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Role of ARK5 in cancer and other diseases (Review)

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Abstract. Malignant tumors are often exposed to hypoxic and glucose-starved microenvironments. AMP-activated protein kinase (AMPK) is an energy sensor that is stimulated during energy-deficient conditions and protects cells from hypoxic injury by regulating metabolism. AMPK-related protein kinase 5 (ARK5) is a member of the catalytic sub-unit of the AMPK family and has an important role in energy regulation and hypoxia. ARK5 is regulated by Akt and liver kinase B1 and is associated with numerous tumor-related molecules to exert the negative effects of tumors. Studies have revealed ARK5 overexpression in cases of tumor invasion and metastasis and a positive association with the degree of cancer cell malignancy, which is regarded as a key element in determining cancer prognosis. Furthermore, ARK5 downregulation improves drug sensitivity through the epithelial-mesenchymal transition pathway, indicating that it may be a potential therapeutic target. In other non-cancer conditions, ARK5 has various roles in neurodegenerative diseases (Alzheimer's and Huntington's disease), renal disorders (diabetic nephropathy and renal fibrosis) and physiological processes (striated muscle generation). In the present review, the upstream and downstream molecular pathways of ARK5 in cancer and other diseases are described and potential therapeutic strategies are discussed.

Contents

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1. Introduction

Malignant tumors often feature inadequate angiogenesis, structure and function as well as over-proliferation and increased energy demand when growing in microenvironments with insufficient blood supply (1). When cancer cells proliferate in these harsh conditions, they must adapt by regulating their cell cycles to improve blood flow and adjust the balance of energy metabolism to maintain proliferation. This is a hypoxic reaction and various molecules are involved in this process (2). Cancer cell response in hypoxic conditions has been extensively studied and hypoxia is widely accepted as a specific marker indicating poor cancer prognosis (3).

AMPK-related protein kinase 5 (ARK5) is a serine/threonine kinase that has been identified as the fifth member of the AMP-activated protein kinase (AMPK) family (4). ARK5 serves a role in the metastasis and invasion of colorectal (CRC) cancer, pancreatic cancer (PC), gastric cancer, hepatic cancer and squamous cell carcinoma (5-8). Akt, the most important ARK5 upstream regulator, phosphorylates ARK5 at the Ser600 residue (a C-terminal site outside the catalytic domain) ans activates 74 kDa kinases (9). Akt is also an important mediator of cancer proliferation, survival and oncogenesis (9,10).

ARK5-mediated Akt was established as a key element that functions as a survival factor in the complex tumorigenesis network (9). ARK5 prevents cell death under hypoxic and glucose-starved conditions by avoiding death receptor (RAS) activation in cells and inhibiting caspase-8 activation by inducing cellular Fas-associated protein with death domain-like interleukin (IL) 1β-converting enzyme-inhibitory protein (c-FLIP) in cancer cells (9,11). Previous studies have revealed that ARK5 is involved in hypoxia-induced cancer cell tolerance to glucose starvation by regulating the transforming growth factor-β (TGF-β) signaling pathway (12,13). Furthermore, ARK5 causes drug resistance by inducing certain cellular morphology transformations, including myosin filament reorganization (13).

As an important and recently investigated intermediate molecule, ARK5 has promising long-term research value. In the current review, the molecular interactions, physical progress and different functions of ARK5 in cancer and

other diseases, as well as potential therapeutic strategies, are discussed (Fig. 1).

2. Relevance of ARK5 in cancer

Multiple myeloma. Multiple myeloma is a common cancer where ~20,000 new cases are diagnosed annually in the United States (14,15). The introduction of autologous stem cell transplantation and novel drugs with various mechanisms of action (including proteasome inhibition and immunomodulation) have fundamentally changed the treatment strategy for multiple myeloma and have significantly prolonged the overall survival of patients (16-19). However, despite these advances, patients only survive for 7-8 years after diagnosis due to drug resistance and minimal residual disease (20).

c-Musculoaponeurotic fibrosarcoma (MAF), which is transcription factor that involved in immune responses and works as a T cell stimulator that induces IL-4 and IL-10 release to control T cell, was revealed to participate in the regulation of fiber cell differentiation (21-23). c-MAF translocation and overexpression have been reported in numerous cases of multiple myeloma (24-26). In various clinical trials, 351 clinical specimens exhibited clear ARK5 and c-MAF overexpression in multiple myeloma-derived cell lines. Sequence analysis of the ARK5 gene promoter further revealed that the gene contained two putative MAF-recognition elements and that ARK5 mRNA acts as a regulator in c-MAF-induced multiple myeloma (8). These results suggested that ARK5 may be a transcriptional target of the large MAF family.

