

Original Article

Up-regulation of Polo-like Kinase 1 in nasopharyngeal carcinoma tissues: a comprehensive investigation based on RNA-sequencing, gene chips, and in-house tissue arrays

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Abstract: Background: Nasopharyngeal carcinoma (NPC) is a highly invasive malignancy which has unique characteristics when found among individuals from certain ethnic or geographic populations. The role and molecular mechanism of Polo-like kinase 1 (PLK1) in NPC remain yet to be clarified. Hence, the aim of this study is to identify the clinical implications of PLK1 in NPC based on gene chip, tissue microarray, and other silico approaches. Methods: Relevant data related to PLK1 levels in NPC was screened for by searching in SRA, GEO, ArrayExpress, OncoPrint and throughout the existing literature on this topic. The raw data about gene chips were normalized by using an RMA algorithm provided by “Limma” package. Furthermore, the “SVA” package of R software was used to remove the batch effect and data from the same platform were merged into one part. The differential expression levels of PLK1 between NPC and non-NPC tissues were extracted and analyzed with the Student’s t-test. Meta-analyses were used to calculate the standard mean difference and sROC. Furthermore, in-house immunohistochemistry was performed with tissue microarrays. Weighted correlation network analysis was used to identify the PLK1-related genes. Several bioinformatic evaluations, including the Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and protein-protein interactions, were also performed to assess the PLK1-related pathways. Results: The tissue microarray and gene chips indicated that the PLK1 levels clearly had an up-regulating trend as compared to the non-cancerous controls. These trends were observed in both the single study and the comprehensive meta-analysis. The area under the sROC curve in the NPC tissues was 0.87, with pooled sensitivity and specificity at 0.950 and 0.710, respectively, based on 393 NPC tissues and 83 non-cancerous controls. A total of 144 genes were identified as co-expressed genes of PLK1 in NPC and were mainly enriched in the “cell cycle” pathway. Among the genes related to the cell cycle, CDK1, CCNA2 and CCNB2 were all closely related to PLK1 expression level. Conclusions: PLK1 may play a potential oncogenic role in the tumorigenesis and development of NPC. Since several PLK1 inhibitors have been developed, it is believed that the PLK1 inhibitors have great therapeutic potential in clinic applications for NPC patients.

Keywords: Polo-like Kinase 1, nasopharyngeal carcinoma, RNA-sequencing, gene chip, tissue microarrays

Introduction

Nasopharyngeal carcinoma (NPC) is a highly invasive malignancy which is disproportionately found among certain ethnic and geographic populations [1, 2]. A predominantly high occurrence of NPC takes place in the Southeast

regions of Asian countries, especially in the Southern areas of China, including the districts of Guang Dong, Guang Xi and Fu Jian [3, 4]. NPC has complex pathogenic mechanisms and a series of molecular events that occur during the process of the tumorigenesis. However, the exact molecular mechanisms of NPC remain

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broadly abstruse, which greatly restrains the clinical manipulation of NPC [5-7]. Thus, there is an urgent need to carry out studies that explore the genetic levels of NPC.

Among all the cancer-related genes, the family of Polo-like kinases (PLKs) are principally serine/threonine kinase enzymes that regulate the cell cycles of different cells. Polo-like kinase 1 (PLK1), one member of the PLKs family, has been reported to play an essential role in the intracellular processes in different cancers [8]. By far, the relationship between PLK1 and the progression and efficient treatment of NPC has been reported only by one group. That other study found that a decrease in PLK1 led to mitotic catastrophe with substantial cytotoxicity when using both in vitro and in vivo experiments. Clinically, PLK1 protein expression levels were detected through immunohistochemistry on 40 cases of NPC samples, of which 28 cases showed an over-expressed pattern [9]. However, the sample size of 40 in that study was quite small, and the findings still need to be validated. Additionally, the exact molecular mechanism of PLK1 in the development of NPC still needs to be clarified. Hence, to identify the clinical role of PLK1 in NPC, we performed data-mining based on RNA-sequencing and gene chip data, further validated by an in-house tissue microarray and immunohistochemistry. We also collected the relative genes of PLK1 in NPC and determined the possible signaling pathways of PLK1 via in silico approaches.

