KIF4A Promotes Clear Cell Renal Cell Carcinoma (ccRCC) Proliferation in vitro and in vivo

This article was published in the following Dove Press journal: OncoTargets and Therapy

Guang-Hua Yang, Zhi-Xing Ren, Xiong Yang, Yan-Gang Zhang

1Department of Urology, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Taiyuan City, Shanxi Province 030032, People’s Republic of China; 2Education and Research Center, Taiyuan Radio and Television University, Taiyuan City, Shanxi Province 030024, People’s Republic of China; 3Department of Urolith Center, Tianjin Institute of Urology, The Second Hospital of Tianjin Medical University, Tianjin 300211, People’s Republic of China

*These authors contributed equally to this work

**Purpose:** To evaluate the expression in human clear cell renal cell carcinoma (ccRCC) tissues and explore the effects of kinesin family member 4A (KIF4A) on ccRCC progression.

**Methods:** GEPIA was used to evaluate the mRNA levels of KIF4A in human ccRCC tissues from TCGA database, and Immunohistochemistry (IHC) assays were performed to assess its expression in human ccRCC tissues collected in our hospital. The clinical-pathological analysis was performed to explore the correlation with KIF4A expression. The effects of KIF4A on ccRCC cell proliferation were detected through colony formation and MTT assays. Finally, the effects of KIF4A on tumor growth were measured using a mice model.

**Results:** Bioinformation results showed the expression of KIF4A mRNA was upregulated in ccRCC tissues and high expression of KIF4A was related with poor prognosis in ccRCC patients. We also found a high expression of KIF4A in human ccRCC tissues collected in our hospital. We also found its expression level was correlated with clinical characteristics, including T stage (P=0.035*) and lymphatic metastasis (P=0.028*). We further confirmed that knockdown of KIF4A suppressed cell proliferation in HTB-47 and CRL-1932 cells. Furthermore, KIF4A contributes to tumor growth of ccRCC cells in mice.

**Conclusion:** We found the abnormal high expression of KIF4A in human ccRCC tissues and demonstrated that KIF4A could serve as a tumor induction gene.

**Keywords:** clear cell renal cell carcinoma, ccRCC, KIF4A, proliferation, prognosis, clinicopathological characteristics

**Introduction**

Renal cell carcinoma is a common urinary disease with a high incidence. In the United States, more than 65, 340 newly diagnosed RCC patients and approximately 14, 970 deaths in 2018. While in 2019, there were 73, 820 new diagnosed RCC patients and approximately 14, 770 deaths. Clear cell renal cell carcinoma (ccRCC) represents the highly aggressive renal malignant tumor which accounts for nearly 80% of renal cell carcinoma. Existing traditional treatments, such as surgical resection, radiation and chemotherapy, seem to be ineffective against this highly aggressive tumor. Recently targeted therapy for ccRCC is promising. VHL was reportedly considered a potential molecular target for ccRCC, but more mutations were subsequently discovered, such as these mutations in ccRCC leading to further identification of their possible therapeutic role in this cancer.

In order to fight this disease in the future, the development of new molecular rars has potential clinical value. The kinesin proteins (KIFs) that are mainly involved in cargo transportation belong to the microtubule-based kinesin family. More than 45 kinesins have...
already been identified in human cells. Kinesins carry out critical functions in the processes of mitosis and cytokinesis. Additionally, previous studies demonstrated that kinesins could promote the separation of sister chromatin. KIF4A also associates with the regulation of DSB repair-related proteins. In recent years, the key role of KIF4A in cancer development has gradually been revealed. KIF4A is widely expressed in a variety of human tissues. Various studies have reported that KIF4A promoted the progression of several cancers, such as cervical cancer and oral cancer. In addition, KIF4A promotes cell proliferation via cell cycle regulation and metastasis in colorectal cancer. However, it is unclear whether KIF4A is involved in the occurrence and development of ccRCC in highly malignant tumors.

Here, we declared that KIF4A is involved in the development of ccRCC and found that KIF4A is highly expressed in ccRCC tissue samples. Our data further confirmed that KIF4A is correlated with the clinical pathology of ccRCC patients such as tumor stage and tumor size. Furthermore, our results indicated that KIF4A depletion dramatically inhibited ccRCC cell proliferation and inhibited tumor growth in mice. Therefore, KIF4A may become a promising therapy for ccRCC.

