

CKS2 in human cancers: Clinical roles and current perspectives (Review)

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Abstract. Cyclin-dependent kinase subunit 2 (CKS2) is indicated in the processes of cell cycle and cell proliferation. Through these processes, *CKS2* is identified as a cancer gene, but its role has not been well reviewed. The aim of the present study was to summarize the clinicopathological significance and the molecular mechanisms of *CKS2* in human cancers. Its expression was upregulated in the majority of the types of cancer studied. *CKS2* was shown to have a function in cancers of the digestive tract, genital tract, thyroid, nerve and certain other types of cancer. *CKS2* can promote progression of certain cancers via positive control of proliferation, invasion and migration. Downregulation of *CKS2* induces cancer cell apoptosis. *CKS2* can change a multitude of cellular mechanisms in cancer pathogenesis by regulating the gene translation of numerous validated targets, such as *p53*, *CDK1*, cyclin A, cyclin B1, caspase-3 and *Bax*. In addition, the molecular mechanism that causes aberrant expression of *CKS2* was epigenetic modification of *miR-26a* and the Y-box-binding protein 1 (*YB-1*) gene. In conclusion, *CKS2* is commonly elevated in cancer, most likely due to its ability to promote cancer cell growth, invasion and migration through regulating certain significant genes. Understanding the mechanisms by which *CKS2* is involved with cancer pathogenesis will be useful in the development of tumor therapy for patients with cancer.

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1. Introduction

The mammalian cyclin kinase subunit (CKS) family has two members; cyclin-dependent kinase subunit 1 (CKS1) and CKS2. CKS1 and CKS2 consist of 79 amino acids, and show 81% homology (1). Richardson *et al* (2) cloned the gene of human CKS for the first time in 1990; identifying human *CKS1* and *CKS2*. Human *CKS1* is located in chromosome 8q21 and coding human *CKS2* gene is located in chromosome 9q22 (3). Studies have shown that CKS1 and CKS2 proteins are associated with cell proliferation (4). Human CKS1 is required for SCF^{Skp2}-mediated ubiquitination and degradation of P27^{kip1} that is essential for the G₁/S transition during the cell cycle (5). Human CKS2 is essential for the first metaphase/anaphase transition of mammalian meiosis (6-8). The function of human CKS1 has been researched thoroughly, while the function of human CKS2 remains poorly understood. Increasing research is focusing on the association between human CKS2 and tumors. However, there is a lack of review on the clinical and functional roles of human CKS2 in human cancers. The present study is a review of the recent progress of human CKS2.

2. *CKS2* is highly expressed in malignant tumors

Hepatocellular carcinoma. Shen *et al* (9) applied the technology of reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and analyzed the mRNA level of *CKS2* in HCC and its adjacent liver tissues that either had cirrhosis or hepatitis, as well as in normal liver tissues. RT-qPCR revealed that the *CKS2* mRNA level was significantly elevated in HCC. This study also used immunostaining analyses to determine the CKS2 protein expression in the tissues. High CKS2 protein expression was evident in 38 out of 48 HCC cases.

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Esophageal carcinoma. Our previous study investigated *CKS2* in esophageal carcinoma (10). Wang *et al* (10) collected 26 paired esophageal carcinoma and its adjacent normal tissues. RT-qPCR revealed that in 65% of esophageal carcinoma tissues, the *CKS2* mRNA level was highly expressed. Immunohistochemical staining analyses were performed to assess the protein levels of *CKS2* in 56 esophageal carcinoma tissues. The results showed that 61% of 56 cases had high *CKS2* protein levels. Kita *et al* (11) also conducted studies of *CKS2* expression in esophageal squamous cell carcinoma. In the mRNA analysis, 93.5% of patients (58/62) showed higher expression of *CKS2* in cancerous compared to non-cancerous tissues.

Gastric cancer. Using RT-qPCR assays on eight human gastric cancer cell lines, Tanaka *et al* (12) observed the expression of *CKS2* mRNA. A significantly higher expression of *CKS2* mRNA was verified in the eight human gastric cancer cells compared to normal cells. RT-qPCR analysis of 109 paired clinical samples showed that 62 of 109 cases (56.9%) exhibited higher levels of *CKS2* mRNA in the tumor tissues compared to the paired normal tissues.

