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The role of the adenylate kinase 5 gene in various diseases and cancer

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Abstract

Adenylate kinases (AKs) are important enzymes involved in cellular energy metabolism. Among AKs, AK5 (adenylate kinase 5), a cytosolic protein, is emerging as a significant contributor to various diseases and cellular processes. This comprehensive review integrates findings from various research groups on AK5 since its discovery, shedding light on its multifaceted roles in nucleotide metabolism, energy regulation, and cellular differentiation. We investigate its implications in a spectrum of diseases, including autoimmune encephalitis, epilepsy, neurodegenerative disorders such as Alzheimer's and Parkinson's, diabetes, lower extremity arterial disease, celiac disease, and various cancers. Notably, AK5's expression levels and methylation status have been associated with cancer progression and patient outcomes, indicating its potential as a prognostic indicator. Furthermore, AK5 is implicated in regulating cellular processes in breast cancer, gastric cancer, colorectal carcinoma, prostate cancer, and colon adenocarcinoma, suggesting its relevance across different cancer types. However, a limitation lies in the need for more robust clinical validation and a deeper understanding of AK5's precise mechanisms in disease pathogenesis, despite its association with various pathophysiological conditions. Nonetheless, AK5 holds promise as a therapeutic target, with emerging evidence suggesting its potential in therapy development.

Introduction

Adenine nucleotides are interconverted by phosphotransferase enzymes called adenylate kinases (AKs). The AKs play a crucial role in maintaining cellular energy homeostasis and high levels of ATP through a combination of AK activity and mitochondrial oxidative phosphorylation [1]. Adenylate kinase 5 (AK5) gene expression was initially found in brain tissue [2]. Research accumulated over time indicates that in addition to the brain, AK5 also appears in endometrial tissues, cardiac tissue, Wharton's jelly cells, pancreatic beta cells, etc [3–6]. Furthermore, according to data from The Human Protein Atlas (www.proteinatlas.org), which employs stringent evaluation methods including immunohistochemical staining patterns and ribonucleic acid sequencing (RNA-seq) data analysis, AK5 is also expressed prominently in kidney, testis, breast, stomach, duodenum, and small intestine, with particularly high expression observed in the neurons of the hippocampus and cerebral cortex [7].

Van Rompay *et al.* [2] found a novel human AK complementary deoxyribonucleic acid (cDNA), designated AK5, by searching the Expressed Sequence Tag (EST) database. This protein has a glycine-rich region that can bind nucleoside triphosphates and a distinct site that can bind nucleoside monophosphates. Additionally, it possesses a lid domain that covers the substrate when it binds [2] The AK5 gene is 278 kb long and has 14 exons. It has three variants that are different in how start codons are used, and the full-length protein has 562 amino acids. The two catalytic domains are known as AK5p1 and AK5p2, and they both contain a glycine-rich p-loop, a nucleoside monophosphate (NMP)-binding domain, and a LID domain. One of the variants encodes a 536 amino acid protein with both AK domains, but it has a shorter N-terminus than full-length AK5. A third transcript encodes a deduced 198-amino acid protein that only contains AK5p2 and is identical to the transcript described earlier [8].

Along with the other AKs, AK5's main job is to maintain the homeostasis of nucleotide metabolism and energy metabolism. Recent research has uncovered its involvement in various cellular processes, including the differentiation of cardiac cells, the stemness of Wharton's jelly cells, and the inhibition of proteasome activity in mantle cell lymphoma [3,4,9,10]. However, the significance of AK5 extends far beyond these functions encompassing a wide range of pathophysiological conditions, including autoimmune encephalitis, epilepsy, neurodegenerative diseases, diabetes, lower extremity arterial disease, celiac disease (CD), and several cancers (Table 1). Given this broad spectrum of involvement, understanding the role of AK5 in various diseases has emerged as an area of intense research interest. In this review, we aim to provide a comprehensive summary of studies investigating the role of the AK5 since its discovery. Furthermore, we highlight the significance of AK5 in disease pathogenesis and its implications

