Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) *2024 25(7):541-556*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2300457

Roles of THEM4 in the Akt pathway: a double-edged sword

Wen XIE[1,2,3], Weidong LIU[1,2,3], Lei WANG[1,2,3], Bin ZHU[1,2,3], Cong ZHAO[1,2,3], Ziling LIAO[1,2,3], Yihan LI[1,2,3], Xingjun JIANG[1], Jie LIU[4]✉, Caiping REN[1,2,3]✉

[1]*NHC Key Laboratory of Carcinogenesis, Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha 410008, China*
[2]*National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha 410008, China*
[3]*The Key Laboratory of Carcinogenesis and Cancer Invasion of the Ministry of Education, Cancer Research Institute, School of Basic Medical Science, Central South University, Changsha 410078, China*
[4]*Department of Critical Care Medicine, Hainan Hospital of Chinese PLA General Hospital, Hainan Province Clinical Medical Center, Sanya 572013, China*

Abstract: The protein kinase B (Akt) pathway can regulate the growth, proliferation, and metabolism of tumor cells and stem cells through the activation of multiple downstream target genes, thus affecting the development and treatment of a range of diseases. Thioesterase superfamily member 4 (THEM4), a member of the thioesterase superfamily, is one of the Akt kinase-binding proteins. Some studies on the mechanism of cancers and other diseases have shown that THEM4 binds to Akt to regulate its phosphorylation. Initially, THEM4 was considered an endogenous inhibitor of Akt, which can inhibit the phosphorylation of Akt in diseases such as lung cancer, pancreatic cancer, and liver cancer, but subsequently, THEM4 was shown to promote the proliferation of tumor cells by positively regulating Akt activity in breast cancer and nasopharyngeal carcinoma, which contradicts previous findings. Considering these two distinct views, this review summarizes the important roles of THEM4 in the Akt pathway, focusing on THEM4 as an Akt-binding protein and its regulatory relationship with Akt phosphorylation in various diseases, especially cancer. This work provides a better understanding of the roles of THEM4 combined with Akt in the treatment of diseases.

Key words: Protein kinase B (Akt); Thioesterase superfamily member 4 (THEM4); Tumor proliferation; Tumor metastasis

1 Introduction

Protein kinase B (Akt/PKB) is a serine/threonine protein kinase of the AGC family (named after PKA, PKG, and PKC), which consists of three main isoforms, Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ) (Revathidevi and Munirajan, 2019), as shown in Fig. 1. Akt1 is expressed mainly in tissues and is involved in phylogeny, inducing protein synthesis and promoting cell growth. Akt2 is widely expressed in muscle and adipocytes and is involved in glucose metabolism, which is an important protein in the insulin signal transduction pathway. Akt3 is highly expressed in the brain, as well as in the testis, and plays a key role in the development of the nervous system. Akt contains three main structural domains: the N-terminal pleckstrin homology (PH) domain, the intermediate kinase structural domain, and the C-terminal regulatory structural domain. The PH and catalytic structural domains are relatively conserved. The activation pathway of Akt is closely related to the structural domain and can be classified into three types: phosphatidylinositol-3-kinase (PI3K)-dependent, PI3K-independent, and protein factor regulation. After stimulation by external cellular stimuli, such as hormones, chemotherapeutic agents, and growth factors, PI3K is activated and produces phosphatidylinositol-(3,4,5)-trisphosphate (PIP3). The PH domain has great affinity for PIP3 and, upon binding to PIP3, the Akt conformation is altered, resulting in the phosphorylation of its Thr308 and Ser473 sites. The Thr308 site is located in the catalytic domain, and the Ser473 site is located in the C-terminal

✉ Caiping REN, rencaiping@csu.edu.cn
Jie LIU, braindream@163.com
Caiping REN, https://orcid.org/0000-0001-6880-7394
Jie LIU, https://orcid.org/0009-0000-3807-226X
Wen XIE, https://orcid.org/0009-0003-9093-9627

Received June 26, 2023; Revision accepted Oct. 10, 2023;
Crosschecked June 18, 2024

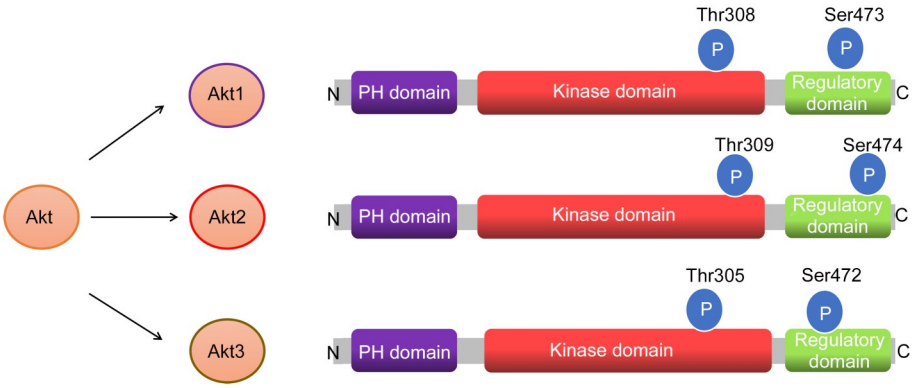

Fig. 1 Schematic diagram of the protein kinase B (Akt) isoforms' domains. Akt consists of three isoforms: Akt1, Akt2, and Akt3. Each isoform contains an N-terminal pleckstrin homology (PH) domain, an intermediate kinase structural domain, and a C-terminal regulatory structural domain. The difference is that they have diverse phosphorylation (P) sites.

regulatory domain. Activated Akt can further activate numerous downstream proteins, such as protein 21 (P21), P27, mechanistic target of rapamycin (mTOR), and murine double minute 2 (MDM2), thereby regulating cell proliferation, cycling, glucose metabolism, and growth, and further participating in the development of a series of cancers (Shariati and Meric-Bernstam, 2019; Li et al., 2023), nervous systems (Maiese et al., 2012), and cardiovascular diseases (Abeyrathna and Su, 2015). The Akt pathway is regulated at multiple levels in the cell, forming a vast regulatory network that plays a crucial role in cancer progression and stem cell development (Jafari et al., 2019; Song et al., 2019).

Thioesterase superfamily member 4 (THEM4), also known as C-terminal modulator protein (CTMP), is a 240-amino acid (aa) protein that belongs to the thioesterase family (Caswell et al., 2022). Its protein structure primarily comprises helices, loops, and β-strands. THEM4 has been found to contain a C-terminal hotdog-fold acyl-coenzyme A (CoA) thioesterase domain. This domain can hydrolyze the thioester bond of acyl-CoA molecules, converting them into their respective acids and CoA. The hotdog-fold acyl-CoA thioesterase domain plays a crucial role in regulating fatty acid metabolism and other related metabolic pathways. Furthermore, THEM4 possesses an N-terminal mitochondrial localization signal. This signal functions as a targeting signal, guiding THEM4 to be specifically localized within the mitochondria of cells (Zhao et al., 2012). Maira et al. (2001) discovered THEM4 and demonstrated its binding to the COOH terminus of Akt. Subsequent research has further explored this interaction and confirmed the binding between the N-terminal regions (1–64 aa) of THEM4 and Akt (Liu et al., 2013).

Basic research has shown that THEM4 acts as an endogenous inhibitor of Akt in certain diseases (Maira et al., 2001; Kao et al., 2023), while promoting the phosphorylation of Akt and activating the Akt pathway in other diseases (Liu et al., 2013; Chang et al., 2016). This indicates that the role of THEM4 in the Akt pathway is double-sided and it is not fully understood. Therefore, the aim of this study is to further investigate the negative/positive regulation relationship between THEM4 and Akt, to elucidate the mechanisms by which THEM4 affects the Akt pathway in different diseases. This work is critical for exploring the therapeutic potential of THEM4 in clinical settings, aiding in the identification of new treatment targets and targeted drugs. Additionally, it can contribute to a better understanding of the molecular mechanisms underlying tumor cell growth, proliferation, and metastasis, thus advancing disease research.

2 THEM4 functions as a negative regulator of Akt

2.1 Lung cancer

Lung cancer is one of the most common cancers worldwide due to poor lifestyle habits such as smoking, a high-phosphorus diet, and working in dusty or smoke-filled workplaces. Currently, it is mainly treated through surgery, radiation therapy, chemotherapy, and the use of inhibitors targeting epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase

(ALK), and c-ros oncogene 1, receptor tyrosine kinase (ROS1). However, millions of patients still die from lung cancer each year (Duma et al., 2019; Thai et al., 2021). Phosphorus is a common additive in food, and high phosphorus can improve the storage capacity of food. However, Jin et al. (2009) reported that a high phosphate content in food is not beneficial to the body. The K-*ras*^{LA1} lung cancer model mice fed a phosphate-rich diet produced significantly more tumor nodules than control mice. Further studies revealed that phosphorylated Akt (p-Akt) (Ser473) was activated in mice fed a high-phosphorus diet, while p-Akt (Thr308) remained unchanged. Moreover, the expression of phosphatase and tensin homolog (PTEN) and THEM4 was inhibited, and the downstream proteins p-mTOR and phosphorylated eIF4E-binding protein 1 (p-4E-BP1) were activated. These results indicate that a high-phosphate diet increases the activity of Akt/mTOR-4E-BP1 and promotes the development of lung cancer, and this pathway affects the expression of the tumor suppressor PTEN and the Akt-binding partner THEM4 (Jin et al., 2009). This study revealed that Akt and THEM4 were negatively correlated in the development of lung carcinogenesis in mice, but did not further explore the relationship between them. In the same year, Hwang et al. (2009) reported a regulatory relationship between THEM4 and Akt in a mouse model of lung cancer. THEM4 was delivered via nebulized administration to the lungs of K-*ras*^{LA1} mice, which are non-small cell lung cancer (NSCLC) experimental animals. Researchers have explored the effects of single nebulization of THEM4 (Hwang et al., 2007) and multiple sequential nebulization (Hwang et al., 2009), both of which alter Akt phosphorylation. However, single nebulization inhibits only Akt phosphorylation at the Thr308 site, causing a decrease in phosphorylation at the Ser9 site of downstream glycogen synthase kinase-3β (GSK3β), leaving p-Akt (Ser473) and total Akt unchanged. Multiple sequential nebulization inhibits the phosphorylation of Akt at the Ser473 and Thr308 sites, which inhibits the activity of Akt1 and decreases downstream p-mTOR (Ser2448), phosphorylated 70 kDa ribosomal protein S6 kinase 2 (p-p70S6K) (Thr389), and p-4E-BP1 (Thr69) activity, ultimately leading to inactivation of the Akt-mTOR-p70S6K pathway and inhibition of cancer cell proliferation (Zhang et al., 2017; Suo et al., 2021). In addition, repeated continuous atomization changed the expression of cyclins in nine-week-old K-*ras*^{LA1} mice; for example, it increased the protein levels of p27 and p21 and inhibited the expression of the cyclins cyclin-dependent kinase 4 (CDK4) and CDK2. The apoptosis of lung cells in 13-week-old K-*ras*^{LA1} mice inhibited pulmonary angiogenesis and cell proliferation. These experimental data indicate that THEM4 can be used as a novel atomizing agent in the treatment of lung cancer. Although the two experiments used the same disease animal model and drug therapy, different durations of animal models produced different experimental results. Therefore, when treating patients of different ages, we should consider the different dosages of drugs and the method and frequency of drug administration.

2.2 Colon cancer and colorectal cancer

To date, colon cancer, one of the most common malignant and high-incidence tumors of the human digestive system, has become one of the four deadliest cancers in the world due to the difficulty of its clinical diagnosis. The Akt pathway has been reported to be involved in different forms of colorectal cancer and affects epithelial-mesenchymal transition (EMT), cell proliferation, and autophagy (Narayanankutty, 2019; Ma et al., 2020). Zhang et al. (2021) reported that in colon cancer, THEM4 could function as a target of microRNA-183-5p (miR-183-5p), which is present in exosomes produced by M2-polarized macrophages. MiR-183-5p activates the Akt and nuclear factor-κB (NF-κB) pathways by targeting THEM4 and inhibiting its messenger RNA (mRNA) and protein expression, leading to the rapid proliferation and growth of colon cancer. The overexpression of THEM4 can reverse the malignant proliferation and metastasis of colon cancer cells induced by miR-183-5p in exosomes (Zhang et al., 2021). However, the relationship between THEM4 and Akt was not confirmed in this study, and whether THEM4 is involved in Akt pathway regulation through protein interactions remains to be further investigated.

2.3 Liver cancer

Along with superior living conditions, obesity, diabetes, and hypertension have become the main underlying diseases in middle-aged and elderly people, and further deterioration of these conditions will lead to the occurrence of liver cancer. Although some drugs can adjust the Akt pathway to treat liver diseases, such

as scutellarin, which can prevent acute alcohol-induced liver injury by regulating the Akt pathway to inhibit inflammation (Zhang et al., 2023), liver cancer is a common and poorly diagnosed tumor worldwide, and the number of patients with liver cancer continues to increase annually (Gravitz, 2014; Anwanwan et al., 2020). Ni et al. (2020) discovered through bioinformatics that THEM4 interacts with the mRNAs of eight other genes, such as regulator of cell cycle gene (*RGCC*), cadherin-15 (*CDH15*), and 5'→3' exoribonuclease 2 (*XRN2*), and can be used as a prognostic indicator of hepatocellular carcinoma. Researchers have found that abnormal mitochondrial metabolism is closely related to the occurrence and development of tumors (Zong et al., 2016; Porporato et al., 2018; Missiroli et al., 2020). Leucine-zipper EF-hand-containing transmembrane 1 (LETM1) is a mitochondrial inner membrane protein that maintains mitochondrial homeostasis (Austin and Nowikovsky, 2019; Natarajan et al., 2021) and serves as a biomarker for predicting colorectal cancer (Piao et al., 2019b; Che et al., 2021), gastric cancer (Piao et al., 2019a), and hepatocellular carcinoma (Zhou et al., 2020). 2A peptides are short sequences that are commonly found in viral genomes and possess unique functions. During protein synthesis, these peptides can undergo self-cleavage, leading to fragmentation of the polypeptide chain. This process allows for the translation of a single polypeptide chain into multiple functionally-independent proteins within a single transcription unit (de Lima and Lanza, 2021; Wang and Marchisio, 2021). Shin et al. (2013) utilized the self-cleaving activity of 2A peptides to connect LETM1 and THEM4 in a cytomegalovirus-enhanced green fluorescent protein (CMV-eGFP) vector. This approach enabled the simultaneous overexpression of LETM1 and THEM4 in the livers of hepatocellular carcinoma model mice, without any limitations on the order of expression or interference between the two genes. The results demonstrated a synergistic therapeutic effect of LETM1 and THEM4 in hepatocellular carcinoma model mice. To further investigate the underlying mechanisms, the researchers conducted western blot experiments, which revealed that the expression of LETM1-2A-THEM4 in hepatocellular carcinoma model mice led to a reduction in the expression levels of phosphorylated adenosine 5'-monophosphate (AMP)-activated protein kinase (p-AMPK) (Thr172), p-Akt (Ser473), and p-Akt (Thr308) (Shin et al., 2013). These findings not only highlight the role of THEM4 as a negative regulator of Akt, but also emphasize the limitations of targeting a single gene for effective cancer treatment. This study suggested that a combinatorial approach targeting multiple targets may be more effective in achieving therapeutic outcomes. Unfortunately, Shin et al. (2013) did not further explore whether the binding ability between Akt and THEM4 changes when LETM1-2A-THEM4 is formed and, if so, what the implications are. This is thought-provoking.

2.4 Pancreatic cancer

Pancreatic cancer is a type of tumor with an increased risk of occurrence with age, and is mainly distributed in Europe and the United States (Vincent et al., 2011; Collisson et al., 2019). The incidence of pancreatic cancer in males is higher than that in females, and epidemiological statistical analysis shows that this may be related to men's preference for smoking and high-fat food (Klein, 2021). Simon et al. (2009) first discovered that the protein structure of THEM4 mainly contains a C-terminal thioesterase domain and an N-terminal domain. According to the prediction of the N-terminal secondary structure of THEM4, four cell permeability peptides were designed: TAT-THEM4-1, TAT-THEM4-2, TAT-THEM4-3, and TAT-THEM4-4. The ability of these four peptides to induce apoptosis in pancreatic cancer cells (AsPC-1, BxPC-3, CFPAC-1, and Panc-1) was detected by flow cytometry, and it was found that TAT-THEM4-4 induced the strongest apoptosis with the highest expression of Caspase-3. Then, inactive variants of the peptide were synthesized and used as negative controls. Western blot analysis showed that, after treatment with the TAT-THEM4-4 polypeptide, the levels of p-Akt (Ser473) and p-GSK3β (Ser9) in pancreatic cancer cells were downregulated, and the proliferation rates of cancer cells decreased (Simon et al., 2009). This is the first report of a short peptide from THEM4 that can inhibit Akt phosphorylation and inactivate Akt. The key amino acid sequence of THEM4 regulating Akt activity has been identified.

2.5 Myogenic differentiation

In addition to playing an important role in the occurrence and development of cancer and various other diseases, Akt protein kinase also plays a crucial role

in the maintenance of self-renewal and the pluripotent differentiation of stem cells (Sumi et al., 2008; Zhao et al., 2020). Akt can promote the proliferation and differentiation of skeletal muscle satellite cells (SMSCs) (Li et al., 2019; Yin et al., 2020), and the activation of PI3K/Akt plays an indispensable role in myoblast differentiation. As one of the downstream target genes of Akt, cyclic adenosine monophosphate response element-binding protein (*CREB*) has the ability to regulate the self-renewal of muscle stem cells (Li and Fan, 2017; Fallatah et al., 2021). To investigate whether the Akt/CREB pathway is involved in myoblast differentiation, Zhu et al. (2017) mined the Gene Expression Omnibus (GEO) database and found that N-myc downstream-regulated gene 4 (NDRG4) (Wen et al., 2019; Vaes et al., 2021), a member of the NDRG family, is associated with cell growth, development, and proliferation, and is most likely involved in the differentiation of myogenic cells. By studying the important role of the NDRG4 and Akt/CREB pathways in muscle regeneration via website prediction, NDRG4 was found to bind to THEM4, and the binding and colocalization of these two proteins were confirmed by co-immunoprecipitation (co-IP) and immunofluorescence. Co-IP experiments further revealed that, when NDRG4 binds to THEM4, the binding of THEM4 to Akt in myoblasts is weakened, the phosphorylation of Akt is increased, the Akt/CREB pathway is activated, and the differentiation of myoblasts is promoted (Zhu et al., 2017). In addition, the Akt/CREB pathway can also be used to treat the complications of diabetes mellitus (Zhang et al., 2020) and nerve damage (Wu et al., 2018).

2.6 Other diseases

In addition to the negative regulation of THEM4 on Akt in cancers, a number of studies have reached similar conclusions about the development of other diseases.