A previous *in vitro* study has demonstrated that ARK5 expression is a key element in multiple myeloma invasion and metastasis and participates in these processes by modulating insulin-like growth factor (IGF)-1 expression (8). Schiller *et al* and Perumal *et al* (27,28) demonstrated that the ratio of AMP/ATP increased during energy deficiency, leading to phosphoric acid-dependent AMPK activation. Eventually, the level of ATP returned to normal due to the inhibition of energy-consuming pathways.

The overexpression of ARK5 has been previously found to exert negative effects on the apoptosis regulation of multiple myeloma by inducing glucose starvation tolerance (29). ARK5 is directly stimulated by Akt, which regulates cancer cell survival and proliferation (9-11,30). Inhibition of ARK5 can lead to the reduction in ATP levels in cells that abnormally express MYC, leading to a variety of pro-apoptotic reactions (31). MYC is widely known as a strong factor in tumorigenesis (29,32).

In conclusion, AKR5 has become a more promising choice to inhibit malignant tumors and prolong patient survival due to high reversal of drug resistance. ARK5 is closely associated with multiple myeloma and may be an effective therapeutic target.

CRC. CRC is one of the most lethal cancers worldwide (15). An available treatment method for all stages of CRC is tumor resection, with chemotherapy and radiotherapy commonly used as neoadjuvants in locally advanced CRC (33). However, the prognosis of patients with CRC remain poor (33). A total of 241 pairs of cDNA from normal tissues and 13 different tumor specimens from patients with CRC were sequenced via DNA array analysis. The results revealed that ARK5 was overexpressed in CRC. Additionally, a total of 56 clinical specimens of primary CRC and liver metastasis demonstrated high ARK5 expression (31).

Poor clinical prognosis caused by ARK5 is primarily associated with a hypoxic microenvironment (34). Hypoxia is very common in tumors and is associated with proliferation, invasiveness, metastasis and drug resistance (35). Hypoxia-inducible factor (HIF)1 serves a regulatory role and can be used as a key prognostic indicator of tumor hypoxia in CRC (36). HIF-1 is a dimer composed of HIF1-α and HIF1-β subunits (37). HIF1-α is a regulator that is triggered by hypoxia and subsequently regulates the activity of the entire complex (37). The expression and absence of HIF1-α is associated with poor prognosis in patients with CRC (38).

Kusakai *et al* (31) revealed that ARK5 was overexpressed in malignant CRC tumors and that this expression dynamically increased in hypoxic conditions. Additionally, it was determined that ARK5 and HIF1-α were overexpressed in CRC, demonstrating a clear linear correlation. ARK5 is regulated by HIF1-α, which amplifies the ARK5 signal and promotes cancer cell survival in hypoxic conditions (37). Due to this, ARK5 is highly expressed in hypoxic solid tumor-associated blood vessels, which is an another important factor in cancer cell angiogenesis and metastasis (37).

HIF1-α expression downstream of ARK5 is associated with tumor stage, tumor grade, lymph node metastasis and liver metastasis (35). However, further research is required to assess the degree of malignancy in solid tumors.

PC. *PC* is the most fatal cancer and is the eighth leading cause of cancer-related death worldwide (39). Prognosis is often poor due to high invasiveness, rapid proliferation and limited treatment (15,39).

By analyzing a rank-based meta-analysis of individual histological features associated with pancreatic ductal adenocarcinoma (40), 8 genes associated with PC progression were identified: ARK5, E2F transcription factor 3, high mobility group AT-Hook 2, RAS P21 protein activator 1, insulin receptor substrate 1, actinin α1, Sloan-Kettering oncogene and Δ-like protein 1 pre-cursor. ARK5 is one of the potential regulators that was significantly differentially expressed (40). These results may indicate that AKR5 serves a significant role in PC.

Gemcitabine (GEM) is the sole first-line drug for advanced PC, although it only prolongs patient survival for a few months due to clinical multi-drug resistance (41). Further research into this resistance mechanism is required to improve PC treatment (15,42,43). Studies have demonstrated that hypoxia increases PC cell resistance to GEM-induced apoptosis (44,45). Additionally, under hypoxic conditions, ARK5 inhibition significantly increases the sensitivity of PC cells to GEM (44). In a previous study, the inhibition of ARK5 reversed the effects of hypoxia on E-cadherin and vimentin expression, whichh are markers of epithelial-mesenchymal transition (EMT) (46). Therefore, ARK5 regulates GEM resistance under hypoxic conditions through the EMT process (47).

