编号：F2014-001

学术跟踪报告

课题名称: LSD1 和 CoREST 以及 LSD1 小分子抑制剂的共结晶

委托人: ***

委托日期: 2014 年 8 月 1 日

检索机构: 深圳市科技图书馆/深圳大学城图书馆

完成日期: 2014 年 8 月 20 日

深圳市科技图书馆(深圳大学城图书馆)

二〇一四年制

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一、 基本信息

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<tr><td rowspan="2">课题名称</td><td colspan="6">中文：LSD1 和 CoREST 以及 LSD1 小分子抑制剂的共结晶</td></tr>
<tr><td colspan="6">英文：The Cocrystallization of LSD1-CoREST with LSD1 inhibitor</td></tr>
<tr><td>涉及学科</td><td colspan="2">结构生物学</td><td colspan="2">课题目的</td><td colspan="2">开题</td></tr>
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1.1 数据库

检索数据库	检索时间范围
中国学术期刊网络出版总库	2012-2014
Derwent Innovation Index(DII)	2012-2014
Web of Science	2012-2014
MEDLINE	2012-2014

1.2 检索词

1. (lysine specific demethylase-1) OR LSD1
2. (赖氨酸 or 组蛋白) and 去甲基化酶 or LSD1

3. crystal*
4. 结构 or 结晶 or 晶体 or 纯化

1.3 检索式

1. ((lysine specific demethylase-1) OR LSD1) and crystal*
2. ((赖氨酸 or 组蛋白)and 去甲基化酶 or LSD1) and 抑制剂 and (结构 or 结晶 or 晶体 or 纯化)

二、 检索结果

2.1 中文文献

2.1.1 检索结果

按上述检索范围和检索策略，共检索到论文 4 篇。涉及药理学等 3 个研究方向，8 家研究机构在进行相关研究，有 6 个基金支持此研究。引用次数最高的是发表在中国药理学通报及中国科学技术大学学报的两篇综述文章。具体信息如下：

2.1.2 所检论文信息(按引用次数降序排列)

序号	标题	来源出版物名称	合计引用次数
1	表观遗传学药物的研究进展	中国药理学通报	7
2	组蛋白去甲基化酶 LSD1 的结构和功能研究进展	中国科学技术大学学报	7

3	抗肿瘤药物新靶点:表观遗传组蛋白赖氨酸特异性去甲基化酶 1	国际药学研究杂志	0
4	组蛋白去甲基化酶家族中组蛋白去甲基化酶 4 作用机制及其应用的研究进展	转化医学杂志	0

2.1.3 研究方向

研究方向	记录数
药理学药学	2
生物化学与分子生物学	1
医学	1

2.1.4 机构信息

机构	记录数
北京协和医学院比较医学中心	1
中国医学科学院医学实验动物研究所	1
卫生部人类疾病比较医学重点实验室	1
中国科学技术大学生命科学学院	1
郑州大学药学院	1
郑州大学新药研发中心	1
江苏师范大学生命科学学院	1
吉林大学白求恩医学院病理生物学教育部重点实验室	1

2.1.5 基金支持信息

基金资助机构	记录数
国家科技重大专项	1
北京协和医学院博士创新基金	1
国家重点基础研究发展计划(973 计划)	1
国家自然科学基金资助项目	1
Karmanos Cancer Institute-SRIG	1
江苏师范大学校基金	1

2.2 英文文献

2.2.1 检索结果

按上述检索范围和检索策略，共检索到论文 20 篇，涉及药理学药学、生物化学与分子生物学、化学、细胞生物学等 5 个研究方向，有意大利帕维亚大学等 8 家研究机构在进行相关研究，包括中国国家自然科学基金在内的 6 个基金资助机构在支持此研究。引用次数最高的文章是发表在 EXPERT OPINION ON THERAPEUTIC TARGETS 上的一篇综述文章。具体信息如下：

2.2.2 所检论文信息(按引用次数降序排列)

序号	标题	来源出版物名称	合计引用次数
1	LSD1 inhibition: a therapeutic strategy in cancer?	EXPERT OPINION ON THERAPEUTIC TARGETS	14
2	LSD1/CoREST is an allosteric nanoscale clamp regulated by H3-histone-tail molecular recognition	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA	12
3	Low Molecular Weight Amidoximes that Act as Potent Inhibitors of Lysine-Specific Demethylase 1	JOURNAL OF MEDICINAL CHEMISTRY	9
4	The role of histone demethylases in cancer therapy	MOLECULAR ONCOLOGY	7
5	Lysine-specifiic histone demethylase 1 (LSD1): A potential molecular target for tumor therapy	CRITICAL REVIEWS IN EUKARYOTIC GENE EXPRESSION	7
6	Molecular Insights into Human Monoamine Oxidase B Inhibition by the Glitazone Antidiabetes Drugs	ACS MEDICINAL CHEMISTRY LETTERS	6
7	Protein Recognition by Short Peptide Reversible Inhibitors of the Chromatin-Modifying LSD1/CoREST Lysine Demethylase	ACS CHEMICAL BIOLOGY	5
8	LSD2/KDM1B and Its Cofactor NPAC/GLYR1 Endow a Structural and Molecular Model for Regulation of H3K4 Demethylation	MOLECULAR CELL	5
9	Structure-function analysis reveals a novel mechanism for regulation of histone demethylase LSD2/AOF1/KDM1b	CELL RESEARCH	5