Table 1. The role of AK5 in different pathophysiological conditions

Diseases	Association with AK5 gene	References
Alzheimer	Downregulated expression. AK5-14-3-3ζ complex.	[11,12]
Asthma	Downregulated expression.	[13]
Autoimmune limbic encephalitis	Anti-AK5 IgG antibodies target neuronal cytosolic AK5 protein.	[14–19]
Breast cancer	Aberrant methylation of AK5 gene.	[20]
Celiac disease	3 bp ATT insertional mutation in gene.	[21]
Colon adenocarcinoma	Control tumor suppressor proteins p16 and p21. Regulate proliferation and metastases.	[22]
Colorectal Carcinoma	AK5 gene hypermethylated. Modulate AMPK/mTOR metastatic signaling pathway.	[23]
Gastric cancer	Regulate autophagy, proliferation & apoptosis.	[24]
Lower extremity arterial disease	Downregulated expression.	[25]
Parkinson	Upregulated expression.	[26]
Prostate Cancer	High expression better survival.	[27]
Temporal lobe epilepsy	Downregulated expression. AK5-CPNE6 complex.	[28]

for developing novel diagnostic and therapeutic strategies. Through this review, we seek to elucidate the potential of AK5 as a therapeutic target and prognostic indicator and we aim to underscore the importance of further research into AK5 and its multifaceted roles in health and disease.

Role in cancers

Breast cancer

In most nations around the world, breast cancer is the most frequently diagnosed cancer in women, accounting for one-fourth of all cancer diagnoses in females and it also ranks first among factors that lead to the death of women from cancer [29,30]. The landscape of cancer genome methylation has been examined in a variety of tumor types, with microarrays serving as the primary analytical tool to date. DNA methylation has been the most thoroughly characterized [31,32].

It has long been understood that CpG islands, which are typically unmethylated, can become methylated in cancer, which causes gene repression [33]. CpG islands in the 5' upstream promoter region of the AK5 gene were found to be aberrantly methylated in primary breast cancer samples, and in MCF-7, and MDA-MB-231 breast cancer cell lines (Fig. 1a). It was also found to be expressed in normal human mammary epithelial cells and adjacent non-cancerous tissues from a breast cancer patient in stage 1 and stage 2. Twenty genes were identified to be aberrantly methylated in human breast cancer cells [20]. Based on this, Miyamoto et al. proposed that AK5 might serve as a promising biomarker for breast cancer. However, data from The Human Protein Atlas (www.proteinatlas.org), suggest AK5 does not act as a prognostic indicator in breast cancer. This data is based on the antibody staining of more than 1000 human breast cancer samples [34,35]. Interestingly, AK2 was found to be highly expressed in breast cancer tissues, with its overexpression associated with increased malignancy [36]. The Human Protein Atlas data also support AK2 as a prognostic factor in breast cancer, where high expression is linked to a favorable outcome [34,35]. Therefore, AK2 may be more relevant as a therapeutic target for breast cancer than AK5.

Gastric cancer

In terms of cancer-related mortality and incidence globally, gastric cancer (GC) comes in third and fifth positions, respectively [37]. The disease is frequently diagnosed at an advanced stage due to the late onset of clinical symptoms, which limits the available therapeutic approaches in more than 50% of cases [29].

In GC patients, a high level of AK5 protein was suggested as an indicator of a significantly unfavorable prognostic factor and is associated with T-stage and N-stage cancer rather than with metastasis and differentiation. AK5 knockdown using AK5 siRNA in human GC cell lines AZ521 and MKN74 drastically inhibited proliferation and autophagy and promoted apoptosis. AK5 is, therefore, a potential oncogene, prognostic marker, and therapeutic target for GC (Fig. 1b) [24].

Colorectal carcinoma (CRC)

CRC is a disorder that only affects the colon or rectum and is brought on by the colon's abnormally high rate of glandular epithelial cell proliferation [30]. In 2023, CRC accounted for 10% of all cancer-related cases, being the second leading cause of cancer-related deaths worldwide [38]. For patients with metastatic lesions, the prognosis for CRC has never been satisfactory [39].

Comparing the AK5 gene in CRC patients' samples to nearby normal tissue and controls revealed significant hypermethylation in tumor DNA. Real-time PCR analysis further indicates that the relationship between AK5 DNA methylation and expression in CRC samples is inverse. In CRC cell lines, the AK5 promoter commonly undergoes hypermethylation, which causes methylation-mediated gene silencing and AK5 gene expression inhibits cell migration and invasion via modulating the adenosine monophosphate-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) signaling pathway, which strongly suggests its possible association with metastasis (Fig. 2) [23].

Prostate cancer

Prostate cancer (PCa) affects millions of men worldwide and is a complex heterogeneous disease. The progression of PCa is strongly associated with the accumulation of mutations leading to genome instability over a patient's lifetime [40,41]. The multidisciplinary field of research into prostate cancer is very active and now includes computational biology in addition to laboratory and clinical science [41].