Furthermore, *in vivo* and *in vitro* ARK5 overexpression was demonstrated in PC metastasis and invasion models

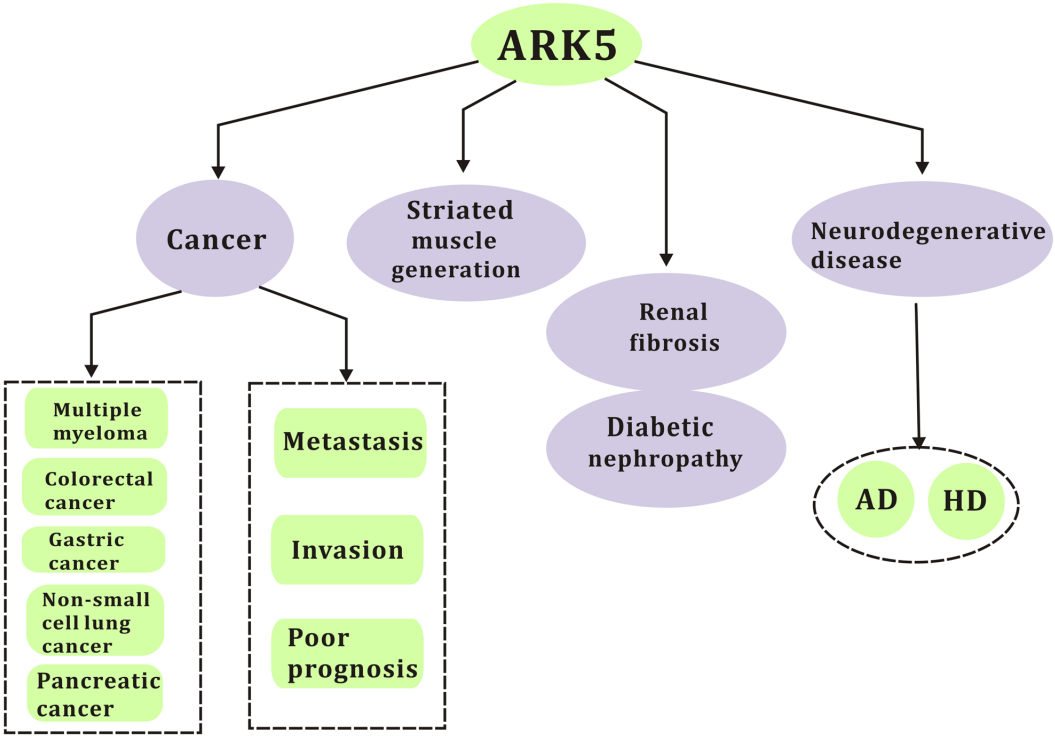

Figure 1. ARK5 serves a role in various diseases. ARK5, AMP-activated protein kinase-related protein kinase 5. AD, Alzheimer's disease; HD, Huntington's disease.

through EMT (9,10). The detection and intervention of ARK5 expression may therefore improve drug sensitivity and improve prognosis and survival time in patients with PC.

Other cancers. ARK5 expression was confirmed in various types of cancer, including osteosarcoma, ovarian, hepatic, CRC, gastric, breast and non-small cell lung cancer (NSCLC). NSCLC is one of the most common malignant tumors worldwide and accounts for ~1/3 of all cancer-related deaths (48,49). Currently, chemotherapy is one of the most effective treatments for NSCLC, with cisplatin being the standard first-line drug, though its long-term therapeutic efficacy reduced by drug resistance (49).

Although various factors are known to induce chemical resistance, the mechanism of cisplatin resistance remains unclear (50). A report has previously indicated that epithelial phenotype NSCLC is more sensitive to chemotherapy compared with a mesenchymal phenotype (51). Mesenchymal tumors that express E-cadherin regain chemical sensitivity (51). In ARK5-knockdown cells, sensitivity to cisplatin increased significantly, suggestomg that it may serve as a potential strategy to improve NSCLC drug resistance. Furthermore, in head and neck squamous cell carcinoma (HNSCC), a study has demonstrated that the expression of micro RNA (miRNA or miR) associated with the invasion and metastasis of melanoma. This was also observed in epithelial ovarian cancer (52).

ARK5 overexpression is associatied with poor prognosis (53-55) and has been identified in various solid tumors. Numerous studies have also demonstrated that ARK5 activation can induce the survival of cancer cells during nutritional deficiency (8,56).

3. Emerging role of ARK5 in cancer genesis and progression

ARK5 and Akt. ARK5 is a member of the AMPK family and its highly conserved T loop is phosphorylated by two molecules: Akt kinase, which acts on serine600 and liver kinase (LK) B1, which acts on threonine200 (57). Research has revealed that ARK5 transcription is regulated by sp1 transcription-activated protein, metabolic pressure and cofactors required at the sp-1 site (58,59). This covers almost all the pathways that involve ARK5.

Akt is a serine-threonine protein kinase that functions as a key regulator of cell survival and serves an important role in tumor genesis, cell survival, proliferation and differentiation (60). Accelerated Akt activation in malignant tumors (including invasion and metastasis) is associated with gene amplification in various types of cancer, including CRC, PC, gastric and ovarian cancer (61-63). Therefore, Akt is essential to malignant tumors.