10	Loss of LSD1 (lysine-specific demethylase 1) suppresses growth and alters gene expression of human colon cancer cells in a p53- and DNMT1 (DNA methyltransferase 1)-independent manner	BIOCHEMICAL JOURNAL	5
11	Triazole-Dithiocarbamate Based Selective Lysine Specific Demethylase 1 (LSD1) Inactivators Inhibit Gastric Cancer Cell Growth, Invasion, and Migration	JOURNAL OF MEDICINAL CHEMISTRY	4
12	Amine Oxidation Mediated by Lysine-Specific Demethylase 1: Quantum Mechanics/Molecular Mechanics Insights into Mechanism and Role of Lysine 661	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY	3
13	Lysine-specific demethylase 1-selective inactivators: protein-targeted drug delivery mechanism	Angewandte Chemie (International ed. in English)	3
14	Expanding the druggable space of the LSD1/CoREST epigenetic target: new potential binding regions for drug-like molecules, peptides, protein partners, and chromatin	PLoS computational biology	2
15	Nonpeptidic Propargylamines as Inhibitors of Lysine Specific Demethylase 1 (LSD1) with Cellular Activity	JOURNAL OF MEDICINAL CHEMISTRY	2
16	Phosphorylation of neuronal Lysine-Specific Demethylase 1LSD1/KDM1A impairs transcriptional repression by regulating interaction with CoREST and histone deacetylases HDAC1/2	JOURNAL OF NEUROCHEMISTRY	1
17	Design of small molecule epigenetic modulators	BIOORGANIC & MEDICINAL CHEMISTRY LETTERS	1
18	A Hypomorphic Lsd1 Allele Results in Heart Development Defects in Mice	PLOS ONE	1
19	Crystal structure of the histone lysine specific demethylase LSD1 complexed with tetrahydrofolate	PROTEIN SCIENCE	0
20	The lysine-specific demethylase 1 is a novel substrate of protein kinase CK2	BIOCHIMICA ET BIOPHYSICA ACTA-PROTEINS AND PROTEOMICS	0

2.2.3 研究方向

研究方向	记录数
PHARMACOLOGY PHARMACY（药理学药学）	6
BIOCHEMISTRY MOLECULAR BIOLOGY（生物化学与分子生物学）	5
SCIENCE TECHNOLOGY OTHER TOPICS（其他科学技术主题）	2
CHEMISTRY（化学）	2
CELL BIOLOGY（细胞生物学）	2

2.2.4 机构信息

机构	记录数
UNIV PAVIA（意大利帕维亚大学）	3
MED UNIV S CAROLINA（南卡罗来纳医药大学）	3
UNIV UTAH（美国犹他大学）	2
UNIV TEXAS MD ANDERSON CANC CTR（德克萨斯大学安德森癌症中心）	2
UNIV HALLE WITTENBERG（哈雷-威登堡大学）	2
UNIV FREIBURG（德国佛雷堡大学）	2
JOHNS HOPKINS UNIV（美国约翰斯霍普金斯大学）	2
EMORY UNIV（艾莫利大学）	2

2.2.5 基金支持信息

基金资助机构	记录数
NATIONAL NATURAL SCIENCE FOUNDATION OF CHINA（中国国家自然科学基金）	3
NATIONAL INSTITUTES OF HEALTH（美国国立卫生研究院）	3
SAMUEL WAXMAN CANCER RESEARCH FOUNDATION（华克斯曼抗癌研究基金会）	2
NIH（美国国立卫生研究院）	2
DEUTSCHE FORSCHUNGSGEMEINSCHAFT（德国科学基金会）	2
ASSOCIAZIONE ITALIANA RICERCA SUL CANCRO（意大利癌症研究协会）	2

三、 附件

3.1 中文文献摘要

[1]张玲,盛树力,秦川.表观遗传学药物的研究进展[J].中国药理学通报,2013,03:297-303.
摘要：随着表观遗传学研究的不断深入,表观遗传学药物的研究取得了巨大进展。目前已有研究并批准上市的表观遗传学药物主要针对DNA异常甲基化和组蛋白的异常修饰。潜在的药物有DNA甲基转移酶抑制剂、赖氨酸去甲基化酶抑制剂、蛋白质甲基转移酶抑制剂、组蛋白去乙酰化酶抑制剂、组蛋白乙酰基转移酶抑制剂、含溴结构域蛋白抑制剂及甲基化组蛋白结合蛋白的抑制剂等。该文综述了近年来表观遗传学治疗在药理学上的进展,以期为疾病防治和基础研究提供一些新的思路。

