Yang *et al.*
European Journal of Medical Research (2025) 30:458
https://doi.org/10.1186/s40001-025-02735-9

European Journal of Medical Research

REVIEW

Open Access

Biological interpretation of DNA/RNA modification by ALKBH proteins and their role in human cancer

Siqi Yang[1], Jixiang Xing[1], Yancheng Song[1], Jiafu Cui[1], Lingling Bao[1], Qilemuge Xi[1*] and Yongchun Zuo[1*]

Abstract

AlkB homolog (ALKBH) belongs to 2-oxoglutarate (2OG) and ferrous iron-dependent oxygenases, which can catalyze the demethylation of DNA/RNA and play an important role in human diseases represented by cancer. Although ALKBH has been extensively studied, the biological explanation for its specific recognition of DNA/RNA and its mechanism of action in cancer remains unclear. This review explores the molecular mechanism of ALKBH protein binding to DNA/RNA through structural fitting and ChIP-Seq analysis and summarizes the structural biology factors that lead to its specific substrate recognition. Furthermore, the expression of ALKBH protein across different tissues is systematically compiled, with a tissue-specific expression map generated for 15 organs and 50 tissues. These maps provide valuable insights into the protein's function in various physiological contexts. Additionally, this review systematically summarizes the regulatory role of the ALKBH protein in the occurrence and development of cancer. It highlights the protein's involvement in key signaling pathways, including Wnt, AKT, AMPK, NF-κB, Hippo, and Notch, revealing its potential for application in early diagnosis and targeted cancer therapy. Overall, this review comprehensively expounds the biological functions of the ALKBH protein and its important role in cancer, which has important theoretical value and application prospects.

Keywords Structural topology, Omics analysis, DNA/RNA recognition, Tissue-specific expression, Signaling pathways

Introduction

ALKBH protein has nine homologous enzymes (ALKBH 1–8 and FTO). In 1983, the AlkB gene was discovered by Sekiguhi et al. in a mutant of *Escherichia coli* [1]. In 1996, bioinformatics discovered the first mammalian ALKBH homolog [2]. Despite a lot of efforts, the function of the AlkB gene has not been determined. It was not until 2001 that Aravind and Koonin identified AlkB as a member of the 2OG-Fe (II) oxygenase superfamily through sequence profile analysis and indicated that it might be involved in RNA demethylation [3]. This greatly promoted the study of the ALKB family. In 2002 and 2003, ALKBH2-3 and ALKBH4-8 were identified, respectively [4, 5]. With the addition of the FTO gene associated with obesity, members of the ALKB family were identified [6]. The discovery history of the ALKBH protein is shown (Fig. 1).

Diverse substrate recognition is a prominent feature of the ALKB family and a classic research topic in this field. ALKBH has a catalytic domain with a double-stranded β-helix (DSBH; a.k.a. jelly-roll, cumin, or jumonjiC) as the core. The DSBH domain consists of two layers of β-sheets and four antiparallel β-sheets, which can play a role in the histone demethylation mechanism. The distribution location and binding substrates of ALKBH

*Correspondence:
Qilemuge Xi
qlmgxi@imu.edu.cn
Yongchun Zuo
yczuo@imu.edu.cn
[1] State Key Laboratory of Reproductive Regulation and Breeding of Grassland Livestock, Institutes of Biomedical Sciences, College of Life Sciences, Inner Mongolia University, Hohhot 010021, China

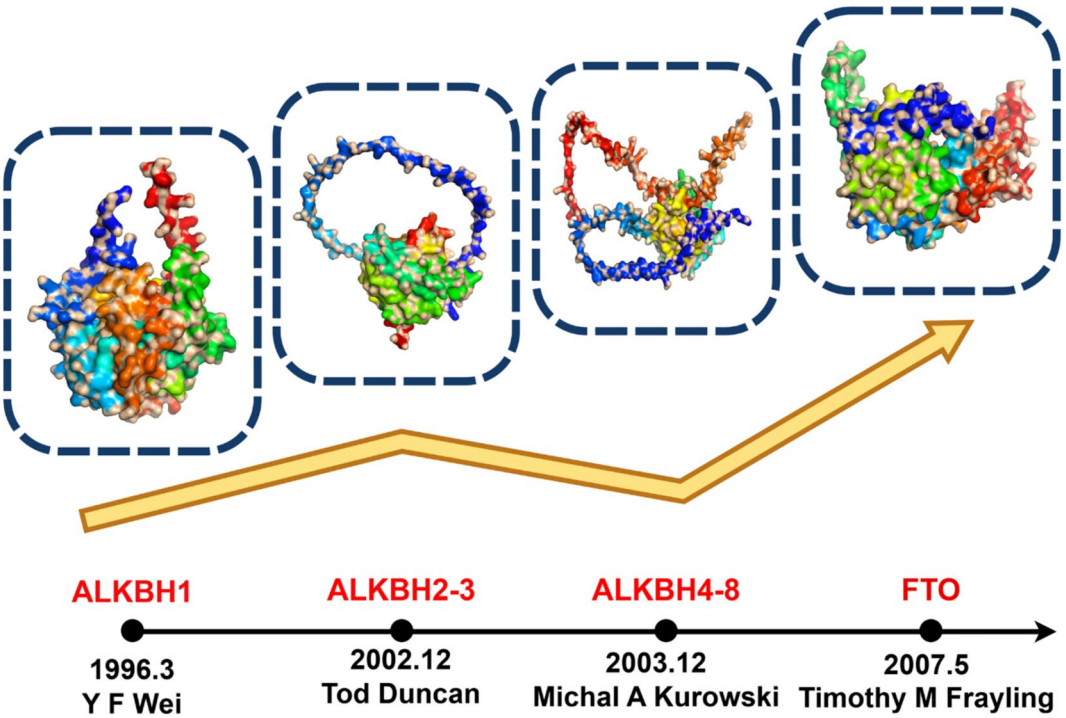

Fig.1 Time and person who discovered the ALKBH protein

Table 1 Distribution and binding sites of ALKBH protein

Protein name	Location	Binding substrate
ALKBH1	Nucleus, mitochondrion	tRNA, ssDNA, dsDNA, mRNA
ALKBH2	Nucleus	dsDNA
ALKBH3	Nucleus, cytoplasm	ssDNA, mRNA
ALKBH4	Nucleus, cytoplasm, midbody	Actin Lys-84
ALKBH5	Nucleus	mRNA
ALKBH6	Nucleus, cytoplasm	bulge DNA, ssDNA, RNA
ALKBH7	Mitochondrion	Self-hydroxylate Leu-110,polycistronic RNA
ALKBH8	Nucleus, cytoplasm	tRNA
FTO	Nucleus	snDNA, mRNA, tRNA

protein are shown (Table 1). ALKBH6 has catalytic activity on various substrates. ALKBH1-3 mainly binds single-stranded or double-stranded DNA and RNA [7–10]. ALKBH4 catalyzes actin demethylation [11]. ALKBH5 and FTO mainly act on mRNA and are considered "erasers" of m6 A [12–14]. ALKBH7 works on proteins and RNA [15, 16]. ALKBH8 catalyzes tRNA modification [17, 18].

As a class of Fe (II)/α-ketoglutarate-dependent dioxygenases, the ALKBH protein family is widely involved in the removal of epigenetic modifications and plays a vital role in maintaining genome stability and affecting the occurrence and development of tumors [19, 20]. The enzymatic activity of ALKBH1 on various substrates such as m6 A, m1 A, m3 C, m5 C, N6-mA, and H2 A is currently a hot topic of research [21, 22]. The functions of ALKBH5 and FTO for m6 A demethylation have been widely reported [23]. Zhao et al.'s study on pancreatic cancer showed that ALKBH6 is essential for maintaining genome stability [24]. FTO can inhibit osteoblast differentiation and function of glycosylated bone marrow mesenchymal stem cells [25]. The signaling pathway regulated by the ALKBH protein plays a key role in a variety of human diseases, such as gastric cancer, lung cancer, and pancreatic cancer, which is closely related to its catalytic function [24, 26–28]. It is a hot topic of current research.

