

Deciphering the role of AMPK-related kinase 5 in human cancer progression and metastasis

Alfredo Bambang¹, Caroline Tanadi², Anton Sumarpo^{1,*} 



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ABSTRACT

Malignancies related mortality is currently growing at an alarming rate. Early detection of cancer is vital in order to improve survival rates of cancer patients, and biomarker detection is regarded as one of the most promising approaches. Recent studies have reported that elevated ARK5 expression is associated with tumor progression and metastasis in several human malignancies. Several pathways are also influenced by the presence of ARK5, most notably the PI3k-Akt pathway, m-TOR phosphorylation, and several pathways that induce increased tumor invasion activity. Additionally, ARK5 expression are linked to miR-1181 down-regulation, which promotes epithelial mesenchymal transformation in ovarian cancer cells. Furthermore, ARK5 gene transcription is also affected by the interaction of c-MAF and MAFB with both MARE core sequences present in the ARK5 promoter. Based on the current evidences, ARK5 might be the master regulator of cancer progression and metastasis, which could potentially serve as a novel target for cancer therapies.

Key words: ARK5, biomarker, cancer, epigenetics, invasion, tumor proliferation

¹Department of Chemistry and Biochemistry, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, 14440, Indonesia

²Undergraduate Medical Program, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta 14440, Indonesia

Correspondence

Anton Sumarpo, Department of Chemistry and Biochemistry, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, 14440, Indonesia

Email: anton.sumarpo@atmajaya.ac.id

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INTRODUCTION

Noncommunicable diseases (NCDs) are the largest contributor to mortality in the world and its prevalence is growing at an alarming rate in the developing countries¹. Cancer is currently regarded as one of the most important NCDs as the number of its occurrences and burden are expected to rise rapidly by the year 2030, in line with the growth of aging population worldwide^{2,3}. The rise in cancer incidences and mortality is attributed to the shift in the prevalence and distribution of several risk factors associated with cancer due to socioeconomic development across the world⁴.

According to the status report on global cancer burden from GLOBOCAN 2018, in 2018, there were 18.1 million new cases (17.0 million excluding non-melanoma skin cancer) and 9.6 million cancer-related deaths (9.5 million excluding non-melanoma skin cancer) worldwide⁵. Furthermore, nearly half of cancer mortality across the globe occur in Asia, as the majority of global population resides there, followed by Europe and America for the incidence (23.4% and 21% of total cancer cases respectively) and mortality (20.3% and 14.4% respectively)⁵.

Early detection of cancer is an important order to increase the survival rate of cancer patients. Currently, cancer biomarkers detection is regarded as one of the most promising approaches. Cancer biomarkers comprise of a wide range of biochemical entities

that can be used for risk assessment, diagnosis, prognosis, and monitoring of disease progression, regression and recurrence⁶. Unfortunately, no biomarker has been considered as an ideal cancer screening tool; thus, more studies are needed to discover new cancer biomarkers for efficient cancer diagnosis and monitoring.

Altered expression of unique proteins is one of the most promising cancer biomarkers, which are often produced by cancer cells or by other cells in response to cancer. One of them is the AMP-activated protein kinase (AMPK) family⁷⁻⁹. The AMP-activated protein kinase (AMPK) family consists of 13 proteins that are further divided into five subfamilies: AMP-activated protein kinase (AMPK), salt-induced kinase (SIK), microtubule-affinity-regulating kinase (MARK), brain-specific kinase (BRSK), and SNF1-like kinase 1 (NUAK). These proteins are essential in several cellular activities, such as metabolism, polarity, cell proliferation or cell death, probably in a sub-family specific manner¹⁰⁻¹².