Materials and methods

mRNA expression level of PLK1 in NPC tissues

mRNA expression level of PLK1 in NPC tissues based on RNA-sequencing and microarray data: To collect the mRNA expression level of PLK1 in NPC tissues assessed by RNA-sequencing and gene chip technique, related studies were screened for in SRA, GEO, ArrayExpress and Oncomine. The search keywords used were “nasopharyngeal carcinoma” OR “NPC”. The inclusion criteria were as follows: (1) the PLK1 expression profiles in NPC and non-NPC controls could be achieved and (2) more than five tumors and controls were enrolled in the study. Four authors (Xia Yang, Wei-jia Mo, Min-da Wei and Dan-ming Wei) carried out the screening independently and discussed all discrepant opinions to reach a consistent agreement about the inclusion of possible data.

The raw data of gene chips were produced by Affymetrix or other platforms and were downloaded and normalized by using the RMA algorithm provided by “Limma” package (<http://bioconductor.org/packages/limma/>). Furthermore, the “SVA” package of R software was used to remove the batch effect.

The microarray data were normalized according to each platform, and the PLK1 mRNA level was extracted. The differential expression level of PLK1 between NPC and non-NPC tissues were analyzed with the Student's t-test by using the SPSS software version 22.0 for each study. Scatterplots were drawn by using GraphPad version 7.0. The area under the curve (AUC) of receiver operating characteristic (ROC) was further calculated to examine the potential for PLK1 to separate NPC from non-NPC tissues.

mRNA expression level of PLK1 in NPC tissues based on previous reports: To gather the reported data of PLK1 mRNA in NPC, a literature search and data extractions were performed as previously reported [10]. The keywords for NPC mentioned above were used. The keywords “PLK1” or “Polo-like Kinase 1” were added to the search. The inclusion and exclusion criteria followed was consistent with the previous studies performed by our group [10, 11]. The PLK1 mRNA levels were presented using enumeration data, instead of ranked data.

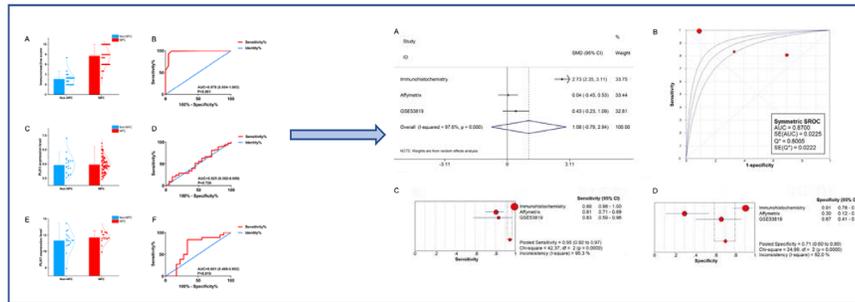
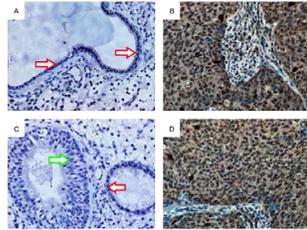
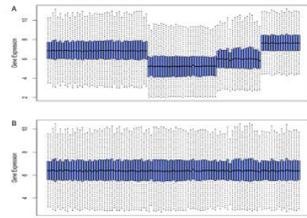
mRNA expression level of PLK1 in NPC tissues based on meta-analyses: To have a comprehensive depiction of the expression levels of PLK1 in NPC, meta-analyses calculating the standard mean difference (SMD) and sROC were performed using Stata software version 14.0. The heterogeneity status of the data decided whether a random- or fixed-model was preferred. The heterogeneity of $I^2 > 50\%$ or $P < 0.05$ led to a random-effects model, and vice versa. Publication bias was detected by using the Begg's and Egger's tests. Sensitivity, specificity and sROC were also calculated [12, 13].