Numerous studies have confirmed the role of Akt in promoting. tumor invasion and metastasis (31,64) during nutritional starvation. However, the downstream factors of Akt in these processes have not been determined.

A previous study has indicated that Akt-1 and Akt-2 expression in CRC and liver metastasis is higher compared with normal tissue and that ARK5 is expressed in highly malignant clinical specimens, especially in those with invasive morphology (29). Since it has been demostrated that Akt is the most direct upstream pathway of ARK5 and ARK5 is the only known protein of the AMPK familiy assocuated with the Akt pathway, this would indicate a close regulatory effect between Akt and ARK5 (55).

Furthermore, Akt mediates several cellular responses induced by insulin and IGF, such as glycogen synthesis via

the phosphorylation of glycogen synthase kinase 3 (65). In a previous study, ARK5 was revealed to mediate the invasion of PC and CRC and to promote cancer cell survival by activating the Akt signaling pathway via IGF-1 (66). Furthermore, nuclear DBF-related kinase 2 (NDR2) was revealed to be activated by IGF-1 treatment, phosphorylating threonine211 on the active T loop of ARK5 (56). ARK5 was also demonstrated to be downstream of NDR2 during activation of IGF-1 signaling (67). Therefore, ARK5 promotes cancer cell survival under the regulation of Akt and ARK5 serves an important role in hypoxia and the Akt pathway in apoptosis in various types of cancer.

The RAF-MEK-ERK and PI3K-Akt-HIF-α pathways serve an important role in cancer development as they are downstream of RAS (68), a protein that regulates cancer cell survival. Akt, as the most direct downstream regulator of RAS, regulates the downstream factor HIF1-α (69). Previous studies have determined a close association between the HIF1-α-mediated RAS pathway and ARK5 (68-70).

A linear correlation was reported between ARK5 and HIF1-α expression (68,70). In a previous study, short interfering RNA suppressed HIF1-α expression under hypoxic conditions. The results revealed that the protein and mRNA levels of ARK5 were significantly decreased indicating that ARK is regulated by HIF1-α and that ARK5 lie downstream of HIF1-α under hypoxic conditions where HIF1-α amplifies the role of ARK5 in hypoxia (57). In conclusion, ARK5 is the key gene of HIF1-α-mediated cancer proliferation and migration under hypoxic conditions.

ARK5 overexpression was demonstrated to significantly stimulate the invasiveness and metastasis of PC cells *in vitro* and *in vivo* by activating matrix metallopeptidase (MMP) and matrix metalloproteinase 1 (MT1)-MMP (7). MMPs, especially MMP-2 and MMP-9, participate in tumor metastasis and MT1-MMP is the most common activating agent of these (34,71). Research has demonstrated that ARK5 increased MMP-2 and MMP-9 production levels and induced their activation via MT1-MMP production (7).

Davis *et al* (58) previously hypothesized that ARK5 activation served as a metabolic checkpoint in the regulation of apoptosis, cell cycle progression and arrest, which confirmed ARK5-modulated 'glucose metabolism' as the most significantly aberrantly affected cellular signaling pathway in a model system for highly metastatic tumors.

ARK5 and EMT. EMT is involved in numerous biological and pathological processes, is associated with chemotherapeutic resistance and has an invasive and anti-apoptotic role in cancer tissues (72). EMT is the process by which polar epithelial cells with firm cell-cell adhesion transform into mesenchymal cells with highly invasive capacity (73). At the molecular level, the gene expression of these cells undergoes numerous changes: The expression of epithelial genes (E-cadherin, tight junction protein 1 and occludin) decreases and the expression of mesenchymal genes (N-cadherin, vimentin and fibronectin) increases (74).

In a study on epithelial ovarian cancer, ARK5 was highly expressed in cancer cells compared with normal tissue and was revealed to be strongly associated with EMT (51). Furthermore, ARK5 was reported to regulate the progression of EMT in various solid tumors (74-78). When ARK5 expression was decreased in cells, the resultant inhibition of E-cadherin expression and the downregulation of vimentin expression were related to ARK5 activation (79).

Since the recurrence of E-cadherin expression was demonstrated to increase the sensitivity of cancer tissues to chemotherapeutic agents in various studies, reducing the expression of ARK5 may increase sensitivity to drugs such as doxorubicin (dox) and cisplatin (75,78).