AMPK-RELATED KINASE 5 (ARK5) STRUCTURE AND FUNCTIONS

AMPKs are a group of metabolite-sensing serine/threonine protein kinases that are activated by an increased AMP:ATP ratio in the cell during metabolic stress¹³. It is frequently activated in hypoxic conditions and plays a vital role in protecting cells from

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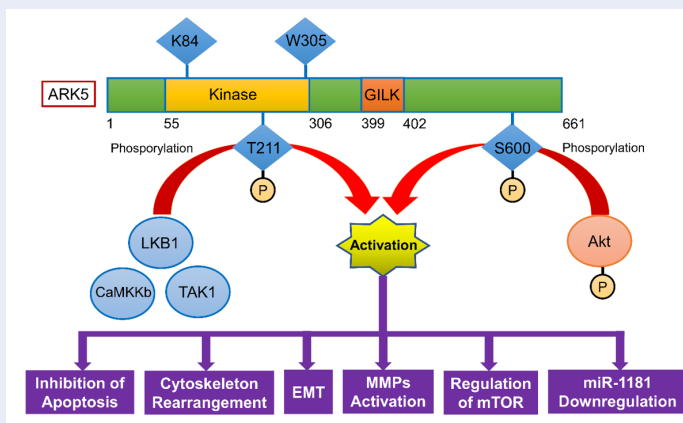


Figure 1: ARK5 sequence and pathways affected by its activation. ARK5 can be activated by the phosphorylation of either Ser-600 by Akt or Thr-211 by LKB1, CaMKKb, or TAK1. Over-expression of ARK5 can influence several pathways that induce increased tumor proliferation and invasion, most notably the PI3k-Akt pathway, mTOR phosphorylation and EMT. Furthermore, ARK5 expression are associated with decreased miR-1181 expression which induces es epithelial mesenchymal transformation in ovarian cancer cells.

apoptosis by inducing changes in energy metabolism through the phosphorylation and inhibition of acetyl-coenzyme A (CoA) carboxylase (ACC), and the stimulation of glucose transporters^{14,15}.

Lately, a novel AMPK family member, Novel (NUA) kinase family 1 (NUAK1) or more commonly addressed as ARK5 was identified and found to be involved in Akt dependent cancer cell survival and migration during glucose starvation in human colon and pancreatic cancer cell lines¹⁶. The analysis of ARK5 amino acid sequence found 47, 45.8, 42.4, and 55% homology to other known members of AMPK family (AMPK- α 1, AMPK- α 2, MELK, and SNARK, respectively), denoting that it is a member of the AMPK family¹⁶.

Human ARK5 gene, also known as KIAA0537, is located on chromosome 12q23.3 and consists of seven exons, approximately 76.7 kbp of genomic DNA. It encodes a 6828-bp mRNA with four splice variants¹⁷. ARK5 protein comprises of 661 amino acids and has a molecular mass of approximately 74 kDa¹⁸. The latest evidence showed that ARK5 could also be found in the cytoplasm and imported into the nucleus by importin- β members. Additionally, it is found that ARK5 might accumulate in the cytoplasm due to increased oxidative stress, which may impair the nuclear import process¹⁹. ARK5 consists of the putative consensus catalytic peptide sequence at amino acids 55 – 305, an Akt-phosphorylation site at Ser-600 near the C-terminus, and a conserved serine/threonine protein kinase domain in the N-terminus (Figure 1)²⁰. Comparing to other AMPK

related kinases, ARK5 can be activated by LKB1 via the phosphorylation of Thr-211 in the T loop of the catalytic domain²¹. ARK5 is stimulated by increased AMP:ATP ratio and phosphorylates SAMS peptide^{16,22,23}. Several factors, such as insulin or insulin-like growth factor 1 (IGF1) could also activate ARK5 in a downstream manner^{16,22,23}.

Numerous studies have reported that elevated ARK5 expression is associated with tumor progression and metastasis in several human malignancies, such as breast, colorectal, gastric, glioma, pancreatic, hepatocellular carcinomas, head and neck cancers, melanomas, nasopharyngeal, non-small cell lung cancer, ovarian and T-cell lymphomas²⁴⁻²⁷. The list of studies describing the role of ARK5 in several cancer pathways are summarized in Table 1. The pathways involved in the pathogenesis of those malignancies varied substantially and are not fully elucidated.