Protein expression level of PLK1 in NPC tissues

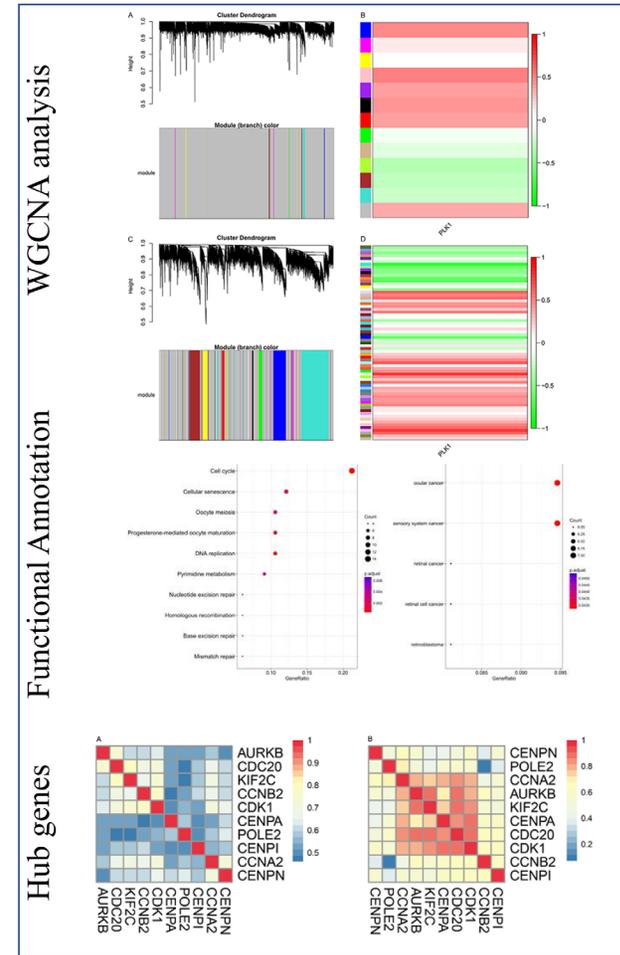
In-house immunohistochemistry with tissue microarray and clinical samples: Two tissue arrays (NPC1507 and NPC1504) were purchased from Pantomics, Inc (Richmond, CA). These arrays included 18 cases of non-cancerous na-

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GEO DataSets



Validation of the expression level of PLK1



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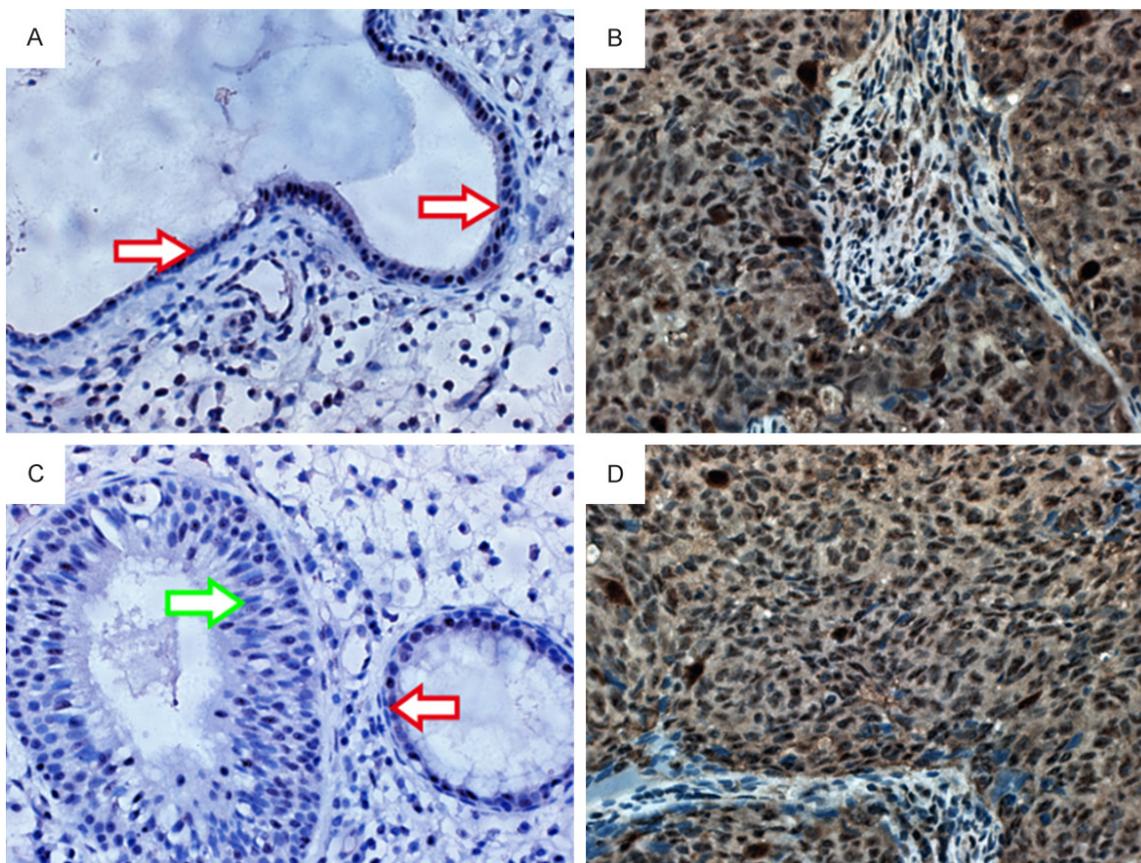