A recent study on prostate cancer (PCa) using bioinformatics analysis based on high-throughput next-generation sequencing technology proposed a novel risk model: "a three-gene signature model (KCNK3, AK5, and ARHGEF38)" strongly associated with cancer-related pathways, overall survival (OS), and tumor microenvironment-related immune cells in PCa. A reduced chance of survival was linked to high expression of ARHGEF38, whereas a higher chance of survival was linked to high expression of KCNK3 and AK5 (Fig. 3). Gene expression alterations in the

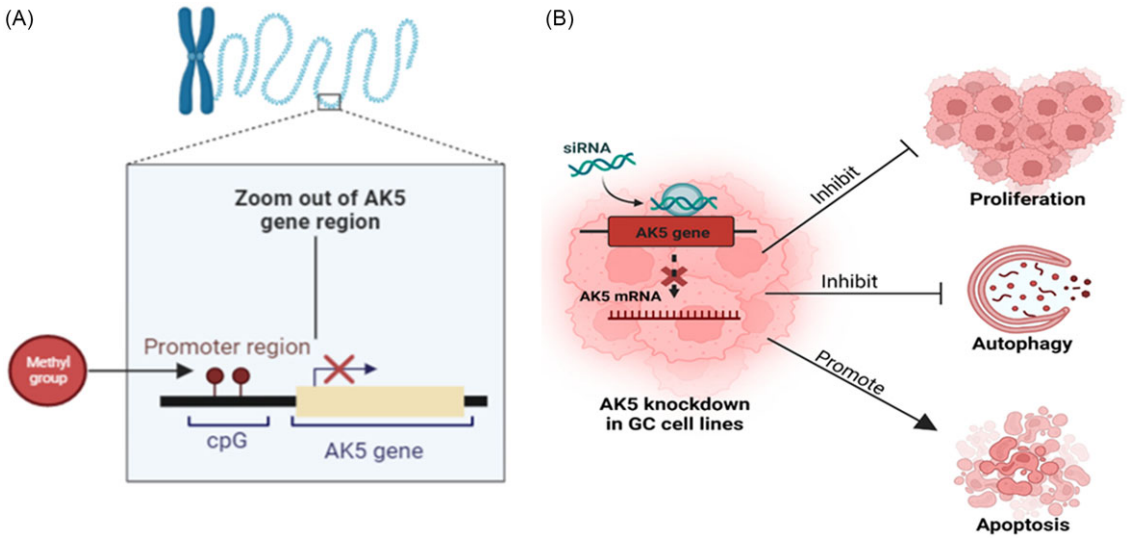

Figure 1. (***a***) in breast cancer patients, the promoter region AK5 gene was found aberrantly methylated. (***b***) AK5 knockdown using small interfering ribonucleic acid (siRNA) in human gastric cancer cell lines drastically inhibits cell proliferation and autophagy and promotes apoptosis at the molecular level.

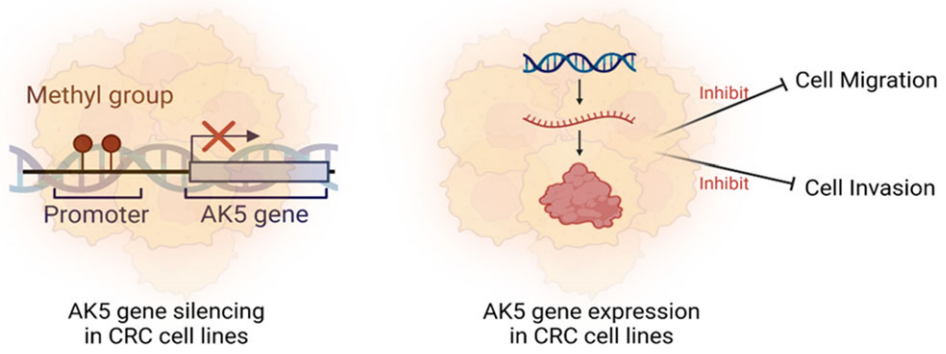

Figure 2. AK5 gene promoter region in colorectal carcinoma cell lines is hypermethylated which inhibits the expression of the AK5 gene. AK5 gene expression inhibits cell migration and cell invasion.

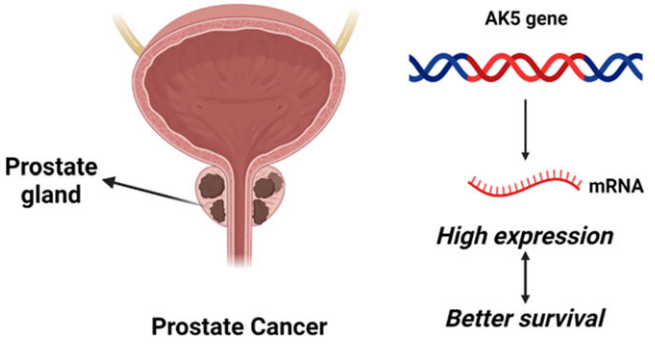

Figure 3. High AK5 gene expression is associated with better survival probability of prostate cancer patients.

model have clinical importance since they are strikingly related to PCa patients' OS [27].