This review delves into the intrinsic mechanisms behind ALKBH's specific recognition of RNA, drawing on structural biology and omics analyses. It outlines the structural and biological factors that govern ALKBH's differential recognition of DNA and RNA, examines its tissue-specific expression, and clarifies its regulatory role in the onset and progression of cancer. Ultimately, it provides a theoretical foundation for understanding the ALKBH protein's specific recognition function and its pivotal role in cellular physiological processes and disease development.

Motif composition of ALKBH protein binding to DNA/RNA

The reasons why ALKBH proteins bind specifically to DNA/RNA were explored at both the gene and protein levels. At the gene level, due to data limitations, we only analyzed ChIP-Seq of ALKBH1/3/5 and FTO (Fig. 2A). One of the canonical functions of ALKBH5 and FTO is to demethylate m6 A, which is a common modification in mRNA and noncoding RNA [29]. Abnormal regulation of m6 A modification plays a key role in the occurrence and development of many diseases, including preeclampsia, thyroid cancer, gastric cancer, and heart failure [30–33]. Early studies have shown that a large number of m6 A modifications are located in the RRACH motif (R,A/G; H, A/C/U) [34]. The raw data of ALKBH1, ALKBH3, ALKBH5, and FTO were obtained from the National Center for Biotechnology Information (NCBI) with accession numbers GSM3357602, GSM1385219, GSM4287404, and GSM271942, respectively.

The study by Kaur et al. in 2022 demonstrated the preference of ALKBH5 for substrates containing the (A/G) m6 AC consensus sequence motif [35]. Li et al.'s study in 2019 showed that FTO binds to GAC and RRACH motifs in m6 A-modified mRNA/lncRNA in a cell type-dependent manner [36]. Consistent with previous studies, from our ChIP-Seq results, whether it is ALKBH5 or FTO, their sequence centers are composed of a motif with A as the core. Other motifs of the two also have similar features, such as continuous U/A bases. We speculate that these features may provide a genetic basis for their specific recognition of m6 A. Furthermore, we present the ChIP-Seq analysis results of ALKBH1 and ALKBH3, which will guide experimental disciplines in deciphering the identified motifs.

At the structural level, we mapped the full-length structural evolution of ALKBH proteins (Fig. 2B). Consistent with previous studies, 10 distinct β-folds constitute the basic skeleton of ALKBH proteins [9] (Fig. 2C). At the same time, β3-β10 is defined as the DSBH domain in traditional studies [9]. The more positively charged arrangement is a significant feature shared by the catalytic cavity of ALKBH proteins (Fig. 2D). Based on the principle of physics that like charges repel and opposite charges attract, and combined with Chao et al.'s earlier studies on DNA-binding TET proteins, which pointed out that the pronounced positive charge creates conditions for DNA binding, we speculate that the widespread positive charge on the surface of the ALKBH protein is one of the reasons for its ability to bind to DNA/RNA [37–40].

In addition to the above factors, the protein's spatial distribution, the role of co-chaperone proteins, and post-translational modifications may also influence substrate binding. The cellular distribution of FTO varies across different cell lines, which affects its interaction with different RNA substrates [41]. The interaction between ALKBH8 and the small auxiliary protein TRM112 is essential for forming a functional tRNA methyltransferase [42]. Reactive oxygen species (ROS) can promote the SUMOylation of ALKBH5 by activating the ERK/JNK signaling pathway, inhibiting ALKBH5's m6 A

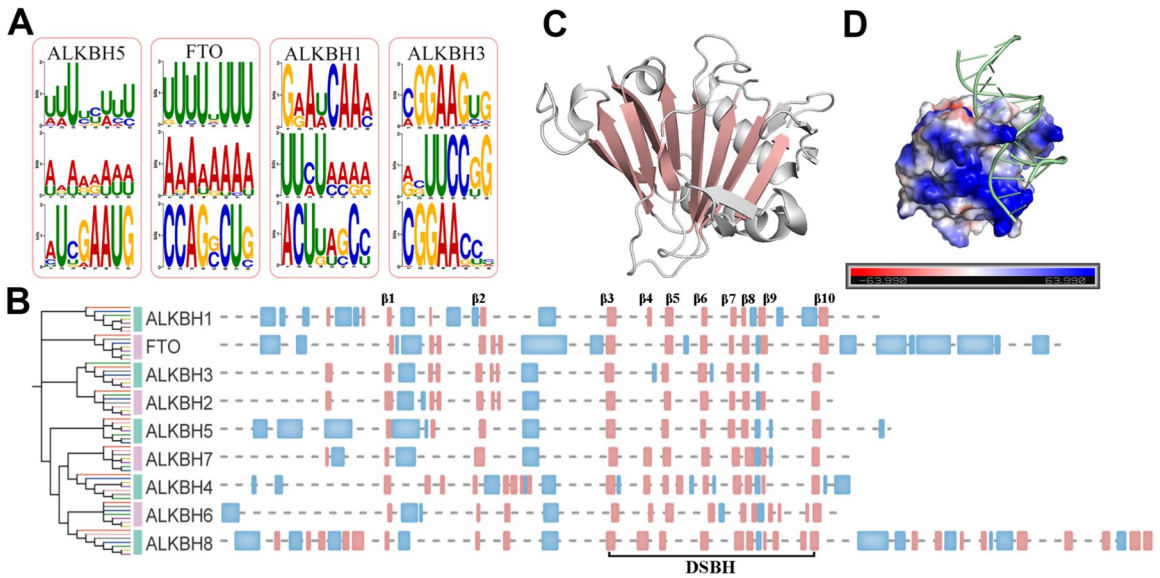

Fig. 2 Motif composition of ALKBH protein binding to DNA/RNA. **A** ChIP-Seq profiles of ALKBH1/3/5 and FTO. **B** Full-length structural arrangement of ALKBH protein. Sky blue: α-helix, Pink: β-sheet. **C** Common binding cavity of ALKBH protein, example protein ALKBH2 (PDB:3s57). Pink: β-sheet. **D** Potential diagram of the ALKBH protein binding cavity, example protein ALKBH2 (PDB:3s57). Blue: positive charge, red: negative charge

demethylase activity by blocking substrate accessibility [43].

Structural preference of ALKBH proteins for differential recognition of DNA and RNA

After summarizing and sorting, we divide the recognition specificity of ALKBH into three categories: (1) prefer to recognize DNA; (2) preferred to recognize RNA, and (3) good recognition ability for DNA,RNA or protein. The first category includes ALKBH2. The second category includes ALKBH5/7/8 and FTO. The third group consists of ALKBH1/3/4/6. Whether these protein members with similar binding preferences share a unified structural mechanism remains unclear. This is mainly because the current studies are still fragmented and lack evidence of broad structural commonality. Therefore, we have summarized the structural biology principles of each member separately as follows (Table 2 and Fig. 3).

ALKBH2 prefers to bind dsDNA. ALKBH2's F102 can be inserted into double-strand stacks and fill the DNA gap, which is an important reason why ALKBH2 can bind dsDNA (Fig. 3A) [44]. In addition, Gly204 and Lys205 of ALKBH2 bind to complementary strands of the methylated strand, which also contributes to ALKBH2 binding to dsDNA (Fig. 3A) [44, 45].