Colorectal cancer (CRC). Notterman *et al* (13) applied semi-quantitative RT-PCR reactions to compare *CKS2* expression levels between colon adenocarcinomas and paired normal colon tissues. The mean expression of *CKS2* in 18 cancerous tissues to normal tissues was >4-fold. Jung *et al* (14) verified that the *CKS2* gene expression level was significantly enhanced in CRC tissues compared to normal tissues in 29 patient cases by RT-qPCR analysis. In another study of *CKS2* in CRC, *CKS2* was significantly augmented in the CRC tissues compared to the adjacent non-cancerous and normal colorectal tissues, using RT-qPCR analyses in combination with western blot (15). Li *et al* (16) also found that *CKS2* was highly expressed in colon cancer.

Bladder cancer (BC). Using microarray analysis, it was shown that *CKS2* expression was altered in BC. *CKS2* was upregulated >1.5-fold in BC compared to normal bladder, and RT-qPCR verified the microarray results. Investigators identified that the *CKS2* gene showed a higher expression in BC (2.1 ± 0.64) compared to the normal bladder (0.1 ± 0.01) (17). Chen *et al* (18) measured the *CKS2* mRNA level of 38 cases in superficial BC samples. The mean level, 2.4 ± 0.2 , was equivalent to 10 times to normal samples (0.2 ± 0.3).

In other malignant tumors, such as glioma (19,20), multiple myeloma (21), follicular thyroid carcinoma (22), prostate cancer (23), malignant melanoma (24), cervical cancer (25), laryngeal squamous cell carcinoma (26) and meningioma (27), *CKS2* was also highly expressed.

3. *CKS2* expression correlates to clinicopathological features

CKS2 was upregulated in numerous malignant tumors and investigators have researched the association between *CKS2* expression and clinicopathological parameters.

Association between CKS2 expression with tumor size, histological tumor differentiation and pathological tumor-node-metastasis (pTNM) stage

Gastric cancer. Kang *et al* (28) performed immunohistochemical staining analyses and identified that *CKS2* was more strongly expressed in the less differentiated area compared to the well differentiated area, even in the same tumor tissue of each patient, occasionally. The study divided 60 cases of gastric cancer tissues into two groups; low and high *CKS2* expression levels. Subsequently, the SPSS® software (SPSS, Inc., Chicago, IL, USA) package was used to determine the association between the results of the immunohistochemical study and the clinicopathological parameters. The analyses showed that a high level of *CKS2* strongly correlated with tumor size, histological tumor differentiation and pTNM stage. A total of 82.1% of the patients in the high *CKS2* expression group had tumor diameters of >4 cm, whereas 73.3% were poorly differentiated and 83.3% were pTNM stage IV. Multivariate logistic analysis was used to assess the predictive value of the *CKS2* expression status for clinicopathological significance. The results corroborated that histological tumor differentiation, pTNM stage and a high level of *CKS2* were significant covariables. A high *CKS2* expression level was also found to be closely associated with tumor size and pTNM stage in gastric cancer tissues (12).

Hepatocellular carcinoma. Shen *et al* (9) applied RT-qPCR and immunohistochemical staining analyses to determine the *CKS2* mRNA and protein expression levels in 48 hepatocellular carcinoma tissues. The SPSS® statistical software package was used to study the associations between *CKS2* expression and various clinicopathological features. The analyses showed that a high *CKS2* expression level strongly correlated with poor differentiation. No association was found between *CKS2* overexpression and tumor size. In another study by Shen *et al* (29), the same results were exhibited in cholangiocarcinoma, and *CKS2* overexpression was associated with poor differentiation.

