Cyclin H predicts a poor prognosis and promotes the proliferation in ovarian cancer

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Cyclin H, CDK2, ovarian cancer, cell cycle
Abstract
Background
Cell cycle dysregulations play a key role in the pathogenesis of malignant tumor. Cyclin H is a part of CDK-activating kinase (CAK) trimeric complex, which is necessary for the regulation of the cell cycle and proliferation. The objective of this investigation was to characterize the clinical significance and biological functions of cyclin H in ovarian cancer.

Methods
Immunohistochemical staining was performed on 60 cases of ovarian cancer and the correlation between cyclin H expression and clinical characteristics was analyzed. The function of cyclin H in ovarian cancers was explored using HO8910 cells and animal model.

Results
Cyclin H was slightly expressed in grade 1 ovarian cancer and highly expressed in high grade 2 and grade 3 tissues. The result of spearman rank correlation analysis showed the expression of cyclin H positively correlated with tumor grade, the FIGO stage, histologic grade and peritoneal metastasis of ovarian cancer. Cyclin H was positively correlates with Ki67 and p-CDK2 in ovarian cancer. In addition, we found the five-year survival rate of cyclin H low expressed patients was higher than that in the cyclin H high expressed patients. Furthermore, knockdown of cyclin H was then achieved using a shRNA in HO8910 ovarian cancer cell line. Silencing of cyclin H resulted in G1/S cell cycle arrest of ovarian cancer cells and suppressed the growth of ovarian cancer.

Conclusions
These results suggest high expression of cyclin H predicts a poor prognosis and promotes the growth of ovarian cancer via regulating cell cycle.

Background
Ovarian cancer is the seventh most common tumor in women and the most lethal gynecological malignancy with high recurrence and mortality rate[1–3]. Five year survival rate of ovarian cancer patient is less than 35% [4]. Most patients with ovarian cancer don’t get a diagnosis until the advanced stage because they do not show symptoms in the early stage [3, 5, 6]. Therefore,
investigating the pathogenesis of ovarian cancer and finding new targeting proteins that closely related to the occurrence, development and prognosis of ovarian cancer will increase the early diagnosis rate of ovarian cancer and provide new ideas for the treatment of ovarian cancer.

Deregulation of cell cycle and abnormal signaling pathways caused by various reasons have been considered as general features of tumor cells [7]. Excessive cell proliferation, blocked differentiation, and impaired apoptosis play a key role in the occurrence and development of tumors. It’s believed that clarifying the molecular mechanism of cell cycle regulation in tumor cells will provide the basis for the development of tumor treatment strategies [8, 9]. There are three main types of molecules that have been found to be involved in cell cycle regulation: cyclin, cyclin-dependent kinase (CDK), and cyclin-dependent kinase inhibitor (CDKI). Expression of cell cycle regulators has been linked to tumor development, prognosis and response to treatment [10]. Cyclin H, a member of the cyclin family, together with CDK7 and MAT1 form CDK-activating kinase (CAK) trimeric complex, which is necessary for the regulation of the cell cycle and proliferation. Abnormal expression of cyclin H has been reported in a variety kinds of tumors such as breast cancer[11], esophageal cancer[12], endometrial cancer[13] and gastrointestinal stromal tumors[14]. However, the clinical significance and biological functions of cyclin H in ovarian cancer still remains unclear.

In this study, the immunohistochemical staining was used to evaluate the expression of cyclin H in 60 ovarian cancer pathological specimens with different grades. The correlation between cyclin H and clinical characteristics, prognosis, and proliferation parameters of tumor patients were analyzed. In addition, we down-regulated the expression of cyclin H in ovarian cancer cells using shRNA and explored its effect on cell cycle and tumor growth. This study revealed that cyclin H highly expressed in high-grade ovarian cancer and promotes the growth of ovarian cancer by regulating cell cycle.

Methods

Pathological Samples

Ovarian cancer specimens embedded in paraffin were obtained from 60 patients who underwent surgery in the Department of Obstetrics and Gynecology at Affiliated Hospital of Nantong University from June 2009 to April 2013. All tumors were from patients newly diagnosed with ovarian cancer and
patients did not receive any treatment before surgery. Among them, four patients’ survival information was missing. All investigations described in this study were performed after informed consent was obtained. Approval for the study was obtained from the Research Ethics Committee of Affiliated Hospital of Nantong University.

