 Commentary

The Mechanism and Influence of AKAP12 in Different Cancers

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The development of cancer is a complex process that requires the participation of many factors, including mutations in genes, regulation of signaling pathways, disruption of homeostasis, and failure of self-monitoring mechanisms. Sufficient evidence has shown that the A kinase anchoring protein (AKAP) family plays an important role in signal transduction in cancer. AKAP12 and AKAP12-regulated signaling pathways are critically involved in the development and progression of a variety of tumors.

AKAP12 (also known as AKAP250, Gravin, or SSeCKS) is located at 6q24-q25 and was first isolated from the serum of patients with myasthenia gravis. The protein localizes to the nucleus of the protoplast membrane, cell margins, and cytoplasm[1]. AKAP12 belongs to the family of kinase scaffolding proteins whose function is to anchor protein kinase C (PKC), protein kinase A (PKA), and cyclins to the plasma membrane[2]. It is also a member of the AKAP family of protein kinases that function as tumor suppressors. The expression of AKAP12 has been found to be down-regulated in various cancers including prostate cancer[3], colon cancer[4], childhood acute lymphoblastic leukemia[5], hepatocellular carcinoma[6], skin cancer[7], and seminoma[8] (Table 1). Furthermore, several previous studies have suggested that down-regulation of AKAP12 expression in advanced cancer is a result of increased hypermethylation of the 5’ CpG island located in the promoter region of AKAP12[9]. What’s more, down-regulated expression of AKAP12 is associated with poor prognosis and high recurrence rate after treatment. Although expression of AKAP12 is decreased in many human cancers, the mechanism by which AKAP12 regulates the cell cycle and inhibits tumor growth has not yet been elucidated. In this context, we will discuss how AKAP12 plays a role in controlling cell signaling pathways and oncogenic progression in cancer. This review will focus on the expression and mechanism of AKAP12 in different cancers as well as its potential to serve as a target for clinical treatment.

Three isoforms of AKAP12 have been detected, known as AKAP12/A, AKAP12/B, and AKAP12/C (305, 287, and 250 kD, respectively)[9,10]. Previous studies have found that AKAP12 is a critical regulator of intracellular signaling. It is involved in the formation of PKA and PKC complexes, remodeling of the cytoskeleton, cell cycle regulation, and is also an important regulator of the β2 adrenergic receptor complex (Table 2). Moreover, several studies have suggested that AKAP12 regulates signal transduction, including G protein coupled receptor-mediated signaling and apoptosis[9]. Importantly, AKAP12 changes the cytoplasmic expression level and cellular localization of cyclin D1 by inhibiting PKC activity, resulting in the separation of the PKC isoenzyme from the plasma membrane[12]. It blocks synthesis of cyclin D1, a G1 phase cyclin, and terminates the cell cycle[10]. In addition, AKAP12 influences cell cycle progression by regulating cytokinesis, which may be involved in regulation of the cell cycle by controlling the formation of actin-myosin rings via scaffolding of PKC[12,12]. Several studies from the last decade have shown that AKAP12-regulated signal transduction complexes are involved in varied aspects of cancer

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development and progression\textsuperscript{[10].} Furthermore, AKAP12 can prevent cancer cell growth by inhibiting the cell cycle protein CDK1 (cyclin-dependent kinase 1), thereby halting cell division and stopping the initiation of apoptosis\textsuperscript{[21].} In line with these findings, expression of AKAP12 is decreased or absent in a variety of cancers, suggesting its potential role as a cancer suppressor gene\textsuperscript{[12,13]} (Figure 1).

In China, colorectal cancer is a primary cause of cancer mortality. Although progress has been made in comprehensive surgical treatments, the 5-year survival rate has not been improved\textsuperscript{[14].} The main reason for the poor survival rate is the postoperative recurrence rate, which is as high as 40%-70\%\textsuperscript{[15].} There is an urgent need to understand mechanisms of colorectal cancer formation and progression, because the survival rates of patients with colorectal cancer have shown only modest improvements over the past decade. In this respect, experiments screening gene clusters in patients with colorectal cancer have been completed to discriminate between high and low risk, to determine the recurrence rate and to predict postoperative effects\textsuperscript{[16,17].}