[2]阮建彬,臧建业.组蛋白去甲基化酶 LSD1 的结构和功能研究进展[J].中国科学技术大学学报,2008,08:930-940.
摘要：组蛋白去甲基化酶 LSD1 的发现是表观遗传学领域的重要进展,揭示了组蛋白赖氨酸甲基化和其他化学修饰如乙酰化、磷酸化、泛素化等一样是一个动态调节的过程.结构和功能研究显示 LSD1 调控着基因转录的激活和抑制以及 p53 的活性,在癌症的发生和发展中起着重要的作用,是一个潜在的抗癌药物开发靶蛋白.

[3]郑一超,马金莲,王志茹,李金凤,赵文,刘宏民.抗肿瘤药物新靶点:表观遗传组蛋白赖氨酸特异性去甲基化酶 1[J].国际药学研究杂志,2014,01:30-36+4.
摘要：组蛋白赖氨酸特异性去甲基化酶 1(histonelysinespecificdemethylase1,LSD1)是一个黄素腺嘌呤二核苷酸(FAD)依赖的氨基氧化酶,能够特异性去除组蛋白 H3K4 和 H3K9 的单、双甲基化。利用 RNA 干扰技术和小分子 LSD1 抑制剂调节 LSD1 的表达量和活性,能够控制肿瘤细胞的增殖、转移和侵袭。同时,由于 LSD1 在多种肿瘤中高表达,靶向 LSD1 的抗肿瘤治疗方案表现出较高的选择性和较低的毒副作用。因此,LSD1 可能成为表观遗传学抗肿瘤药物的新靶点。本文对近年来 LSD1 的结构、功能研究及最新的 LSD1 抑制剂研究进展做一综述和分析。

[4]叶覃,AndreanaHOLOWATYJ,RoselyneM.LABBE,刘辉,ZengquanYANG.组蛋白去甲基化酶家族中组蛋白去甲基化酶 4 作用机制及其应用的研究进展[J].转化医学杂志,2014,01:13-18.
摘要：组蛋白甲基化是一种重要的表观遗传性修饰方式,是一个可逆的动态调节过程。组蛋白去甲基化酶家族中组蛋白去甲基化酶 4 能催化去除组蛋白赖氨酸残基甲基标记,调节染色质的结构,参与精细调控基因转录,维持染色质的活性和非活性平衡。组蛋白去甲基化酶 4 异常可能导致细胞增殖、分化、个体发育、能量代谢及肿瘤发生发展等多种生物进程异常。研究显示组蛋白去甲基化酶 4 可作为新的药物靶标。本文就组蛋白去甲基化酶 4 家族的结构、作用机制、在疾病发生发展进程中的生物学功能及特异性抑制剂开发的最新研究进展作一综述。

3.2 英文文献摘要

Luka, Z. and S. Pakhomova, et al. (2014). "Crystal structure of the histone lysine specific demethylase LSD1 complexed with tetrahydrofolate." PROTEIN SCIENCE **23**(7): 993-998.

An important epigenetic modification is the methylation/demethylation of histone lysine residues. The first histone demethylase to be discovered was a lysine-specific demethylase 1, LSD1,

a flavin containing enzyme which carries out the demethylation of di- and monomethyllysine 4 in histone H3. The removed methyl groups are oxidized to formaldehyde. This reaction is similar to those performed by dimethylglycine dehydrogenase and sarcosine dehydrogenase, in which protein-bound tetrahydrofolate (THF) was proposed to serve as an acceptor of the generated formaldehyde. We showed earlier that LSD1 binds THF with high affinity which suggests its possible participation in the histone demethylation reaction. In the cell, LSD1 interacts with co-repressor for repressor element 1 silencing transcription factor (CoREST). In order to elucidate the role of folate in the demethylating reaction we solved the crystal structure of the LSD1-CoREST-THF complex. In the complex, the folate-binding site is located in the active center in close proximity to flavin adenine dinucleotide. This position of the folate suggests that the bound THF accepts the formaldehyde generated in the course of histone demethylation to form 5,10-methylene-THF. We also show the formation of 5,10-methylene-THF during the course of the enzymatic reaction in the presence of THF by mass spectrometry. Production of this form of folate could act to prevent accumulation of potentially toxic formaldehyde in the cell. These studies suggest that folate may play a role in the epigenetic control of gene expression in addition to its traditional role in the transfer of one-carbon units in metabolism.