**Immunohistochemistry (IHC) and quantification**

Formalin-fixed, paraffin-embedded sections were prepared for all tissues and reviewed by 3 pathologists. The protein expression of cyclin H, Ki67 and p-CDK2 in ovarian cancer was determined by IHC. In short, ovarian cancer sections were dewaxed in xylene and rehydrated through gradient ethanol, and then heated in Tris-EDTA buffer (pH 8.0) for 15 min in a microwave oven to retrieve the antigen. After cooling naturally and rinsing with phosphate-buffered saline, slides were then incubated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity and 2% bovine serum albumin (Beyotime Biotechnology, Shanghai, China) was applied for 2 h at room temperature to block nonspecific reactions. Tissue sections were then incubated with cyclin H monoclonal antibody (1:50, MA5-32331, Thermo Fisher Scientific, Waltham, MA, USA), Ki67 monoclonal antibody (1:300, ab92742, Abcam, Cambridge, MA, USA) or p-CDK2 polyclonal antibody (1:100, PA5-38128, Thermo Fisher Scientific) at 4°C overnight. All slides were finally visualized using the Envision kit (Dako, Glostrup, Denmark) according to the instructions and counterstained with hematoxylin, dehydrated, and cover slipped. Sections were imaged under an optical microscope and evaluated in a blinded manner. More than 500 cells were counted for each slide to determine the mean positive percent and the expression range was scored as follows: 0 (<20%), 1 (20% to 50%), 2 (51% to 75%) and 3 (>75% points).

**Cell culture and transfection**

Human ovarian carcinoma HO8910 cell line used in this study was obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). HO8910 cell line was cultured in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 0.11g/L Sodium Pyruvate, 100 U/mL penicillin-streptomycin mixture (Gibco) at 37°C and 5% CO₂ incubator. shRNA of cyclin H (sequence: 5’-CCG GCG ACC TGG TAG AAT CTC TCT ACT CGA GTA GAG AGA TTC
TAC CAG GTC GTT TTT G-3’) was synthesized by Sangon Biotech (Shanghai, China) and cloned to pLenti-CMV-puro vector and transfected to HO8910 cells using FuGENE transfection reagent (Promega, Madison, WI, USA). Stable transfected cell lines were obtained after screened with 1 μg/mL puromycin. Empty plasmid was transfected into HO8910 cells as control group.

**Cell viability assay**

Cells were seeded in 96 well plated (3000 cells per well) and culture in complete RPMI 1640 medium containing 10% fetal bovine serum. Cell viability was measured by cell counting kit-8 (C0038, Beyotime, Shanghai, China) according to the manufacture’s instruction at 0, 12, 24, 48 and 72 h after seeding. Five wells were performed for each time point. Cell viability at each time point was compared to the initial cell viability.

**Western blot**

HO8910 cells digested with trypsin, washed with PBS and collected by centrifugation. Cell pellets were resuspended with cell lysate and incubate on ice for 20 min. Samples were blotted using SDS-PAGE gel and transferred to polyvinylidene fluoride membrane (0.2 μm, Millipore, USA). After blocking with 5% non-fat milk, membrane was incubated with cyclin H antibody (1:1500, MA5-32331, Thermo Fisher Scientific), MAT1 antibody (1:1000, ab129176, Abcam), CDK7 antibody (1:1000, 2916, Cell Signaling Technology, Danvers, MA, USA), p-CDK2 antibody (1:100, PA5-38128, Thermo Fisher Scientific) or CDK2 antibody (1:1000, 2546, Cell Signaling Technology) and then HRP goat anti-rabbit or mouse IgG (ABclonal, Wuhan, China). Membranes were finally visualized using an enhanced chemiluminescence reagent (34095, Thermo Pierce). GAPDH (1:2000, Abcam) was used as control.