Experiments have confirmed that the AKAP12 regulates tissue remodeling and contraction and that it controls disease progression by regulating macrophages during recovery\textsuperscript{[18].} In inflammation, the structural environment is created through the activation of M2 macrophages by AKAP12\textsuperscript{[14,15]} mesenteric cells of the colon. Macrophages demonstrate plasticity in colon, and have two phenotypes: M1 and M2. M1 macrophages are pro-inflammatory\textsuperscript{[14]}, while M2 macrophages are anti-inflammatory and activated by AKAP12\textsuperscript{[14]} colonic mesenchymal cells (Figure 2). During inflammation, the number of M1 macrophages is increased in the extracellular matrix (ECM), which becomes less translucent. During the convalescent phase of inflammation, M2 macrophages gradually increase, causing the ECM to become more compact and remodeling tissues\textsuperscript{[14].} Furthermore, Tokoro et al. confirmed that the expression of CD206, an M2 macrophage marker, was positively correlated with AKAP12 expression in both human and mice\textsuperscript{[14].} Furthermore, AKAP12 gene expression is absent or decreased by more than 50\% in colorectal cancer\textsuperscript{[15].} Patients with colorectal cancer that re-expressing AKAP12 are less likely to suffer from recurrence after surgery and have a significant improvement in quality of life\textsuperscript{[14].} Enhanced expression of AKAP12 has been shown to decrease the invasion and migration of cancer cells, significantly inhibiting the metastatic potential of colorectal cancer cells\textsuperscript{[18].}

Hepatocellular carcinoma (HCC) is one of the most intractable cancers. Gradually, genetic testing of many HCC patients has begun to focus on AKAP12. AKAP12 is down-regulated in HCC tissues as a result of DNA methylation\textsuperscript{[6,19].} AKAP12 has two splice variants, AKAP12a and AKAP12\textsuperscript{[19].} Expression of AKAP12a in primary liver cancer is mainly regulated by DNA methylation, whereas AKAP12\textsuperscript{[19]} expression is affected by MicroRNA (miRNA)\textsuperscript{[20].} AKAP12 has been found to act as a cancer suppressor in HCC\textsuperscript{[21]}. Bioinformatics analysis has revealed that miR-103 binds to the AKAP12 3’UTR (untranslated regions) area, and expression of miR-103 is associated with AKAP12\textsuperscript{[6].} Moreover, studies have shown that miR-103 expression promotes cell proliferation, inhibits apoptosis and decreases AKAP12 expression in HCC\textsuperscript{[6,20].} In line with these findings, re-expression of AKAP12 inhibits cancer cell growth, while miR-103

### Table 1. The Reports of AKAP12 in Various Cancers

<table>
<thead>
<tr>
<th>Cancer</th>
<th>References</th>
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<tbody>
<tr>
<td>Colon cancer</td>
<td>[4,14-18]</td>
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<tr>
<td>Gastric cancer</td>
<td>[13,22,23]</td>
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<td>Adolescent chronic myeloid leukemia</td>
<td>[23,24]</td>
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<td>Children acute lymphocytic leukemia</td>
<td>[5]</td>
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<tr>
<td>Acute leukemia</td>
<td>[24,25]</td>
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<td>Prostate cancer</td>
<td>[3,8,26]</td>
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<td>Seminoma</td>
<td>[8]</td>
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<tr>
<td>Breast cancer</td>
<td>[27-29]</td>
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<td>Cutaneous squamous cell carcinoma</td>
<td>[30,31]</td>
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<tr>
<td>Skin cancer</td>
<td>[7]</td>
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<tr>
<td>Melanoma</td>
<td>[32,33]</td>
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<tr>
<td>Hepatocellular carcinoma</td>
<td>[6,20,21]</td>
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### Table 2. Signaling Pathways Involving AKAP12 and Their Biological Functions

<table>
<thead>
<tr>
<th>Pathway/complexs</th>
<th>Major Function</th>
<th>References</th>
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<tbody>
<tr>
<td>PKA</td>
<td>Negative regulation of cell cycle and subsequent sequestration of Ras/MEK/ERK</td>
<td>[2,9,35]</td>
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<tr>
<td>PKC</td>
<td>Inhibition of the JNK and Raf/MEK/ERK signaling pathways</td>
<td>[1,9,11]</td>
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<td>Cyclin D1</td>
<td>Inhibition of nucleotide translocation</td>
<td>[11]</td>
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<td>β2 adrenergic receptor</td>
<td>Conduction signal</td>
<td>[34]</td>
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Figure 1. AKAP12 acts as a tumor suppressor by inhibiting cancer cell migration, proliferation, and angiogenesis. AKAP12 is localized to the nucleus of the protoplast membrane, cell margins, and cytoplasm. (a) AKAP12 is bound to cyclin D1 to inhibit nuclear translocation. Cyclin D1 is synthesized during the G1-S phase transition and is rapidly degraded as cells enter the S phase. (b) AKAP12 supports completion of cytokinesis by regulating actin-myosin rings via scaffolding of PKC. (c) The Src-FAK complexes also modulate cell proliferation and the G1-S phase transition by stimulating the Raf/MEK/ERK2 cascade and the nuclear translocation of cyclin D1. (d) AKAP12 is bound to PKC. AKAP12 sequesters Src and PKC resulting in the inhibition of the JNK and Raf/MEK/ERK signaling pathways, which regulate angiogenesis and control cancer cell proliferation.