Pachaiyappan, B. and P. M. Woster(2014). "Design of small molecule epigenetic modulators." BIOORGANIC & MEDICINAL CHEMISTRY LETTERS **24**(1): 21-32.

The field of epigenetics has expanded rapidly to reveal multiple new targets for drug discovery. The functional elements of the epigenomic machinery can be categorized as writers, erasers and readers, and together these elements control cellular gene expression and homeostasis. It is increasingly clear that aberrations in the epigenome can underly a variety of diseases, and thus discovery of small molecules that modulate the epigenome in a specific manner is a viable approach to the discovery of new therapeutic agents. In this Digest, the components of epigenetic control of gene expression will be briefly summarized, and efforts to identify small molecules that modulate epigenetic processes will be described. (C) 2013 The Authors. Published by Elsevier Ltd. All rights reserved.

Toffolo, E. and F. Rusconi, et al. (2014). "Phosphorylation of neuronal Lysine-Specific Demethylase 1LSD1/KDM1A impairs transcriptional repression by regulating interaction with CoREST and histone deacetylases HDAC1/2." JOURNAL OF NEUROCHEMISTRY **128**(5): 603-616.

Epigenetic mechanisms play important roles in brain development, orchestrating proliferation, differentiation, and morphogenesis. Lysine-Specific Demethylase 1 (LSD1 also known as KDM1A and AOF2) is a histone modifier involved in transcriptional repression, forming a stable core complex with the corepressors corepressor of REST (CoREST) and histone deacetylases (HDAC1/2). Importantly, in the mammalian CNS, neuronal LSD1-8a, an alternative splicing isoform of LSD1 including the mini-exon E8a, sets alongside LSD1 and is capable of enhancing neurite growth and morphogenesis. Here, we describe that the morphogenic properties of neuronal LSD1-8a require switching off repressive activity and this negative modulation is mediated in vivo by phosphorylation of the Thr369b residue coded by exon E8a. Three-dimensional crystal structure analysis using a phospho-mimetic mutant (Thr369bAsp), indicate that phosphorylation affects the residues surrounding the exon E8a-coded amino acids, causing a local conformational change. We suggest that phosphorylation, without affecting demethylase activity, causes in neurons CoREST and HDAC1/2 corepressors detachment from LSD1-8a and impairs neuronal LSD1-8a repressive

activity. In neurons, Thr369b phosphorylation is required for morphogenic activity, converting neuronal LSD1-8a in a dominant-negative isoform, challenging LSD1-mediated transcriptional repression on target genes.

Costa, R. and G. Arrigoni, et al. (2014). "The lysine-specific demethylase 1 is a novel substrate of protein kinase CK2." BIOCHIMICA ET BIOPHYSICA ACTA-PROTEINS AND PROTEOMICS **1844**(4): 722-729.

Protein kinase CK2 is a pleiotropic serine/threonine kinase responsible for the generation of a substantial proportion of the human phosphoproteome. CK2 is generally found as a tetramer with two catalytic, alpha and alpha' and two non catalytic beta subunits. CK2 alpha C-terminal tail phosphotylation is regulated during the mitotic events and the absence of these phosphosites in alpha' suggests an isoform specialization. We used a proteomic approach to identify proteins specifically phosphorylated by a CK2 alpha phosphomimetic mutant, CK2 alpha T344ET360ES362ES370E (CK2 alpha 4E), in human neuroblastoma SKNBE cellular extract. One of these proteins is lysine-specific demethylase 1 (LSD1 or KDM1A), an important player of the epigenetic machinery. LSD1 is a FAD-dependent amine oxidase and promotes demethylation of lysine 4 and lysine 9 of mono- and di-methylated histone H3. We found that LSD1 is a new substrate and an interacting partner of protein kinase CK2. Three CK2 phosphosites, (Ser131, Ser137 and Ser166) in the N-terminal region of LSD1 have been identified. This domain is found in all chordates but not in more ancient organisms and it is not essential for LSD1 catalytic event while it could modulate the interaction with CK2 and with other partners in gene repressing and activating complexes. Our data support the view that the phosphorylation of the N-terminal domain by CK2 may represent a mechanism for regulating histone methylation, disclosing a new role for protein kinase CK2 in epigenetics. (C) 2014 Elsevier B.V. All rights reserved.

Nicholson, T. B. and A. K. Singh, et al. (2013). "A Hypomorphic Lsd1 Allele Results in Heart Development Defects in Mice." PLOS ONE **8**(e609134).