**mRNA level of cyclin H detection**

The total RNA of HO8910 cells were obtained using Trizol (Invitrogen) and reverse transcription was performed using a commercial kit (RR047B, TaKaRa, Tokyo, Japan) containing gDNA Eraser to eliminate genomic DNA contamination. Relative mRNA level of cyclin H was determined by real time quantitative PCR (RR420L, TaKaRa). β-actin was used as control. Following primers was used: cyclin H forward, 5’-TGT TCG GTG TTT AAG CCA GCA-3’; cyclin H reverse, 5’-TCC TGG GGT GAT ATT CCA TTA CT-3’; β-actin forward, 5’-TCG AGC ACG GCA TCG TCA CCA-3’; β-actin reverse, 5’-ATA GCA ACG TAC
ATG GCT-3’.

**Cell cycle detection**

HO8910 cells were synchronized by deprivation of serum over 72 h. The serum-free medium was replaced by complete medium and cells were cultured for another 48 h before collection. Cells were digested with trypsin and single cell suspension was prepared before fixation and staining. Propidium staining was performed using a detection kit (C1052, Beyotime) and detected using BD FACSCanto II (San Diego, CA, USA). The data was analyzed using Modfit software and the percentage of cells in each phase was counted.

**Tumor model**

Female BALBC/c nude mice (5–6 weeks old, 14–16 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and maintained in specific pathogen-free conditions. HO8910 cells (5 × 10^5/mouse) in 100 μL PBS were inoculated subcutaneously of the nude mice in the flank. Tumor diameters are measured with digital calipers every other day, and the tumor volume is calculated by the following formula: Volume = (width)^2 × length/2.

**Statistical Analysis**

IBM SPSS was used for all statistical analyses. Chi-square test was used for clinicopathological categorical variables. Overall survival was analyzed by the Kaplan-Meier method. T test was used to study the differences between two groups. Spearman rank correlation analysis was used to measure the association between the expression of cyclin H, Ki67 and p-CDK2. Differences were considered statistically significant at \( p < 0.05 \).

**Results**

**High expression of cyclin H in ovarian cancer.**

To investigate the expression and potential role of cyclin H in ovarian cancer, immunohistochemical staining was performed on 60 cases of ovarian cancer, and cyclin H expressions were scored. Representative results of immunohistochemistry are shown in Figure 1. In the ovarian cancer tissues, cyclin H localized in the nucleus and cytoplasm. The mean percentage of cyclin H positive cells was 33.16% (ranged from 3.10% to 77.48%). Cyclin H was slightly expressed in grade 1 ovarian cancer
and highly expressed in high grade 2 and grade 3 samples (Fig. 1 A-C). High cyclin H expression is significantly correlated with increased tumor grade (Fig.1 D and Table1).

**Correlation between cyclin H expression and Clinicopathologic Variables of ovarian cancer.**

In order to further explore the potential physiological or pathological role of cyclin H in ovarian cancer, the correlation of cyclin H with clinicopathological parameters were analyzed and summarized in Table 1. The result of spearman rank correlation analysis showed the expression level of cyclin H in ovarian cancer was not associated with age, histologic subtype, residual tumor size and chemotherapy. Interestingly, Cyclin H positively correlated with the FIGO (International Federation of Gynecology and Obstetrics) stage (P=0.002), histologic grade (P=0.0017) and peritoneal metastasis. Compared with patients without peritoneal metastasis, cyclin H expression was significantly increased in individuals with malignant tumor cells in peritoneal fluid (P=0.016). These data suggested that abnormal expression of cyclin H is closely related to the pathological characteristics of ovarian cancer.

**Cyclin H positively correlates with Ki67 and p-CDK2 in ovarian cancer**

Cyclin H is well known as a component of CDK-activating kinase that contributes to cell cycle via mediating CDK activation [15]. Therefore, the expression of cell cycle-related molecules Ki67 and p-CDK2 was determined by immunohistochemical staining. As expected, Ki67 was highly expressed in grade 2 and grade 3 ovarian cancer when compared with grade 1 specimens (Fig. 2 A-C). The expression trend of p-CDK2 (Fig. 2 D-F) was similar to Ki67 and cyclin H. In addition, the result showed that Cyclin H was positively correlated with Ki-67 (r = 0.907; P <0.001) (Fig. 2 G) and p-CDK2 (r = 0.788; P <0.001) (Fig. 2 H) expression in 60 ovarian cancer tissues, demonstrating the involvement of cyclin H in ovarian cancer cell cyclin regulation.