Materials and Methods
Bioinformatical Analyze
GEPIA (http://gepia.cancer-pku.cn/detail.php?gene=KIF4A) was used to analyze data from TCGA (The Cancer Genome Atlas) for differential expressed genes, and the median was utilized as the threshold to separate the patients into two groups for Kaplan-Meier survival analysis.

Antibodies, Primers and shRNA Plasmids
Anti-KIF4A (for IHC assays, 1:400 dilution, for immunoblot assays, 1:1000 dilution, for immunohistochemistry (IHC) assays were subsequently performed. Briefly, the samples were

| Feature     | All n=78 | KIF4A Expression | χ² | P   |
|-------------|----------|------------------|----|-----|
| Age (year)  |          |                  |    |     |
| < 55        | 48       | 21               | 27 | 2.834 | 0.092 |
| ≥ 55        | 30       | 19               | 11 |     |
| Gender      |          |                  |    |     |
| Male        | 44       | 25               | 19 | 1.238 | 0.266 |
| Female      | 34       | 15               | 19 |     |
| T stage     |          |                  |    |     |
| T1–2        | 52       | 21               | 31 | 4.468 | 0.035* |
| T3–4        | 46       | 19               | 27 |     |
| Tumor grade |          |                  |    |     |
| Low         | 34       | 21               | 13 | 2.651 | 0.103 |
| High        | 44       | 19               | 25 |     |
| Tumor size  |          |                  |    |     |
| < 4 cm      | 28       | 19               | 9  | 4.803 | 0.028* |
| ≥ 4 cm      | 50       | 21               | 29 |     |

Note: *P < 0.05.
fixed with 4% PFA, then cut into 5-μm slices and blocked with 2% BSA in PBS for 30 mins. The slides were then incubated with an antibody targeting KIF4A at 4°C overnight. Subsequently, after rinsing in PBS several times, the slides were maintained in a biotinylated secondary antibody conjugated to horseradish peroxidase (HRP). Subsequently, a staining reaction was performed by using a DAB kit.

KIF4A is mainly located in the nucleus of ccRCC cells. The percentage of positively stained cells was scored as follows: 0, negative staining; 1, 10–50% positive tumor cells and 2, >50% positive-stained cells. The intensity of KIF4A staining was evaluated as 0 (no staining), 1 (moderate staining) and 2 (strong staining). The expression level of KIF4A was analysed based on the staining index: the staining intensity score was multiplied by the positive tumor cell staining score. KIF4A staining scored 0, 1 and 2 were considered low-expression, other scores of 3 and 4 were evaluated as high-expression.

Cell Culture and Transfection
Human ccRCC cell lines, HTB-47 and CRL-1932, were obtained from ATCC. Both HTB-47 and CRL-1932 cells were maintained in RPMI1640 medium with 10% fetal bovine serum addition (Gibico, CA, USA) in a 5% CO₂ incubator.

KIF4A shRNA plasmids were transfected into HTB-47 and CRL-1932 cells were conducted using lipofectamine 2000 (11668019, Invitrogen, Carlsbad, CA, USA). Stable knockdown of HTB-47 cells was obtained by its shRNA lentivirus infection and the selection was performed by puromycin supplementation and used in animal experiments.

Quantitative PCR Assay
Total RNA from human ccRCC cells were extracted via Trizol reagent (15596026, Invitrogen, Carlsbad, CA, USA). Then, the RNA was reverse-transcribed into cDNA by M-MLV reverse transcriptase (M1701, Promega, Madison, Wisconsin, USA).

SYBR Ex Taq kit (638319, Takara, Japan) was utilized to conduct Quantitative RT-PCR and the relative KIF4A expression level was normalized to the level of GAPDH.

Immunoblot Assays
Extract ccRCC cells or tissue proteins with RIPA buffer (9800, Cell Signaling, Danvers, MA). Then, the protein was separated by SDS-PAGE, and subsequently transferred protein onto polyvinylidene fluoride (PVDF) membranes and blocked with 5% BSA. Membranes were incubated with primary antibodies targeting KIF4A, Ki67, PCNA, and β-actin, respectively, and then incubated with HRP-conjugated secondary antibodies for 1 hr. Visualize signals with ECL kit. To analyze the intensity of each band, ImageJ software was used.