CRC. Jung *et al* (14) studied the correlation between the *CKS2* gene expression level and clinical characteristics in CRC tissues. The analysis of variance statistical test was used and the results showed that a high level of *CKS2* expression was associated with early tumor stage. The *CKS2* mRNA levels of 16 patients in stage II were higher compared to eight patients in stage III. Yu *et al* (15) also reported results that the overexpression of *CKS2* was correlated with the pathological stage in CRC tissues. Using western blot analyses, this study reported that tumor tissues from 15 stage III CRC patients demonstrated significantly higher *CKS2* protein levels compared to those from 15 stage II patients. *CKS2* expression was also correlated with tumor size and differentiation. The reason for the results differing from Jung *et al* (14) may be due to the smaller number of patients from stage III in the latter.

CKS2 promotes cancer invasion and metastasis
CKS2 promotes cancer invasion

BC. In 2006, Kawakami *et al* (17) first reported that *CKS2* expression is strongly correlated with the progression of human BC. This study subjected 21 BC samples to the RT-qPCR assay for *CKS2*, and identified a considerable difference between superficial and invasive BC. *CKS2* had a significantly greater level of upregulation in invasive compared to superficial BC ($P=0.04$).

Chen *et al* (18) used the RT-qPCR analysis on 45 patient samples with a diagnosis of superficial BC (of these, seven patients developed muscle-invasive BC during the follow-up period) and 10 samples from normal bladders. Expression of the *CKS2* gene in superficial BC tissues was >10-fold higher compared to normal bladder tissues, and was ~4-fold higher following disease transition in patients developing muscle-invasive cancer. *CKS2* had significantly increased expression levels following transition from superficial BC to muscle-invasive cancer.

Gastric cancer. Tanaka *et al* (12) reported that 31 cases exhibited serosal invasion among 40 cases of high *CKS2* expression in gastric cancer tissues.

From these outcomes it can be inferred that *CKS2* may function as a tumor oncogene. *CKS2* may promote cancer invasion and it may be a useful biomarker for predicting disease outcome and the requirement for early preventive treatments.

CKS2 promotes lymph node metastasis. Lymph node metastasis is extremely common for cancers, and almost all will develop to lymph node metastasis of tumors (30).

Esophageal carcinoma. Kita *et al* (11) examined the mRNA level of *CKS2* in esophageal squamous cell carcinoma tissues from 62 esophageal cancer patients. The patients with values below the median expression level in tumor tissues were assigned to the low expression group, whereas those with values above the median were assigned to the high expression group. Of the 31 patients assigned to the high expression group, 26 (83.9%) presented with lymphatic invasion. This result suggested that high expression of *CKS2* was significantly associated with the incidence of lymphatic invasion. Wang *et al* (10) also observed the correlation between high *CKS2* expression and lymph node metastasis in esophageal carcinoma tissues.

Gastric cancer. Tanaka *et al* (12) showed the results that 40 cases had high *CKS2* expression and 69 cases had low *CKS2* expression in 109 cases of gastric cancer tissues. Of the 40 cases with high *CKS2* expression, 32 (80%) had lymph node metastasis, indicating that *CKS2* promotes lymph node metastasis. Kang *et al* (28) also observed the correlation between high *CKS2* expression and lymph node metastasis in gastric cancer tissues.

Cervical cancer. Lyng *et al* (31) identified the gene expression in 29 cervical cancer patients with and 19 patients without lymph node metastases using a genomic microarray technique. *CKS2* was noted to be elevated in node-positive compared to node-negative tumors.

From these outcomes it can be suggested that *CKS2* may function as a tumor oncogene and it promotes lymph node metastasis from carcinoma *in situ*.

CKS2 is associated with liver metastasis. The liver is the most common site of metastases from the digestive tract tumors. Tanaka *et al* (12) reported that five cases (12.5%) had an incidence of liver metastasis in 40 gastric cancer tissues with a high level of *CKS2* mRNA expression. In 69 cases with a low

level of *CKS2* mRNA expression, just one case (1.4%) had the incidence of liver metastasis, indicating that high expression of *CKS2* was associated with liver metastasis.

Lin *et al* (32) identified conflicting results to Tanaka *et al* (12). The mRNA profiling of *CKS2* was undertaken by RT-qPCR to confirm the differential expression between liver metastases and primary tumors. *CKS2* was noted to be downregulated in 28 metastases tissues relative to 40 primary colon tumors.