Figure 2. Upon stimulation by inflammation, macrophages can differentiate into M1 or M2 phenotypes in the colon. M1 macrophages release pro-inflammatory cytokines, which cause the ECM to become less translucent and can worsen disease. M2 macrophages are anti-inflammatory factors that are driven by AKAP12+ colonic mesenchymal cells and allow the ECM to become more compact and remodel tissues.
re-expression promotes cancer growth and inhibits AKAP12 expression.

On the contrary, a recent study by Liu et al. investigated the relationship between AKAP12 methylation and recurrence or prognosis of primary HCC and found that methylation of AKAP12 reduced the risk of recurrence of primary hepatocellular carcinoma and prolonged patient survival. Therefore, AKAP12 can serve as an independent factor with which to determine the prognosis of patients with HCC. As mentioned above, AKAP12 is down-regulated in HCC tissues and its expression is regulated by DNA methylation. Liu et al. showed that recurrence of liver cancer was associated with DNA methylation. Methylation of the AKAP12 gene has been found to be higher in non-recurrent hepatoma cells than in relapsed HCC cell lines from the same patient. Although the conclusion of this study differs from previous studies, it also provides a baseline for future research.

Gastric cancer is a malignant tumor that originates from the gastric mucosa epithelium. This disease has a low cure rate, poor prognosis, and high recurrence rate. The pathogenesis of gastric cancer is still unclear, but it can roughly be divided into two aspects: genetic variation and environmental impact. Choi et al. showed that AKAP12 encodes two major isoforms, weighing 305 and 287 kD, and is divided into two subtypes, AKAP12α and AKAP12β, which independently control the activation of different promoters. A large number of studies have shown that high expression of AKAP12 has an inhibitory effect on the occurrence of gastric cancer which improves the prognosis with treatment and reduces the recurrence rate. Hypermethylation of CpG islands in the AKAP12 promoter region of gastric cancer cells has been reported, suggesting that DNA methylation is directly involved in silencing of the AKAP12 gene in gastric cancer.

Previous research has demonstrated that decreased expression of AKAP12 was connected with poor overall survival in acute leukemia. Yildirim et al. showed that AKAP12 expression was 11-fold lower in acute leukemia compared with a control group and 77% of patients demonstrated a decrease in AKAP12 expression. Interestingly, the experiment also found that increased AKAP12 expression was positively correlated with poor prognosis and was an indicator of poor prognosis in acute leukemias. This conclusion is totally different from later experimental results. Childhood acute lymphocytic leukemia is the most common malignant cancer of the blood system in childhood. Abnormal methylation of CpG islands in the promoter region of AKAP12 has been reported in childhood acute lymphocytic leukemia, which resulted in reduced AKAP12 expression or loss of function. Therefore, AKAP12 is the regulatory signal of PKA and PKC, which activates the downstream target Ras in JMML. The epigenetic regulation of three AKAP12 promoters (α, β, and r), has been detected in several studies. In the JMML sample, hypermethylation of the AKAP12 promoter was associated with decreased AKAP12 expression. These findings demonstrate that AKAP12α is epigenetically silenced in JMML, and emphasized the importance of the abnormal regulation of Ras signaling.

Prostate cancer is a unique human disease and AKAP12 can effectively inhibit malignant cells at an early stage. Several studies have proved that the loss of AKAP12 expression is associated with prostate cancer metastasis and demonstrated the presence of three copy number variations (CNV) in the 6q13-22 region of most prostate cancers. Moreover, loss of AKAP12 expression promotes apoptosis and leads to higher levels of cell proliferation in prostate cells. This process is considered to be the result of cancer suppressor function deficiency. Patients with high expression of AKAP12 had higher postoperative survival rates and better quality of life. Irwin et al. have summarized the evidence that AKAP12 acts to suppress metastasis in vivo. Although its expression has little effect on cancer growth, it effectively inhibits metastasis. Taken together, these findings indicate the potential of AKAP12 as a potential therapeutic target for clinical treatment.