Colon adenocarcinoma

The most frequent malignant tumor of the digestive tract is colon adenocarcinoma (COAD), whose etiology is currently unclear [42,43].

In recent research that used both patient samples and cell lines, the role of AK5 in the onset and progression of COAD was examined. It was found that low AK5 expression can serve as a standalone prognostic biomarker in clinical diagnosis and that low AK5 protein also promoted proliferation and metastasis by controlling the tumor-suppressor proteins p16 and p21, which are involved in the cell-cycle pathway (Fig. 4). These findings open new perspectives on COAD and could have a significant impact on the creation of novel COAD therapeutics and clinical diagnostic approaches [22].

Role in brain disorders

Alzheimer

Alzheimer's disease (AD) is the most prevalent form of dementia and is characterized by neuritic plaques and neurofibrillary tangles because of amyloid-beta peptide (Aβ) buildup in the medial temporal lobe and neocortical structures, the area of the brain most affected [44]. All eukaryotic cells express the 14-3-3 protein family of regulatory molecules, but they are particularly abundant in the cytoplasm of neurons in cerebral tissue [45]. There are numerous brain functions and various brain disorders that are closely

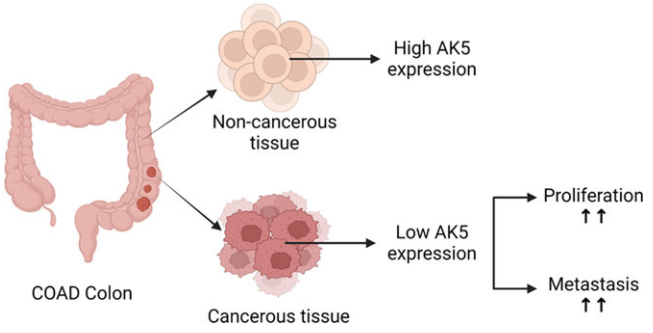

Figure 4. In colon adenocarcinoma, higher AK5 protein expression is found in adjacent non-cancerous tissue compared to cancerous tissue. Low AK5 expression promotes proliferation and metastasis.

associated with 14-3-3 proteins, most of which are expressed in the brain [46]. Evidence suggests that these proteins are involved in brain development, memory formation, and several neurological disorders [47]. These proteins are associated with tau deposition in neurofibrillary tangles [48]. In AD, there is an abnormal increase in the phosphorylation of the tau protein [49]. In a study, it was discovered that a specific isoform of 14.3.3 proteins, namely 14-3-3 ζ, actively facilitated the glycogen synthase kinase-3 beta-dependent phosphorylation of tau [50].

In vivo proteomic analysis of brain tissue from transgenic mice using tandem affinity purification and LC-MS method identified almost 40 novel 14-3-3**ζ** binding proteins including AK5, which had consensus sequences for 14-3-3 binding. Further, co-immunoprecipitation studies confirmed the association of 14-3-3**ζ** with AK5 (Fig. 5b). AK5 interacts directly in the brain with the 14.3.3 **ζ** complex [11]. In humans, whole-transcript expression assays found deregulated expression of genes involved in purine metabolism in AD samples compared to controls. Further, transcriptome analysis of these genes found differential expression of messenger ribonucleic acid (mRNAs) with regional variations. AD stages V-VI, AK5 mRNA was found to be differentially downregulated in the frontal cortex (Fig. 5a) [12].

Parkinson

Parkinson's disease (PD) is a progressive neurodegenerative condition marked by tremors, rigidity, postural instability, and bradykinesia in motor function. The fastest-growing neurological disease in terms of deaths, years lived with a disability, and prevalence is the second most prevalent neurodegenerative condition after AD [51]. A similar study like Alzheimer's was performed on human cases of PD brains, which explored alterations in gene expression at mRNA level, and encoding enzymes involved in purine metabolism, finding upregulation of AK5 mRNA in the frontal cortex area 8 at stages 5–6 (Fig. 5c) [26].

Autoimmune limbic encephalitis

Autoimmune limbic encephalitis (ALE) is a syndrome of rapidly progressive neurological disease characterized by psychiatric disturbances, memory deficits, and seizure activity caused by antibodies that target antigens of the central nervous system either intracellularly or on the cellular surface [18,52]. Initially considered paraneoplastic, the condition was later found, with the discovery of disease-causing antibodies, to be non-paraneoplastic in many cases [53].