The size of the binding region and the role of key amino acids affect RNA recognition by ALKBH5 and FTO. The tendency of ALKBH7 and ALKBH8 to bind RNA is very prominent. Studies have shown that ALKBH7 does not show any demethylation activity against m1 A and m3 C damage in ssDNA/dsDNA, closely related to its structure [46, 47]. Studies on the binding of ALKBH7 to RNA are mostly speculative and require further structural biology investigations [46, 48]. ALKBH8 consists of the N-terminal RNA recognition motif (RRM) domain, the C-terminal methyltransferase (MTase) domain, and its central

Table 2 Binding site and substrate information for ALKBH6

Protein name	PDB/ALPHAFold	Binding site	Substrate
ALKBH2	3s57	F102	dsDNA
ALKBH8	AF-Q96BT7-F1	RRM domain	tRNA
ALKBH1	6KSF	R24 and R159/tRNA binding domain	dsDNA/tRNA
ALKBH6	7VJV	E139-P158	bulge DNA, ssDNA, RNA

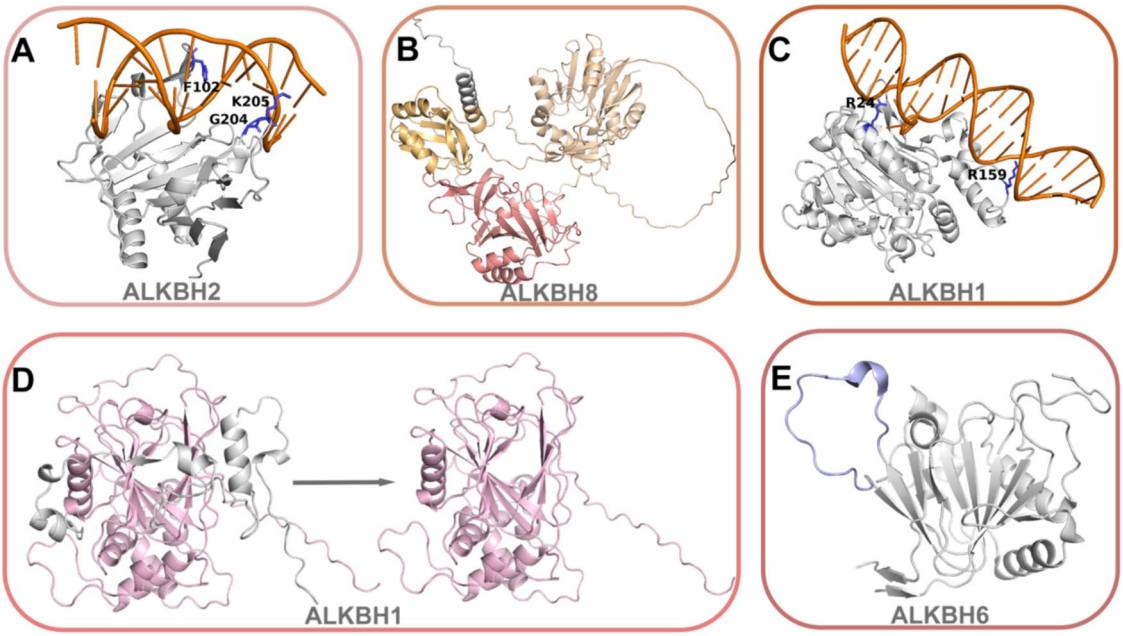

Fig. 3 Structural preference of ALKBH proteins for differential recognition of DNA and RNA. **A** ALKBH2 (PDB:3 s57) key amino acid for dsDNA binding. **B** Distribution of structural domains of ALKBH8 (AF-Q96BT7-F1). Dark yellow structure on the left: RRM. Rose-pink: alkB-like domain. Orange-yellow structure on the right: MTase. **C** Key amino acid for ALKBH1 (PDB:6 KSF) binding to dsDNA. **D** Regions of ALKBH1 (AF-Q13686-F1) binding tRNA. Pink: binding region. **E** Structure of ALKBH6 (PDB:7 VJV). Purple: combined with loop

AlkB-like domain (Fig. 3B) [49]. The RRM domain is connected to the AlkB domain through an ordered structure, maintaining the geometric invariance of the interaction between the two domains, which helps regulate tRNA's binding and catalytic activity. Both the AlkB-like domain and the MTase domain can catalyze covalent super modification of swinging nucleotide bases in specific tRNA anticodon loops [17].

Two arginine residues, R24 and R159 of ALKBH1, can be inserted into small grooves of DNA from both ends as two "fingers" to fix DNA substrates (Fig. 3C). This recognition pattern may help locate DNA substrates so that the flipped bases are properly inserted into the active pocket [10]. Additionally, ALKBH1 has a tRNA binding domain that eukaryotic tRNA ligase uses to identify tRNA (Fig. 3D) [50–52].

Xu et al. showed that the narrow binding region and the blocking effect of amino acids at some positions were responsible for the binding of ALKBH3, ALKBH5, and FTO to single-stranded nucleic acids such as mRNA and ssDNA [16]. ALKBH6 binds to ssDNA, foamy DNA, highlighted DNA, and RNA, but not dsDNA. Chen et al. proposed that the loop region of E139-P158 acts as a barrier to prevent the entry of paired double-stranded nucleic acids into the active site, a unique property that allows ALKBH6 to differentiate between nucleic acids (Fig. 3E) [45]. Since the structure of ALKBH4 has not been resolved, the reason for its specific identification of substrates is still unclear.

The above summary highlights that, beyond the conserved dioxygenase catalytic core, subtle structural features play a critical role in determining substrate specificity. However, due to the limited number of resolved protein–nucleic acid complex structures, a unified model of substrate recognition across the ALKBH family is still lacking. In particular, it remains challenging to systematically analyze the recognition differences among ssDNA, dsDNA, and RNA substrates. We believe that future structural and biochemical investigations will be essential to advance our understanding of these differential recognition mechanisms.

Tissue-specific expression analysis of ALKBH

ALKBH proteins play a role in many biological processes. Tissue-specific expression data were obtained from The Human Protein Atlas (https://www.proteinatlas.org/). We compiled the specific expression profiles of ALKBH proteins in 50 tissues of 15 organs (Fig. 4). It can be seen that ALKBH5, ALKBH7, and FTO have higher relative expression levels in each tissue than other members

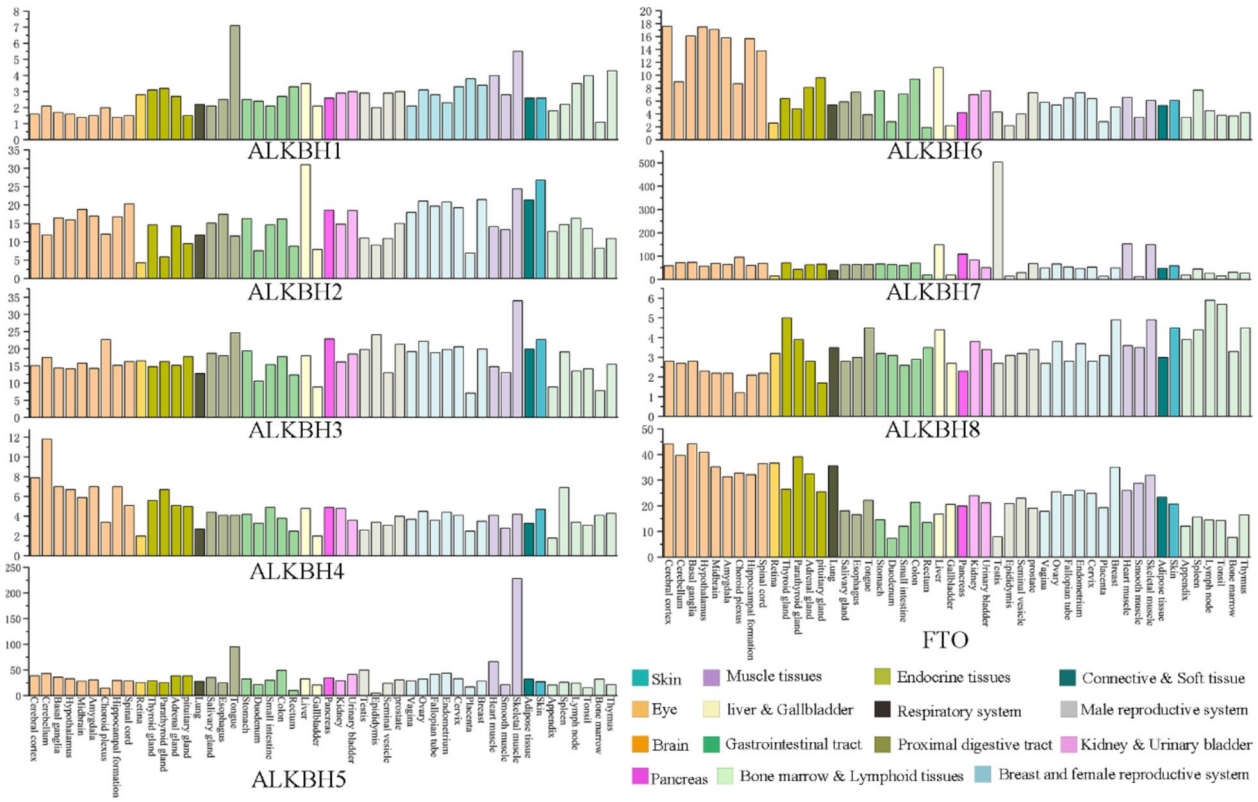

Fig. 4 Tissue-specific expression profile of ALKBH protein. The horizontal axis represents different organizations, and the vertical axis unit is nTPM