Lysine-specific demethylase 1 (Lsd1/Aof2/Kdm1a), the first enzyme with specific lysine demethylase activity to be described, demethylates histone and non-histone proteins and is essential for mouse embryogenesis. Lsd1 interacts with numerous proteins through several different domains, most notably the tower domain, an extended helical structure that protrudes from the core of the protein. While there is evidence that Lsd1-interacting proteins regulate the activity and specificity of Lsd1, the significance and roles of such interactions in developmental processes remain largely unknown. Here we describe a hypomorphic Lsd1 allele that contains two point mutations in the tower domain, resulting in a protein with reduced interaction with known binding partners and decreased enzymatic activity. Mice homozygous for this allele die perinatally due to heart defects, with the majority of animals suffering from ventricular septal defects. Molecular analyses revealed hyperphosphorylation of E-cadherin in the hearts of mutant animals. These results identify a previously unknown role for Lsd1 in heart development, perhaps partly through the control of E-cadherin phosphorylation.

Karasulu, B. and M. Patil, et al. (2013). "Amine Oxidation Mediated by Lysine-Specific Demethylase 1: Quantum Mechanics/Molecular Mechanics Insights into Mechanism and Role of Lysine 661." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY **135**(36): 13400-13413.

We report classical molecular dynamics (MD) simulations and combined quantum mechanics/molecular mechanics (QM/MM) calculations to elucidate the catalytic mechanism of the

rate-determining amine oxidation step in the lysine-specific demethylase 1 (LSD1)-catalyzed demethylation of the histone tail lysine (H3K4), with flavin adenine dinucleotide (FAD) acting as cofactor. The oxidation of substrate lysine (sLys) involves the cleavage of an alpha-CH bond accompanied by the transfer of a hydride ion equivalent to FAD, leading to an imine intermediate. This hydride transfer pathway is shown to be clearly favored for sLys oxidation over other proposed mechanisms, including the radical (or single-electron transfer) route as well as carbanion and polar-nucleophilic mechanisms. MD simulations on six NVT ensembles (covering different protonation states of sLys and K661 as well as the K661M mutant) identify two possible orientations of the reacting sLys and FAD subunits (called "downward" and "upward"). Calculations at the QM(B3LYP-D/6-31G*)/CHARMM22 level provide molecular-level insights into the mechanism, helping to understand how LSD I achieves the activation of the rather inert methyl-CH bond in a metal-free environment. Factors such as proper alignment of sLys (downward orientation), transition-state stabilization (due to the protein environment and favorable orbital interactions), and product stabilization via adduct formation are found to be crucial for facilitating the oxidative alpha-CH bond cleavage. The current study also sheds light on the role of important active-site residues (Y761, K661, and W695) and of the conserved water-bridge motif. The steric influence of Y761 helps to position the reaction partners properly,K661 is predicted to get deprotonated prior to substrate binding and to act as an. active-site base that accepts a proton from sLys to enable the subsequent amine oxidation, and the water bridge that is stabilized by K661 and W695 mediates this proton transfer.

Robertson, J. C. and N. C. Hurley, et al. (2013). "Expanding the druggable space of the LSD1/CoREST epigenetic target: new potential binding regions for drug-like molecules, peptides, protein partners, and chromatin." PLoS computational biology **9**(7).

Lysine specific demethylase-1 (LSD1/KDM1A) in complex with its corepressor protein CoREST is a promising target for epigenetic drugs. No therapeutic that targets LSD1/CoREST, however, has been reported to date. Recently, extended molecular dynamics (MD) simulations indicated that LSD1/CoREST nanoscale clamp dynamics is regulated by substrate binding and highlighted key hinge points of this large-scale motion as well as the relevance of local residue dynamics. Prompted by the urgent need for new molecular probes and inhibitors to understand LSD1/CoREST interactions with small-molecules, peptides, protein partners, and chromatin, we undertake here a configurational ensemble approach to expand LSD1/CoREST druggability. The independent algorithms FTMap and SiteMap and our newly developed Druggable Site Visualizer (DSV) software tool were used to predict and inspect favorable binding sites. We find that the hinge points revealed by MD simulations at the SANT2/Tower interface, at the SWIRM/AOD interface, and at the AOD/Tower interface are new targets for the discovery of molecular probes to block association of LSD1/CoREST with chromatin or protein partners. A fourth region was also predicted from simulated configurational ensembles and was experimentally validated to have strong binding propensity. The observation that this prediction would be prevented when using only the X-ray structures available (including the X-ray structure bound to the same peptide) underscores the relevance of protein dynamics in protein interactions. A fifth region was highlighted corresponding to a small pocket on the AOD domain. This study sets the basis for future virtual screening campaigns targeting the five novel regions reported herein and for the design of LSD1/CoREST mutants to probe LSD1/CoREST binding with chromatin and various protein partners.

Jin, L. H. and C. L. Hanigan, et al. (2013). "Loss of LSD1 (lysine-specific demethylase 1) suppresses growth and alters gene expression of human colon cancer cells in a p53- and DNMT1 (DNA methyltransferase 1)-independent manner." BIOCHEMICAL JOURNAL **449**(2): 459-468.