Cell Proliferation Assays
For colony formation assay, about 500 ccRCC cells transfected with control or KIF4A shRNA plasmids were seeded into 6-well culture plates. After culturing for 2 weeks, the cells were fixed with PFA and dyed with 0.2% crystal violet for 20 mins at room temperature, and then manually counted the number of colonies.

For MTT assays, cells were seeded in 96-well culture dishes at a density of about 1000 cells. After adherence, incubated cells with MTT. After washing with PBS, dimethyl sulfoxide (DMSO) was added to each well, and the absorbance was measured at 570 nm using a microplate reader.

Tumor Growth Assays
Nude BalB/c mice (6–8 weeks, 18–22 g, female) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). For tumor growth assay, HTB-47 cells transfected with control or KIF4A shRNA lentivirus were injected subcutaneously into athymic nude BalB/c mice. After 2 weeks, the tumor of each mouse was isolated and photographed. And the tumor volume was monitored every 3 days and marked the growth curve of tumor growth. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The animal study was carried out in accordance with the guidelines approved by the Animal Experimentation Ethics Committee of Shanxi Bethune Hospital. The protocol was approved by the Committee, and all surgery and all efforts were made to minimize suffering.

Semi-Quantitative Analysis Assay for the Results of Immunohistochemistry in vivo
The expression of KIF4A or Ki67 was determined with anti-KIF4A or anti-Ki67 antibody in paraffin tumor using an immunohistochemistry kit (ZSGB-BIO, pv6000, Chian) according to the manufacture’s protocols. Briefly, isolated tumor tissues were fixed into 10% neutral-buffered formalin and then embedded in paraffin blocks. Then, the embedded tissues were cut into 4 μm sections. After antigens retrieval in a microwave oven for 15 mins and endogenous
peroxidase blockage, sections were added with indicated antibodies overnight at 4°C. And then, goat anti-rabbit antibody was added at 37°C for 1 hr. Added DAB to visualize signals. Images were taken under a microscope (Olympus BX43).

Statistics
GraphPad 6 was used for data analysis in this study. All results were represented as mean ± SD. For statistical significance, Student’s t-test was used. The Kaplan–Meier curve method was performed for survival analysis. Additionally, \( P<0.05 \) was considered significantly different.

Results
KIF4A Expression Correlates with ccRCC
KIF4A has been reported to be important in the promotion of cancer development. Bioinformatic analysis was conducted to analyze the expression level of KIF4A in ccRCC tissues and normal kidney tissues. As shown in Figure 1, KIF4A expression in ccRCC tissues (n = 523) was increased at mRNA levels compared to normal tissues (n = 100) (Figure 1A). According to the expression pattern KIF4A in patients, all ccRCC patients can be divided into high group (n=254, from TCGA dataset) and low group (n=257, from TCGA data set), as shown in Figure 1B. We noticed patients with high KIF4A expression had poor overall survival (OS) and disease-free survival (DFS). These data suggest that KIF4A may be an adverse prognosis in ccRCC patients. To further verify the potential role of KIF4A in ccRCC, we detected its expression level in ccRCC tissue samples by IHC assays. We found KIF4A mainly located in the nucleus of ccRCC cells (Figure 2A). Consistently, adjacent tissues exhibited low or no KIF4A expression compared with ccRCC tissues (Figure 2A and B).

Then, 78 patient samples were divided into KIF4A low and high-expression groups based on the staining scored listed in Materials and methods (Figure 2A and Table 1). Forty patients were evaluated as low expression of KIF4A, whereas 38 of them exhibited high-expression levels (Table 1).

We then analysed the clinical-pathological characteristics, such as age, gender, tumor stage, tumor grade and size in ccRCC patients. It is worth noting that no significant correlations were found between patient age, gender and tumor grade between KIF4A low and high-expression groups (Table 1). Interestingly, the expression level of KIF4A was correlated with tumor stage (P=0.034) and tumor size (P=0.028) in ccRCC patients (Table 1).

Taken together, our results demonstrated that KIF4A correlates with ccRCC.