In severe non-paraneoplastic ALE refractory to immunotherapy, adenylate kinase 5 (AK5) immunoglobulin G (IgG) antibodies were found either in the serum or cerebrospinal fluid (CSF) targeting neuronal cytosolic AK5 protein. IgG is considered a biomarker of the disease (Fig. 6) [14–19]. As of 2023 more than 30 cases of anti-AK5 encephalitis have been recognized and they were administrated with immunotherapeutic agents [54–56]. The specific etiology of anti-AK5 encephalitis is yet to be determined, and the precise pathogenic mechanism leading to its development remains ambiguous. Presently, there are no available AK5-blocking antibodies or compounds for therapeutic intervention.

Temporal lobe epilepsy

Among epilepsy, TLE is the most prevalent type, which is frequently resistant to anti-epileptic drugs (AEDs) [57]. Chronic spontaneous recurrent seizures are the most common hallmarks of TLE, and epileptic patients show frequent symptoms of memory loss, psychiatric disorders, and behavioral problems [57,58].

AK5 protein considerable downregulation was reported in temporal lobe epilepsy patients and in epileptic rat models (Fig. 7a). Co-immunoprecipitation analysis confirmed the interaction of AK5 protein with a brain-specific protein, calcium-dependent phospholipid-binding protein (CPNE6), and this AK5-CPNE6 complex could play an important role in controlling epileptic seizures and could be involved in the development of refractory epilepsy (Fig. 7b) [28]. Studies on CPNE6 provide evidence of its link to brain development, memory formation, long-term potentiation and its upregulation is closely related to axonal growth and epilepsy [59–62].

Possible association with type 2 diabetes

In many tissues, ATP-sensitive potassium (K-ATP) channels – so named because intracellular ATP inhibits them – play important physiological roles. These channels control glucose-dependent insulin secretion in pancreatic cells and are the target of sulfonylurea medications used to treat type 2 diabetes [63]. K-ATP channels are membranous proteins found in many organs and play important roles in linking metabolism with the electrical activity of the cell [64].

In the substantia nigra, cerebral cortex, hippocampus, basal ganglia, and thalamic nucleus, K-ATP channels are highly expressed. These channels can be found in presynaptic membranes, axons, and cell bodies [65]. The K-ATP channels are hetero-octamer protein complexes having four Kir6.2 subunits, which make pores and four regulatory sulphonylurea receptor subunits in the islet cells [66,67].

Food intake leads to an increase in glucose metabolism which inhibits β-cell K-ATP channel activity due to rise in intracellular adenosine triphosphate/adenosine diphosphate [ATP]/[ADP] ratio, leading to insulin secretion by affecting the membrane electrical activity [67]. Patch clamp study on AK1 gene knockout mice found reduction in β-cells K-ATP channel activity though the activity was not completely suppressed. Increase in the concentration of ADP in pancreatic beta cells favors insulin secretion and decreases K-ATP channel activity. When AMP concentration increases, AK1 catalyzes the formation of ADP in presence of ATP

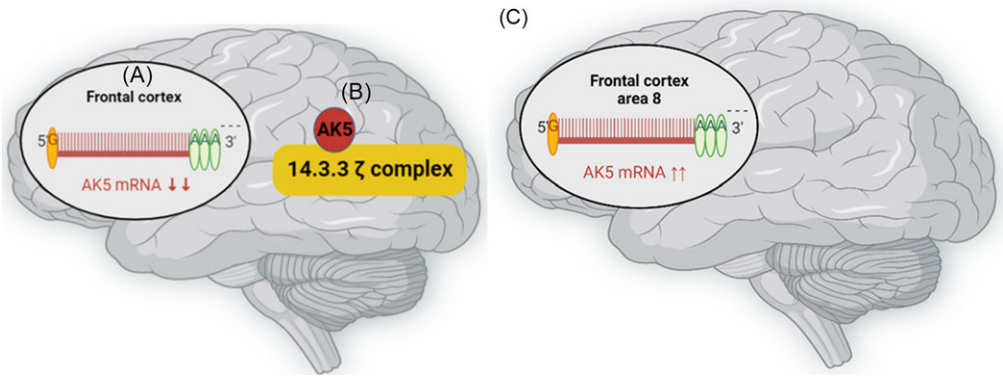

Figure 5. (***a*** and ***b***) AK5 association with Alzheimer: (***a***) AK5 gene showing downregulation in the frontal cortex; (***b***) AK5 protein co-immunoprecipitated with 14-3-3ζ complex. (***c***) AK5 association with Parkinson: AK5 gene showing upregulation at transcriptional level in the frontal cortex area eight of the brain.