Epigenetic silencing of gene expression is important in cancer. Aberrant DNA CpG island hypermethylation and histone modifications are involved in the aberrant silencing of tumour-suppressor genes. LSD1 (lysine-specific demethylase 1) is a H3K4 (histone H3 Lys(4)) demethylase associated with gene repression and is overexpressed in multiple cancer types. LSD1 has also been implicated in targeting p53 and DNMT1 (DNA methyltransferase 1), with data suggesting that the demethylating activity of LSD1 on these proteins is necessary for their stabilization. To examine the role of LSD1 we generated LSD1 heterozygous (LSD1(+/-)) and homozygous (LSD1(-/-)) knockouts in the human colorectal cancer cell line HCT116. The deletion of LSD1 led to a reduced cell proliferation both in vitro and in vivo. Surprisingly, the knockout of LSD1 in HCT116 cells did not result in global increases in its histone substrate H3K4me2 (dimethyl-H3K4) or changes in the stability or function of p53 or DNMT1. However, there was a significant difference in gene expression between cells containing LSD1 and those null for LSD1. The results of the present study suggested that LSD1 is critical in the regulation of cell proliferation, but also indicated that LSD1 is not an absolute requirement for the stabilization of either p53 or DNMT1.

Fang, R. and F. Chen, et al. (2013). "LSD2/KDM1B and Its Cofactor NPAC/GLYR1 Endow a Structural and Molecular Model for Regulation of H3K4 Demethylation." MOLECULAR CELL **49**(3): 558-570.

Dynamic regulation of histone methylation represents a fundamental epigenetic mechanism underlying eukaryotic gene regulation, yet little is known about how the catalytic activities of histone demethylases are regulated. Here, we identify and characterize NPAC/GLYR1 as an LSD2/KDM1b-specific cofactor that stimulates H3K4me1 and H3K4me2 demethylation. We determine the crystal structures of LSD2 alone and LSD2 in complex with the NPAC linker region in the absence or presence of histone H3 peptide, at resolutions of 2.9, 2.0, and 2.25 angstrom, respectively. These crystal structures and further biochemical characterization define a dodecapeptide of NPAC (residues 214-225) as the minimal functional unit for its cofactor activity and provide structural determinants and a molecular mechanism underlying the intrinsic cofactor activity of NPAC in stimulating LSD2-catalyzed H3K4 demethylation. Thus, these findings establish a model for how a cofactor directly regulates histone demethylation and will have a significant impact on our understanding of catalytic-activity-based epigenetic regulation.

Ogasawara, D. and Y. Itoh, et al. (2013). "Lysine-specific demethylase 1-selective inactivators: protein-targeted drug delivery mechanism." Angewandte Chemie (International ed. in English) **52**(33).

Schmitt, M. L. and A. T. Hauser, et al. (2013). "Nonpeptidic Propargylamines as Inhibitors of Lysine Specific Demethylase 1 (LSD1) with Cellular Activity." JOURNAL OF MEDICINAL CHEMISTRY **56**(18): 7334-7342.

Lysine demethylases play an important role in epigenetic regulation and thus in the development of diseases like cancer or neurodegenerative disorders. As the lysine specific demethylase 1 (LSD1/KDM1) has been strongly connected to androgen and estrogen dependent

gene expression, it serves as a promising target for the therapy of hormone dependent cancer. Here, we report on the discovery of new small molecule inhibitors of LSD1 containing a propargylamine warhead, starting out from lysine containing substrate analogues. On the basis of these substrate mimicking inhibitors, we were able to increase potency by a combination of similarity-based virtual screening and subsequent synthetic optimization resulting in more druglike LSD1 inhibitors that led to histone hypermethylation in breast cancer cells.

Tortorici, M. and M. T. Borrello, et al. (2013). "Protein Recognition by Short Peptide Reversible Inhibitors of the Chromatin-Modifying LSD1/CoREST Lysine Demethylase." ACS CHEMICAL BIOLOGY **8**(8): 1677-1682.

The combinatorial assembly of protein complexes is at the heart of chromatin biology. Lysine demethylase LSD1(KDM1A)/CoREST beautifully exemplifies this concept. The active site of the enzyme tightly associates to the N-terminal domain of transcription factors of the SNAIL1 family, which therefore can competitively inhibit the binding of the N-terminal tail of the histone substrate. Our enzymatic, crystallographic, spectroscopic, and computational studies reveal that LSD1/CoREST can bind to a hexapeptide derived from the SNAIL sequence through recognition of a positively charged alpha-helical turn that forms upon binding to the enzyme. Variations in sequence and length of this six amino acid ligand modulate affinities enabling the same binding site to differentially interact with proteins that exert distinct biological functions. The discovered short peptide inhibitors exhibit antiproliferative activities and lay the foundation for the development of peptidomimetic small molecule inhibitors of LSD1.