**Cyclin H expression is associated with poor patient outcome in ovarian cancer.**

To determine whether the expression of cyclin H predicts the survival of ovarian cancer patients, we analyzed the relationship between cyclin H protein and mRNA expression and the survival of ovarian cancer patients. Carcinoma specimens were divided into cyclin H low expressers (score ≤ 2, n = 40) and cyclin H high expressers (score > 2, n = 16) according to the immunohistochemical staining
results. According to Kaplan-Meier analysis, the five-year survival rate of patients in the cyclin H low-expression group was significantly higher than that in the cyclin H high-expression group ($P = 0.0014$) (Fig. 3 A). In addition, the relationship between the cyclin H mRNA expression level and the survival of ovarian cancer patients was analyzed using publicly available datasets ($n=1656$, http://www.kmplot.com) according to the instructions. Patients with low mRNA expression of cyclin H have a significant improvement in survival time (Fig. 1A). These data demonstrated that high expression of cyclin H correlates with poor prognosis of ovarian cancer.

**Knockdown of cyclin H results in G1/S cell cycle arrest of ovarian cancer cells.**

To clarify the role of cyclin H in proliferation and cell cycle of ovarian cancer cells, expression of cyclin H was knocked down in HO8910 cells. Knock down efficiency of cyclin H shRNA was determined by quantitative PCR (Fig. 4 A) and western blot (Fig. 4B). The expression level of cyclin H was greatly reduced in cyclin H shRNA transfected cells compared with control group. Cell viability and cell cycle of HO8910 cells were measured by CCK8 and flow cytometry, respectively. Cyclin H silenced HO8910 cells showed weaker proliferative capacity, indicated by the lower cell viability than the control group cells (Fig. 4 C). G1/S cell cycle arrest was observed in cyclin H interfered cells, the percentage of cells in G1 phase was increased and percentage of cells in S phase was decreased compared with the control group (Fig. 4 D-F). The expression of cyclin H, MAT1, CDK7 and p-CDK2 in HO8910 cells was gradually increased after serum stimulation (Fig. 4 G, H). These results suggested that cyclin H is an important positive regulator of ovarian cancer cell proliferation.

**Cyclin H regulates the growth of ovarian cancer.**

Using a nude mouse subcutaneous tumor model, we confirmed the role of cyclin H in ovarian cancer in vivo. Cyclin H shRNA or control shRNA transfected HO8910 cells were implanted s.c. and the development of tumor was monitored every another day. Obvious tumors can be observed on day 8 and the tumor volume was determined. Mice was sacrificed and tumor was removed and weighed on day 20. The tumor weight and size in cyclin H shRNA group were much lighter and smaller than those in control group, indicating cyclin H knockdown significantly reduced ovarian cancer growth (Fig. 5 A-C). No obvious changes in body weight and daily behavior could be found between the two groups.
Discussion
In the past decade, the incidence and mortality of ovarian cancer in China has been increasing rapidly[16, 17]. Currently, the clinical treatment of ovarian cancer is surgery combined with chemotherapy drugs (cisplatin, carboplatin, etc.)[5, 18]. However, ovarian cancer is susceptible to chemotherapy resistance and prone to recurrence and metastasis after surgery[19, 20]. Ovarian cancer has become a great challenge to the gynecological oncologist and it’s urgent to identify new prognostic factors and develop new strategies to improve the outcome of ovarian cancer patients. In this study, we demonstrated the high expression of cyclin H in ovarian cancer and cyclin H was associated with unfavorable clinicopathologic variables of patients.