TGF-β1 is a key induction factor of the EMT pathway in tumors (80) and induces EMT in NSCLC (81). ARK5 knockdown was reported to decrease TGF-β1-induced EMT and invasion and metastasis of cells under hypoxic conditions (82). The results indicated that ARK5 may be involved in the hypoxia-induced TGF-β1 pathway in cancer cells, which contributes to glucose starvation tolerance (82). In a liver study, ARK5 expressed lipid fibers and ultimately induced hepatic cell necrosis (52,83). This process is also observed in PC (84). ARK5 inhibits cancer cell death stimulation and promotes normal tissue necrosis. Additionally, another study reported that the suppression of ARK5 reversed hypoxia-induced EMT in hepatic cancer cells (81). The results suggested that ARK5 overexpression is indicative of hypoxia. An additional protein, zinc finger E-box-binding homeobox 1 (ZEB1), also acts as an activator of EMT in mantle cell lymphoma cells and determines resistance to different chemotherapeutic drugs (81). ARK5 was demonstrated to suppress ZEB1 to improve drug sensitivity (81,85).

In conclusion, ARK5 is associated with drug resistance in solid tumors and mediates the EMT pathway and numerous EMT-related molecules such as TGF-β1 and ZEB1. Since ARK5 knockdown improves resistance to clinical drugs, ARK5 may serve as a new target to reverse drug resistance.

ARK5 and Fas. Fas is a member of the tumor necrosis factor receptor family (86). As a transmembrane protein, it transmits apoptotic signals in cells and induces apoptosis when Fas ligands bind to the Fas receptor (87). Fas is widely expressed in normal tissues and tumors.

Fas-cell mediated apoptosis serves an important role in various biological processes as it triggers a series of downstream pathways (86,87), suggesting that ARK5 inhibits the activation of caspase-8 and the expression of caspase-6 containing two putative ARK5 phosphorylation sites: Ser80 and Ser257 (29), and ultimately inhibits the phosphorylation induced by Fas ligand and Fas. This could promote the survival of cancer cells under conditions of nutritional starvation (12,86).

In further studies, ARK5 was revealed to inhibit caspase-8 activation by preserving c-FLIP in response to Akt stimulation (4,88), preventing cell death caused by glucose starvation and RAS activation in cancer cells (8,86). RAS-induced apoptosis was demonstrated to be inhibited by increased ARK5 expression (11). These results suggested that ARK5 is associated with energy metabolism and cell apoptosis.

ARK5 and MYC. MYC is an important inducer protein in cancer that interferes with cell cycle metabolism and ribosome synthesis (89). When combined with MYC-associated factor X, MYC regulates transcription and oncogenic activity (90). MYC

overexpression has been demonstrated to induce cell apoptosis as it cannot maintain an adequate ratio of ATP/ADP (91,92). Kusakai *et al* and Cox and Der (67,68) previously demonstrated that, using synthetic lethal RNAi screening, ARK5 kinases regulated protein expression by activating various pathways that ultimately maintained or triggered cancer cell survival, particularly when MYC was overexpressed.

Activation of ARK5 and AMPK in response to metabolic stress combats apoptosis in cancer cells and they are markers for most solid tumors (93,94). AMPK is activated by the tumor inhibitor LKB1 (95). In the absence of LKB1, AMPK responds to calcium ions by phosphorylating calcium/calmodulin-dependent protein kinase kinase 2 (95,96). As a member of the AMPK family, ARK5 is also primarily activated by LKB1 (55).

However, Ciccarese *et al* (97) revealed a second pathway that maintains ARK5 activity in the absence of LKB1: ARK5 responds to calcium signaling through protein kinase C α (PKCα) to regulate AMPK activation (98) and mTORC1-dependent protein translation, protecting cells from MYC-driven apoptosis.

The Calcium-AMPK-mTORC1 metabolic checkpoint-dependent activation requires PKCα and ARK5 while in the absence of ARK5, activated mTOR increases ATP consumption and impairs MYC response to AMPK (98). Therefore, in the presence of ARK5, ATP synthesis is enhanced through the MYC pathway. The depletion of PKCα and ARK5 leads to apoptosis, which suggests that this pathway serves an active role in tumor maintenance (29). The results indicated a novel role for calcium ions in supporting cancer cell viability and elucidated the synthetic lethal interaction between ARK5 and MYC (98). Similarly, PKCα and -β have been demonstrated to phosphorylate Akt (99,100), preventing typical MYC-induced apoptosis by inhibiting the expression and function of apoptotic Bcl-2 homology protein (100-102). Therefore, ARK5 expression is necessary for MYC overexpression, even if Akt is overexpressed (29), suggesting that ARK5 may be a potential target for treating MYC-driven cancer (4,103).

Overall, ARK5 and PKCα may control multiple pathways that promote cancer cell survival. The targeted suppression of these pathways may therefore have potential therapeutic benefits in numerous types of cancer in which MYC is deregulated (103).

At the organelle level, ARK5 is involved in energy regulation by maintaining mitochondrial adaptability and stability and increases the expression of proteins in the respiratory chain by activating MYC, which enhances respiratory capacity (55). When ARK5 is depleted, this phenomenon is eliminated (55). As an important upstream regulator of MYC, ARK5 serves an important role in tumor regulation at the organelle level.