Knockdown of KIF4A Weakened ccRCC Cell Proliferation in vitro
To explore the potential function of KIF4A in ccRCC, the KIF4A shRNA plasmids were transfected into HTB-47 and CRL-1932 cells, to knockdown KIF4A expression.
The reduction of KIF4A in HTB-47 and CRL-1932 cells confirmed the knockdown efficiency by quantitative RT-PCR assays (Figure 3A). The reduction of KIF4A expression was further proved via Immunoblot assays in HTB-47 and CRL-1932 cells (Figure 3B).

To evaluate the role of KIF4A in cell proliferation, colony formation assays were performed. The knockdown of KIF4A dramatically inhibited the proliferation of ccRCC cells, assessed by the significant reduction in colony numbers (Figure 4A). Similarly, through MTT assays, we observed an obvious decreased absorbance value in both HTB-47 and CRL-1932 cells with KIF4A ablation (Figure 4B). We also assessed the expression levels of cell proliferation markers, Ki67 and PCNA. Consistently, KIF4A ablation resulted in an obvious reduction in Ki67 and PpCNA expression levels in HTB-47 and CRL-1932 cells (Figure 4C and D).

In conclusion, our data suggested that KIF4A promotes ccRCC cell proliferation in vitro.

**KIF4A Promotes ccRCC Growth in Mice**

Similar to our previous results, KIF4A ablation led to the inhibition of ccRCC cell proliferation in vitro, and then we speculated that KIF4A stimulated the development of ccRCC in mice.

To confirm our hypothesis, HTB-47 cells with control or KIF4A shRNA lentivirus infection were subcutaneously injected into nude mice. After 2 weeks, the tumor was isolated, photographed, and measured. According to the tumor volume measured every 3 days, we plotted the tumor growth curve in each group. Representative photographs of tumors are displayed in Figure 5A. Interestingly, the volume of tumors in KIF4A-depleted groups was significantly smaller than the control groups (Figure 5A).

Additionally, silencing of KIF4A was confirmed by IHC assays (Figure 5B). We also examined the expression level of Ki67 in control and KIF4A knockdown groups by IHC assays. As expected, Ki67 reduction was observed in tumors in the KIF4A knockdown groups, which indicates that KIF4A knockdown impaired proliferation capacity (Figure 5C). Therefore, we showed that the involvement of KIF4A in the regulation of ccRCC development in mice.

**Discussion**

There are four types of renal carcinoma: clear cell renal carcinoma, granulosa cell renal carcinoma, mixed cell renal carcinoma, and undifferentiated cell renal carcinoma. Among them, the vast majority are renal clear cell carcinoma, accounting for 70%–80% of renal carcinoma. Renal clear cell carcinoma is often asymptomatic in the early stage, or only fever, fatigue and other systemic symptoms; tumor volume was found to increase. However, in the advanced stage, existing treatments have little effect on ccRCC. New treatments are urgently needed to combat this disease. Through the bioinformation analysis, we noticed the high mRNA levels of KIF4A in human ccRCC tissues, and KIF4A expression was correlated with the prognosis of ccRCC patients, indicating that KIF4A can be used as a prognostic indicator of ccRCC.

Performing IHC assays, we noticed 78 human ccRCC tissue samples and their corresponding non-tumor adjacent tissue samples, we found a relatively high-expression level
of KIF4A in tumor tissues, and the expression level of KIF4A was remarkably associated with clinical features including tumor size and the number of tumor nodes. Similarly, another study indicated that high expression of KIF4A is correlated with lymph node metastasis in patients with colorectal cancer. These clinical data testified the possible role of KIF4A in the histopathology and progression of tumorigenesis. Further investigations are consistent with the hypothesis that KIF4A serves as a trigger of ccRCC progression. Performing IHC assays, we also found the decrease expression of Ki67 in tumor tissues isolated from mice of KIF4A depletion groups, which further confirmed the critical role of KIF4A on the regulation of ccRCC cell proliferation.