The Akt pathway plays an important role in nervous system development and the neuronal growth. Miyawaki et al. (2009) reported that hippocampal CA1 neurons die after ischemic injury in rats. Ischemia increases THEM4 expression in CA1 neurons and enhances its binding ability to Akt and p-Akt (Ser473/Thr308), thus inhibiting Akt activity and further inhibiting the phosphorylation of GSK3β and forkhead box transcription factor 3A (FOXO3A), the downstream targets of the Akt pathway, ultimately leading to neuronal cell death. The findings of this study provide new guiding directions and therapeutic targets for the treatment of a range of injuries caused by hippocampal ischemia. Shin et al. (2017) established an intracerebroventricular (i. c. v.) kainic acid (KA) injection mouse model. After treatment with KA, little THEM4 expression was detected in mouse hippocampal astrocytes during the first 3 d, but THEM4 phosphorylation was activated, along with Akt phosphorylation at Thr308 and CREB phosphorylation. On Day 6, THEM4 expression was detected, but the phosphorylation of THEM4 was diminished and the phosphorylation of Akt or CREB was not enhanced. Based on the above results, Shin et al. (2017) hypothesized that the phosphorylation of THEM4 can induce the activation of the Akt/CREB pathway, and that THEM4 produced later inhibits this pathway. However, they did not determine which kinase activates THEM4 phosphorylation in astrocytes and releases THEM4, which binds to Akt, suggesting that THEM4 may be affected by other proteins. This study focused on the expression of THEM4 and p-Akt (Thr308) at different time points, providing a new idea for the clinical treatment of neural damage in the brain at different time points. Although Miyawaki et al. (2009) and Shin et al. (2017) studied neurological damage in the brain, they did not use the same mouse models, so their conclusions are not entirely consistent. Li et al. (2018) further provided evidence confirming that THEM4 negatively regulates Akt expression in rats with cerebral ischemia, and THEM expression increases with age in rats, while the opposite is true of Akt activity. In the treatment of ischemic brain injury, low expression of THEM4 activates the Akt pathway to address the apoptosis induced by ischemic brain injury. Huang et al. (2015) reported that activating transcription factor 3 (ATF3) binds to and negatively regulates THEM4, further activating p-Akt (Ser473) to exert anti-apoptotic effects. Further research from the same laboratory revealed that ATF3/THEM4/Akt not only serves as a therapeutic target for ischemic brain injury but also reduces the occurrence of ischemic stroke (Kao et al., 2023).

With the intake of fast food and various junk foods, the prevalence of overweight and obesity in today's world is gradually increasing, and the prevalence of adolescent obesity is also increasing annually.

Park et al. (2014) used two mouse models of diabetic mice induced by a high-fat diet (HFD) and transgenic obese mice and found that THEM4 maintains a negative regulatory relationship with Akt, and HFD-induced insulin is influenced by translational and transcriptional regulation of the Akt pathway. In conclusion, THEM4 negatively regulates Akt in obese diabetic mice, and HFD-induced insulin is influenced by translational and transcriptional regulation of the Akt pathway.

Diabetic nephropathy is a chronic kidney disease caused by type 1 diabetes or type 2 diabetes due to poor blood glucose control, and is one of the microvascular complications of diabetes. Chen et al. (2016) constructed a diabetic mouse model as well as high glucose-induced HKC cells to simulate ex vivo experiments. Western blot assays revealed that THEM4 expression was decreased in renal cells of diabetic mice and in human HKC cells induced by high glucose, while the expression of p-Akt (Ser473) and transforming growth factor-β1 (TGF-β1) was increased. Overexpression of THEM4 decreased Akt activity in HKC cells induced by high glucose and inhibited extracellular matrix accumulation. The above results suggest that THEM4, an Akt inhibitor, can treat diabetic nephropathy by modulating Akt activity.

Through clinical sample collection, Liu et al. (2018) reported that reduced expression of THEM4 in patients leads to dilated cardiomyopathy and hypertrophic cardiomyopathy. Liu et al. (2018) established a mouse model of hypertrophic cardiomyopathy using aortic banding instead of traditional left anterior descending artery ligation, and further found that knockdown of THEM4 activated Akt and promoted the phosphorylation of its downstream proteins mTOR, GSK3β, and p70S6K. These findings suggest that THEM4, which negatively regulates Akt, can be used as a novel target for the treatment of pathological myocardial hypertrophy.

Pulmonary hypertension is a common cardiovascular disease that is characterized by increased pulmonary vascular load, leading to right ventricular hypertrophy, but its early symptoms are not obvious (Hassoun, 2021). Sun et al. (2016) studied the mechanism of human pulmonary hypertension and found that the addition of asymmetric dimethylarginine (ADMA) compounds increased pulmonary hypertension by inhibiting the binding of THEM4 to Akt, increasing the ubiquitination degradation pathway of THEM4, and stimulating the phosphorylation of Akt. These findings reveal that THEM4 has the potential to be used as a therapeutic target for pulmonary hypertension in human cardiovascular diseases.

As a fat-soluble vitamin, vitamin D regulates the calcium salt balance in the human body, improves immunity and skeletal muscle development, and can even treat diabetes (Angellotti et al., 2019), multiple sclerosis (Jagannath et al., 2018), and cancer (Giustina et al., 2020), making it one of the essential vitamins for the human body. Luciferase reporter assays and chromatin immunoprecipitation assays confirmed that the receptor of vitamin D can interact with the promoter of THEM4 in mouse macrophages. When vitamin D is insufficient, THEM4 is normally transcribed in cells, resulting in the downregulation of phosphorylation of Akt at the Thr308 site and its downstream target protein GSK3β, which ultimately inhibits cyclooxygenase-2 (COX-2) expression and prevents the release of proinflammatory factors. Inactivation of this pathway is partly responsible for the onset of inflammatory responses and contributes to an increased risk of chronic inflammatory diseases (Wang QS et al., 2014). The results of this study reveal the importance of vitamin D for the treatment of chronic diseases, provide a new therapeutic strategy for treating chronic diseases, and show that THEM4 negatively regulates the Akt pathway in these chronic diseases.

Amyotrophic lateral sclerosis (ALS), a neurodegenerative disease that affects movement (Mejzini et al., 2019; Masrori and van Damme, 2020), mainly manifests as muscle weakness and muscle atrophy. In the classical ALS-superoxide dismutase 1 (SOD1) mouse disease model (mSOD1 G93A) (Gurney et al., 1994) and the gastrocnemius muscle tube atrophy model of C2C12 treated with tumor necrosis factor-α (TNF-α) (Wang DT et al., 2014), THEM4 was found to bind to Akt; with muscle atrophy, the higher the expression of THEM4, the lower the phosphorylation of Akt, and an IP experiment confirmed that the combination ability of the two proteins was also enhanced (Wang et al., 2019). Wang et al. (2023) discovered that THEM4 was significantly upregulated in the gastrocnemius muscle of mice 7 d after sciatic nerve injury. Further investigation using *THEM4*-knockout mice with sciatic nerve injury compared to wild-type mice with only sciatic nerve injury revealed the upregulation of p-Akt (Ser473) expression and the activation of the

Akt pathway, leading to enhanced phosphorylation of downstream targets such as GSK3β (Ser9), ribosomal protein S6, and 4E-BP1. These findings emphasize the crucial role of THEM4 in skeletal muscle atrophy and further confirm the negative regulatory relationship between THEM4 and Akt signaling in ALS (Wang et al., 2023).

3 THEM4 functions as a positive regulator of Akt

In 2001, researchers officially began to describe the regulatory relationship between THEM4 and Akt. In the first half of this paper, THEM4, an endogenous inhibitor of Akt, plays a role in cancers and other diseases. However, as an increasing number of studies have been conducted, other researchers have come to the opposite conclusion regarding the regulatory relationship between THEM4 and Akt, suggesting that THEM4 can also act as a positive regulator of Akt. For example, Ono et al. (2007) reported that, in CCOS-1, HeLa and HepG2 cells, and 3T3-L1 adipocytes, transient transfection or lentiviral infection of THEM4 activated the phosphorylation of Akt, and through the Akt pathway, THEM4 was involved in several biological processes, such as anti-apoptosis and glucose uptake. Since then, several other researchers have confirmed this phenomenon in other cancer studies.