ARK5 and aneuploidy. The AMPK family has 13 different sub-groups with different regulatory modes (104). However, all of them are activated by LKB1 under conditions of metabolic pressure, when ATP levels are low (105). Tumor protein P53 is a downstream protein of AMPK and its phosphorylation serves an important role in apoptosis and cell aging (105).

ARK5 is part of the AMPK subfamily. It was demonstrated experimentally that ARK5 regulates P53 phosphorylation *in vivo* and *in vitro*, directly interacting with the P53 nucleus under the regulation of LKB1 (106). Additionally, ARK5 activation via P21 and weak acid resistance protein 1 prevents cells from entering the S phase from the G1 phase (106).

ARK5 was demonstrated to induce premature cell aging, which is closely associated with genetic aneuploidy, which had been previously demonstated in fibroblast (89). ARK5 regulates ploidy and senescence. Decreased ARK5 prevents aneuploidy in cells and enhance their replicative lifespan, while increased ARK5 induces gross aneuploidies and senescence (89).

This ARK5-induced aneuploidy increased the genomic instability of cancer cells and allowed them to overgrow, invade and metastasize, demonstrating the role of ARK5 in tumor regulation (89). Similarly to how cancer develops and exacerbates, genomic instability tends to induce cell senescence (107-109). Genomic instability often manifests as an increase in aneuploidy and a decrease in large tumor suppressor kinase 1 (LATS1) expression, a kinase involved in mitotic exit (109). Decreased LATS1 levels block cell division, affect genomic stability and increase the amount of abnormal DNA in each cell (109). AMPK accelerates P53-mediated cell senescence and ARK5 also leads to genomic aneuploidy changes without the involvement of P53 (110). However, experimental knockdown or overexpression of ARK5 did not affect cellular P53 activity (110).

In summary, in terms of cellular senescence regulation, ARK5 knockdown extends the lifespan of cells, metabolism and slows fibroblast senescence in normal individuals, even in the absence of LKB1. LATS1 is a regulator of stable gene expression and the overexpression of ARK5 weakens the expression of LATS1 (89). Such changes are independent of P53, which highlights the potential role of aneuploidy in ARK5-mediated senescence (89), and suggests a difference between the ARK5 and AMPK families at downstream action sites.

Furthermore, ARK5-induced aneuploidy leads directly to the death of MCF10a immortal cells rather than to senescence in other types of cells (89), indicating that aneuploidy serves a different role in different cells.

ARK5 and miRNA. miRNAs are non-coding RNAs 18-22 nucleotides in length that regulate various physiological activities through specific binding to the 3'-untranslated regions (3'UTR) of mRNA (111). miRNAs are involved in cell proliferation, invasion and metastasis (111). Currently, different miRNA families serve different roles in malignant tumors (112). For example, the miR-200 family (miR-200a, -200b, -200c, -141 and -429) slowed EMT in cancer cells by lowering ZEB1/ZEB2 expression (111,113).

It has been demonstrated that ARK5 was regulated by different miRNAs in different cancers and that ARK5 was negatively associated with the expression of certain miRNAs (112). In a liver cancer study, miR-204 reduced ARK5 expression and invasion, and reversed drug resistance (114). Similarly, miR-145 acted as a negative regulator of intrahepatic cholangiocarcinoma via the regulation of ARK5. miR-145 also reduced MT1-MMP, MMP-2 and MMP-9 expression to alter cell metastasis (115). miR-211 is associated with cell invasion and response to melanoma adhesion (116). In HNSCC, miR-203 regulated EMT by targeting ARK5 and

miR-96 regulated PC malignancy in the same manner (117). These three RNAs function upstream of ARK5 to block cell invasion (112). Overall, ARK5-associated miRNA could be regarded as a potential therapeutic target in the future treatment of cancer.

4. ARK5 as a potential therapeutic target

While chemotherapy is still a major strategy in cancer treatment, drug resistance has become a novel limitation that has led to its failure in long-term use (118). Therefore, there is an urgent requirement to elucidate novel strategies to increase drug efficacy.

Salinomycin. It has been established that ARK5 is highly expressed after treatment with certain clinical therapeutic drugs, including dox, 5-fluorouracil and cisplatin (50). Since ARK5 is associated with cancer cell drug resistance, its downregulation may serve as a potential therapeutic strategy to increase drug sensitivity (50). To increase drug sensitivity, salinomycin may be used as it targets ARK5 (50).