Figure 2. PLK1 protein expression in tissues from nasopharyngeal region. Non-cancerous nasopharyngeal tissues (A, C): Only a small portion of the epithelial cells was positive for PLK1 immunostaining. Red arrow: simple columnar epithelium; Green arrow: squamous metaplasia. Nasopharyngeal carcinoma (NPC: B, D): Major part of NPC were positively stained for PLK1. $\times 400$.

Table 1. General characteristics of included microarrays

First contributor	Year	Country	Data source	Platform	Cancer group	Normal controls
Ahlquist P	2008	USA	GSE12452	Affymetrix GPL570	31	10
Yap L	2009	United Kingdom	GSE13597	Affymetrix GPL96	25	3
Hu C	2012	United Kingdom	GSE34573	Affymetrix GPL570	15	4
Bao YN	2014	China	GSE53819	Agilent GPL6480	18	18
Xiong W	2017	China	GSE64634	Affymetrix GPL570	12	4

sopharyngeal tissues and 282 cases of NPCs. Another 26 cases of non-cancerous nasopharyngeal tissues and 10 NPCs were collected from the Department of Pathology, First Affiliated Hospital of Guangxi Medical University from July 1st to August 1st 2018. The Ethical Committee of First Affiliated Hospital of Guangxi Medical University approved this study. All the patients signed the informed consents for the use of these samples for research. Altogether, the current immunohistochemistry study included 44 non-cancerous controls, including nasopharyngeal polyp and chronic mu-

cosa inflammation, as well as 292 cases of NPCs.

In the immunohistochemistry, the primary antibody used was a rabbit polyclonal antibody, anti-PLK1 antibody (at 1/500 dilution) (Abcam's RabMAb technology), as previously reported [14]. The immunostaining results of PLK1 expression was assessed according to the following two factors: first, the proportion of stained cells among total cells (0: < 5%; 1: 5%-25%; 2: 25%-50%; 3: 50%-75%; 4: > 75%); second, the staining degree of the positive cells

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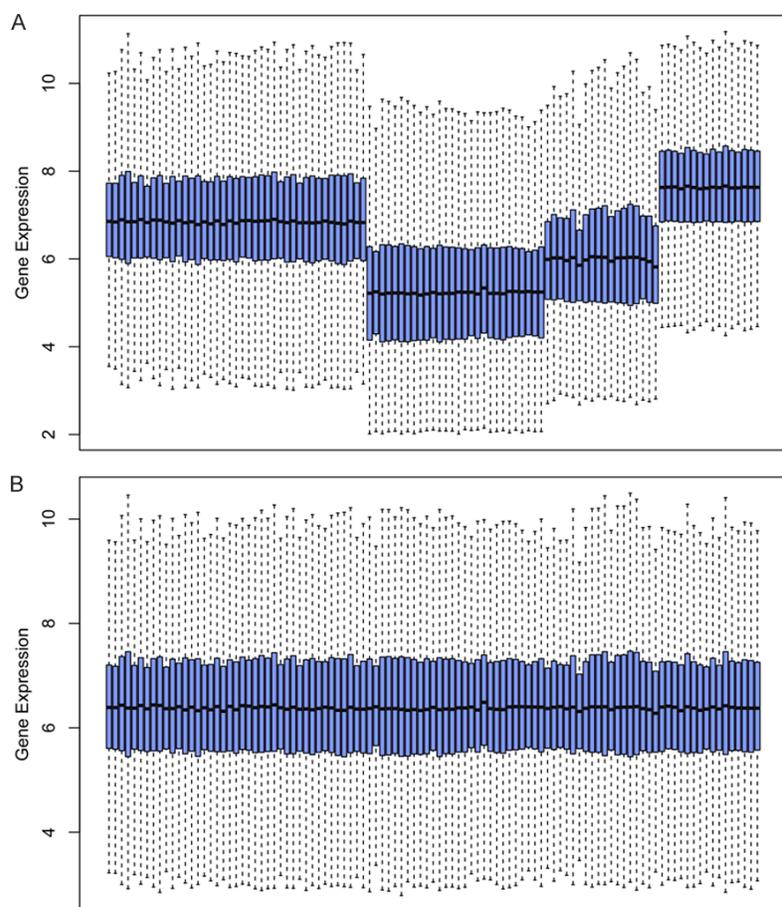