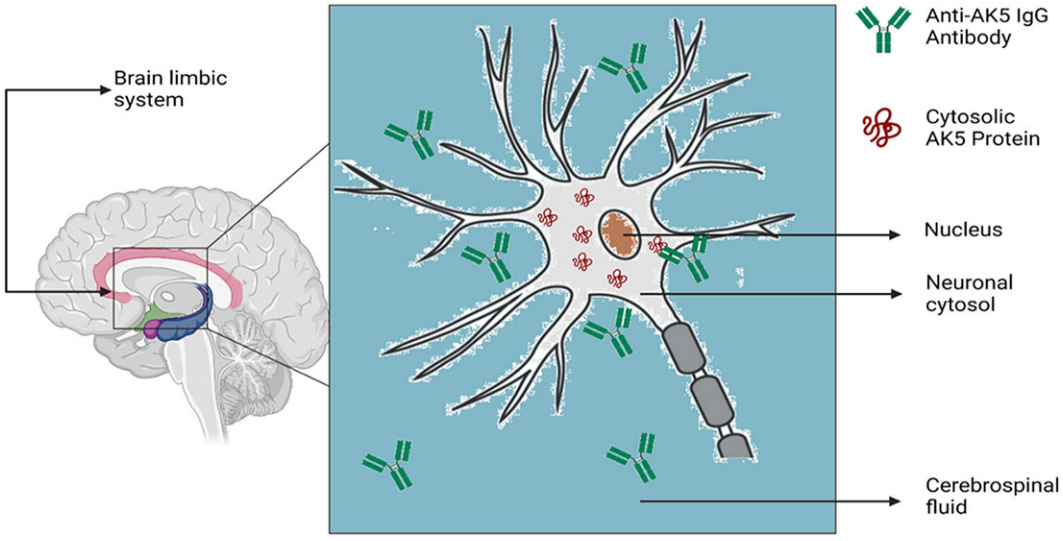

Figure 6. Anti-AK5 IgG antibodies present in cerebrospinal fluid targeting the neuronal cytosolic target, AK5 protein, in the brain limbic area of autoimmune limbic encephalitis.

and thereby reduces K-ATP channel activity [68]. Two cytosolic isoforms of AK, AK1, and AK5 are expressed in human islets and INS-1 cells (mouse islet), and AK1 was immunoprecipitated with the Kir6.2 subunit of K-ATP channel [6]. We propose that these studies raised the possibility that the other cytosolic isoform, AK5, might be involved in compensating the role of cytosolic AK1 in AK1 gene knockout mice by regulating ATP/ADP ratio and K-ATP channel activity (Fig. 8).

Role in other disorders

Lower extremity arterial disease (LEAD)

In atherosclerosis, the wall of the artery builds lesions which often lead to narrowing due to the accumulation of atheromatous plaque [69,70]. The most common appearance of atherosclerosis is peripheral arterial disease (PAD) which is a chronic inflammatory process in which formation of atheromatous plaques in arteries is accelerated [71,72].

LEAD is one of the characteristics of PAD, in which chronic degeneration occurs due to the check-in blood flow caused by stenosis or occlusion of lower limb vessels [73]. In patients with LEAD, high-throughput sequencing was used to study microRNAome and transcriptome of peripheral blood mononuclear cells (PBMCs), finding dysregulation of 26 microRNA and 14 genes (AK5 gene was downregulated), that can be used as novel biomarkers of LEAD in new diagnostic and therapeutic approaches [25].

Celiac disease

CD is a multi-factorial long-term auto-inflammatory disorder, mainly affecting the small intestine where individuals have permanent intolerance to gluten, present in foods such as wheat, rye, and barley and it occurs in both sporadic and familial forms [21,74,75]. Next-generation sequencing of exome of a Saudi Arabia consanguineous family with CD found a rare 3 bp ATT insertional mutation in AK5 gene and showed the autosomal recessive mode of inheritance, which suggests AK5 gene probably playing a role in this disease [21].