Salinomycin is an ionophore antibiotic that kills cancer stem cells and reverses EMT (50). A previous study on lung cancer demonstrated that salinomycin increased dox sensitivity and demonstrated a synergistic effect (combination index, 0.430 with dox) (50). Furthermore, it was revealed that salinomycin suppresses ZEB1, an important EMT pathway molecule (83,84). Therefore, salinomycin may be a novel drug that could be used in drug combinations. However, weight loss and nerve injury are side effects of salinomycin treatment and may affect its application in a clinical setting (119).

Additionally, it has been demonstrated that combination treatment reversed dox-induced EMT morphology, reversed EMT marker protein (vimentin and E-cadherin) expression and inhibited ARK5 expression (75). This process was also demonstrated in breast cancer, gastric cancer, non-small cell lung cancer, cholangiocarcinoma and hepatic cancer (120).

ON123300. ON123300 is a novel second-generation oral drug and CDK inhibitor, which exerts dual inhibitory effects on CDK4 and ARK5 (121). Compared with first-generation drugs, the site-specific ON123300 reverses the poor curative effects exerted by other drugs and the resultant poor cancer prognosis, which was demonstrated in multiple myeloma treatment (120). ON123300 was also revealed to exert beneficial effects in breast cancer, glioma and mantle cell lymphomas *in vivo* and *in vitro* without obvious harm to normal tissue (122).

ARK5 is a molecule that may lead to first-generation drug failure (20,28,122). However, while ARK5 overexpression has been observed in primary multiple myeloma cells, ON123300 caused cell cycle arrest and apoptosis, serving as an ARK5 and CDK4 inhibitor (32,28). MYC-CDK4 binding in multiple myeloma cells is a key interaction in tumorgenesis and ON123300 also has an impact on the MYC pathway as well as on the retinoblastoma/mTOR pathway (122). Furthermore, ARK5 knockdown significantly increased first-generation drug sensitivity of multiple myeloma (123,124).

7x. As 7x is a novel cyanopyridopyrimidine compound that acts as a multi-kinase inhibitor, 7x targets CDK4/cyclin D1 and ARK5 kinases (122). ARK5 is also negatively regulated by 7x (124). A previous study demonstrated that ARK5 targeting resulted in high efficacy when cancer cell proliferation and metastasis were inhibited with no significant signs of toxicity (122).

With the increasing number of compounds that have been discovered as effective drugs targeting the ARK5 protein for cancer treatment, the current study hypothesizes that ARK5 could be considered a significant therapeutic strategy for cancer under 7x treatment.

HTH-01-015. HTH-01-015 is a highly selective protein kinase inhibitor, which mainly affects ARK5 by inhibiting the phosphorylation of myosin phosphatase target subunit 1 (MYPT1), a substrate of ARK5 (125). Experimentally, HTH-01-015 was revealed to slow mitosis by inhibiting the phosphorylation of MYPT1 and preventing the entry of cells into the M phase by regulating DNA replication in the S phase (126). This indicated that HTH-01-015 inhibited ARK5 and modulated cell migration and adhesion. Additionally, HTH-01-015 was revealed to detect the physiological function of ARK5 abnormalities, further elucidating its role (126).

In summary, HTH-01-015 was demonstrated to regulate the physiological functions of cells by acting on ARK5 and could potentially serve as a novel drug to combat clinical drug resistance.

5. Relationship between ARK5 and other diseases

ARK5, diabetic nephropathy and renal fibrosis. ARK5 is associated with Tau protein stability. This may explain the potential link between ARK5 and a range of diseases, including neurodegenerative diseases and diabetes (55).

ARK5 serves an important role in human diabetic nephropathy (DN) along with TGF-β1 (127,128). DN is often accompanied by a series of renal diseases in which TGF-β1 mediates glomerular sclerosis and tubular fibrosis (127).

In vitro, TGF-β1 was used to induce a model of renal tubular fibrosis. The protein changes that occur during the EMT process in epithelial cells include: E-cadherin (epithelial) to N-cadherin (mesenchymal) transformation and increased vimentin, α-smooth muscle actin, connective tissue growth factor and Notch ligand Jagged-1 (129). By silencing ARK5 and thereby lowering TGF-β1 expression, fibrosis was reversed in renal tubular cells. This also influenced downstream proteins of TGF-β1 by preventing the stimulation of Jagged-1, verifying the link between ARK5 and EMT (130,131).

ARK5 and striated muscle generation. ARK5 protein was not previously identified in murine skeletal muscle. However, in later studies, ARK5 was demonstrated to be highly expressed in cardiac muscle and skeletal muscle, and ARK5 mRNA was identified via reverse transcription-quantitative PCR (130). Muscle contraction increased ARK5 phosphorylation (132). However, this did not alter ARK5 activity, indicating that phosphorylation of ARK5 at Ser400 cannot stimulate this kinase (132).