3.1 Breast cancer

Breast cancer is caused by the abnormal proliferation of mammary epithelial cells, and its incidence ranks first among female malignant tumors worldwide, with a certain genetic risk (Trayes and Cokenakes, 2021). Liu et al. (2013) reported that THEM4 expression is upregulated in human breast cancer cell lines and breast cancer specimens, and the total amount of Akt is not changed after the overexpression of THEM4, but the expression of p-Akt (Thr308) and p-Akt (Ser473) is upregulated, demonstrating that the upregulation of THEM4 mediates the activation of the Akt pathway. Immunofluorescence assay revealed that THEM4 is expressed in both the nucleus and the cytoplasm of breast cancer cells, and co-localized with Akt at the plasma membrane. Glutathione-*S*-transferase (GST) pull-down experiments further revealed that the 1–64-aa fragment in the N-terminal domain of THEM4 binds to Akt to promote the phosphorylation of Akt at Thr308 and Ser473, thus activating the Akt pathway and promoting the proliferation of breast cancer cells. The findings of this study by Liu et al. (2013) suggest that THEM4 may function as an oncogene in breast cancer and could be a marker molecule for breast cancer diagnosis. It has been documented that human epidermal growth factor receptor 2 (HER2) activation is influenced by Akt, mitogen-activated protein kinase (MAPK), and other signaling pathways. As one of the treatments for HER2-rich breast cancer patients, trastuzumab is still unable to address the disease conditions of most patients either by itself or in combination. To understand the mechanism of trastuzumab resistance in HER2-enriched patients and screen for new targets for combination therapy, researchers have conducted experiments such as cell counting kit-8 (CCK-8) assays and western blotting. They found that THEM4 can mediate trastuzumab resistance. In the HER2-positive breast cancer cell line SkBR3, overexpression of THEM4 led to increased phosphorylation of Akt at the Thr308 and Ser473 sites. In BT-483 cells, an HER2-positive breast cancer cell line with high endogenous expression of THEM4, the addition of Akt inhibitor IV, PD98059, and rapamycin (Akt, MAPK, and mTOR inhibitors, respectively) showed that trastuzumab resistance induced by THEM4 did not occur in the presence of Akt or mTOR inhibitor, confirming that THEM4 is a positive regulator of Akt (Chen et al., 2017). This study revealed that THEM4 may serve as a specific target for trastuzumab treatment in breast cancer patients, providing new insights and approaches for the treatment of HER2-enriched patients. In addition, THEM4 was also involved in tamoxifen resistance (TamR) in breast cancer and was inversely regulated by elongation of very-long-chain fatty acid protein 2 (ELOVL2) (Jeong et al., 2021). Genome-wide methylation sequencing of MCF-7 cells and MCF-7/TamR cells revealed that *ELOVL2* expression was downregulated by DNA hypermethylation in MCF-7/TamR cells. High-throughput sequencing and in vivo and in vitro experiments revealed that ELOVL2 can inhibit MCF-7/TamR cell proliferation via a resistance mechanism associated with the Akt pathway. THEM4 expression was downregulated at the RNA and protein levels, and the total Akt protein and p-Akt (Thr305, 308, 309) levels were decreased after ELOVL2

overexpression. These results indicate that ELOVL2 is downregulated by hypermethylation and inhibits THEM4 expression in TamR cancer, leading to the inactivation of Akt as well as downstream target genes such as *CREB* and *mTOR*, ultimately leading to TamR and increased cell proliferation (Jeong et al., 2021). These findings reveal the important roles of THEM4 and ELOVL2 in drug resistance in breast cancer and may provide new targets and strategies for breast cancer treatment. A recent study further confirmed that THEM4 is positively correlated with Akt expression in breast cancer and revealed that THEM4 promoted the metastasis and invasion of the triple-negative breast cancer (TNBC) cell lines BT549 and MDA-MB231, mainly using in vitro and in vivo experiments (Lin et al., 2023). The overexpression of THEM4 in MDA-MB231 cells significantly activated the PI3K/Akt pathway, especially the expression of p-Akt S473. Further experiments using the Akt inhibitor IV to block Akt signaling showed that MDA-MB231 cells were transfected with vector control, vector control/Akt inhibitor IV, THEM4, and the THEM4/Akt inhibitor. Transwell assays showed that the number of invasive cells in the THEM4 overexpression group was significantly higher than that in the control group, but the number of invasive cells in the THEM4 overexpression group treated with Akt inhibitor IV was lower than that in the group without the inhibitor. These findings indicate that elevated THEM4 expression in TNBC leads to the activation of the PI3K/Akt pathway, promoting TNBC metastasis through a THEM4/Akt-dependent mechanism.

3.2 Nasopharyngeal carcinoma

Nasopharyngeal carcinoma (NPC) is a special head and neck malignancy originating from the mucosa of the nasopharynx. It is mainly composed of poorly differentiated and undifferentiated carcinomas and is characterized by typical ethnic aggregation and unbalanced distribution (Xu et al., 2022). The survival rate of patients with early-stage NPC after curative treatment is relatively high. However, more than half of NPC patients are diagnosed in the middle and late stages due to the concealed location of the disease and the inapparent early symptoms. Therefore, it is essential to identify early diagnostic markers for NPC (Huang and O'Sullivan, 2017). The mechanism of NPC development is cross-regulated by several signaling pathways, among which the Akt pathway is activated. One study revealed that the Akt/mTOR pathway is activated in NPC cells and is associated with poor prognosis in NPC patients (Wang WY et al., 2014). Chang et al. (2016) investigated the role of THEM4, an Akt-binding protein, in NPC and found that THEM4 is highly expressed at the RNA and protein levels in NPC tissues compared to normal tissues. Akt and THEM4 bind to each other in NPC cell lines, and abnormal THEM4 expression is associated with lymph node metastasis and poor prognosis and promotes NPC progression. Moreover, THEM4 positively regulates the phosphorylation of Akt at Ser473 and increases the phosphorylation of its downstream protein kinase GSK3β at the Ser9 site. This study revealed a positive regulatory relationship between Akt and THEM4 in NPC and the role of THEM4 in NPC, demonstrating that THEM4 may be used as a new marker or target for NPC detection and treatment.

4 Discussion

As an important regulator of intracellular signaling, Akt plays a crucial role in cell growth and development by regulating cell survival, cell proliferation, angiogenesis, energy metabolism, and so on. Through mass spectrometry, co-IP, yeast two-hybrid, and other experiments, researchers have found multiple proteins that bind to Akt to explore their functions in cells, such as mTOR, MDM2, NRH: quinone oxidoreductase 2 (NQO2), and heat shock protein 90 (Hsp90) (Sarbassov et al., 2005; Hsieh et al., 2014; Zeng et al., 2021). After binding to Akt, they regulate Akt activity or alter Akt localization in cells, thereby affecting the functions of Akt in cells. Hsp90 enhances the phosphorylation of Akt and promotes the proliferation and metastasis of tumor cells. The A and B chains of NQO2 bind to the PH and kinase domains of Akt, respectively, and inhibit Akt phosphorylation to regulate cell proliferation. However, there are some controversies about the regulatory relationship between Akt-binding proteins and Akt, as exemplified by THEM4 depicted in this review. In this review, we mainly described that THEM4 is involved in the regulation of various cancers and other diseases through positively/negatively regulating Akt, thereby

affecting the expression of downstream target genes (Fig. 2 and Table 1). It interacts with Akt, inhibits Akt phosphorylation, and functions as an endogenous inhibitor of Akt in many tumors and other diseases, including lung cancer, colorectal cancer, pancreatic cancer, diabetes, cardiovascular disease, and ALS. However, previous studies on breast cancer (Liu et al., 2013; Chen et al., 2017; Lin et al., 2023) and NPC (Chang et al., 2016) have shown that THEM4 binding to Akt does not negatively regulate the activity of Akt but promotes its phosphorylation, which contradicts the above reports (Fig. 3). Faced with the opposite conclusion, the researchers adopted the same experimental approaches, but the conclusion remained unchanged, so they speculated that the reason for the difference in findings may be that even the same genes and pathways function differently in different cancer types and diseases. Moreover, the Akt pathway involves the influence of multiple genes and signaling pathways and is not only regulated by THEM4. Therefore, Akt phosphorylation may be regulated by other genes and signaling pathways in different cancer and disease types. The underlying mechanism is not well elucidated and deserves further exploration.

As a member of the thioesterase superfamily, THEM4 has the opposite effect on the regulation of Akt, and whether it is influenced by its family members is also worth exploring. In the thioesterase superfamily, in addition to THEM4, which regulates the Akt pathway, THEM2 has also been reported to be involved in fatty acid transport and oxidation and the biosynthetic pathway of glycerol by regulating the phosphorylation of Akt (Tillander et al., 2019). Among other family members, THEM1 is involved in mouse metabolism (Li et al., 2021) and THEM5 is involved in cardiolipin remodeling and fatty liver development (Zhuravleva et al., 2012); however, whether they are involved in regulating Akt activation is unknown.

In conclusion, exploring the regulatory relationship between THEM4 and Akt is of great significance for comprehending tumor development mechanisms, devising targeted therapeutic strategies, and evaluating tumor prognosis. These research findings have the potential to offer novel insights and approaches for

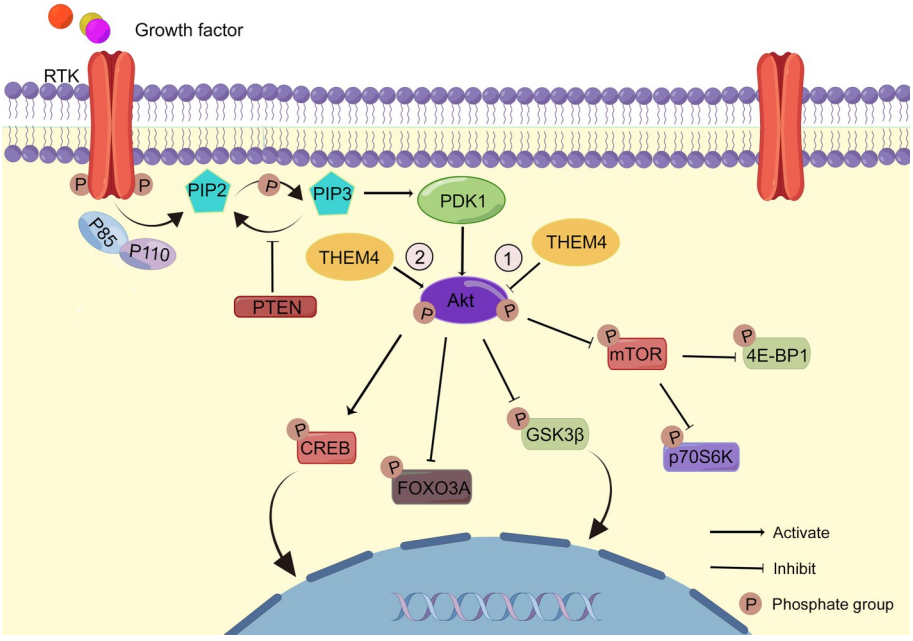

Fig. 2 Akt phosphorylation regulated by THEM4 in a positive/negative manner in various tumors (created by Figdraw). (1) THEM4, as an endogenous inhibitor of Akt, inhibits the activity of Akt and regulates downstream target genes; (2) THEM4 positively regulates Akt phosphorylation and downstream target genes. Akt: protein kinase B; THEM4: thioesterase superfamily member 4; RTK: receptor tyrosine kinase; P85: protein 85; P110: protein 110; PIP2: phosphatidylinositol 4,5-bisphosphate; PIP3: phosphatidylinositol 3,4,5-trisphosphate; PDK: phosphoinositide-dependent kinase; PTEN: phosphatase and tensin homolog; mTOR: mechanistic target of rapamycin; 4E-BP1: eIF4E-binding protein 1; CREB: cyclic adenosine monophosphate response element-binding protein; FOXO3A: forkhead box transcription factor 3A; GSK3β: glycogen synthase kinase-3β; p70S6K: 70 kDa ribosomal protein S6 kinase 2.