Asthma

Asthma (AS) is a heterogeneous clinical syndrome that is defined as a chronic inflammatory disease of the respiratory airways. Airway hyper-responsiveness, or an exaggerated airway-

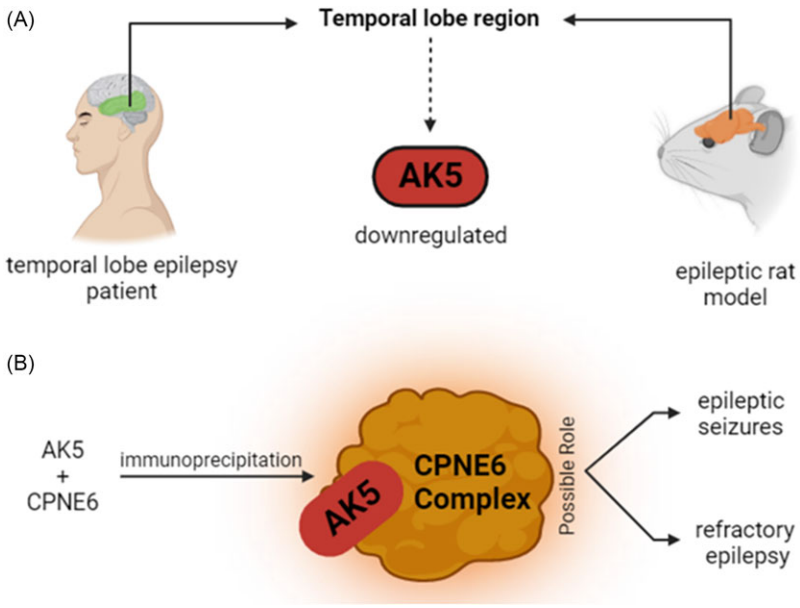

Figure 7. Association of AK5 in temporal lobe epilepsy. (***a***) AK5 protein is downregulated in the temporal lobe area of temporal lobe epilepsy patients and epileptic rat model. (***b***) AK5 protein co-immunoprecipitated with brain specific protein, calcium-dependent phospholipid-binding protein (CPNE6) complex.

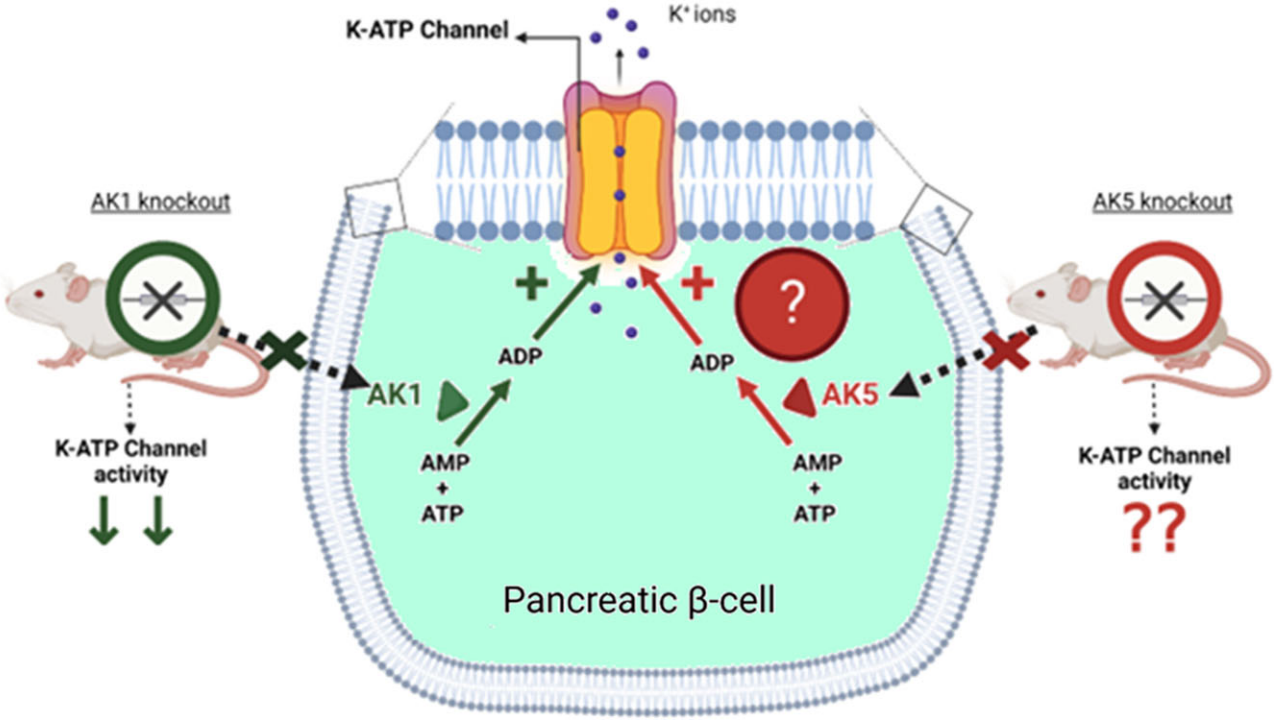

Figure 8. K-ATP channel activity is reduced in pancreatic β-cell cells of AK1 gene knockout mice. ADP production is decreased in the absence of the AK1 enzyme, and ADP is crucial for improving K-ATP channel performance. A similar experiment using mice lacking the AK5 gene to study its role in K-ATP channel activity remains to be done.