Destruction of cell cycle regulatory mechanisms leads to uncontrolled cell growth. Cell cycle is a complicated and delicate process that strictly regulated by the activation and inactivation of cyclin, CDK, and CDKIs. Cyclin and CDK are regulatory and catalytic subunits, play key roles in regulating cell cycle initiation and phase transition. Each Cyclin and CDK is expressed in a specific phase in the cell cycle, controls the progress of that phase and switches to the next phase. Cyclin H is a polypeptide consisting of 323 amino acids, forms CAK with CDK7 and MAT1 [21]. CAK form the core part of TFIIH, phosphorylate the CTD subunit of RNA polymerase II (RNAPII) and participate in the transcription [22, 23]. CAK also phosphorylates CDK2, thereby promoting the cell cycle from G1 to S phase[24].

Emerging evidence has indicated that abnormal expression or genetic polymorphisms of cyclin H are associated with tumor progression and chemo-sensitivity[13, 25, 26]. Here, we observed cyclin H was positively correlated with increased tumor grade of ovarian cancer and the expression of the proliferation marker Ki-67 and p-CDK2. Similar results have been reported by in diffuse large B-cell lymphoma[27], esophageal squamous cell carcinoma[12] and breast cancer[11]. In Prashant Bavi et al.’s study, they concluded that reduced or absent cyclin H expression was significantly associated with poor overall survival of patients with diffuse large B-cell lymphoma [27]. Hetal Patel and colleagues also described that Cyclin H expression was associated with better patient outcome in breast cancer [11]. However, we came to the opposite conclusion in ovarian cancer using the protein and mRNA data, five-year survival rate of cyclin H low patients was significantly higher than cyclin H
high patients. Consistent with our research, cyclin H positivity was proved to be significantly associated with reduced disease-specific survival of patients with gastrointestinal stromal tumor [14]. These findings suggest that cyclin H may play different roles in different types of tumors. The transition from G1 to S phase is a key step in the cell cycle process and plays an important role in the development of most tumors. Suppression of G1/S transition is believed to be an attractive therapeutic target to stop cancer cells from proliferating [28]. Previously study has reported that the down-regulation of cyclin H-CDK7 was implicated in arrest of liver cancer cells in G1 phase [29]. To further investigate the role of cyclin H in ovarian cancer cells, cyclin H in HO8910 cells was knocked down and cell cycle was detected after serum starvation induced cell cycle synchronization and serum releasing. We found cyclin H shRNA resulted in G1/S cell cycle arrest and proliferation inhibition of HO8910 cells. The expression of cyclin H was gradually increased after serum stimulation, as well as the associated cofactors CDK7 and MAT1. The phosphorylation of CDK2 also showed the similar trend with CAK components, suggesting cyclin H might promote the G1/S transition by enhancing the phosphorylation of CDK2. Animal experiment confirmed the positive regulatory role of cyclin H in ovarian cancer, knock down of cyclin H obviously suppressed the growth of tumor in nude mice.

Conclusions
In conclusion, these findings indicate that high expression of cyclin H in ovarian cancer was associated with the poor prognosis of patients and promote tumor growth through cell cycle regulation. This study reveals a candidate for early diagnosis or prognosis prediction of ovarian cancer and provides evidence supporting the therapeutic value of cyclin H in ovarian cancer. In the past decades, tremendous efforts have been made to develop inhibitors of CDKs and many inhibitors have been described, but most of them have failed rigorous clinical testing[30]. Considering the controversial role of cyclin H in different kinds of tumor has been reported, more in-depth, systematic researches are needed to be conducted to confirm the therapeutic value of cyclin H in ovarian cancer.

Abbreviations
CAK: CDK-activating kinase; CDK: Cyclin-dependent kinase; CDKI: Cyclin-dependent kinase inhibitor;
Declarations

**Ethics approval and consent to participate.**

Study protocol of ovarian cancer tissue section was approved by the Research Ethics Committee of Affiliated Hospital of Nantong University (approval No. 20080516-003). All animal procedures were carried out under the approval of the Ethics Committee for Laboratory Animals of Nantong University (approval No. 20150418).

**Consent for publication.**

Not applicable.

**Availability of data and materials**

Not applicable.

**Competing interests**

The authors have declared that no competing interest exists.