KIF4A is a member of KIF4 subfamily, which consists of KIF4A, KIF4B, KIF21A and KIF21B. Various studies have reported that the KIF4 subfamily is critical in tumorigenesis and progression. It is reported that KIF4A was a new component of the chromosome segregation machinery and acted critical roles in mediating spindle organization and cytokinesis. All these functions suggest that KIF4A may have an impact on tumorigenesis and development. In fact, KIF4A plays as an oncogene and participates in the progression of several malignancies, including breast cancer, oral cancer, and cervical cancer. KIF4A stimulates cell migration and invasion in lung cancer, and it was found to regulate cell proliferation by the activation of spindle assembly checkpoint.

Figure 3 KIF4A expression was effectively inhibited in both HTB-47 and CRL-1932 human ccRCC cells caused by its shRNA plasmids. (A) Quantitative RT-PCR assays revealed the dramatically reduced expression level of KIF4A caused by its shRNA in HTB-47 and CRL-1932 cells, respectively. (B) Immunoblot assays confirmed the efficiently silencing of KIF4A caused by its shRNA in HTB-47 and CRL-1932 cells. Results are presented as mean ± SD, *P < 0.05.
KIF4A promotes ccRCC cell proliferation in vitro. (A) HTB-47 and CRL-1932 cells transfected with control or KIF4A shRNA, and the proliferation capacity was quantified by colony formation assays. (B) The results of MTT assays showed the inhibition of cell proliferation caused by KIF4A depletion. (C) Immunoblot assays showed Ki67 expression level in control or KIF4A knockdown HTB-47 and CRL-1932 cells. (D) Immunoblot assays revealed the expression level of PCNA in control or KIF4A ablation ccRCC cells. Results are presented as mean ± SD, *P < 0.05.
Additionally, KIF4A promotes the proliferation of colorectal cancer by the regulation of p21-mediated cell cycle progression, and they found KIF4A knockdown obviously decreased the expression level of pAKT, and the expression level of MEK and ERK was comparable, suggesting that KIF4A promotes CRC proliferation through PI3K/AKT signaling pathway.

Interestingly, we found that KIF4A contributed to cell proliferation of ccRCC in vitro and in mice. Whether KIF4A promotes ccRCC through this signaling pathway also requires further study.

KIF4A was known to be involved in the regulation of mitosis. Previous studies have indicated KIF4A could be phosphorylated by CDK1 at S1186, triggered chromosome compaction. Meanwhile, KIF4A affected the spindle formation and chromosome segregation in oocytes. These studies suggest KIF4A has the potential to affect the cell cycle and proliferation of cancer cells. Interestingly, here we noticed that KIF4A promoted cell proliferation in ccRCC, and therefore thought KIF4A affected the proliferation via mediating mitosis.

In addition to KIF4A, other members of KIF family also play various roles in the tumor development and metastasis. KIF7 could promote the growth of prostate tumor through LKB1-mediated AKT inhibition. KIFC1 was associated with the progression of HCC. Additionally, KIF3A
participated in the development of breast cancer. These studies, together with our results, indicate the possible roles of KIFs as potential therapeutic targets.

Collectively, our results demonstrated that KIF4A was highly expressed in human HCC tissues. We also found a correlation between KIF4A expression level and clinical characteristics in ccRCC patients. Furthermore, KIF4A promoted the proliferation of ccRCC cells in vitro and in mice. Therefore, we have a preliminary mechanism study of KIF4A in ccRCC development and provide a novel therapeutic target for the treatment of ccRCC.

Data Sharing Statement
The dataset supporting the conclusions of this article is included in the article.

Disclosure
The authors declare that they have no competing interests in this work.