(Fig. 4). In fact, ALKBH5 and ALKBH7 have been studied more deeply than other members. ALKBH5 has been reported in articles related to diseases such as the pancreas, stomach, colorectum, and liver, where it is highly expressed [53–56]. FTO has also been widely studied, especially in the endometrium, ovary, prostate, and stomach, where it is highly expressed [57–60]. Research on ALKBH7 started relatively late but has made rapid progress. In 2022, Chen et al.'s pan-cancer analysis revealed that ALKBH7 was highly expressed in 17 cancers and lowly expressed in 5 cancers, indicating that ALKBH7 may serve as a potential prognostic pan-cancer biomarker [61].

ALKBH1 is high in skeletal muscle. It may be a potential target for the treatment of osteoporosis [62]. ALKBH2 primarily functions in the development and progression of colorectal cancer, lung cancer, and bladder cancer [63–65]. Since ALKBH3's tRNA demethylation promotes protein synthesis in cancer cells, it has also been widely considered [27]. There are fewer studies on ALKBH4 and ALKBH6, which are believed to play a role in pancreatic cancer and lung cancer, gastric cancer, and colorectal cancer [66–68]. Although the expression level of ALKBH 8 in brain tissue is not high, its function is important. Its abnormal expression can lead to human neurodevelopmental disorders [69]. In addition, high expression of ALKBH8 is believed to promote the occurrence of human bladder cancer [70].

Regulatory pathways of ALKBH protein in cancer

The mechanisms of ALKBH protein regulating cancer occurrence in a total of 7 signaling pathways, including Wnt, AKT, TGF-β, AMPK, NF-κB, Hippo, and Notch, have been summarized (Table 3 and Fig. 5). Compared with ALKBH1/2/5 and FTO, the research on other members is relatively lacking.

Wnt

Gemcitabine (GEM) is a first-line chemotherapy drug approved for the treatment of advanced pancreatic cancer (PC). SH3BP5-AS1 plays an important role in PC resistance to GEM, and its expression is positively correlated with PC resistance to GEM. SH3BP5-AS1 stability is regulated by ALKBH5/IGF2BP1-mediated m6 A modification. Low expression of ALKBH5 increases the m6 A modification level of SH3BP5-AS1, which further promotes the recognition of SH3BP5-AS1 by the m6 A recognition protein IGF2BP1, thereby promoting the expression of SH3BP5-AS1. SH3BP5-AS1 activates the Wnt signaling pathway through sponge miR-139-5p, upregulates the expression of CTBP1, and promotes GEM resistance [71].

In gastric cancer, increased ALKBH5 and decreased m6 A modification levels may lead to activation of the Wnt/PI3 K-Akt signaling pathway, thereby promoting the malignant phenotype of gastric cancer. This suggests that ALKBH5 may play an important role in the occurrence and development of gastric cancer [72]. AXIN2, a Wnt suppressor, was identified as a target of ALKBH5. ALKBH5 binds and demethylates AXIN2 messenger RNA, leading to its dissociation and degradation from the N+ +6-methyladenosine reader IGF2BP1, thereby overactivating Wnt/β-catenin. Subsequently, ALKBH5-induced Wnt/β-catenin targets,

Table 3 Roles of ALKBH-associated signaling pathways in cancer

Protein	Factor	Signaling pathways	Cancer
ALKBH5	SH3BP5-AS1	Wnt	Pancreatic cancer
ALKBH5	m6 A	Wnt/PI3 K-Akt	Gastric cancer
ALKBH5	AXIN2 falls	Wnt/β-catenin	Colorectal cancer
FTO	HOXB13	WNT	Endometrial cancer
FTO	lncRNA HOXC13-AS	WNT	Cervical cancer
ALKBH5	KCNK15-AS1	AKT	Pancreatic cancer
ALKBH5	PAQR4	PI3 K/AKT	Hepatocellular carcinoma
FTO	FLOT2	PI3 K/Akt/mTOR	Diffuse large B-cell lymphoma
FTO	–	AKT	Ovarian cancer
FTO	miR-139-5p/ZNF217	PI3 K/Akt/mTOR	Prostate cancer
FTO	MOXD1	TGF-β	Gastric cancer
ALKBH1	NRF1	AMPK	Gastric cancer
ALKBH2	BMI1	NF-κB	Colorectal cancer
ALKBH5	TLR4	NF-κB	Ovarian cancer
ALKBH5	TRAF1	NF-κB	Myeloma
FTO	MOXD1 mRNA	MAPK, TGF-β, NOTCH and JAK/STA	Gastric cancer

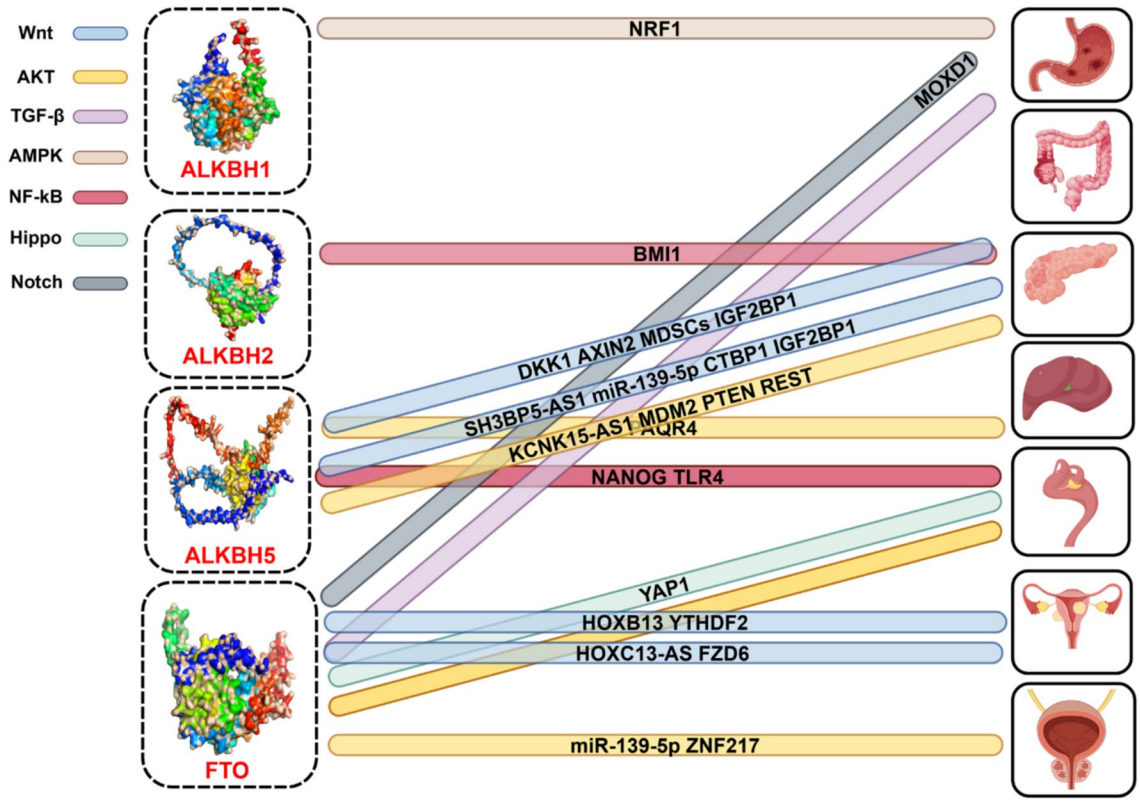

Fig. 5 Regulatory pathways of ALKBH protein in cancer. Different pathways are represented by different colors, and core factors are labeled