Uncordinated-82 (UNC-82) kinase serves a key role in *Caenorhabditis elegans*, a nematode, and is an orthologue of human ARK5 and SNF1/AMP kinase-regulated kinase (SNARK) that is necessary for myosin filament reorganization

during cellular elongation (27). Research has suggested that ARK5 may exert a similar function to UNC-82 in striated muscle development (130). ARK5 knockout could lead to alterations in contractile apparatus protein (the actin-myosin cytoskeleton) phosphorylation and SNARK reduction may lead to muscle mass disruption with increasing age. These results revealed the conserved role of UNC-82/ARK5/SNARK in muscle generation across diverse animal lineages (27).

Additionally, ARK5 was demonstrated to be a direct target of large musculoaponeurotic fibrosarcoma proteins and its activation was mediated by MAF-recognition element sequences (133). Activation of ARK5 in muscle allows it to interact with several types of myosin phosphatases (134). Furthermore, the ARK5-MYPT1-serine/threonine-protein phosphatase 1B complex promoted the interaction between ARK5 and 1433 protein, which inhibited phosphatase activity (134).

Ultimately, ARK5 controls cell adhesion and as a regulator of myosin phosphatase compounds; it prevents the phosphorylation of AMPK targets by LKB1 and controls phosphatase compounds to influence the phosphorylation of its targets (135).

Furthermore, experiments have demonstrated that ARK5 is involved in the negative feedback regulation of insulin signaling transduction and inhibits insulin-mediated glucose uptake in skeletal muscle (136).

ARK5 and neurodegenerative diseases. The effects of ARK5 in neurological diseases primarily manifest in three aspects: Promoting the polarization and migration of neurons, affecting the expression and accumulation of Tau protein and affecting neuron apoptosis through CASP6 (137).

Neuronal axons need energy to grow and branch, and this energy comes from the large consumption of ATP in the mitochondria (138). As a mediating factor, ARK5 regulates the growth of axons and cortical neuron branches. The LKB1-ARK5 pathway controls the fixation of mitochondria in axons and ARK5 mediates axon growth and growth of cortical neuron branches (138). Overexpression of ARK5 can increase the axon branch, and knockout can cause growth stagnation (138).

Numerous neurodegenerative proteinopathies share a common pathogenesis: Abnormal accumulation of disease-related proteins (139). ARK5 was revealed to regulate Tau by stabilizing the protein via specific Ser356 phosphorylation and its inhibition inhibited Tau expression in Drosophila neurodegeneration (139). The results suggested that reducing Tau expression is potentially an effective strategy to alleviate Tau-related neurodegenerative changes. Furthermore, ARK5 may serve as a new entry point for the treatment of Tau-associated diseases (139).

CASP6 was demonstrated to be an important molecule in neurodegenerative diseases, particularly in Alzheimer's disease (AD) and Huntington's disease (137). Research has revealed that avoiding destruction of CASP6 protected mice from neural diseases, such as AD (140). This protection is the result of ARK5 phosphorylating CASP6 at Ser257, which caused CASP6 inhibition and prevented neural cell death (86). The phosphorylation achieved by AKR5 is indirect and is mediated via the downregulation of p53 expression (106) due to the direct relationship between p53 and CASP6 (141). Furthermore, ARK5-mediated CASP6 phosphorylation inhibited its activation, mediating CASP6 activity (86). This phosphorylation site is specific for CASP6 (86,142).

The aforementioned study offered a potential site for future drug discovery by targeting this unique site to gradually inhibit neural death. Since ARK5 is a negative regulator of CASP6, a potential strategy could involve activating ARK5 expression or interfering with CASP6 phosphorylation at this specific site.

6. Conclusion and future perspectives

The current review summarized all known functions of ARK5 and potential drugs from a limited study size. Being a member of the AMPK family, ARK5 serves a metabolic role through its expression: Promoting the survival of cancer cells in harsh microenvironments. Additionally, ARK5 serves an important role in cell adhesion and metastasis (84).

Various processes are involved in cancer cell drug resistance and further research into this resistance mechanism is required to fully elucidate it. As an emerging molecule, ARK5 exerts multiple functions in certain diseases, particularly in malignant tumors. Since AKR5 can be used to evaluate the malignancy of tumors, metastasis and drug restistancy, it is a potential therapeutic target for cancer and drug resistance. Targeting ARK5 in combination with other drugs could potentially improve drug resistance and inhibit tumor metastasis.

The current review hypothesized that in the future, drugs targeting ARK5 will have impactful and specific effects that will improve clinical drug resistance. However, further research into the role of ARK5 in normal cells may be beneficial as it could reduce the targeting side effects of ARK5 therapy. The present review also hypothesized that the relationship between ARK5 and TAU stability is conducive to further elucidating the molecular mechanism of neurodegenerative diseases in future studies.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

GM and QJ contributed to the concept and design of this manuscript. BZ contributed to data reviewing and part of the

analysis GM, QJ and BZ wrote the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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