Spermatocytoma originates from the testicular germ cells and is the most common tumor of the testis. In embryonic tumors, abnormal mitosis is closely associated with the process of carcinogenesis. Hehny et al. found a macromolecular complex containing AKAP12, mitotic kinases, Aurora, and plk1 (polo-like kinase 1), all of which are down-regulated in human soblastoma. Knockout of AKAP12 promoted cell mitosis and caused microtubule disorder. Therefore, the deletion of AKAP12 is closely related to the growth and reproduction of embryonic cancers.

Breast cancer is a disease with high incidence among women. One of the risks factors for breast cancer is still unclear, but it can roughly be divided into two aspects: genetic variation and environmental impact. Choi et al. showed that AKAP12 encodes two major isoforms, weighing 305 and 287 kD, and is divided into two subtypes, AKAP12α and AKAP12β, which independently control the activation of different promoters. A large number of studies have shown that high expression of AKAP12 has an inhibitory effect on the occurrence of gastric cancer which improves the prognosis with treatment and reduces the recurrence rate. Hypermethylation of CpG islands in the AKAP12 promoter region of gastric cancer cells has been reported, suggesting that DNA methylation is directly involved in silencing of the AKAP12 gene in gastric cancer.
cancer is nucleotide polymorphisms, which are mainly derived from the rs2046210 of 6q25.1. A previous study has revealed that AKAP12 and ESR1 are two important regulatory genes. The study found that in breast cancer, AKAP12 gene expression is lower and ESR1 gene expression is higher. AKAP12 expression is also positively associated with disease prognosis and patients with high AKAP12 expression have a better prognosis after treatment.

Cutaneous squamous cell carcinomas (CSCCs) is a common human cancers. Although it has metastatic ability, it usually exhibits lower invasiveness than cancers occurring in other organs, which is mainly due to the presence of metastasis suppressor protein. Many studies using AKAP12-null mice have demonstrated that increased exposure to carcinogens induces the conversion of papilloma to squamous cell carcinoma. Despite the low frequency of cancer metastasis, spindle-cell metastases occurs in several AKAP12-deficient mice after treatment. Interestingly, the increased oncogenic effect observed with the loss of AKAP12 is associated with the induction of FAK (focal adhesion kinase) which is a mediator known to be involved in the progression of skin cancer. Compared with in situ injection of wild-type B16F10 melanoma cells, the trend for spontaneous peritoneal metastasis is significantly increased in AKAP12-deficient mice. Importantly, Irwin et al. demonstrated that AKAP12 deficiency has no effect on primary tumor growth. Together, these findings suggest that AKAP12 controls the occurrence and development of metastasis by changing the tumor microenvironment.

Consistent with these findings, decreased expression of AKAP12 in melanomas may result in a ‘double hit’ that not only makes cells more susceptible to UV-induced (ultraviolet ray) mutations, but also makes AKAP12 more sensitive and unstable within the cell. A previous study uncovered a new pathway for nucleic acid excision. It found that in melanocytes, AKAP12 has resection and repair functions in the nucleus. These findings demonstrate that AKAP12 inhibits the growth and metastasis of cancer cells and functions as a cancer suppressor gene. The mechanism by which AKAP12 inhibits cancer cell growth requires further investigation in order to facilitate the translation of scientific research results into clinical treatments.

In summary, the stable expression of AKAP12 inhibits tumor occurrence and cancer cell proliferation. Therefore, AKAP12 has potential value in the clinical treatment cancer. There are several therapeutic strategies that could be implemented at present. First, patients with mutations in the AKAP12 gene may receive gene therapy to introduce exogenous normal AKAP12-gene into target cells in order to correct defects or abnormalities caused by loss of AKAP12 expression. Second, for cancers caused by hypermethylation of the CpG islands in the AKAP12 promoter region, methylation inhibitors or demethylases could be utilized to stabilize the expression of AKAP12 gene. Finally, to utilize the fully potential of AKAP12 as a tumor suppressor for clinical treatment, its activity could be amplified using activators or inhibitors, such that AKAP12 tumor suppressor function would be more stably expressed and more clinically applicable.

Although there are some contradictory reports in the literature, AKAP12 acts as a tumor suppressor in most cancers. Achieving stable, high-level AKAP12 expression is a promising option for clinical treatment of cancer patients. In most cancers, increased AKAP12 expression inhibits proliferation and metastasis of cancer cells and is negatively correlated with the recurrence rate. This has important implications for clinical treatment and should be further explored as a means to improve patient survival. This review summarizes the effects of AKAP12 expression in several types of tumors. It discusses the role of AKAP12 in various pathways and highlights areas that need further exploration, hoping to inspire more in-depth studies to resolve these problems.

The authors report no conflicts of interest.

These authors contributed equally to this work and should be considered co-first authors.

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