CKS2 is associated with the survival rate. The prognosis for cancer patients is extremely important. Tanaka *et al* (12) used Kaplan-Meier survival curves to evaluate the survival rate in gastric cancer patients according to the levels of *CKS2* mRNA expression. The overall 5-year survival rate was significantly higher in the *CKS2* low expression group (59.9%) compared to the *CKS2* high expression group (23.9%). Thus, *CKS2* mRNA expression was associated with the prognosis.

4. Molecular mechanisms

CKS2 regulates the cell cycle

CKS2 downregulates p53. *CKS2* is a transcription target downregulated by p53 (7). Kang *et al* (28) used SUN638 and AGS gastric cancer cells that were transfected with GFP; GFP-*CKS2* plasmids. The western blot results showed that the protein expression level of p53 in GFP-*CKS2*-overexpressing SUN638 and AGS cells was lower compared to the GFP control cells, suggesting that the increased *CKS2* protein affected the protein level of p53. However, it requires further study to understand how *CKS2* overexpression causes the downregulation of p53.

CKS2 upregulates cyclin A, cyclin B1 and CDK1. Cholangiocarcinoma cells, QBC939, were stably transfected with sh*CKS2* or shCtrl plasmids, and stable *CKS2*-knockdown cells were established. RT-qPCR and western blot analysis were used to determine the cell cycle associated genes. *CKS2* downregulation resulted in a marked reduction in the protein and mRNA levels of cyclin A and cyclin B1 when compared with shCtrl cells. Flow cytometry showed that *CKS2* downregulation increased the cells at the G₂/M phase and decreased the cells at the S and G₁ phases. In this study, *CKS2*-knockdown induced cholangiocarcinoma cell cycle arrest in G₂ phase by decreasing the expressions of cyclin A and cyclin B1. These results suggest that *CKS2* may serve as a cell cycle checkpoint protein for S/G₂ transition and this is one of the mechanisms of how *CKS2* promotes cholangiocarcinoma progression (29).

Tissues from 10 gastric cancer patients were prepared and RT-qPCR analysis was performed (28). Cell cycle regulators, cyclin A, cyclin B1 and *CDK1*, were evidently upregulated in tumor tissues compared to normal tissues. The study also used the small interfering RNA (siRNA)-mediated gene-silencing method to suppress the transcript level of *CKS2* in AGS gastric cancer cells. When the transcript levels of *CKS1* and *CDK1* were compared with the *CKS2* level, the genes were upregulated, possibly by a complicated mechanism in which the proteins that have high similarities, such as CKSs and CDKs, complement each other to maintain their basic role.

CDK1 and cyclin B1 are known to be important players in the cell cycle. Numerous studies have demonstrated that

cyclin B1-CDK1 protein kinase, also known as mitosis promoting factor, is essential for mitosis and that in its absence, cells are unable to progress past the G₂ phase of the cell cycle (33,34). CKS2 binds to CDK1 via interaction with the catalytic subunit of CDK1, and subsequently affects cell cycle.

CKS2 regulates cell apoptosis. Tanaka *et al* (12) used the siRNA-mediated gene-silencing method to suppress the transcript level of CKS2 in MKN74 gastric cancer cells and examined caspase-3 expression by flow cytometry. CKS2-siRNA cells had increased caspase-3 activity (10.8%) compared to the siRNA-negative control (2%), indicating that the suppression of CKS2 expression increased caspase-3 activity by >5-fold. The knockdown of CKS2 expression increased Bax expression at the protein level, shown by western blot analyses. Caspase-3 and Bax proteins are involved in cell apoptosis, these results show that apoptosis is induced by inhibiting CKS2 expression.