Table 1 Akt phosphorylation regulated by THEM4 in various cancers and diseases

Cancer/disease	Research subject	THEM4 expression	Activation state of Akt	Regulation of THEM4 and Akt	Phosphorylation site	Downstream target gene	Function	Reference
Lung cancer	K-*ras*^{LA1} mouse model	High	Inhibited	Negative	p-Akt (Thr308)	p-mTOR (Ser9)	Inhibiting the proliferation of NSCLC cells	Hwang et al., 2007, 2009
					p-Akt (Ser473), p-Akt (Thr308)	p-mTOR (Ser2448), p-p70S6K (Thr389), p-4E-BP1 (Thr69)	Inhibiting the proliferation of lung cancer	Zhang et al., 2017; Suo et al., 2021
Colon cancer	M2-polarized macrophages	Low	Activated	Negative	p-Akt		Rapid proliferation and growth of colon cancer cells	Zhang et al., 2021
Liver cancer	Liver cancer cell	High	Inhibited	Negative	p-Akt (Ser473), p-Akt (Thr308)		Inhibiting the proliferation of liver cancer cells	Shin et al., 2013
Pancreatic cancer	Pancreatic cancer cell	High	Inhibited	Negative	p-Akt (Ser473)	p-GSK3β (Ser9)	Decreasing the proliferation rate of pancreatic cancer	Simon et al., 2009
Myogenic differentiation	Myogenic cell	Low	Activated	Negative	p-Akt (Ser473)	p-CREB	Promoting myoblast differentiation	Zhu et al., 2017
Neurological diseases	Ischemic injury rat model	High	Inhibited	Negative	p-Akt (Ser473), p-Akt (Thr308)	p-GSK3β (Ser9), p-FOXO3A (Ser256)	Leading to neuronal cell death	Miyawaki et al., 2009
	KA injection mouse model	High	Inhibited	Negative	p-Akt (Thr308)	p-CREB	Astrocyte activation	Shin et al., 2017
Ischemic brain injury	Primary mouse cortical neuronal cells	High	Inhibited	Negative	p-Akt (Ser473)	p-Bad	Inhibiting cell apoptosis	Huang et al., 2015
Ischemic brain infarction	Stroke model	Low	Inhibited	Negative	p-Akt (Ser473)		Prevention of ischemic cerebral infarction	Kao et al., 2023
Obesity and diabetes	Obese diabetic mouse model	High	Inhibited	Negative	p-Akt (Ser473)		Impairing insulin signaling in obese mouse adipose tissue	Park et al., 2014

To be continued

Table 1 (continued)

Cancer/disease	Research subject	THEM4 expression	Activation state of Akt	Regulation of THEM4 and Akt	Phosphorylation site	Downstream target gene	Function	Reference
Diabetic nephropathy	Diabetic mouse model, high glucose-induced human HKC cells	Low	Activated	Negative	p-Akt (Ser473)		Accumulating extracellular mechanisms	Chen et al., 2016
Cardiovascular disease	Hypertrophic cardiomyopathy mouse model	Low	Activated	Negative	p-Akt (Ser473)	p-mTOR (Ser2448), p-p70S6K (Thr389), p-GSK3β (Ser9)	Reducing heart damage by inhibiting the Akt pathway	Liu et al., 2018
Pulmonary hypertension	Pulmonary hypertension lamb model	Low	Activated	Negative	p-Akt (Ser473), p-Akt (Thr308)		Increasing pulmonary arterial hypertension	Sun et al., 2016
Chronic inflammation	Mouse macrophage	High	Inhibited	Negative	p-Akt (Thr308)	p-GSK3β (Ser9)	Preventing the release of proinflammatory factors	Wang QS et al., 2014
ALS	ALS-SOD1 mouse disease model (mSOD1 G93A)	High	Inhibited	Negative	p-Akt (Ser473)	p-FOXO3A (Ser256)	Leading to skeletal muscle atrophy	Wang et al., 2019
	THEM4-knockout mice	Low	Activated	Negative	p-Akt (Ser473)	p-GSK3β (Ser9), p-S6, p-4E-BP1	Preventing skeletal muscle atrophy	Wang et al., 2023
Breast cancer	Human breast cancer cell	High	Activated	Positive	p-Akt (Ser473), p-Akt (Thr308)	p-GSK3β (Ser9)	Promoting the proliferation and metastasis of breast cancer cells	Liu et al., 2013; Chen et al., 2017
TNBC	TBNC cell	High	Activated	Positive	p-Akt (Ser473)		Promoting the metastasis of TNBC	Lin et al., 2023
NPC	NPC cell	High	Activated	Positive	p-Akt (Ser473)	p-GSK3β (Ser9)	Promoting the proliferation, invasion, and metastasis of NPC cells	Chang et al., 2016

Akt: protein kinase B; THEM4: thioesterase superfamily member 4; p-: phosphorylated; mTOR: mechanistic target of rapamycin; NSCLC: non-small cell lung cancer; p70S6K: 70 kDa ribosomal protein S6 kinase 2; 4E-BP1: eIF4E-binding protein 1; GSK3β: glycogen synthase kinase-3β; CREB: cyclic adenosine monophosphate response element-binding protein; FOXO3A: forkhead box transcription factor 3A; KA: kainic acid; Bad: B-cell lymphoma 2 (Bcl2)-associated death promoter; ALS: amyotrophic lateral sclerosis; SOD1: superoxide dismutase 1; TNBC: triple-negative breast cancer; NPC: nasopharyngeal carcinoma.