References
1. Bie L, Zhao G, Cheng P, et al. The accuracy of survival time prediction for patients with glioma is improved by measuring mitotic spindle checkpoint gene expression. *PLoS One*. 2011;6(10):e25631. doi:10.1371/journal.pone.0025631
2. Murphy KA, James BR, Wilber A, Griffiths PR. A synthetic mouse model of metastatic renal cell carcinoma for quantitative and longitudinal assessment of preclinical therapies. *J Virol*. 2017;122: e55080.
3. Lane BR, Kattan MW. Predicting outcomes in renal cell carcinoma. *Curr Opin Urol*. 2005;15(5):289–297. doi:10.1097/01.mou.00000178336.94991.17
4. Alsharodi M, Katz H. Check point inhibitors a new era in renal cell carcinoma. *Med Oncol*. 2018;35(6):85. doi:10.1007/s12032-018-1147-y
5. Wang X, Wang T, Chen C, et al. Serum exosomal miR-210 as a potential biomarker for clear cell renal cell carcinoma. *J Cell Biochem*. 2019;120(2):492–502.
6. Ghatalia P, Zibelman MR, Gynnisman DM, Plimack ER. Evolving landscape of the treatment of metastatic clear cell renal cell carcinoma. *Clin Adv Hematol Oncol*. 2018;16(10):677–686.
7. Song Y, Huang J, Shan L, Zhang HT. Analyses of potential predictive markers and response to targeted therapy in patients with advanced clear-cell renal cell carcinoma. *Chin Med J (Engl)*. 2015;128(15):2062–2063. doi:10.4103/0366-6999.161335
8. Ricketts CJ, Linehan WM. Insights into epigenetic remodeling in VHL-deficient clear cell renal cell carcinoma. *Cancer Discov*. 2017;7(11):1221–1223. doi:10.1158/2159-8290.CD-17-0971
9. Hogner A, Krause H, Jandrig B, et al. PBRM1 and VHL expression correlate in human clear cell renal cell carcinoma with differential association with patient’s overall survival. *Urol Oncol*. 2018;36(3):94e9–94e14. doi:10.1016/j.urolonc.2017.10.027
10. Hirokawa N, Tanaka Y. Kinesin superfamily proteins (KIFs): various functions and their relevance for important phenomena in life and diseases. *Exp Cell Res*. 2015;334(1):16–25. doi:10.1016/j.yexcr.2015.02.016
11. Camlin NJ, McLaughlin EA, Holt JE. Motoring through: the role of kinesin superfamily proteins in female meiosis. *Hum Reprod Update*. 2017;23(4):409–420. doi:10.1093/humupd/dmx010
12. Lucanus AJ, Yip GW. Kinesin superfamily: roles in breast cancer, patient prognosis and therapeutics. *Oncogene*. 2018;37(7):833–838. doi:10.1038/onc.2017.406
13. Tang F, Pan MH, Lu Y, Wan X, Zhang Y, Sun SC. Involvement of Kif4a in spindle formation and chromosome segregation in mouse oocytes. *Aging Dis*. 2018;9(4):623–633.
14. Wan Q, Shen Y, Zhao H, et al. Impaired DNA double-strand breaks repair by kinesin family member 4A inhibition renders human H1299 non-small-cell lung cancer cells sensitive to cisplatin. *J Cell Physiol*. 2019;234(7):10360–10371.
15. Hou PF, Jiang T, Chen F, et al. KIF4A facilitates cell proliferation via induction of p21-mediated cell cycle progression and promotes metastasis in colorectal cancer. *Cell Death Dis*. 2018;9(5):477. doi:10.1038/s41419-018-0550-9
16. Matsumoto Y, Saito M, Saito K, et al. Enhanced expression of KIF4A in colorectal cancer is associated with lymph node metastasis. *Oncol Lett*. 2018;15(2):2188–2194. doi:10.3892/ol.2017.7555
17. Taniwaki M, Takano A, Ishikawa N, et al. Activation of KIF4A as a prognostic biomarker and therapeutic target for lung cancer. *Clin Cancer Res*. 2007;13(22 Pt 1):6624–6631. doi:10.1158/1078-0432.CCR-07-1328
18. Araki H, Tsuzuki T, Kimura T, et al. Relationship of pathologic factors to efficacy of sorafenib treatment in patients with metastatic clear cell renal cell carcinoma. *Am J Clin Pathol*. 2015;143(4):492–499. doi:10.1010/ajcpm8rj2ecaWOQQ
19. Hirota E, Yan L, Tsunoda T, et al. Genome-wide gene expression profiles of clear cell renal cell carcinoma: identification of molecular targets for treatment of renal cell carcinoma. *Int J Oncol*. 2006;29(4):799–827.
20. Grande Pulido E, Martin Centeno A, Maroto Centeno A, Solsona E. The multiple functions of KIF4A functions in chromosome condensation and segregation. *Cell Cycle*. 2007;6(10):1221–1223. doi:10.1083/jcb.200401142
21. Tostain J, Li G, Gentil-Perrat A, Gigante M. Carbonic anhydrase 9 in clear cell renal cell carcinoma: a marker for diagnosis, prognosis and treatment. *Eur J Cancer*. 2010;46(18):3141–3148. doi:10.1016/j.ejca.2010.07.020
22. Grepin R, Guyot M, Jacquin M, et al. Acceleration of clear cell renal cell carcinoma growth in mice following bevacizumab/avastin treatment: the role of CXCL cytokines. *Oncogene*. 2012;31(13):1683–1694. doi:10.1038/onc.2011.360
23. Mazumdar M, Sundareshan S, Misteli T. Human chromokinesin KIF4A functions in chromosome condensation and segregation. *J Cell Biol*. 2004;166(5):613–620. doi:10.1083/jcb.200401142
24. Kurasawa Y, Earnshaw WC, Mochizuki Y, Dohmae N, Todokoro K. Human chromokinesin KIF4A: a potential predictor for progression of human oral cancer. *Hum Reprod Update*. 2017;37(11):812–818. doi:10.1093/humupd/dmx010
25. Sheng L, Hao SL, Yang WX, Sun Y. The multiple functions of KIF4A in spindle formation and chromosome segregation in mouse oocytes. *Cancer Discov*. 2017;7(11):1221–1223. doi:10.1158/2159-8290.CD-17-0971
26. Gao J, Sai N, Wang C, et al. Overexpression of chromokinesin KIF4A inhibits proliferation of human gastric carcinoma cells both in vitro and in vivo. *Tunis J Med*. 2011;39(4):314–318. doi:10.1016/j.tjme.2011.08.005
27. Gao J, Sai N, Wang C, et al. Overexpression of chromokinesin KIF4A inhibits proliferation of human gastric carcinoma cells both in vitro and in vivo. *Tunis J Med*. 2011;39(4):314–318. doi:10.1016/j.tjme.2011.08.005
28. Wang H, Lu C, Li Q, et al. The role of Kif4a in doxorubicin-induced apoptosis in breast cancer cells. *Mol Cells*. 2014;37(1):812–818. doi:10.14348/molcells.2014.0210
29. Minakawa Y, Kasamatsu A, Koihe H, et al. Kinesin family member 4A: a potential predictor for progression of human oral cancer. *PLoS One*. 2013;8(12):e85951. doi:10.1371/journal.pone.0085951
29. Wu G, Chen PL. Structural requirements of chromokinesin Kif4A for its proper function in mitosis. Biochem Biophys Res Commun. 2008;372(3):454–458. doi:10.1016/j.bbrc.2008.05.065