Shen *et al* (29) observed the changes in the expression of Bcl-2 family proteins in cholangiocarcinoma cells by western blot analysis. Bax increased following CKS2 knockdown (shCKS2). The study also examined the activation of Bax by immunofluorescence and found that Bax activation increased in shCKS2 cells. In the nude mouse tumorigenesis experiment, the 32 kDa proenzyme caspase-3 was cleaved to obtain its active form of 17 kDa following CKS2 knockdown. These data suggest that CKS2 knockdown enhances the susceptibility of cholangiocarcinoma cells to a Bax-mediated mitochondrial caspase-dependent apoptosis. To further confirm CKS2 knockdown promoting cholangiocarcinoma cells under apoptosis, flow cytometry was used to evaluate the apoptotic susceptibility of QBC939-shCKS2 and shCtrl cells. Data indicated that the apoptosis rate of shCKS2 cells (42.5%) was significantly higher compared to shCtrl cells (12.3%) under hypoxia growth conditions. From these results it is shown that CKS2 downregulation can promote cholangiocarcinoma under apoptosis.

5. CKS2 acts as a target gene

CKS2 has its affect on the expression of downstream genes, mostly cyclin A, cyclin B1 and CDK1. In certain studies, a variety of results indicated that CKS2 is a direct target of specific genes.

CKS2 is a target of miR-26a. Lv *et al* (35) explored the miR-26a-regulated target gene(s), using three publicly-available miRNA target prediction tools: TargetScan, ncRNA and mirecords. CKS2 was selected as a potential target.

To assess whether miR-26a directly altered the expression of CKS2, a fragment of the 3'untranslated region (UTR) of CKS2 mRNA [wild-type (wt) 3'UTR or the mutant sequence (mut 3'UTR)] containing the putative miR-26a binding sequence, was cloned into a luciferase reporter vector. HEK-293T cells were subsequently transfected with the wt or mut 3'UTR of CKS2 and miR-26a mimic. Luciferase expression was decreased by ~50% when the wt 3'UTR and miR-26a mimic were co-transfected, while the mut 3'UTR had no effect on luciferase activity.

This study also verified that CKS2 mRNA and protein levels were decreased by miR-26a overexpression in TPC-1 and CGTH W3 cells, and this effect was attenuated by anti-miR-26a. These results indicate that CKS2 is a direct target of miR-26a, and miR-26a modulates cell growth and tumorigenesis of papillary thyroid carcinoma via regulating CKS2.

Y-box-binding protein 1 (YB-1) gene affects CKS2 expression. YB-1 belongs to a family of RNA-and DNA-binding proteins. It performs pleiotropic cellular functions, including transcriptional regulation, translational regulation, DNA repair and drug resistance (36). To have an improved insight into the role of YB-1 on cell proliferation, Yu *et al* (37) used T-47D breast cancer cells that were transfected with YB-1 siRNA. The CKS2 gene was downregulated in response to siYB-1 treatment by RT-qPCR analysis; however, the investigators did not discuss the interaction between YB-1 and CKS2. There may be a link, and silencing of the YB-1 gene may induce CKS2 downregulation, but more experiments are required to clarify the regulation.

6. Conclusion

A number of human malignancies are characterized by CKS2 overexpression, and it is generally known as an oncogene. The role of the CKS2 protein in tumors has attracted increasing interest. In recent years, investigators have explored the gene function of CKS2 by gene knockout or overexpression studies. CKS2 has abnormal expression in a variety of malignant tumor tissues and was closely associated with certain biological behavior, such as tumor development, progression and metastasis. Certain investigators have conducted a more in-depth research of CKS2, suggesting that CKS2 can increase the cell cycle proteins, cyclin A, cyclin B1 and CDK1, thus promoting cancer cell proliferation. CKS2 was closely associated with cell apoptosis through a Bax-mediated mitochondrial caspase-dependent apoptosis. Simultaneously, CKS2 was also regulated by miR-26a and the YB-1 gene. The present review allows an unobstructed view on the clinicopathological significance and the molecular mechanisms of CKS2 in human cancers. The application is promising in cancer due to the significant role of the CKS2 protein. Investigating the association between CKS2 and tumors, cell cycle and apoptosis depends on the further studies of the CKS2 biological functions. Along with the development of associated experimental studies, the CKS2 protein could become a new biomarker of tumors for tumor therapy.

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