narrowing response to certain triggers like viruses, allergens, and exercise, is linked to chronic inflammation and causes recurrent episodes of wheezing, breathlessness, chest tightness, and/or coughing that can change over time and in intensity [76]. The most prevalent leukocytes among inflammatory cells in alveoli, distal airspaces, and conducting airways are macrophages [77–79]. Macrophages play a variety of roles in AS inflammation, such as changing the production of cytokines or chemokines that are anti-inflammatory and activating inflammasomes to control cellular functions [80].

The screening of differentially expressed genes (DEGs) related to macrophages in AS through samples obtained from a public database to build a risk prediction model and explore its predictive abilities in AS diagnosis suggests a new diagnostic model based on

10 macrophages-related DEG signatures to predict AS risk. Among these novel biomarkers associated with macrophages in AS, AK5 gene was found downregulated when compared to controls [13].

Conclusion

As a member of the AK family, the function of AK5 is not only restricted to preserving the homeostasis of nucleotide and energy metabolism in living cells, but studies suggest it also plays a significant role in cardiac cell differentiation, WJC stemness, and proteasome inhibition in mantle cell lymphoma. A growing body of research has shown that, in addition to the brain, AK5 is expressed at the transcriptional and translational levels in a variety of other tissues, plays a critical role in a wide range of pathophysiological disorders, and has the potential to serve as a diagnostic biomarker and a target for treating the disease.

AK5 is also associated with cell death and cell cycle signaling pathways. It has varying molecular effects on tumorigenesis in various cancers. In a three-gene signature model high AK5 gene expression is related to better OS of prostate cancer patients. In gastric cancer patients, high AK5 protein is considered an unfavorable prognostic marker whereas in COAD low AK5 protein promotes metastasis and proliferation. In breast cancer and colorectal carcinoma tissues, AK5 gene promoter region was found methylated when compared to normal tissue and methylation was inversely proportional to gene expression. By regulating AMPK/mTOR signaling and the tumor suppressor proteins p16 and p21 in colorectal carcinoma and COAD, respectively, the AK5 protein is crucial in preventing metastasis.

Mutated AK5 gene with 3 bp ATT insertional mutation is a potential candidate for CD in Saudi patients. AK5 protein might be involved in regulating ATP/ADP ratio and K-ATP channel activity in type 2 diabetes mellitus. Transcriptional dysregulation of the AK5 gene has been reported in asthma, LEAD, and neurodegenerative diseases like Alzheimer's and Parkinson's, though translational downregulation of the AK5 gene has been reported in Temporal Lobe Epilepsy patients. IgG antibodies of CSF or serum target neuronal cytosolic antigen-AK5 in severe ALE, a rapidly progressing neurological syndrome with unclear etiology. Anti-AK5 encephalitis recognized in over 30 cases, lacks specific treatment, emphasizing the need for further research into its causes and therapeutic options.

It is crucial to conduct additional research studies on AK5 because of its potential role as a prognostic indicator and therapeutic target for several different types of disorders, as well as its relationships with numerous cellular processes. There are many questions that remain to be explored. In AD, the evidence, such as the involvement of the 14.3.3 ζ protein in tau phosphorylation, deregulation of the AK5 gene, and the direct interaction between AK5 and 14.3.3 ζ in the brain, suggests that further exploration of the AK5-14.3.3 ζ complex through expanded in vivo studies and detailed investigation of intricate molecular mechanisms could yield valuable insights into its role, potentially paving the way for therapeutic advancements. A similar approach could be applied to explore the role of the AK5-CPNE6 complex role in temporal lobe epilepsy for a comprehensive understanding and potential therapeutic applications. AK5 gene expression is strongly associated with different stages of cancer by regulating signaling pathways, but its role in many other aggressive cancers like glioblastoma, pancreatic adenocarcinoma, lung cancer, etc remains to be explored.

In conclusion, while AK5 demonstrates promise as a potential diagnostic biomarker and therapeutic target for various diseases, it is essential to recognize that most studies discussed are observational or preclinical, limiting the ability to draw definitive conclusions regarding its clinical utility. Further research is warranted to validate AK5's clinical applications and elucidate its precise mechanisms of action.

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