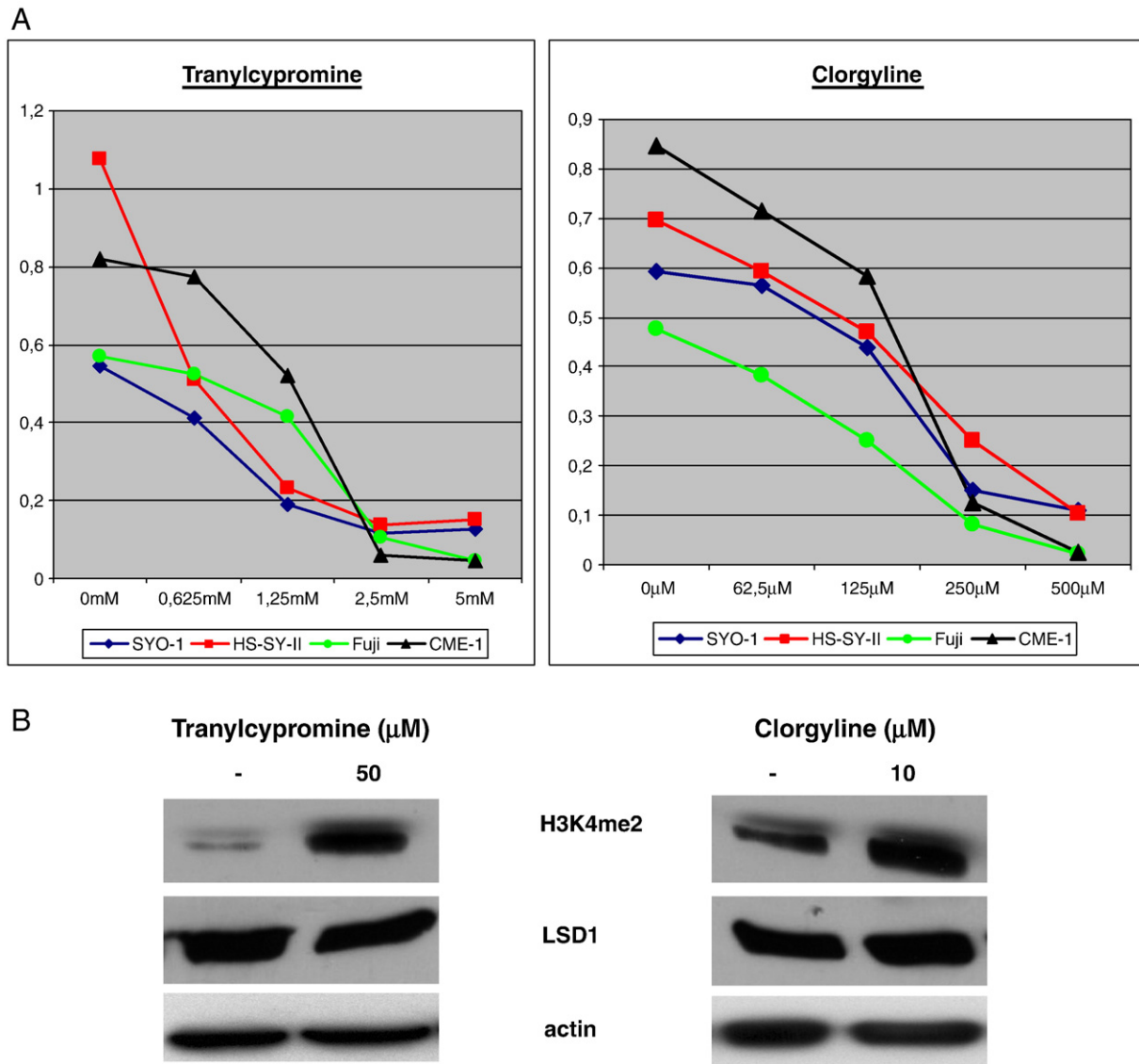


Fig. 3 Reduction in cell growth and increase of global H3K4 methylation upon MAOIs treatment. A, Four synovial sarcoma cell lines were treated with tranylcypromine and clorgyline for 48 hours for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. MAOIs treatment resulted in extensive reduction of cell numbers. B, Western blot analysis confirmed an accumulation of H3K4 dimethylation (H3K4me2) in CME-1 cells upon treatment with 50 µM tranylcypromine and 10 µM clorgyline for 48 h. LSD1 protein levels were not affected. β -Actin served as the loading control.

synergistically as LSD1 inhibitor [27]. A combination of a selective LSD1 inhibitor and inhibitors of other epigenetically acting enzymes might prove useful to prevent the development of resistance to treatment and achieve a maximal effect.

Among the tumors with intermediate biological potential, SFT turned out as an LSD1-positive entity. None of the other fibroblastic/myofibroblastic tumors nor any of the intermediate tumors showed such a homogeneous and strong protein expression. In most cases, which were examined in our institute after the discovery of the phenomenon, strong LSD1 expression turned out as a helpful and consistent diagnostic marker. The histopathological diagnosis of SFT is based on

the growth pattern of tumor cells, that is, the so-called patternless pattern, the presence of characteristic hemangiopericytoma-like vessels and the expression of CD34, bcl-2 and CD99. In a relevant proportion of cases, however, these features can be inconsistently present. Thus, LSD1 might serve as a useful additional diagnostic marker. Further studies are needed to clarify whether LSD1 is also expressed in classic hemangiopericytomas, lipomatous hemangiopericytomas and giant cell angiofibromas, which are closely related to SFT, or in the newly described dedifferentiated solitary fibrous tumors [28].

In summary, we identified LSD1 as a novel potential target for selective therapies in distinct highly malignant

sarcomas, as in rhabdomyosarcomas, synovial sarcomas, DSRCT and MPNST. In addition, we found that LSD1 can be used as a diagnostic marker for SFT.

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