30. Nunes Bastos R, Gandhi SR, Baron RD, Gruneberg U, Nigg EA, Barr FA. Aurora B suppresses microtubule dynamics and limits central spindle size by locally activating KIF4A. J Cell Biol. 2013;202(4):605–621. doi:10.1083/jcb.201301094

31. Bastos RN, Cundell MJ, Barr FA. KIF4A and PP2A-B56 form a spatially restricted feedback loop opposing aurora B at the anaphase central spindle. J Cell Biol. 2014;207(6):683–693. doi:10.1083/jcb.201409129

32. Willemsen MH, Ba W, Wissink-Lindhout WM, et al. Involvement of the kinesin family members KIF4A and KIF5C in intellectual disability and synaptic function. J Med Genet. 2014;51(7):487–494. doi:10.1136/jmedgenet-2013-102182

33. Rath O, Kozierski F. Kinesins and cancer. Nat Rev Cancer. 2012;12(8):527–539. doi:10.1038/nrc3310

34. Wong KY, Liu J, Chan KW. KIF7 attenuates prostate tumor growth through LKB1-mediated AKT inhibition. Oncotarget. 2017;8(33):54558–54571. doi:10.18632/oncotarget.8133

35. Fu X, Zhu Y, Zheng B, et al. KIFC1, a novel potential prognostic factor and therapeutic target in hepatocellular carcinoma. Int J Oncol. 2018;52(6):1912–1922. doi:10.3892/ijo.2018.4348

36. Xia P, Chu S, Liu G, et al. High expression of KIF3A is a potential new parameter for the diagnosis and prognosis of breast cancer. Biomed Rep. 2018;8(4):343–349. doi:10.3892/br.2018.1061