Zhang, Q. and S. K. Qi, et al. (2013). "Structure-function analysis reveals a novel mechanism for regulation of histone demethylase LSD2/AOF1/KDM1b." CELL RESEARCH **23**(2): 225-241.

LSD2/A0F1/KDM1b catalyzes demethylation of mono- and di-methylated H3K4 and plays an important role in transcriptional regulation and genomic imprinting. Here, we report the high-resolution crystal structures of apo-LSD2 and LSD2 in complex with a peptide that mimics H3K4me2. Three structural domains of LSD2, namely, the novel N-terminal zinc finger, the centrally located SWIRM domain, and the C-terminal oxidase domain, closely pack together to form a boot-shaped structure. The active site cavity in the oxidase domain is large enough to accommodate several residues of the histone H3 tail and cannot discriminate between the different states of H3K4 methylation. The N-terminal zinc-finger domain, composed of a novel C4H2C2-type zinc finger and a specific CW-type zinc finger, is required for demethylase activity and, surprisingly, the binding of cofactor flavin adenine dinucleotide (FAD). In fact, a relay of extensive interactions through the zinc finger-SWIRM-oxidase domains is required for LSD2 demethylase activity and the binding of FAD. These results reveal a novel mechanism for the zinc finger and SWIRM domains in controlling LSD2 demethylase activity and provide a framework for elucidating the regulation and function of LSD2.

Zheng, Y. C. and Y. C. Duan, et al. (2013). "Triazole-Dithiocarbamate Based Selective Lysine Specific Demethylase 1 (LSD1) Inactivators Inhibit Gastric Cancer Cell Growth, Invasion, and Migration." JOURNAL OF MEDICINAL CHEMISTRY **56**(21): 8543-8560.

Lysine specific demethylase 1 (LSD1), the first identified histone demethylase, plays an important role in epigenetic regulation of gene activation and repression. The up-regulated LSD 1's expression has been reported in several malignant tumors. In the current study, we designed and synthesized five series of 1,2,3-triazole-dithiocarbamate hybrids and screened their inhibitory

activity toward LSD1. We found that some of these compounds, especially compound 26, exhibited the most specific and robust inhibition of LSD1. Interestingly, compound 26 also showed potent and selective cytotoxicity against LSD1 overexpressing gastric cancer cell lines MGC-803 and HGC-27, as well as marked inhibition of cell migration and invasion, compared to 2-PCPA. Furthermore, compound 26 effectively reduced the tumor growth bared by human gastric cancer cells in vivo with no signs of adverse side effects. These findings suggested that compound 26 deserves further investigation as a lead compound in the treatment of LSD1 overexpressing gastric cancer.

Hazeldine, S. and B. Pachaiyappan, et al. (2012). "Low Molecular Weight Amidoximes that Act as Potent Inhibitors of Lysine-Specific Demethylase 1." JOURNAL OF MEDICINAL CHEMISTRY **55**(17): 7378-7391.

The recently discovered enzyme lysine-specific demethylase 1 (LSD1) plays an important role in the epigenetic control of gene expression, and aberrant gene silencing secondary to LSD1 dysregulation is thought to contribute to the development of cancer. We reported that (bis)guanidines, (bis)biguanides, and their urea- and thiourea isosteres are potent inhibitors of LSD1 and induce the re-expression of aberrantly silenced tumor suppressor genes in tumor cells in vitro. We now report a series of small molecule amidoximes that are moderate inhibitors of recombinant LSD1 but that produce dramatic changes in methylation at the histone 3 lysine 4 (H3K4) chromatin mark, a specific target of LSD1, in Calu-6 lung carcinoma cells. In addition, these analogues increase cellular levels of secreted frizzle-related protein (SFRP) 2, H-cadherin (HCAD), and the transcription factor GATA4. These compounds represent leads for an important new series of drug-like epigenetic modulators with the potential for use as antitumor agents.

Lynch, J. T. and W. J. Harris, et al. (2012). "LSD1 inhibition: a therapeutic strategy in cancer?" EXPERT OPINION ON THERAPEUTIC TARGETS **16**(12): 1239-1249.

Introduction: The role of epigenetic dysfunction in cancer is increasingly appreciated. This has raised the question as to whether enzymes that regulate the structure and function of chromatin might represent novel therapeutic targets. The histone demethylase LSD1 is one such candidate and novel, potent inhibitors are under development. Areas covered: The literature on LSD1 (also known as KDM1A, AOF2, BHC110 or KIAA0601) was identified in Pubmed and is herein discussed. Areas covered include the structure and enzymatic activity of LSD1, its role in chromatin regulatory complexes, its functional roles in normal and malignant tissue, pharmacological inhibitors of its activity and their putative therapeutic roles. Expert opinion: Pre-clinical data supporting a therapeutic role for LSD1 inhibitors are most encouraging in acute myeloid leukaemia, although optimal dosing strategies and beneficial combinations with other agents remain unclear. Studies making use of potent, selective LSD1 inhibitors active in the nanomolar range are required to establish therapeutic indications in other subtypes of haematological malignancy, and in solid tumours.