Figure 3. Integration of included Affymetrix gene chips. A. Box plot displays the overall expression profiles of four Affymetrix gene chips before normalization. B. The batch effect of the four gene chips was removed by using “SVA” package of R software.

(0: no staining; 1: light yellow or yellow; 2: brown; 3: dark brown). Two numbers were multiplied to generate the final score for each sample, which was shown as enumeration data. The minimum of the immunohistochemical score was 0, and the maximum was 12. We also catalyzed the samples into positive staining (≥ 6) and negative staining (< 6) based on the medium value of the score. All the assessments for immunohistochemical staining were carried out by three authors independently (Wei-jia Mo, Gang Chen and Dan-ming Wei).

All the statistical analysis for IHC was performed using SPSS 22.0. A student's t-test was performed to analyze the enumeration data. The frequencies and percentages were calculated for the classified variables, and a Chi-square test was used to assess the difference of PLK1 expression between two groups. All the *P*-values in the statistical analysis were two-

tailed, and $P < 0.05$ was considered to be statistically significant.

Protein expression level of PLK1 in NPC tissues based on previous reports: The literature was searched to collect data related to PLK1 protein expression levels in NPC. The procedure was followed as previously described [10]. The search strategies used were consistent with the aforementioned procedures. The protein expression level of PLK1 was divided into positive and negative groups based on detailed rules. Hence the positive and negative ratios were obtained or calculated.

Protein expression level of PLK1 in NPC tissues based on meta-analyses: Similar to the meta-analyses for the mRNA level mentioned above, a meta-analysis calculating odds ratios (OR) was performed to summarize the comprehensive protein level of PLK1 in NPC. The procedure was performed as previously described [15].

Potential relative genes and signaling pathways of PLK1 in NPC

Relative genes of PLK1 in NPC as assessed by using weighted correlation network analysis (WGCNA): To explore the potential molecular mechanisms of PLK1 in NPC, “WGCNA” package of R software was conducted to identify the PLK1-related genes [16]. Genes with correlation coefficient more than 0.4 and *P*-value < 0.05 were identified as the PLK1-related genes. Then, PLK1-related genes were submitted to “clusterprofiler” for gene functional enrichment analysis [17].

Relative signaling pathways of PLK1 in NPC: The PLK1-related genes in NPC underwent several bioinformatic evaluations, including the