ROLE OF ARK5 IN PI3K/AKT PATHWAY

Akt pathway plays an important role in tumor malignancy, and it is well established that the PI3K pathway regulates Akt activity²⁸. Suzuki *et al.* demonstrated that overexpression of endogenous ARK5 showed strong Akt-mediated activity and increased invasion activity in matrigel assays²⁹. Currently, ARK5 is the only known Akt-direct downstream factor in the AMPK family. It is postulated that Akt is activated by phosphorylation of threonine 308 and serine 473, which will then phosphorylate ARK5 at Ser600 and

inhibit apoptosis and induces invasion activity as a consequence²⁴.

Activated Akt also exhibits anti-apoptotic properties by phosphorylating several apoptosis-associated factors, such as Bad, caspase 9, and forkhead, which further accentuates the problem³⁰. Cheng et al. found that the Akt pathway was involved in mTOR/p70S6k signaling pathway, which is crucial in epithelial mesenchymal transformation (EMT)³¹. Furthermore, Chen *et al.* also provided an evidence of ARK5 involvement in Akt/mTOR/p70S6k signaling pathway, which induced invasion and metastasis of gastric cancer³².

ARK5 AND THE REGULATION OF CELL DEATH PATHWAY

Several studies indicated that some cancer cells have high tolerance to nutrient starvation during hypoxia³³. This ability is commonly addressed as *austerity* and was found to be an important factor in cancer cell survival and tumor formation. Interestingly, altered ARK5 expression seems to play a central role in the development of austerity³⁴.

A study done by Suzuki *et al.* (2004) discovered that overexpression of ARK5 in tumors is correlated with a significant reduction in the ratio of necrotic cell death and a significant increase in invasion activity²⁹. It is postulated that these effects are caused by the inhibition of caspase-8 and negative regulation of caspase-6-associated FasL/Fas system²². These findings matched the results of previous studies that observed an increased tolerance against apoptosis by glucose starvation as a consequence of inhibition of caspase 8 activation due to the preservation of c-Flip^{22,35}.

LKB1/ARK5 PATHWAY

Most of AMPKs, as well as ARK5, are activated by an upstream kinase, which phosphorylates the threonine residue in the catalytic domain³⁶. Until recently, three upstream kinases have been identified, including Liver Kinase B1 (LKB1), Ca²⁺/calmodulin-dependent protein kinase kinase b (CaMKKb), and transforming growth factor- β -activated kinase 1 (TAK1)^{37,38}. Currently, LKB1 is regarded as an essential AMPK upstream kinase³⁹.

Although ARK5 is commonly associated with cancer cell survival and invasion, a study done by Hou *et al.* found that ARK5 directly phosphorylates p53 in the presence of LKB1, which in turns induces cell cycle arrest at G1 phase and exhibits tumor suppression properties⁴⁰. Similar to these findings, a recent study also

reported the role of ARK5 in the control of cellular senescence and ploidy, indicating the tumor suppressive properties of ARK5⁴¹. Hou *et al.* observed that LKB1/ARK5 activity induces cell cycle arrest by up-regulating p21/WAF expression. It can be postulated that LKB1 and ARK5 form a complex with p53 in the nucleus, and binds to the p21/WAF promoter, which stimulates gene expression and leads to cell cycle arrest⁴⁰.