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**Authors’ contributions**

Chen Peng and Yuquan Zhang; Data curation, Yansong Yang; Formal analysis, Yansong Yang; Investigation, Chen Peng and Yuquan Zhang; Methodology, Chen Peng, Yansong Yang, Li Ji and Panpan Yang; Project administration, Xiaoqing Yang and Yuquan Zhang; Resources, Li Ji, Xiaoqing Yang and Yuquan Zhang; Software, Panpan Yang; Supervision, Xiaoqing Yang and Yuquan Zhang; Validation, Li Ji; Writing – original draft, Chen Peng; Writing – review & editing, Yansong Yang, Panpan Yang and Yuquan Zhang.

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**References**

1. Herzog TJ, Pothuri B. Ovarian cancer: a focus on management of recurrent disease. *Nat Clin Pract Oncol.* 2006; 3(11):604-611.
2. Webb PM, Jordan SJ. Epidemiology of epithelial ovarian cancer. Best Pract Res Clin Obstet Gynaecol. 2017; 41:3-14.

3. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med. 2017; 14(1):9-32.

4. Kossai M, Leary A, Scoazec JY, Genestie C. Ovarian Cancer: A Heterogeneous Disease. Pathobiology. 2018; 85(1-2):41-49.

5. Doubeni CA, Doubeni AR, Myers AE. Diagnosis and Management of Ovarian Cancer. Am Fam Physician. 2016; 93(11):937-944.

6. Stewart C, Ralyea C, Lockwood S. Ovarian Cancer: An Integrated Review. Semin Oncol Nurs. 2019; 35(2):151-156.

7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5):646-674.

8. Casimiro MC, Crosariol M, Loro E, Li Z, Pestell RG. Cyclins and cell cycle control in cancer and disease. Genes Cancer. 2012; 3(11-12):649-657.

9. Law ME, Corsino PE, Narayan S, Law BK. Cyclin-Dependent Kinase Inhibitors as Anticancer Therapeutics. Mol Pharmacol. 2015; 88(5):846-852.

10. Bai J, Li Y, Zhang G. Cell cycle regulation and anticancer drug discovery. Cancer Biol Med. 2017; 14(4):348-362.

11. Patel H, Abduljabbar R, Lai CF, Periyasamy M, Harrod A, Gemma C, Steel JH, Patel N, Busonero C, Jerjees D et al. Expression of CDK7, Cyclin H, and MAT1 Is Elevated in Breast Cancer and Is Prognostic in Estrogen Receptor-Positive Breast Cancer. Clin Cancer Res. 2016; 22(23):5929-5938.

12. Zhang J, Yang X, Wang Y, Shi H, Guan C, Yao L, Huang X, Ding Z, Huang Y, Wang H et al. Low expression of cyclinH and cyclin-dependent kinase 7 can decrease the proliferation of human esophageal squamous cell carcinoma. Dig Dis Sci. 2013;
13. Kayaselcuk F, Erkanli S, Bolat F, Seydaoglu G, Kuscu E, Demirhan B. Expression of cyclin H in normal and cancerous endometrium, its correlation with other cyclins, and association with clinicopathologic parameters. Int J Gynecol Cancer. 2006; 16(1):402-408.

14. Dorn J, Spatz H, Schmieder M, Barth TF, Blatz A, Henne-Bruns D, Knippschild U, Kramer K. Cyclin H expression is increased in GIST with very-high risk of malignancy. BMC Cancer. 2010; 10:350.

15. Schneider E, Kartarius S, Schuster N, Montenarh M. The cyclin H/cdk7/Mat1 kinase activity is regulated by CK2 phosphorylation of cyclin H. Oncogene. 2002; 21(33):5031-5037.

16. Jiang X, Tang H, Chen T. Epidemiology of gynecologic cancers in China. J Gynecol Oncol. 2018; 29(1):e7.

17. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin. 2016; 66(2):115-132.

18. Pozzar RA, Berry DL. Patient-centered research priorities in ovarian cancer: A systematic review of potential determinants of guideline care. Gynecol Oncol. 2017; 147(3):714-722.

19. Karakashev S, Aird KM. Ovarian cancer: how can resistance to chemotherapy be tackled? Future Oncol. 2017; 13(30):2737-2739.