Baron, R. and N. A. Vellore(2012). "LSD1/CoREST is an allosteric nanoscale clamp regulated by H3-histone-tail molecular recognition." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA **109**(31): 12509-12514.

The complex of lysine-specific demethylase-1 (LSD1/KDM1A) with its corepressor protein CoREST is an exceptionally relevant target for epigenetic drugs. Here, we provide insight into the local and global changes of LSD1/CoREST conformational dynamics that occur upon H3 binding

on the basis of a total cumulative time of one microsecond molecular dynamics simulation. The LSD1/CoREST complex functions as an allosteric nanoscale-binding clamp, which is regulated by substrate binding. In the unbound state, LSD1/CoREST reversibly visits clamp states that are more open or significantly more closed compared with the available X-ray crystal structures. The Lys triad of residues Lys355, Lys357, and Lys359 gates the entrance of the H3 pocket. H3 binding shifts the pocket breathing dynamics toward open, higher-volume states while reducing the overall flexibility of the LSD1/CoREST nanoscale clamp. We show that the H3 pocket is an allosteric site for the regulation of the rotation of the amino oxidase domain with respect to the Tower domain. The allosteric mechanism relies on the specific reduction of nanoscale domain rotation upon local H3-tail binding. Instead, clamp opening/closing motions that do not involve domain rotation only reduce in amplitude yet are dominant in the bound state. Overall, our data suggest that the H3 binding pocket is a central target site to (i) switch off LSD1 amino oxidase activity, thus H3-tail demethylation; (ii) block the competitive binding of transcription factors; and (iii) prevent chromatin anchoring to LSD1/CoREST. This study underscores the importance of receptor flexibility for future epigenetic drug discovery.

Chen, Y. W. and W. Jie, et al. (2012). "Lysine-specifiic histone demethylase 1 (LSD1): A potential molecular target for tumor therapy." CRITICAL REVIEWS IN EUKARYOTIC GENE EXPRESSION **22**(1): 53-59.

Lysine-specific demethylase 1 (LSD I), the first identified histone demethylase, was belonged to the superfamily of the flavin adenine dinucleotide (FAD)-dependent amine oxidases. LSD1 specifically demethylates mono- or dimethylated dimethylated histone H3 lysine4 (H3K4) and H3 lysine 9 (H3K9) via a redox process. Recently evidences showed that LSDI played an important role in a broad spectrum of biological processes, including cell proliferation, adipogenesis, spermatogenesis, chromosome segregation and embryonic development. Furthermore, LSD1 also could promote progress of tumor by inhibiting the tumor suppressor activity of p53. To date, as a potential drug for discovering anti-tumor drugs, the medical significance of LSD1 inhibitors have been greatly appreciated. Here, we reviewed the remarkable progress being made in understanding of LSDI, mainly on its structure, basic function and medical application in tumor therapy..

Binda, C. and M. Aldeco, et al. (2012). "Molecular Insights into Human Monoamine Oxidase B Inhibition by the Glitazone Antidiabetes Drugs." ACS MEDICINAL CHEMISTRY LETTERS **3**(1): 39-42.

The widely employed antidiabetic drug pioglitazone (Actos) is shown to be a specific and reversible inhibitor of human monoamine oxidase B (MAO B). The crystal structure of the enzyme-inhibitor complex shows that the R-enantiomer is bound with the thiazolidinedione ring near the flavin. The molecule occupies both substrate and entrance cavities of the active site, establishing noncovalent interactions with the surrounding amino acids. These binding properties differentiate pioglitazone from the clinically used MAO inhibitors, which act through covalent inhibition mechanisms and do not exhibit a high degree of MAO A versus B selectivity. Rosiglitazone (Avandia) and troglitazone, other members of the glitazone class, are less selective in that they are weaker inhibitors of both MAO A and MAO B. These results suggest that pioglitazone may have utility as a "repurposed" neuroprotectant drug in retarding the progression of disease in Parkinson's patients. They also provide new insights for the development of reversible isoenzyme-specific MAO inhibitors.

Hoffmann, I. and M. Roatsch, et al. (2012). "The role of histone demethylases in cancer therapy." MOLECULAR ONCOLOGY **6**(6SI): 683-703.

Reversible histone methylation has emerged in the last few years as an important mechanism of epigenetic regulation. Histone methyltransferases and demethylases have been identified as contributing factors in the development of several diseases, especially cancer. Therefore, they have been postulated to be new drug targets with high therapeutic potential. Here, we review histone demethylases with a special focus on their potential role in oncology drug discovery. We present an overview over the different classes of enzymes, their biochemistry, selected data on their role in physiology and already available inhibitors. (C) 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.