including Dickkopf-related protein 1 (DKK1), were activated. ALKBH5-induced DKK1 recruited myeloid-derived suppressor cells (MDSCs) to drive immunosuppression in colorectal cancer (CRC), an effect that could be blocked by anti-DKK1 in vitro and in vivo. Finally, vesicle-like nanoparticles encapsulating ALKBH5 small interfering RNA or anti-DKK1 enhanced anti-PD-1 therapy and inhibited CRC growth [73].

The mRNA level of FTO is increased in cervical squamous cell carcinoma (CSCC) tissues compared with corresponding adjacent normal tissues [74]. FTO can catalyze the demethylation modification of the 3'UTR region of HOXB13 mRNA, thereby eliminating the recognition of m6 A modification by YTHDF2 protein. Reduced HOXB13 mRNA decay and increased HOXB13 protein expression are accompanied by activation of the WNT signaling pathway and expression of downstream proteins, leading to endometrial cancer metastasis and invasion [75]. FTO-stabilized lncRNA HOXC13-AS epigenetically upregulated FZD6 and activated Wnt/β-catenin signaling to drive cervical cancer proliferation, invasion, and EMT [76].

PTEN/AKT

Upregulation of ALKBH5 may inhibit the progression of pancreatic cancer by increasing the stability and function of the K member 15 and WISP2 antisense RNA 1 (KCNK15-AS1) factor, which can inhibit pancreatic cancer. Upregulation of ALKBH5 leads to a decrease in the m6 A modification level of KCNK15-AS1, which may increase the stability of KCNK15-AS1. KCNK15-AS1 promotes the ubiquitination of RE1 silencing transcription factor (REST) by recruiting MDM2 proto-oncogene (MDM2) protein, thereby upregulating phosphatase and tensin homolog (PTEN) at the transcriptional level, thereby inhibiting the AKT signaling pathway and inhibiting the migration and invasion of PC cells [77]. ALKBH5 downregulates progestin and AdipoQ Receptor 4 (PAQR4) expression in an m6 A-dependent manner, thereby inhibiting PI3 K/AKT pathway activation and suppressing hepatocellular carcinoma (HCC) growth [78].

FTO-mediated m6 A demethylation upregulates FLOT2 to activate the downstream PI3 K/Akt/mTOR signaling pathway, leading to the aggressiveness of diffuse

large B-cell lymphoma (DLBCL) [79]. FTO overexpression promotes AKT phosphorylation and significantly increases the viability and autophagy of ovarian cancer cells [80]. FTO inactivates the tumor-accelerated PI3K/AKT/mTOR pathway by regulating the downstream miR-139-5p/ZNF217 axis to hinder prostate cancer development [81]. FTO targets MOXD1 mRNA and promotes its expression through m6 A methylation. MOXD1 upregulation activates the cancer-related signaling pathway TGF-β and promotes the occurrence of gastric cancer [82].

AMPK and NF-κB

ALKBH1-induced 6 mA demethylation inhibits NRF1-driven transcription of downstream targets, including multiple genes involved in the AMPK signaling pathway. ALKBH1 inhibits AMPK signaling, leading to a metabolic shift toward the Warburg effect, thereby promoting gastric carcinogenesis [83].

ALKBH2 inhibition alleviates malignancy in colorectal cancer by regulating BMI1-mediated activation of the NF-κB pathway [64]. TLR4 activates the NF-κB pathway, upregulates ALKBH5 expression, increases m6 A levels and NANOG expression, all of which contribute to ovarian carcinogenesis [84]. ALKBH5 regulates TRAF1 expression by reducing the m6 A abundance in the 3′ untranslated region (3′-UTR) of TRAF1 transcripts, activates NF-κB, and promotes the growth and survival of multiple myeloma cells [85].

Hippo and Notch

ALKBH5 can affect the occurrence of esophageal cancer and glioblastoma by affecting the Hippo pathway [86]. YAP1 is a major downstream target of the Hippo signaling pathway and is negatively regulated by it. The overexpression of FTO disrupts the Hippo signaling pathway, reduces YAP1 phosphorylation, subsequently increasing YAP1 translocation to the nucleus, and promotes the proliferation of human ovarian granulosa cells [87].

FTO targets MOXD1 mRNA and promotes its expression through m6 A methylation, which activates cancer-related signaling pathways (MAPK, TGF-β, NOTCH, and JAK/STAT) and promotes the progression of gastric cancer [82].

It is worth noting that in the above review of signaling pathways, we can observe some seemingly contradictory phenomena. For example, in the Wnt pathway, the downregulation of ALKBH5 activates the Wnt signal in prostate cancer, while its upregulation also activates the Wnt signal in gastric cancer. Similarly, in the PTEN/AKT pathway, overexpression of FTO activates the AKT signal in diffuse large B-cell lymphoma but inhibits the AKT signal in prostate cancer. These phenomena may be closely related to epigenetic changes and interactions with other regulatory factors. We hypothesize that this may be due to the regulation of protein expression by epigenetic or post-translational modifications in different cellular environments and cancer types, leading to opposing biological effects in different tissues or cancer models.

Conclusion

Since the first ALKB gene was discovered, more than 40 years of research have passed. People have conducted a lot of exploration and made many progress in this field. The structure and catalytic mechanism of the ALKBH protein have been gradually resolved. However, the reason for its specific recognition of DNA/RNA is still not completely clear.

This review investigates the molecular mechanisms underlying ALKBH protein's binding to DNA/RNA through structural fitting and ChIP-Seq analysis, summarizes the structural and biological factors that contribute to its differential recognition of DNA and RNA, examines the expression of ALKBH protein across various tissues, and discusses its regulatory role in the initiation and progression of cancer. These insights will enhance our understanding of the ALKBH protein's specific recognition function and its involvement in diverse cellular physiological processes and disease development.

Notably, AlphaFold has supplemented structural data for ALKBH protein monomers, and experimental complex structures of several ALKBH family members have also been resolved [88]. However, the structure of ALKBH4 in complex with its binding partners remains experimentally uncharacterized, and its precise conformation and functional mechanisms require further investigation. In addition, the experimental structure of ALKBH6 was resolved relatively late, and its related research is still relatively limited, more biological studies are needed to reveal its specific role in cellular processes [45]. Meanwhile, the relevant omics data for ALKBH2, ALKBH4, ALKBH6, ALKBH7, and ALKBH8 still require further supplementation and refinement.

At present, high-resolution X-ray crystal diffraction experiments are still one of the core means to analyze the structure of protein–nucleic acid complexes, which is of great significance for verifying predicted structures and discovering key interaction interfaces [89]. However, some ALKBH family members have poor stability when binding to substrates or only form transient complexes under specific physiological conditions, which makes traditional crystallographic methods face certain challenges. Therefore, alternative structural biology techniques such as cryo-electron microscopy (cryo-EM) and nuclear magnetic resonance (NMR) are also urgently needed to

capture the structural state of these dynamic complexes [90, 91]. At the omics level, existing studies have focused on the regulation of m6 A modification at the transcriptome and epitranscriptome levels, but systematic identification of ALKBH protein targets is still insufficient.