20. Pujade-Lauraine E, Combe P. Recurrent ovarian cancer. Ann Oncol. 2016; 27 Suppl 1:i63-i65.

21. Larochelle S, Chen J, Knights R, Pandur J, Morcillo P, Erdjument-Bromage H, Tempst P, Suter B, Fisher RP. T-loop phosphorylation stabilizes the CDK7-cyclin H-MAT1 complex in vivo and regulates its CTD kinase activity. EMBO J. 2001; 20(14):3749-
22. Fuss JO, Tainer JA. XPB and XPD helicases in TFIIH orchestrate DNA duplex opening and damage verification to coordinate repair with transcription and cell cycle via CAK kinase. DNA Repair (Amst). 2011; 10(7):697-713.

23. Shiekhattar R, Mermelstein F, Fisher RP, Drapkin R, Dynlacht B, Wessling HC, Morgan DO, Reinberg D. Cdk-activating kinase complex is a component of human transcription factor TFIIH. Nature. 1995; 374(6519):283-287.

24. Desai D, Wessling HC, Fisher RP, Morgan DO. Effects of phosphorylation by CAK on cyclin binding by CDC2 and CDK2. Mol Cell Biol. 1995; 15(1):345-350.

25. Palugulla S, Devaraju P, Kayal S, Narayan SK, Mathaiyan J. Genetic polymorphisms in cyclin H gene are associated with oxaliplatin-induced acute peripheral neuropathy in South Indian digestive tract cancer patients. Cancer Chemother Pharmacol. 2018; 82(3):421-428.

26. Murali A, Nalinakumari KR, Thomas S, Kannan S. Association of single nucleotide polymorphisms in cell cycle regulatory genes with oral cancer susceptibility. Br J Oral Maxillofac Surg. 2014; 52(7):652-658.

27. Bavi P, Abubaker J, Hussain A, Sultana M, Al-Dayel F, Uddin S, Al-Kuraya KS. Reduced or absent cyclin H expression is an independent prognostic marker for poor outcome in diffuse large B-cell lymphoma. Hum Pathol. 2008; 39(6):885-894.

28. Liu M, Liu H, Chen J. Mechanisms of the CDK4/6 inhibitor palbociclib (PD 0332991) and its future application in cancer treatment (Review). Oncol Rep. 2018; 39(3):901-911.

29. Dai QS, Liu W, Wang XB, Lu N, Gong DD, Kong LY, Guo QL. NCPMF-60 induces G2/M cell cycle arrest and apoptosis in human hepatocellular carcinoma HepG2 cells. Anticancer Drugs. 2011; 22(1):46-57.
30. Sanchez-Martinez C, Lallena MJ, Sanfeliciano SG, de Dios A. Cyclin dependent kinase (CDK) inhibitors as anticancer drugs: Recent advances (2015-2019). Bioorg Med Chem Lett. 2019; 29(20):126637.

Tables
Table 1. Relationship between clinicopathological characteristics and Cyclin H protein expression in ovarian cancer.
| Clinicopathological characteristic | No. Cases | Cyclin H Expression | R    | P     |
|-----------------------------------|-----------|---------------------|------|-------|
|                                   |           |                     |      |       |
|                                   |           | 0 | 1 | 2 | 3 |
| Age (years)                       |           | 0 | 1 | 2 | 3 |
| <Mean                            | 27        | 1 | 8 | 10 | 8   | -0.012 | 0.92 |
| ≥Mean                            | 33        | 3 | 6 | 16 | 8   |       |       |
| FIGO stage, n (%)                 |           | 0 | 1 | 2 | 3 |
| I                                | 15        | 3 | 5 | 6  | 1   | 0.389  | 0.00 |
| II                               | 6         | 0 | 3 | 1  | 2   |       |       |
| III                              | 31        | 1 | 5 | 16 | 9   |       |       |
| IV                               | 8         | 0 | 1 | 3  | 4   |       |       |
| Histologic subtype, n (%)        |           | 0 | 1 | 2 | 3 |
| Serous papillary adenocarcinoma  | 42        | 2 | 9 | 17 | 14  | -0.228 | 0.07 |
| Endometrioid adenocarcinoma      | 7         | 1 | 2 | 2  | 2   |       |       |
| Clear cell carcinoma             | 3         | 0 | 1 | 2  | 0   |       |       |
| Mucinous carcinoma               | 8         | 1 | 2 | 5  | 0   |       |       |
| Histologic grade, n (%)          |           | 0 | 1 | 2 | 3 |
| G1                               | 20        | 4 | 7 | 7  | 2   | 0.466  | 0.001|
| G2                               | 16        | 0 | 3 | 11 | 2   |       |       |
| G3                               | 24        | 0 | 4 | 8  | 12  |       |       |
| Residual tumor (cm), n (%)       |           | 0 | 1 | 2 | 3 |
| ≤1                               | 45        | 3 | 12| 20 | 10  | 0.166  | 0.15 |
| >1                               | 15        | 1 | 2 | 6  | 6   |       |       |
| Chemotherapy, n (%)              |           | 0 | 1 | 2 | 3 |
| Platinum only                    | 1         | 0 | 1 | 0  | 0   | 0.146  | 0.26 |
| Other/platinum                   | 40        | 2 | 7 | 20 | 11  |       |       |
| None                             | 10        | 2 | 6 | 6  | 5   |       |       |
| Malignant tumor cells in peritoneal fluid |          | 0 | 1 | 2 | 3 |
| Present                          | 31        | 3 | 10| 13 | 5   | 0.308  | 0.01 |
| Absent or rare                   | 29        | 1 | 4 | 13 | 11  |       |       |