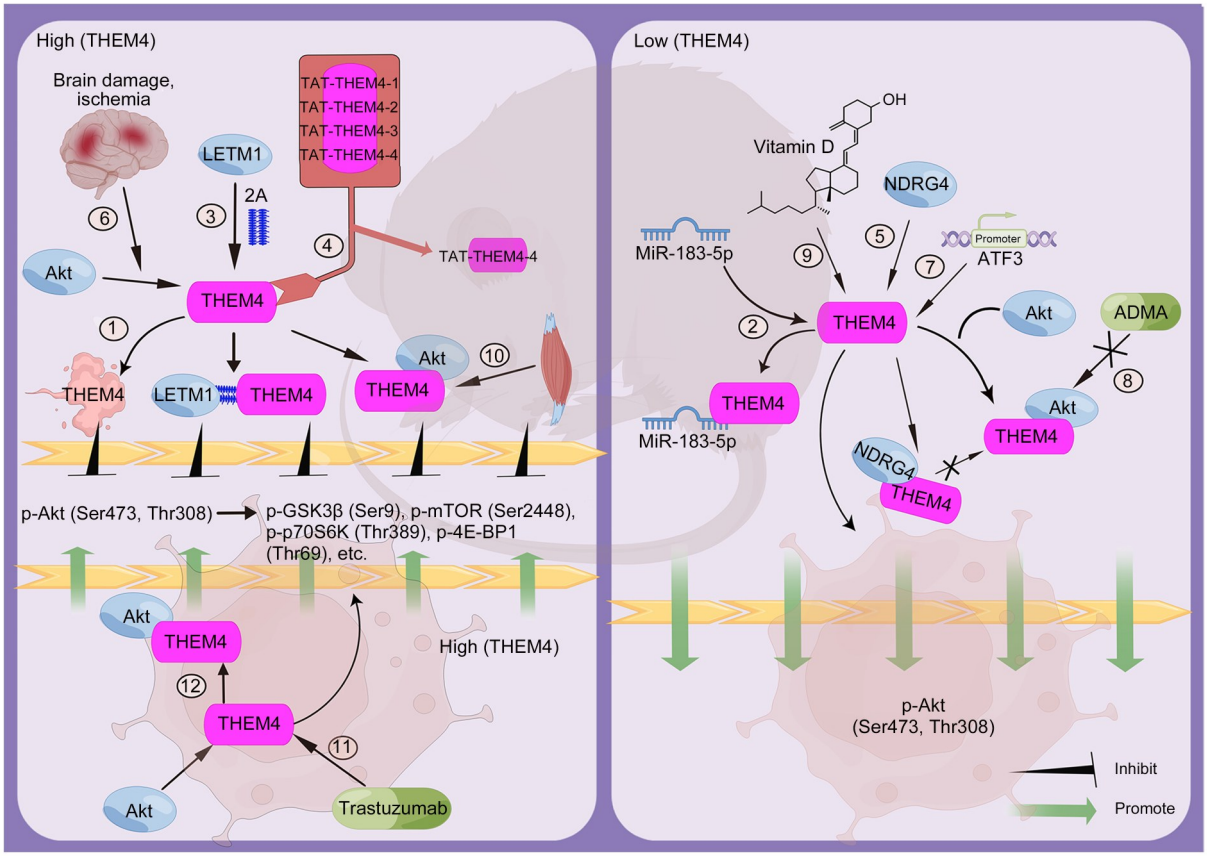

Fig. 3 Examples illustrating the regulatory relationship between THEM4 and Akt in various diseases (created by Figdraw). (1) In a lung cancer mouse model, THEM4 is administered as an aerosol and eventually inhibits cancer cell proliferation by inhibiting the phosphorylation of Akt (Hwang et al., 2007, 2009). (2) MiR-183-5p inhibited the mRNA and protein expression of THEM4 and activated Akt in colorectal cancer, leading to the rapid proliferation and growth of colon cancer (Zhang et al., 2021). (3) Shin et al. (2013) used the 2A peptides to link LETM1 and THEM4 in vitro, enabling the simultaneous overexpression of LETM1 and THEM4 in the liver of a mouse liver cancer model, and subsequently found that the expression levels of p-Akt (Ser473) and p-Akt (Thr308) were decreased in vivo. (4) Simon et al. (2009) designed four cell-penetrating peptides based on the predicted secondary structure of the N-terminal region of THEM4. TAT-THEM4-4 treatment decreased p-Akt (Ser473) and p-GSK3β (Ser9) levels in pancreatic cancer cells. (5) NDRG4 in myogenic cells binds to THEM4, leading to a weakened binding between THEM4 and Akt. This results in increased phosphorylation of Akt, ultimately promoting myogenic cell differentiation (Zhu et al., 2017). (6) Miyawaki et al. (2009) reported that cerebral damage and ischemia in rats led to increased THEM4 expression, which enhanced its ability to bind to Akt and inhibited Akt phosphorylation. (7) In a mouse model of ischemic brain injury, the transcription factor ATF3 binds and inhibits THEM4 transcription, which further activates p-Akt (Ser473) to inhibit cell apoptosis (Huang et al., 2015). (8) Sun et al. (2016) discovered that the addition of ADMA compounds inhibited the binding between THEM4 and Akt, stimulating Akt phosphorylation and eventually leading to pulmonary arterial hypertension. (9) In mouse macrophages, vitamin D receptors can interact with the promoter of THEM4, preventing its normal transcription. This interaction activates p-Akt (Thr308) and GSK3β, releasing proinflammatory cytokines (Wang et al., 2014). (10) In mSOD1 G93A and TNF-α-treated C2C12 gastrocnemius muscle atrophy model, as muscle atrophy occurs, the expression of THEM4 increases, its binding capacity to the Akt protein strengthens, and Akt phosphorylation decreases (Wang et al., 2019). (11) THEM4 can mediate resistance to trastuzumab. In the HER2-positive SkBR3 breast cancer cell line, the overexpression of THEM4 leads to increased phosphorylation of Akt at the Thr308 and Ser473 sites (Chen et al., 2017). (12) In breast cancer and nasopharyngeal carcinoma studies, THEM4 was found to interact with Akt and positively regulate Akt phosphorylation, ultimately promoting cell proliferation (Liu et al., 2013; Chang et al., 2016). THEM4: thioesterase superfamily member 4; Akt: protein kinase B; p-: phosphorylated; miR: microRNA; mRNA: messenger RNA; LETM1: leucine-zipper EF-hand-containing transmembrane 1; GSK3β: glycogen synthase kinase-3β; mTOR: mechanistic target of rapamycin; p70S6K: 70 kDa ribosomal protein S6 kinase 2; 4E-BP1: eIF4E-binding protein 1; NDRG4: N-myc downstream-regulated gene 4; ATF3: activating transcription factor 3; ADMA: asymmetric dimethylarginine; SOD1: superoxide dismutase 1; mSOD1 G93A: SOD1 mouse disease model; TNF-α: tumor necrosis factor-α; HER2: human epidermal growth factor receptor 2.

personalized and precise cancer treatment. Nonetheless, further experimental and clinical investigations are required to validate these discoveries and translate them into clinical practice. It is worth noting that the regulatory interplay between THEM4 and Akt remains incompletely characterized. For example, Wang et al. (2021) reported that THEM4 interacts with enolase-phosphatase 1 (*ENOPH1*), but neither knockdown nor overexpression of *ENOPH1* in gliomas alters THEM4 protein expression. Transcriptome sequencing showed that *ENOPH1* is related to the TGF-β and mTOR signaling pathways, which seriously affects the survival of high-grade glioma patients. Its high expression in U251 cells promotes cell proliferation, migration, EMT, and other malignant phenotypes. BEZ235, an Akt inhibitor, rescues the activation of the PI3K/Akt/mTOR pathway induced by *ENOPH1* overexpression (Wang et al., 2021). However, the relationships among ENOPH1, THEM4, and the PI3K/Akt/mTOR pathway in glioma and the regulatory relationship between THEM4 and the PI3K/Akt/mTOR pathway have not been studied. Furthermore, the involvement of THEM4 in myoblast differentiation has thus far been reported in just one study (Zhu et al., 2017). Given its significance as a regulator in the Akt pathway, it is anticipated that researchers will delve deeper into its role in stem cells in the future. This exploration may pave the way for a new theoretical foundation and therapeutic approach toward utilizing stem cell therapy in disease treatment.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 82071399 and 81773179), the Key Research and Development Program of Hunan Province (No. 2022SK2055), and the Natural Science Foundation of Hunan Province (No. 2023JJ30733), China. We thank Figdraw.com for drawing figures.

Author contributions

Caiping REN and Jie LIU conceived and designed the study. Cong ZHAO, Ziling LIAO, and Yihan LI collected the data. Wen XIE wrote the manuscript. Xingjun JIANG, Weidong LIU, Lei WANG, and Bin ZHU revised the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Wen XIE, Weidong LIU, Lei WANG, Bin ZHU, Cong ZHAO, Ziling LIAO, Yihan LI, Xingjun JIANG, Jie LIU, and Caiping REN declare that they have no conflicts of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Abeyrathna P, Su YC, 2015. The critical role of Akt in cardiovascular function. *Vascul Pharmacol*, 74:38-48. https://doi.org/10.1016/j.vph.2015.05.008

Angellotti E, D'Alessio D, Dawson-Hughes B, et al., 2019. Effect of vitamin D supplementation on cardiovascular risk in type 2 diabetes. *Clin Nutr*, 38(5):2449-2453. https://doi.org/10.1016/j.clnu.2018.10.003

Anwanwan D, Singh SK, Singh S, et al., 2020. Challenges in liver cancer and possible treatment approaches. *Biochim Biophys Acta (BBA) Rev Cancer*, 1873(1):188314. https://doi.org/10.1016/j.bbcan.2019.188314

Austin S, Nowikovsky K, 2019. *LETM1*: essential for mitochondrial biology and cation homeostasis? *Trends Biochem Sci*, 44(8):648-658. https://doi.org/10.1016/j.tibs.2019.04.002

Caswell BT, de Carvalho CC, Nguyen H, et al., 2022. Thioesterase enzyme families: functions, structures, and mechanisms. *Protein Sci*, 31(3):652-676. https://doi.org/10.1002/pro.4263

Chang JW, Jung SN, Kim JH, et al., 2016. Carboxyl-terminal modulator protein positively acts as an oncogenic driver in head and neck squamous cell carcinoma via regulating Akt phosphorylation. *Sci Rep*, 6:28503. https://doi.org/10.1038/srep28503

Che N, Yang ZT, Liu XZ, et al., 2021. Suppression of LETM1 inhibits the proliferation and stemness of colorectal cancer cells through reactive oxygen species-induced autophagy. *J Cell Mol Med*, 25(4):2110-2120. https://doi.org/10.1111/jcmm.16169

Chen N, Hao J, Li LS, et al., 2016. Carboxy-terminal modulator protein attenuated extracellular matrix deposit by inhibiting phospho-Akt, TGF-β1 and α-SMA in kidneys of diabetic mice. *Biochem Biophys Res Commun*, 474(4):753-760. https://doi.org/10.1016/j.bbrc.2016.05.032

Chen YC, Li HY, Liang JL, et al., 2017. CTMP, a predictive biomarker for trastuzumab resistance in HER2-enriched breast cancer patient. *Oncotarget*, 8(18):29699-29710. https://doi.org/10.18632/oncotarget.10719

Collisson EA, Bailey P, Chang DK, et al., 2019. Molecular subtypes of pancreatic cancer. *Nat Rev Gastroenterol Hepatol*, 16(4):207-220. https://doi.org/10.1038/s41575-019-0109-y

de Lima JGS, Lanza DCF, 2021. 2A and 2A-like sequences: distribution in different virus species and applications in biotechnology. *Viruses*, 13(11):2160. https://doi.org/10.3390/v13112160

Duma N, Santana-Davila R, Molina JR, 2019. Non-small cell lung cancer: epidemiology, screening, diagnosis, and treatment. *Mayo Clin Proc*, 94(8):1623-1640. https://doi.org/10.1016/j.mayocp.2019.01.013

Fallatah B, Shuaib M, Adroub S, et al., 2021. Ago1 controls myogenic differentiation by regulating eRNA-mediated

CBP-guided epigenome reprogramming. *Cell Rep*, 37(9): 110066. https://doi.org/10.1016/j.celrep.2021.110066

Giustina A, Adler RA, Binkley N, et al., 2020. Consensus statement from 2nd international conference on controversies in vitamin D. *Rev Endocr Metab Disord*, 21(1):89-116. https://doi.org/10.1007/s11154-019-09532-w