Future studies can combine single-cell sequencing, multi-omics integrated analysis (such as transcriptome, epigenetic group, proteome, and methylation group), and CRISPR–Cas9-mediated functional screening to further reveal the specific mechanism of action of ALKBH protein in different tissues and disease states [92, 93]. In addition, most of the current data are derived from in vitro models and lack validation in clinical samples. In the future, more primary sample studies derived from patient tissues are needed to enhance the clinical relevance of the conclusions.

Author contributions
All authors listed have made a substantial and intellectual contribution to the work and approved it for publication. Q.X. and Y.Z. Conceptualization. S.Y. Visualization, Formal analysis, Writing—Original Draft. J.X., Y.S., J.C., and L.B. Writing—Review & Editing. All authors commented on previous versions of the manuscript.

Funding
This research was funded by the National Natural Scientific Foundation of China (62171241, 62461046), the Group Project of Developing Inner Mongolia through Talents (2025TEL25), the China Postdoctoral Science Foundation under Grant Number (2024MD763987).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 14 April 2025 Accepted: 28 May 2025
Published online: 06 June 2025

References

1. Kataoka H, Yamamoto Y, Sekiguchi M. A new gene (alkB) of *Escherichia coli* that controls sensitivity to methyl methane sulfonate. J Bacteriol. 1983;153(3):1301–7.
2. Wei YF, Carter KC, Wang RP, Shell BK. Molecular cloning and functional analysis of a human cDNA encoding an *Escherichia coli* AlkB homolog, a protein involved in DNA alkylation damage repair. Nucleic Acids Res. 1996;24(5):931–7.
3. Aravind L, Koonin EV. The DNA-repair protein AlkB, EGL-9, and leprecan define new families of 2-oxoglutarate- and iron-dependent dioxygenases. Genome Biol. 2001;2(3):Research0007.
4. Duncan T, Trewick SC, Koivisto P, Bates PA, Lindahl T, Sedgwick B. Reversal of DNA alkylation damage by two human dioxygenases. Proc Natl Acad Sci U S A. 2002;99(26):16660–5.
5. Kurowski MA, Bhagwat AS, Papaj G, Bujnicki JM. Phylogenomic identification of five new human homologs of the DNA repair enzyme AlkB. BMC Genomics. 2003;4(1):48.
6. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007;316(5826):889–94.
7. Fu D, Samson LD. Direct repair of 3, N(4)-ethenocytosine by the human ALKBH2 dioxygenase is blocked by the AAG/MPG glycosylase. DNA Repair (Amst). 2012;11(1):46–52.
8. Liefke R, Windhof-Jaidhauser IM, Gaedcke J, Salinas-Riester G, Wu F, Ghadimi M, et al. The oxidative demethylase ALKBH3 marks hyperactive gene promoters in human cancer cells. Genome Med. 2015;7(1):66.
9. Yang S, Xing J, Liu D, Song Y, Yu H, Xu S, et al. Review and new insights into the catalytic structural domains of the Fe(II) and 2-Oxoglutarate families. Int J Biol Macromol. 2024;278(Pt 4): 134798.
10. Zhang M, Yang S, Nelakanti R, Zhao W, Liu G, Li Z, et al. Mammalian ALKBH1 serves as an N(6)-mA demethylase of unpairing DNA. Cell Res. 2020;30(3):197–210.
11. Yu K, Qi TF, Miao W, Liu X, Wang Y. Quantitative proteomics revealed new functions of ALKBH4. Proteomics. 2022;22(7): e2100231.
12. Huang Y, Su R, Sheng Y, Dong L, Dong Z, Xu H, et al. Small-molecule targeting of oncogenic FTO demethylase in acute myeloid leukemia. Cancer Cell. 2019;35(4):677-91.e10.
13. Qu J, Yan H, Hou Y, Cao W, Liu Y, Zhang E, et al. RNA demethylase ALKBH5 in cancer: from mechanisms to therapeutic potential. J Hematol Oncol. 2022;15(1):8.
14. Ye G, Li J, Yu W, Xie Z, Zheng G, Liu W, et al. ALKBH5 facilitates CYP1B1 mRNA degradation via m6A demethylation to alleviate MSC senescence and osteoarthritis progression. Exp Mol Med. 2023;55(8):1743–56.
15. Wang G, He Q, Feng C, Liu Y, Deng Z, Qi X, et al. The atomic resolution structure of human AlkB homolog 7 (ALKBH7), a key protein for programmed necrosis and fat metabolism. J Biol Chem. 2014;289(40):27924–36.
16. Xu B, Liu D, Wang Z, Tian R, Zuo Y. Multi-substrate selectivity based on key loops and non-homologous domains: new insight into ALKBH family. Cell Mol Life Sci. 2021;78(1):129–41.
17. Fu D, Brophy JA, Chan CT, Atmore KA, Begley U, Paules RS, et al. Human AlkB homolog ABH8 Is a tRNA methyltransferase required for wobble uridine modification and DNA damage survival. Mol Cell Biol. 2010;30(10):2449–59.
18. Zdżalik D, Vågbø CB, Kirpekar F, Davydova E, Puścian A, Maciejewska AM, et al. Protozoan ALKBH8 oxygenases display both DNA repair and tRNA modification activities. PLoS ONE. 2014;9(6): e98729.
19. Li H, Zhang Y, Guo Y, Liu R, Yu Q, Gong L, et al. ALKBH1 promotes lung cancer by regulating m6A RNA demethylation. Biochem Pharmacol. 2021;189: 114284.
20. Schmidl D, Lindlar Né Jonasson NSW, Menke A, Schneider S, Daumann LJ. Spectroscopic and in vitro investigations of Fe(2+) /α-ketoglutarate-dependent enzymes involved in nucleic acid repair and modification. ChemBioChem. 2022;23(11):e202100605.
21. Xiong L, Li F, Guo Y, Zhang J, Xu K, Xiong Z, et al. Discovery of a potent and cell-active inhibitor of DNA 6mA demethylase ALKBH1. J Am Chem Soc. 2024;146(10):6992–7006.
22. Zhong J, Xu Z, Ding N, Wang Y, Chen W. The biological function of demethylase ALKBH1 and its role in human diseases. Heliyon. 2024;10(13): e33489.
23. Zhou C, Zhang Y, Shi SM, Yin D, Li XD, Shi YH, et al. FTO downregulation-mediated m6A modification resulting in enhanced hepatocellular carcinoma invasion. Cell Biosci. 2025;15(1):58.
24. Zhao S, Devega R, Francois A, Kidane D. Human ALKBH6 is required for maintenance of genomic stability and promoting cell survival during exposure of alkylating agents in pancreatic cancer. Front Genet. 2021;12: 635808.
25. Feng L, Zhao W, Fan Y, Yuan C, Zhang X. RNA N6-methyladenosine demethylase FTO inhibits glucocorticoid-induced osteoblast differentiation and function in bone marrow mesenchymal stem cells. J Cell Biochem. 2023;124(11):1835–47.
26. Hu Y, Gong C, Li Z, Liu J, Chen Y, Huang Y, et al. Demethylase ALKBH5 suppresses invasion of gastric cancer via PKMYT1 m6A modification. Mol Cancer. 2022;21(1):34.