ARK5 AND MTOR PATHWAY

Some cancer progression was influenced by the mTOR pathway such as gastric cancer, glioma and non-small cell lung carcinoma (NSCLC)⁴². Suzuki *et al.* (2004) and Lu *et al.* (2013) observed that mTOR phosphorylation induced by IGF-1 is regulated by ARK5 downstream of Akt pathway^{29,43}. Contradictory to these findings, the phosphorylation site of mTOR (Ser2448) is not compatible with AMPK family phosphorylation motif. Hence, it is reasonable that ARK5 regulates several factors associated with mTOR phosphorylation or it could phosphorylate mTOR directly at a site other than Ser2448, which in turn causes a conformational change that grants the ability to phosphorylate Ser244²⁹. A recent study by Chen *et al.* (2017) revealed that ARK5 could directly phosphorylate p70S6k and induces the downregulation of mTOR³².

ARK5 AND INCREASED TUMOR INVASIVENESS

ARK5 overexpression is related to increased tumor invasiveness in several cancer cell lines. Several studies have shown that ARK5 was significantly associated with several cancer pathways that are vital in the development of tumor invasion. These pathways include cytoskeleton rearrangement, EMT, and the activation of several matrix metalloproteinases (MMP)^{32,43,44}. Cytoskeleton rearrangement was one of the hallmarks of increased tumor invasion activity. Previously, it was found that phosphorylation of the cofilin at Ser3 by LIM kinase 1 (LIMK1) could affect F-actin activity, and in turn, increased invasion activity and chemotaxis⁴⁵. The results of a study done by Lu *et al.* suggested that ARK5 could interact with LIMK1 directly, and the knockdown of ARK5 resulted in decreased phosphorylation of LIMK1. Thus, it was proposed that there is a possible interplay between IGF-1 induced activation of PI3K/Akt/ARK5 pathways and cytoskeleton rearrangement⁴³. Another study done by Shi *et al.* showed that suppression of ARK5 could inhibit the F-actin polymerization stimulated by EGF, further elucidating the importance of ARK5 in EGF-induced cofilin recycling and actin polymerization⁴⁴.

Table 1: The effects of ARK5 in the pathogenesis of several cancer types

Pathways /Process Affected	Cancer Type	Effects of ARK5	Reference
Cytoskeleton rearrangement	Glioma	ARK5 regulates cytoskeleton rearrangement (IGF-1-induced activation of LIMK1 and cofilin).	43
	NSCLC	Knockdown of ARK5 inhibits EGF-induced F-actin polymerization.	44
EMT	Gastric	Knockdown of ARK5 upregulated E-cadherin and downregulated Slug, SIP1, and vimentin.	32
	HCC	ARK5 stimulated EMT, and ARK5 suppression restored E-cadherin and vimentin expression.	46
	HCC	Knockdown of ARK5 reverse the process of EMT.	47
	HNSCC	ARK5 is involved in the invasion and EMT induction of HNSCC. However, altered ARK5 expression did not change the cell morphology or the expression of EMT-related molecules including E-cadherin, N-cadherin, vimentin, SNAI1, and SNAI2.	48
	NPC	Knockdown of ARK5 suppressed EMT and reduces N-cadherin.	49
MMPs	Ovarian	ARK5 overexpression stimulates EMT, downregulates the epithelial markers and upregulates the mesenchymal expression.	48
	Breast	Increased MMP activity.	50
	Glioma	Increased MMP activity.	43
	NSCLC	Knockdown of ARK5 inhibits the activation of MMP-2 and MMP-9.	44
	NSCLC	Knockdown of ARK5 inhibits the activation of MMP-2, MMP-9 and its transcription regulator (NF-kB).	51
	NPC	Knockdown of ARK5 reduces MMP-2, MMP-9 and its regulatory factor MT1-MMP.	52
mTOR	Gastric	Knockdown of ARK5 downregulated the mTOR/p70S6k signals.	32
	Glioma	Regulates m-TOR phosphorylation induced by IGF-1	43
	NSCLC	Phosphorylation of m-TOR and induced the phosphorylation of two mTOR downstream (p70S6K1 and 4E-binding protein 1 (4E-BP1))	44
PI3K-Akt	Breast	PI3K/Akt/ARK5 pathways activity increased the invasiveness of MDA-MB-231	53
	Colorectal	Invasion activity was further increased by Akt expression and decreased by PI-3K inhibitor LY294002 treatment	54