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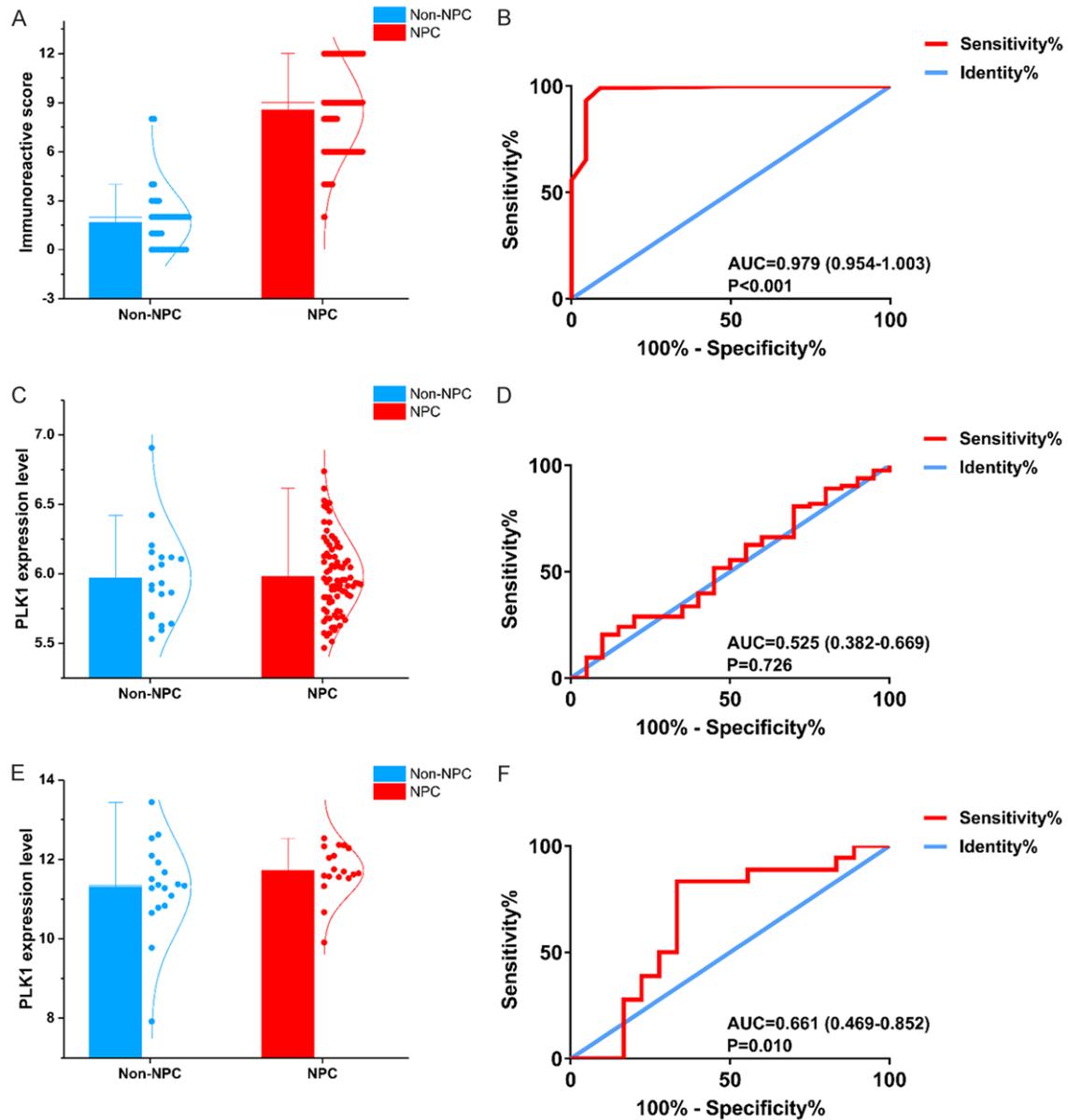


Figure 4. Up-regulation of PLK1 in nasopharyngeal carcinoma (NPC) and non-NPC tissues based on different data source. A. The expression of PLK1 in NPC tissues and adjacent non-NPC tissues based on immunohistochemistry (IHC). B. Receiver operating characteristic (ROC) curve analysis of PLK1 in NPC according to the results of NPC. C. The expression of PLK1 in NPC and adjacent non-NPC tissues from the merged Affymetrix chips from the Gene Expression Omnibus (GEO) database. D. ROC curve analysis of PLK1 in NPC from merged Affymetrix chips. E. The expression level of PLK1 in NPC and adjacent non-NPC tissues from the GSE53819 dataset from GEO database. F. ROC curve of PLK1 in NPC based on GSE53819 dataset.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The ggplot2 package of R software was used for the visualization. The protein-protein interactions (PPI) was also performed as previously reported [13, 18]. The relationship between the expression of PLK1 and several relative genes were analyzed using a Spearman's correlation.

Results

The flowchart of the present study

The present study mainly includes two parts: investigation of the expression profiles of PLK1 and the exploration of the potential molecular mechanism in NPC (**Figure 1**).