27. Ueda Y, Ooshio I, Fusamae Y, Kitae K, Kawaguchi M, Jingushi K, et al. AlkB homolog 3-mediated tRNA demethylation promotes protein synthesis in cancer cells. Sci Rep. 2017;7:42271.
28. Wang L, Feng X, Jiao Z, Gan J, Meng Q. Characterization of the prognostic and diagnostic values of ALKBH family members in non-small cell lung cancer. Pathol Res Pract. 2022;231: 153809.
29. Smolin EA, Buyan AI, Lyabin DN, Kulakovskiy IV, Eliseeva IA. RNA-Seq data of ALKBH5 and FTO double knockout HEK293T human cells. Data Brief. 2022;42: 108187.
30. Wang J, Zhao G, Zhao Y, Zhao Z, Yang S, Zhou A, et al. N(6)-methylation in the development, diagnosis, and treatment of gastric cancer. J Transl Int Med. 2024;12(1):5–21.
31. Xu X, Zhao J, Yang M, Han L, Yuan X, Chi W, et al. The emerging roles of N6-methyladenosine RNA modifications in thyroid cancer. Eur J Med Res. 2023;28(1):475.
32. Yang Y, Muisha MB, Zhang J, Sun Y, Li Z. Research progress on N(6)-adenosylate methylation RNA modification in heart failure remodeling. J Transl Int Med. 2022;10(4):340–8.
33. Zhang Y, Guo X, Chen Z, Guo R. Low m6A modification-mediated upregulation of PLAC8 promotes trophoblast cell invasion and migration in preeclampsia. Eur J Med Res. 2023;28(1):466.
34. Imanishi M. Mechanisms and strategies for determining m(6) A RNA modification sites by natural and engineered m(6) a effector proteins. Chem Asian J. 2022;17(16): e202200367.
35. Kaur S, Tam NY, McDonough MA, Schofield CJ, Aik WS. Mechanisms of substrate recognition and N6-methyladenosine demethylation revealed by crystal structures of ALKBH5-RNA complexes. Nucleic Acids Res. 2022;50(7):4148–60.
36. Li Y, Wu K, Quan W, Yu L, Chen S, Cheng C, et al. The dynamics of FTO binding and demethylation from the m(6)A motifs. RNA Biol. 2019;16(9):1179–89.
37. Bigman LS, Levy Y. Protein diffusion on charged biopolymers: DNA versus microtubule. Biophys J. 2020;118(12):3008–18.
38. Chao L, Yang S, Li H, Long C, Xi Q, Zuo Y. Competitive binding of TET1 and DNMT3A/B cooperates the DNA methylation pattern in human embryonic stem cells. Biochim Biophys Acta Gene Regul Mech. 2022;1865(7): 194861.
39. Rajavelu A, Jurkowska RZ, Fritz J, Jeltsch A. Function and disruption of DNA methyltransferase 3a cooperative DNA binding and nucleoprotein filament formation. Nucleic Acids Res. 2012;40(2):569–80.
40. Xu C, Liu K, Lei M, Yang A, Li Y, Hughes TR, et al. DNA sequence recognition of human CXXC domains and their structural determinants. Structure. 2018;26(1):85-95.e3.
41. Wei J, Liu F, Lu Z, Fei Q, Ai Y, He PC, et al. Differential m(6)A, m(6)A(m), and m(1)A demethylation mediated by FTO in the cell nucleus and cytoplasm. Mol Cell. 2018;71(6):973-85.e5.
42. Songe-Møller L, van den Born E, Leihne V, Vågbø CB, Kristoffersen T, Krokan HE, et al. Mammalian ALKBH8 possesses tRNA methyltransferase activity required for the biogenesis of multiple wobble uridine modifications implicated in translational decoding. Mol Cell Biol. 2010;30(7):1814–27.
43. Yu F, Wei J, Cui X, Yu C, Ni W, Bungert J, et al. Post-translational modification of RNA m6A demethylase ALKBH5 regulates ROS-induced DNA damage response. Nucleic Acids Res. 2021;49(10):5779–97.
44. Yang CG, Yi C, Duguid EM, Sullivan CT, Jian X, Rice PA, et al. Crystal structures of DNA/RNA repair enzymes AlkB and ABH2 bound to dsDNA. Nature. 2008;452(7190):961–5.
45. Ma L, Lu H, Tian Z, Yang M, Ma J, Shang G, et al. Structural insights into the interactions and epigenetic functions of human nucleic acid repair protein ALKBH6. J Biol Chem. 2022;298(3): 101671.
46. Fu D, Jordan JJ, Samson LD. Human ALKBH7 is required for alkylation and oxidation-induced programmed necrosis. Genes Dev. 2013;27(10):1089–100.
47. Lee DH, Jin SG, Cai S, Chen Y, Pfeifer GP, O'Connor TR. Repair of methylation damage in DNA and RNA by mammalian AlkB homologues. J Biol Chem. 2005;280(47):39448–59.
48. Zhang LS, Xiong QP, Peña Perez S, Liu C, Wei J, Le C, et al. ALKBH7-mediated demethylation regulates mitochondrial polycistronic RNA processing. Nat Cell Biol. 2021;23(7):684–91.
49. Pastore C, Topalidou I, Forouhar F, Yan AC, Levy M, Hunt JF. Crystal structure and RNA binding properties of the RNA recognition motif (RRM) and AlkB domains in human AlkB homolog 8 (ABH8), an enzyme catalyzing tRNA hypermodification. J Biol Chem. 2012;287(3):2130–43.
50. Ghosh A, Vishveshwara S. A study of communication pathways in methionyl- tRNA synthetase by molecular dynamics simulations and structure network analysis. Proc Natl Acad Sci U S A. 2007;104(40):15711–6.
51. Liu F, Clark W, Luo G, Wang X, Fu Y, Wei J, et al. ALKBH1-mediated tRNA demethylation regulates translation. Cell. 2016;167(3):816-28.e16.
52. Nakanishi K, Ogiso Y, Nakama T, Fukai S, Nureki O. Structural basis for anticodon recognition by methionyl-tRNA synthetase. Nat Struct Mol Biol. 2005;12(10):931–2.
53. Fang Y, Wu X, Gu Y, Shi R, Yu T, Pan Y, et al. LINC00659 cooperated with ALKBH5 to accelerate gastric cancer progression by stabilising JAK1 mRNA in an m(6) A-YTHDF2-dependent manner. Clin Transl Med. 2023;13(3): e1205.
54. He Y, Hu H, Wang Y, Yuan H, Lu Z, Wu P, et al. ALKBH5 inhibits pancreatic cancer motility by decreasing long non-coding RNA KCNK15-AS1 methylation. Cell Physiol Biochem. 2018;48(2):838–46.
55. Wang J, Yang Y, Sun F, Luo Y, Yang Y, Li J, et al. ALKBH5 attenuates mitochondrial fission and ameliorates liver fibrosis by reducing Drp1 methylation. Pharmacol Res. 2023;187: 106608.
56. Yan G, An Y, Xu B, Wang N, Sun X, Sun M. Potential impact of ALKBH5 and YTHDF1 on tumor immunity in colon adenocarcinoma. Front Oncol. 2021;11: 670490.
57. Jiang ZX, Wang YN, Li ZY, Dai ZH, He Y, Chu K, et al. The m6A mRNA demethylase FTO in granulosa cells retards FOS-dependent ovarian aging. Cell Death Dis. 2021;12(8):744.
58. Li M, Wu X, Li G, Lv G, Wang S. FTO promotes the stemness of gastric cancer cells. DNA Cell Biol. 2023;42(7):411–20.
59. Wang Z, Sun H, Zhu H, Gu D, Chen X, Pan Y, et al. Demethylase FTO inhibits the development of prostate cancer by upregulating EGR2 expression in an m6A manner. Turk J Biol. 2022;46(6):426–38.
60. Zhai J, Li S, Sen S, Opoku-Anane J, Du Y, Chen ZJ, et al. m(6)A RNA methylation regulators contribute to eutopic endometrium and myometrium dysfunction in adenomyosis. Front Genet. 2020;11:716.
61. Chen K, Shen D, Tan L, Lai D, Han Y, Gu Y, et al. A pan-cancer analysis reveals the prognostic and immunotherapeutic value of ALKBH7. Front Genet. 2022;13: 822261.
62. Cai GP, Liu YL, Luo LP, Xiao Y, Jiang TJ, Yuan J, et al. Alkbh1-mediated DNA N6-methyladenine modification regulates bone marrow mesenchymal stem cell fate during skeletal aging. Cell Prolif. 2022;55(2): e13178.
63. Fujii T, Shimada K, Anai S, Fujimoto K, Konishi N. ALKBH2, a novel AlkB homologue, contributes to human bladder cancer progression by regulating MUC1 expression. Cancer Sci. 2013;104(3):321–7.
64. Ke B, Ye K, Cheng S. ALKBH2 inhibition alleviates malignancy in colorectal cancer by regulating BMI1-mediated activation of NF-κB pathway. World J Surg Oncol. 2020;18(1):328.
65. Wu SS, Xu W, Liu S, Chen B, Wang XL, Wang Y, et al. Down-regulation of ALKBH2 increases cisplatin sensitivity in H1299 lung cancer cells. Acta Pharmacol Sin. 2011;32(3):393–8.
66. Jiang X, Zhu Z, Ding L, Du W, Pei D. ALKBH4 impedes 5-FU Sensitivity through suppressing GSDME induced pyroptosis in gastric cancer. Cell Death Dis. 2024;15(6):435.
67. Jingushi K, Aoki M, Ueda K, Kogaki T, Tanimoto M, Monoe Y, et al. ALKBH4 promotes tumourigenesis with a poor prognosis in non-small-cell lung cancer. Sci Rep. 2021;11(1):8677.
68. Shen C, Yan T, Tong T, Shi D, Ren L, Zhang Y, et al. ALKBH4 functions as a suppressor of colorectal cancer metastasis via competitively binding to WDR5. Front Cell Dev Biol. 2020;8:293.
69. Saad AK, Marafi D, Mitani T, Du H, Rafat K, Fatih JM, et al. Neurodevelopmental disorder in an Egyptian family with a biallelic ALKBH8 variant. Am J Med Genet A. 2021;185(4):1288–93.
70. Shimada K, Nakamura M, Anai S, De Velasco M, Tanaka M, Tsujikawa K, et al. A novel human AlkB homologue, ALKBH8, contributes to human bladder cancer progression. Cancer Res. 2009;69(7):3157–64.
71. Lin C, Wang Y, Dong Y, Lai S, Wang L, Weng S, et al. N6-methyladenosine-mediated SH3BP5-AS1 upregulation promotes GEM chemoresistance in pancreatic cancer by activating the Wnt signaling pathway. Biol Direct. 2022;17(1):33.
72. Zhang C, Zhang M, Ge S, Huang W, Lin X, Gao J, et al. Reduced m6A modification predicts malignant phenotypes and augmented Wnt/PI3K-Akt signaling in gastric cancer. Cancer Med. 2019;8(10):4766–81.