HCC: Hepatocellular carcinoma; HNSCC: Head and neck squamous cellcarcinoma; NPC: Nasopharyngealcancer; NSCLC: Non-small cell lungcarcinoma

THE ROLE OF ARK5 IN EMT REGULATION

EMT is known to trigger cancer metastasis, in which epithelial cells gain mesenchymal features that induce tumor invasion and metastasis. Epithelial cells are identified by various characteristics, such as tight connections between the cells, polarized dissemination of multiple organelles and cytoskeleton components, immotile relative to their surroundings, and expressed several epithelial markers such as E-cadherin and α -catenin. In contrast, during the EMT process, cells gain mesenchymal properties, for examples, they loose connections between the cells, no polarization of organelles and cytoskeleton component, gain invasive features, and express mesenchymal markers, such as N-cadherin, vimentin, β -catenin, α -SMA, SNAI1, TWIST, TGFB1, ZEB1, and TGFB2⁵⁵. During EMT, numerous regulatory pathways are altered at multiple levels. The alterations are mainly observed in the transcription and translation process, including the expression of non-coding RNAs, altered splicing and protein stability⁵⁶.

Numerous studies showed that EMT was affected by ARK5 in multiple ways. A study done by Zhang *et al.* demonstrated that ARK5 downregulates the epithelial markers (E-cadherin) and upregulates the mesenchymal markers (β -catenin, fibronectin, vimentin, α -SMA, SNAI1, TWIST, TGFB1, ZEB1), thus, inducing EMT⁵¹. Consistent with the previous findings, Xu *et al.* and Chen *et al.* found that ARK5 suppression can upregulate E-cadherin and downregulate vimentin^{32,46}. Xu *et al.* noted that EMT induced by overexpression of ARK5 was responsible for the development of doxorubicin resistance in HCC cell lines, and another study by Ye *et al.* showed that suppression of ARK5 could reverse the EMT process^{46,47}. Knockdown of ARK5 expression was associated with reduced invasion and metastasis of GC cell, further confirming the role of EMT in tumor invasion and metastasis³².

Similarly, Obayashi *et al.* also concluded that ARK5 was involved in EMT induction and invasion in HNSCC. Interestingly, the results of the study also indicated that ARK5 itself was incapable of inducing EMT as the alterations in ARK5 expression did not affect the cell morphology or the expression level of E-Cadherin, N-Cadherin, vimentin, SNAI1, and SNAI2⁴⁸. According to these findings, perhaps ARK5 and EMT related markers are both upregulated during EMT, which in turn induces tumor invasion.

ROLE OF ARK5 IN MMPs ACTIVATION

Tumor invasion in numerous cancers is also induced by the degradation of extracellular matrix and basement membrane by MMPs. MMPs comprises of more than 20 members of zinc-dependent neutral endopeptidases, and amongst them, numerous studies identified that MMP-2 and MMP-9 are associated with tumor invasiveness. These MMPs should be activated before they could induce extracellular matrix degradation. In addition, MT1-MMP is known to be involved in the process⁵⁷.

Several studies have observed an association between ARK5 expression and MMP activation^{29,43}. Suzuki *et al.* was the first to demonstrate that transfection with Ca-Akt1 or exposure to IGF-1 is associated with an increase in MMP2 and MMP9 level and upregulation of MT1-MMP, potentially confirming the critical role of PI3K-Akt pathway in the activation of MMPs regulated by IGF-1. MT1-MMP upregulation is attributed to increased ARK5 levels, which in turn stimulates MMP2 and MMP9 secretion. These findings indicate that ARK5 probably modulates MT1-MMP expression at the translational level²⁹.