Gravitz L, 2014. Liver cancer. *Nature*, 516(7529):S1. https://doi.org/10.1038/516S1a

Gurney ME, Pu HF, Chiu AY, et al., 1994. Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science*, 264(5166):1772-1775. https://doi.org/10.1126/science.8209258

Hassoun PM, 2021. Pulmonary arterial hypertension. *N Engl J Med*, 385(25):2361-2376. https://doi.org/10.1056/NEJMra2000348

Hsieh TC, Lin CY, Bennett DJ, et al., 2014. Biochemical and cellular evidence demonstrating AKT-1 as a binding partner for resveratrol targeting protein NQO2. *PLoS ONE*, 9(6):e101070. https://doi.org/10.1371/journal.pone.0101070

Huang CY, Chen JJ, Wu JS, et al., 2015. Novel link of anti-apoptotic ATF3 with pro-apoptotic CTMP in the ischemic brain. *Mol Neurobiol*, 51(2):543-557. https://doi.org/10.1007/s12035-014-8710-0

Huang SH, O'Sullivan B, 2017. Overview of the 8th edition TNM classification for head and neck cancer. *Curr Treat Options Oncol*, 18(7):40. https://doi.org/10.1007/s11864-017-0484-y

Hwang SK, Kwon JT, Park SJ, et al., 2007. Lentivirus-mediated carboxyl-terminal modulator protein gene transfection via aerosol in lungs of K-*ras* null mice. *Gene Ther*, 14(24): 1721-1730. https://doi.org/10.1038/sj.gt.3303042

Hwang SK, Lim HT, Minai-Tehrani A, et al., 2009. Repeated aerosol delivery of carboxyl-terminal modulator protein suppresses tumor in the lungs of K-*ras*^{LA1} mice. *Am J Respir Crit Care Med*, 179(12):1131-1140. https://doi.org/10.1164/rccm.200810-1553OC

Jafari M, Ghadami E, Dadkhah T, et al., 2019. PI3K/Akt signaling pathway: erythropoiesis and beyond. *J Cell Physiol*, 234(3):2373-2385. https://doi.org/10.1002/jcp.27262

Jagannath VA, Filippini G, do Nascimento IJB, et al., 2018. Vitamin D for the management of multiple sclerosis. *Cochrane Database Syst Rev*, 9(9):CD008422. https://doi.org/10.1002/14651858.CD008422.pub3

Jeong D, Ham J, Kim HW, et al., 2021. ELOVL2: a novel tumor suppressor attenuating tamoxifen resistance in breast cancer. *Am J Cancer Res*, 11(6):2568-2589.

Jin H, Xu CX, Lim HT, et al., 2009. High dietary inorganic phosphate increases lung tumorigenesis and alters Akt signaling. *Am J Respir Crit Care Med*, 179(1):59-68. https://doi.org/10.1164/rccm.200802-306OC

Kao MH, Huang CY, Cheung WM, et al., 2023. Activating transcription factor 3 diminishes ischemic cerebral infarct and behavioral deficit by downregulating carboxyl-terminal modulator protein. *Int J Mol Sci*, 24(3):2306. https://doi.org/10.3390/ijms24032306

Klein AP, 2021. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. *Nat Rev Gastroenterol Hepatol*, 18(7):493-502. https://doi.org/10.1038/s41575-021-00457-x

Li J, Shan WR, Zuo ZY, 2018. Age-related upregulation of carboxyl terminal modulator protein contributes to the decreased brain ischemic tolerance in older rats. *Mol Neurobiol*, 55(7):6145-6154. https://doi.org/10.1007/s12035-017-0826-6

Li L, Fan CM, 2017. A CREB-MPP7-AMOT regulatory axis controls muscle stem cell expansion and self-renewal competence. *Cell Rep*, 21(5):1253-1266. https://doi.org/10.1016/j.celrep.2017.10.031

Li Y, Imai N, Nicholls HT, et al., 2021. Thioesterase superfamily member 1 undergoes stimulus-coupled conformational reorganization to regulate metabolism in mice. *Nat Commun*, 12:3493. https://doi.org/10.1038/s41467-021-23595-x

Li Y, She WY, Xu XR, et al., 2023. AAZ2 induces mitochondrial-dependent apoptosis by targeting PDK1 in gastric cancer. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 24(3): 232-247. https://doi.org/10.1631/jzus.B2200351

Li ZH, Cai BL, Abdalla BA, et al., 2019. *LncIRS1* controls muscle atrophy via sponging miR-15 family to activate IGF1-PI3K/AKT pathway. *J Cachexia Sarcopenia Muscle*, 10(2):391-410. https://doi.org/10.1002/jcsm.12374

Lin CH, Lin WD, Huang YC, et al., 2023. Carboxyl-terminal modulator protein facilitates tumor metastasis in triple-negative breast cancer. *Cancer Gene Ther*, 30(3):404-413. https://doi.org/10.1038/s41417-022-00559-x

Liu XX, Yang Q, Zhu LH, et al., 2018. Carboxyl-terminal modulator protein ameliorates pathological cardiac hypertrophy by suppressing the protein kinase B signaling pathway. *J Am Heart Assoc*, 7(13):e008654. https://doi.org/10.1161/JAHA.118.008654

Liu YP, Liao WC, Ger LP, et al., 2013. Carboxyl-terminal modulator protein positively regulates Akt phosphorylation and acts as an oncogenic driver in breast cancer. *Cancer Res*, 73(20):6194-6205. https://doi.org/10.1158/0008-5472.CAN-13-0518

Ma Z, Lou SP, Jiang Z, 2020. PHLDA2 regulates EMT and autophagy in colorectal cancer via the PI3K/AKT signaling pathway. *Aging (Albany NY)*, 12(9):7985-8000. https://doi.org/10.18632/aging.103117

Maiese K, Chong ZZ, Wang SH, et al., 2012. Oxidant stress and signal transduction in the nervous system with the PI 3-K, Akt, and mTOR cascade. *Int J Mol Sci*, 13(11): 13830-13866. https://doi.org/10.3390/ijms131113830

Maira SM, Galetic I, Brazil DP, et al., 2001. Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. *Science*, 294(5541):374-380. https://doi.org/10.1126/science.1062030

Masrori P, van Damme P, 2020. Amyotrophic lateral sclerosis:

a clinical review. *Eur J Neurol*, 27(10):1918-1929. https://doi.org/10.1111/ene.14393

Mejzini R, Flynn LL, Pitout IL, et al., 2019. ALS genetics, mechanisms, and therapeutics: where are we now? *Front Neurosci*, 13:1310. https://doi.org/10.3389/fnins.2019.01310

Missiroli S, Perrone M, Genovese I, et al., 2020. Cancer metabolism and mitochondria: finding novel mechanisms to fight tumours. *EBioMedicine*, 59:102943. https://doi.org/10.1016/j.ebiom.2020.102943

Miyawaki T, Ofengeim D, Noh KM, et al., 2009. The endogenous inhibitor of Akt, CTMP, is critical to ischemia-induced neuronal death. *Nat Neurosci*, 12(5):618-626. https://doi.org/10.1038/nn.2299

Narayanankutty A, 2019. PI3K/Akt/mTOR pathway as a therapeutic target for colorectal cancer: a review of preclinical and clinical evidence. *Curr Drug Targets*, 20(12): 1217-1226. https://doi.org/10.2174/1389450120666190618123846

Natarajan GK, Mishra J, Camara AKS, et al., 2021. LETM1: a single entity with diverse impact on mitochondrial metabolism and cellular signaling. *Front Physiol*, 12:637852. https://doi.org/10.3389/fphys.2021.637852

Ni FB, Lin Z, Fan XH, et al., 2020. A novel genomic-clinicopathologic nomogram to improve prognosis prediction of hepatocellular carcinoma. *Clin Chim Acta*, 504:88-97. https://doi.org/10.1016/j.cca.2020.02.001

Ono H, Sakoda H, Fujishiro M, et al., 2007. Carboxy-terminal modulator protein induces Akt phosphorylation and activation, thereby enhancing antiapoptotic, glycogen synthetic, and glucose uptake pathways. *Am J Physiol Cell Physiol*, 293(5):C1576-C1585. https://doi.org/10.1152/ajpcell.00570.2006

Park J, Li YW, Kim SH, et al., 2014. New players in high fat diet-induced obesity: LETM1 and CTMP. *Metabolism*, 63(3):318-327. https://doi.org/10.1016/j.metabol.2013.10.012

Piao L, Yang ZT, Feng Y, et al., 2019a. LETM1 is a potential biomarker of prognosis in lung non-small cell carcinoma. *BMC Cancer*, 19:898. https://doi.org/10.1186/s12885-019-6128-9

Piao L, Feng Y, Yang ZT, et al., 2019b. LETM1 is a potential cancer stem-like cell marker and predicts poor prognosis in colorectal adenocarcinoma. *Pathol Res Pract*, 215(7): 152437. https://doi.org/10.1016/j.prp.2019.152437

Porporato PE, Filigheddu N, Pedro JMBS, et al., 2018. Mitochondrial metabolism and cancer. *Cell Res*, 28(3):265-280. https://doi.org/10.1038/cr.2017.155

Revathidevi S, Munirajan AK, 2019. Akt in cancer: mediator and more. *Semin Cancer Biol*, 59:80-91. https://doi.org/10.1016/j.semcancer.2019.06.002

Sarbassov DD, Guertin DA, Ali SM, et al., 2005. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*, 307(5712):1098-1101. https://doi.org/10.1126/science.1106148