Statistical analyses were performed by Pearson χ² test.

* P < 0.05, ** P < 0.01
Immunohistochemistry staining of Cyclin H in ovarian cancer tissues. Expression of Cyclin H in ovarian cancer of grade 1 (A), grade 2 (B) and grade 3 (C) was measured by Immunohistochemistry staining and representative images were displayed. Scale bar, 20 \( \mu m \). (D) Quantification of Cyclin H expression in ovarian cancer (\( P < 0.001 \)).
Figure 2

Cyclin H correlates with Ki67 and p-CDK2 in ovarian cancer. (A-C) Immunohistochemistry staining of Ki67 in ovarian cancer of grade1 (A), grade2 (B) and grade3 (C). (D-F) Immunohistochemistry staining of p-CDK2 in ovarian cancer. Scale bar, 20 μm. (G) Correlation between the expression of Ki67 and Cyclin H in ovarian cancer. (H) Cyclin H was positively correlated with p-CDK2 in ovarian cancer.
Survival analysis in ovarian cancer patients. (A) Analysis of patients showing low level of cyclin H (score \( \leq 2 \), \( n = 40 \)) and those with high expression of cyclin H (score \( > 2 \), \( n = 16 \)).

(B) Analysis of 1656 ovarian cancer patients showed that high expression of cyclin H predicted poor prognosis. The Kaplan-Meier plots were generated by Kaplan-Meier Plotter (http://www.kmplot.com).
Cyclin H regulates the proliferation and cell cycle of ovarian cancer cells. (A) mRNA level of cyclin H in vector and cyclin H shRNA transfected ovarian cancer HO8910 cells. (B) The protein expression of cyclin H was detected by Western blot. (C) Proliferation difference between control and cyclin H silencing HO8910 cells. PI staining was performed to measure the percentage cells in each cell cycle phase and analyzed by Modfit software (D and E). (F) Quantification of the percentage cells in each cell cycle phase after transfected with cyclin H shRNA. (G) Expression of cyclin H, CDK7, MAT1, p-CDK2 and CDK2 in HO8910 cells after serum deprivation and refeeding. Serum-starved HO8910 cells were cultured in serum
containing medium for 4, 8, 12, 24 and 48 h and cell lysates were analyzed by western blot.

(H) Relative level of p-CDK2 was normalized total level of CDK2 at each time point. *

\[ P<0.05, **P<0.01, ***P<0.001. \]
Cyclin H promotes the growth of ovarian cancer. Nude mice were implanted with cyclin H silenced HO8910 cells or control cells and tumor volume was monitored regularly (A). (B) Tumor weight was measured at day 20 after mice sacrificed. (C) Tumors from control or cyclin H shRNA group. Data are means ± SEM. Representative results of one of three independent experiments. **P<0.01.