Another study done by Chen *et al.* showed a reduction in the level of the active form of NF- κ B (p-p65) in the nucleus of siNUAK1 cells, which are treated with TNF- α before the experiment⁵⁸. NF- κ B is a ubiquitous transcription factor and was recognized as a critical regulator in malignant transformation involving MMP upregulation⁵⁹. From these findings, we could assume that ARK5 might also serve as an essential factor in NF- κ B activation, which in turn triggers MMP expression.

EPIGENETICS REGULATION OF ARK5 EXPRESSION

MicroRNA plays an important role in carcinogenesis. Some microRNAs are reported to have tumor-suppressor or oncogene activity, and influence tumor cell survival, proliferation, apoptosis, invasion, and metastasis in various types of human cancers. The expression of microRNAs was found to be dysregulated in most cancer and commonly interact with other genes and proteins, including ARK5⁶⁰.

Several studies have found that the expression of numerous microRNAs could downregulate ARK5 expression in numerous cancer cell types, which inhibited tumor invasion and progression. Currently, there are five microRNAs, including miR-96, miR-145, miR-203, miR-204, and miR-211, which can inhibit ARK5 expression in vitro. Additionally, it is

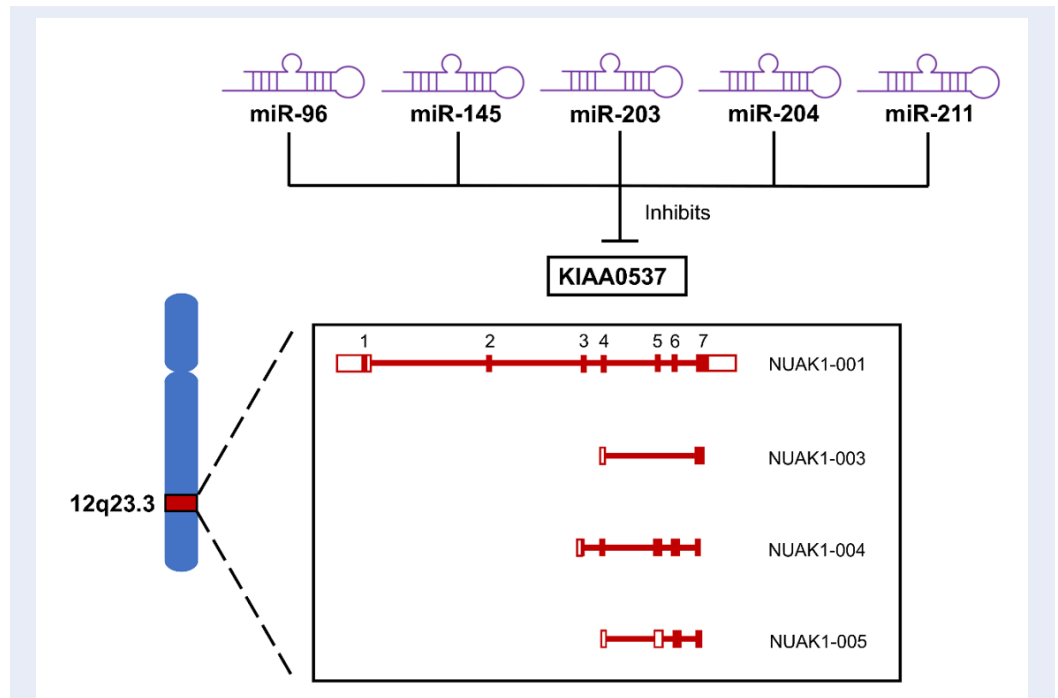


Figure 2: MicroRNAs associated with ARK5 down-regulation. Human ARK5 gene (KIAA0537) is located on chromosome 12q23.3 and encodes a 6828-bp mRNA with four splice variants. Recent studies have identified several microRNAs which influence ARK5 expression. Currently, there are five microRNAs (miR-96, miR-145, miR-203, miR-204, and miR-211) which could down-regulate ARK5 expression *in vitro*.

found that the expression of these microRNAs is often decreased in various cancer cell lines, which leads to the activation of numerous pathways that induce tumor invasion and progression^{44,48,61-63}.