Shariati M, Meric-Bernstam F, 2019. Targeting AKT for cancer therapy. *Expert Opin Investig Drugs*, 28(11):977-988. https://doi.org/10.1080/13543784.2019.1676726

Shin JY, Chung YS, Kang B, et al., 2013. Co-delivery of LETM1 and CTMP synergistically inhibits tumor growth in H-ras12V liver cancer model mice. *Cancer Gene Ther*, 20(3):186-194. https://doi.org/10.1038/cgt.2013.6

Shin N, Yi MH, Kim S, et al., 2017. Astrocytic expression of CTMP following an excitotoxic lesion in the mouse hippocampus. *Exp Neurobiol*, 26(1):25-32. https://doi.org/10.5607/en.2017.26.1.25

Simon PO, McDunn JE, Kashiwagi H, et al., 2009. Targeting AKT with the proapoptotic peptide, TAT-CTMP: a novel strategy for the treatment of human pancreatic adenocarcinoma. *Int J Cancer*, 125(4):942-951. https://doi.org/10.1002/ijc.24424

Song MQ, Bode AM, Dong ZG, et al., 2019. AKT as a therapeutic target for cancer. *Cancer Res*, 79(6):1019-1031. https://doi.org/10.1158/0008-5472.CAN-18-2738

Sumi T, Tsuneyoshi N, Nakatsuji N, et al., 2008. Defining early lineage specification of human embryonic stem cells by the orchestrated balance of canonical Wnt/β-catenin, Activin/Nodal and BMP signaling. *Development*, 135(17): 2969-2979. https://doi.org/10.1242/dev.021121

Sun XT, Kellner M, Desai AA, et al., 2016. Asymmetric dimethylarginine stimulates Akt1 phosphorylation via heat shock protein 70-facilitated carboxyl-terminal modulator protein degradation in pulmonary arterial endothelial cells. *Am J Respir Cell Mol Biol*, 55(2):275-287. https://doi.org/10.1165/rcmb.2015-0185OC

Suo CJ, Gui ZP, Wang ZJ, et al., 2021. Bortezomib limits renal allograft interstitial fibrosis by inhibiting NF-κB/TNF-α/Akt/mTOR/P70S6K/Smurf2 pathway via IκBα protein stabilization. *Clin Sci (Lond)*, 135(1):53-69. https://doi.org/10.1042/CS20201038

Thai AA, Solomon BJ, Sequist LV, et al., 2021. Lung cancer. *Lancet*, 398(10299):535-554. https://doi.org/10.1016/S0140-6736(21)00312-3

Tillander V, Miniami A, Alves-Bezerra M, et al., 2019. Thioesterase superfamily member 2 promotes hepatic insulin resistance in the setting of glycerol-3-phosphate acyltransferase 1-induced steatosis. *J Biol Chem*, 294(6): 2009-2020. https://doi.org/10.1074/jbc.RA118.005184

Trayes KP, Cokenakes SEH, 2021. Breast cancer treatment. *Am Fam Physician*, 104(2):171-178.

Vaes N, Schonkeren SL, Rademakers G, et al., 2021. Loss of enteric neuronal *Ndrg4* promotes colorectal cancer via increased release of Nid1 and Fbln2. *EMBO Rep*, 22(6): e51913. https://doi.org/10.15252/embr.202051913

Vincent A, Herman J, Schulick R, et al., 2011. Pancreatic cancer. *Lancet*, 378(9791):607-620. https://doi.org/10.1016/S0140-6736(10)62307-0

Wang B, Xu X, Liu X, et al., 2021. Enolase-phosphatase 1 acts as an oncogenic driver in glioma. *J Cell Physiol*, 236(2):1184-1194.

https://doi.org/10.1002/jcp.29926

Wang DT, Yin Y, Yang YJ, et al., 2014. Resveratrol prevents TNF-α-induced muscle atrophy via regulation of Akt/mTOR/FoxO1 signaling in C2C12 myotubes. *Int Immunopharmacol*, 19(2):206-213. https://doi.org/10.1016/j.intimp.2014.02.002

Wang JM, Fry CME, Walker CL, 2019. Carboxyl-terminal modulator protein regulates Akt signaling during skeletal muscle atrophy *in vitro* and a mouse model of amyotrophic lateral sclerosis. *Sci Rep*, 9:3920. https://doi.org/10.1038/s41598-019-40553-2

Wang JM, Tierney L, Wilson C, et al., 2023. Carboxyl-terminal modulator protein (CTMP) deficiency mitigates denervation-induced skeletal muscle atrophy. *Biochem Biophys Res Commun*, 644:155-161. https://doi.org/10.1016/j.bbrc.2023.01.023

Wang QS, He YH, Shen YJ, et al., 2014. Vitamin D inhibits COX-2 expression and inflammatory response by targeting thioesterase superfamily member 4. *J Biol Chem*, 289(17):11681-11694. https://doi.org/10.1074/jbc.M113.517581

Wang WY, Wen QY, Xu LN, et al., 2014. Activation of Akt/mTOR pathway is associated with poor prognosis of nasopharyngeal carcinoma. *PLoS ONE*, 9(8):e106098. https://doi.org/10.1371/journal.pone.0106098

Wang XK, Marchisio MA, 2021. Synthetic polycistronic sequences in eukaryotes. *Synth Syst Biotechnol*, 6(4):254-261. https://doi.org/10.1016/j.synbio.2021.09.003

Wen L, Liu L, Tong LY, et al., 2019. NDRG4 prevents cerebral ischemia/reperfusion injury by inhibiting neuronal apoptosis. *Genes Dis*, 6(4):448-454. https://doi.org/10.1016/j.gendis.2019.01.001

Wu X, Liang YR, Jing XN, et al., 2018. Rifampicin prevents SH-SY5Y cells from rotenone-induced apoptosis via the PI3K/Akt/GSK-3β/CREB signaling pathway. *Neurochem Res*, 43(4):886-893. https://doi.org/10.1007/s11064-018-2494-y

Xu HJ, Yan XJ, Zhu HC, et al., 2022. TBL1X and Flot2 form a positive feedback loop to promote metastasis in nasopharyngeal carcinoma. *Int J Biol Sci*, 18(3):1134-1149. https://doi.org/10.7150/ijbs.68091

Yin HD, He HR, Shen XX, et al., 2020. MiR-9-5p inhibits skeletal muscle satellite cell proliferation and differentiation by targeting IGF2BP3 through the IGF2-PI3K/Akt signaling pathway. *Int J Mol Sci*, 21(5):1655. https://doi.org/10.3390/ijms21051655

Zeng J, He SL, Li LJ, et al., 2021. Hsp90 up-regulates PD-L1 to promote HPV-positive cervical cancer via HER2/PI3K/AKT pathway. *Mol Med*, 27:130. https://doi.org/10.1186/s10020-021-00384-2

Zhang EY, Shi HL, Yang L, et al., 2017. Ginsenoside Rd regulates the Akt/mTOR/p70S6K signaling cascade and suppresses angiogenesis and breast tumor growth. *Oncol Rep*, 38(1):359-367. https://doi.org/10.3892/or.2017.5652

Zhang SS, Xue R, Geng YP, et al., 2020. Fisetin prevents HT22 cells from high glucose-induced neurotoxicity *via* PI3K/Akt/CREB signaling pathway. *Front Neurosci*, 14:241. https://doi.org/10.3389/fnins.2020.00241

Zhang SX, Li DG, Zhao M, et al., 2021. Exosomal miR-183-5p shuttled by M2 polarized tumor-associated macrophage promotes the development of colon cancer *via* targeting THEM4 mediated PI3K/AKT and NF-κB pathways. *Front Oncol*, 11:672684. https://doi.org/10.3389/fonc.2021.672684

Zhang X, Dong ZC, Fan H, et al., 2023. Scutellarin prevents acute alcohol-induced liver injury via inhibiting oxidative stress by regulating the Nrf2/HO-1 pathway and inhibiting inflammation by regulating the AKT, p38 MAPK/NF-κB pathways. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 24(7):617-631. https://doi.org/10.1631/jzus.B2200612

Zhao H, Lim K, Choudry A, et al., 2012. Correlation of structure and function in the human hotdog-fold enzyme hTHEM4. *Biochemistry*, 51(33):6490-6492. https://doi.org/10.1021/bi300968n

Zhao SJ, Kong FQ, Jie J, et al., 2020. Macrophage MSR1 promotes BMSC osteogenic differentiation and M2-like polarization by activating PI3K/AKT/GSK3β/β-catenin pathway. *Theranostics*, 10(1):17-35. https://doi.org/10.7150/thno.36930

Zhou BY, Yang CH, Yan X, et al., 2020. LETM1 knockdown promotes autophagy and apoptosis through AMP-activated protein kinase phosphorylation-mediated Beclin-1/Bcl-2 complex dissociation in hepatocellular carcinoma. *Front Oncol*, 10:606790. https://doi.org/10.3389/fonc.2020.606790

Zhu MF, Zheng R, Guo YW, et al., 2017. NDRG4 promotes myogenesis via Akt/CREB activation. *Oncotarget*, 8(60):101720-101734. https://doi.org/10.18632/oncotarget.21591

Zhuravleva E, Gut H, Hynx D, et al., 2012. Acyl coenzyme A thioesterase Them5/Acot15 is involved in cardiolipin remodeling and fatty liver development. *Mol Cell Biol*, 32(14):2685-2697. https://doi.org/10.1128/MCB.00312-12

Zong WX, Rabinowitz JD, White E, 2016. Mitochondria and cancer. *Mol Cell*, 61(5):667-676. https://doi.org/10.1016/j.molcel.2016.02.011