73. Zhai J, Chen H, Wong CC, Peng Y, Gou H, Zhang J, et al. ALKBH5 drives immune suppression via targeting AXIN2 to promote colorectal cancer and is a target for boosting immunotherapy. Gastroenterology. 2023;165(2):445–62.
74. Zhou S, Bai ZL, Xia D, Zhao ZJ, Zhao R, Wang YY, et al. FTO regulates the chemo-radiotherapy resistance of cervical squamous cell carcinoma (CSCC) by targeting β-catenin through mRNA demethylation. Mol Carcinog. 2018;57(5):590–7.
75. Zhang L, Wan Y, Zhang Z, Jiang Y, Lang J, Cheng W, et al. FTO demethylates m6A modifications in HOXB13 mRNA and promotes endometrial cancer metastasis by activating the WNT signalling pathway. RNA Biol. 2021;18(9):1265–78.
76. Wang T, Li W, Ye B, Zhang S, Lei X, Zhang D. FTO-stabilized lncRNA HOXC13-AS epigenetically upregulated FZD6 and activated Wnt/β-catenin signaling to drive cervical cancer proliferation, invasion, and EMT. J buon. 2021;26(4):1279–91.
77. He Y, Yue H, Cheng Y, Ding Z, Xu Z, Lv C, et al. ALKBH5-mediated m(6) A demethylation of KCNK15-AS1 inhibits pancreatic cancer progression via regulating KCNK15 and PTEN/AKT signaling. Cell Death Dis. 2021;12(12):1121.
78. Wang W, Huang Q, Liao Z, Zhang H, Liu Y, Liu F, et al. ALKBH5 prevents hepatocellular carcinoma progression by post-transcriptional inhibition of PAQR4 in an m6A dependent manner. Exp Hematol Oncol. 2023;12(1):1.
79. Zhang Y, Chen Y, Guo Q, Zhang Y, Liu A. Fat mass and obesity-associated protein (FTO)-induced upregulation of flotillin-2 (FLOT2) contributes to cancer aggressiveness in diffuse large B-cell lymphoma (DLBCL) via activating the PI3K/Akt/mTOR signal pathway. Arch Biochem Biophys. 2024;758: 110072.
80. Zhao L, Kong X, Zhong W, Wang Y, Li P. FTO accelerates ovarian cancer cell growth by promoting proliferation, inhibiting apoptosis, and activating autophagy. Pathol Res Pract. 2020;216(9): 153042.
81. Azhati B, Reheman A, Dilixiati D, Rexiati M. FTO-stabilized miR-139-5p targets ZNF217 to suppress prostate cancer cell malignancies by inactivating the PI3K/Akt/mTOR signal pathway. Arch Biochem Biophys. 2023;741: 109604.
82. Lai Y, Dong H, Xu P, Wang J, Feng W, Zhao Z, et al. RNA N6-methyladenosine demethylase FTO targets MOXD1 promoting the malignant phenotype of gastric cancer. BMC Gastroenterol. 2024;24(1):29.
83. Wang X, Wong CC, Chen H, Fu K, Shi L, Su H, et al. The N(6)-methyladenine DNA demethylase ALKBH1 promotes gastric carcinogenesis by disrupting NRF1 binding capacity. Cell Rep. 2023;42(3): 112279.
84. Jiang Y, Wan Y, Gong M, Zhou S, Qiu J, Cheng W. RNA demethylase ALKBH5 promotes ovarian carcinogenesis in a simulated tumour microenvironment through stimulating NF-κB pathway. J Cell Mol Med. 2020;24(11):6137–48.
85. Qu J, Hou Y, Chen Q, Chen J, Li Y, Zhang E, et al. RNA demethylase ALKBH5 promotes tumorigenesis in multiple myeloma via TRAF1-mediated activation of NF-κB and MAPK signaling pathways. Oncogene. 2022;41(3):400–13.
86. Chen P, Li S, Zhang K, Zhao R, Cui J, Zhou W, et al. N(6)-methyladenosine demethylase ALKBH5 suppresses malignancy of esophageal cancer by regulating microRNA biogenesis and RAI1 expression. Oncogene. 2021;40(37):5600–12.
87. Wang R, Cheng F, Yang X. FTO attenuates the cytotoxicity of cisplatin in KGN granulosa cell-like tumour cells by regulating the Hippo/YAP1 signalling pathway. J Ovarian Res. 2024;17(1):62.
88. Hu L, Li Z, Cheng J, Rao Q, Gong W, Liu M, et al. Crystal structure of TET2-DNA complex: insight into TET-mediated 5mC oxidation. Cell. 2013;155(7):1545–55.
89. Von Dreele RB. Protein crystal structure analysis from high-resolution X-ray powder-diffraction data. Methods Enzymol. 2003;368:254–67.
90. Benjin X, Ling L. Developments, applications, and prospects of cryo-electron microscopy. Protein Sci. 2020;29(4):872–82.
91. Rubach P, Majorek KA, Gucwa M, Murzyn K, Wlodawer A, Minor W. Advances in cryo-electron microscopy (cryoEM) for structure-based drug discovery. Expert Opin Drug Discov. 2025;20(2):163–76.
92. Ali A, Azmat U, Khatoon A, Akbar K, Murtaza B, Ji Z, et al. From gene editing to tumor eradication: the CRISPR revolution in cancer therapy. Prog Biophys Mol Biol. 2025;196:114–31.
93. Wang X, Fan D, Yang Y, Gimple RC, Zhou S. Integrative multi-omics approaches to explore immune cell functions: challenges and opportunities. iScience. 2023;26(4):106359.

Publisher's Note