Bell *et al.* demonstrated that miR-211 directly target ARK5, causing its down-regulation, and thus, leads to reduced invasion activity in melanoma⁶¹. The findings of this study also suggested that ARK5 is a downstream target of MITF via miR-211. Another study by Xiong *et al.* found that miR-145 directly targets ARK5, suppressing PI3k-Akt signaling and MMP expression in intrahepatic cholangiocarcinoma cell⁶³. Similarly, Obayashi *et al.* also showed that miR-203 could downregulate ARK5 and suppressed invasion and EMT in HNSCC⁴⁸. Furthermore, the results of several studies have revealed that miR-96 and miR-204 could down-regulate ARK5 expression by targeting its 3' UTR^{44,63}. In contrast, Zhang *et al.* found that ARK5 could down-regulate miR-1181 expression, and thus, leading to increased EMT in ovarian cancer cell lines⁵¹.

Over-expression of ARK5 in cancer cell lines can also be stimulated by c-MAF and MAFB of the Large-MAF family. It was first noted in the study done by Suzuki *et al.* that c-MAF, MAFB, and ARK5 mRNAs

are often expressed together in multiple myeloma cell lines⁶⁴. This observation leads to a hypothesis that ARK5 gene transcription could be affected by Large-MAF family, considering that c-MAF and MAFB are known to stimulate gene transcription through the interaction with MAF-recognition element (MARE) sequences on the promoter region of the gene⁵².

An earlier study done by Motohashi *et al.* indicated that TGCTGAC is regarded as the core sequence of MARE and that the 5'-side thymidine is indispensable for the interaction between c-MAF/MAFB with MARE⁵³. Suzuki *et al.* found that the sequence of ARK5 promoter encompasses two MARE core sequences mentioned in the previous study⁶⁴. It is also observed that the expression of ARK5 gene induced by increased expression of c-MAF and MAFB was significantly lower when either MARE sequences was mutated. Together, these findings demonstrate that ARK5 gene transcription is influenced by the interaction of c-MAF and MAFB with both MARE core sequence present in the ARK5 promoter⁶⁴.

CONCLUSIONS

Early detection of cancer is vital to improve the survival rates of cancer patients, and ARK5 expression is

currently deemed as a promising biomarker of cancer development and progression. Based on the latest evidences, ARK5 might be a master regulator of cancer progression and metastasis due to its involvement in several important cancer pathways. Additionally, ARK5 expression was also affected by several microRNAs, which could down-regulate ARK5 expression *in vitro*, and thus, might be useful in therapeutic application. Further studies are needed to fully elucidate the function of ARK5 and to develop a strategy to inhibit ARK5 expression, which could serve as a novel target for cancer therapies in the future.

ABBREVIATIONS

AMPK: AMP-activated protein kinase

ARK5: AMPK-related kinase 5

CaMKKb: Ca²⁺/Calmodulin-dependent protein kinase kinase b

EMT: Epithelial mesenchymal transformation

GC: Gastric cancer

HCC: Hepatocellular carcinoma

HNSCC: Head and neck squamous cell carcinoma

IGF1: Insulin-like growth factor 1

LKB1: Liver kinase b 1

MARE: MAF-recognition element

MMP: Matrix metalloproteinase

mTOR: Mammalian target of Rapamycin

NCD: Noncommunicable disease

NPC: Nasopharyngeal carcinoma

NSCLC: Non-small cell lung carcinoma

NUAK1: Novel kinase family 1

Tak1: Transforming growth factor b kinase 1

COMPETING INTERESTS

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed to the drafting of this manuscript. AB and CT searched, obtained and summarized the data. AB and AS reviewed and edited the first draft of the manuscript. All authors reviewed, commented and approved the final draft of the manuscript.

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