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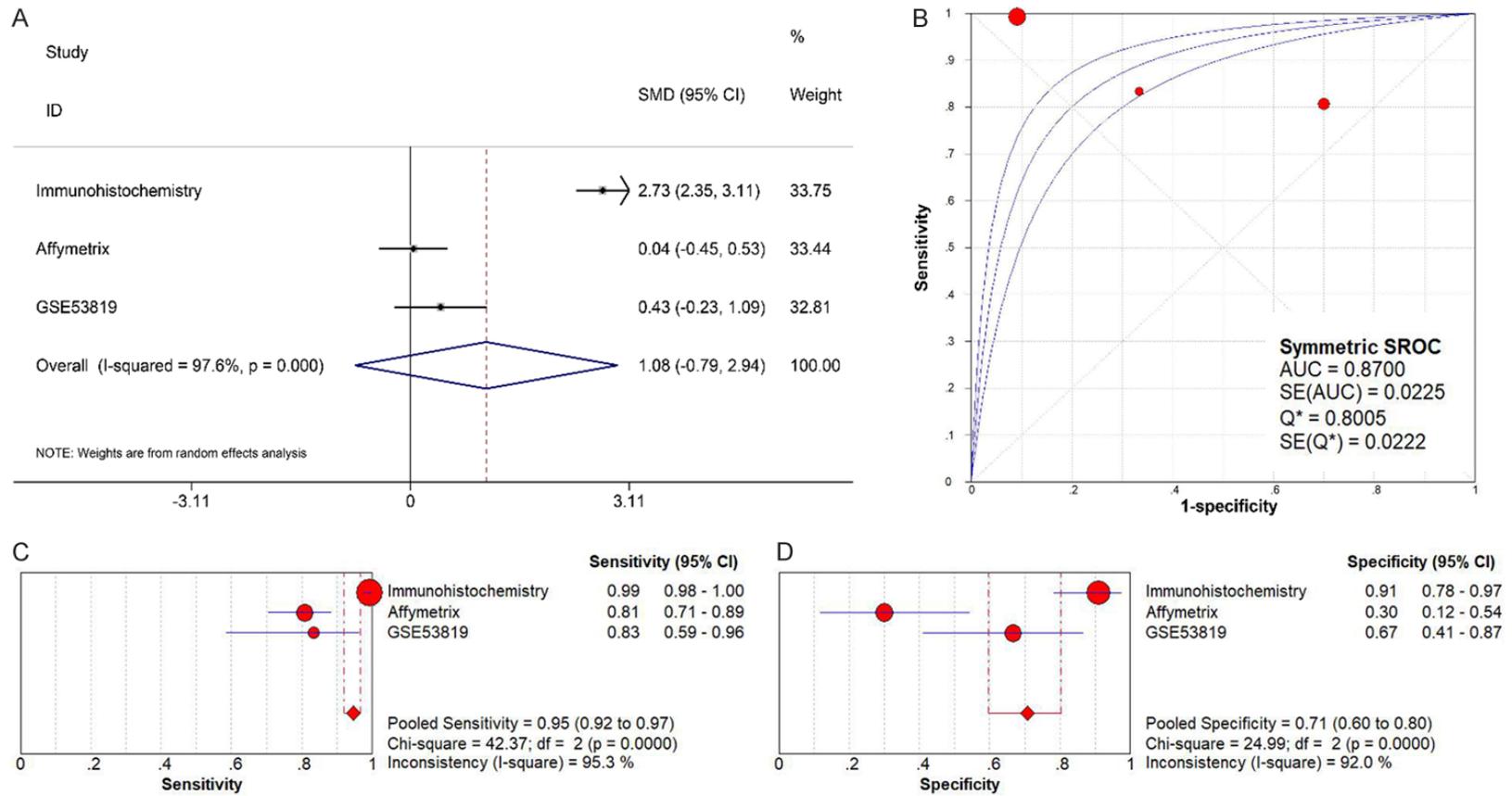


Figure 5. Validation of the upregulation of PLK1 in NPC tissues based on meta-analysis. (A) Forest plot of standard mean difference indicates that PLK1 has a tendency for overexpression in NPC tissues. (B) Area under curve of summary receiver operating characteristic curve was 0.870, which indicates that PLK1 could have ideal performance in distinguishing NPC from non-NPC tissues. Forest plots of pooled sensitivity (C) and specificity (D) are also used to show the pooled result.

Up-regulation of PLK1 in NPC

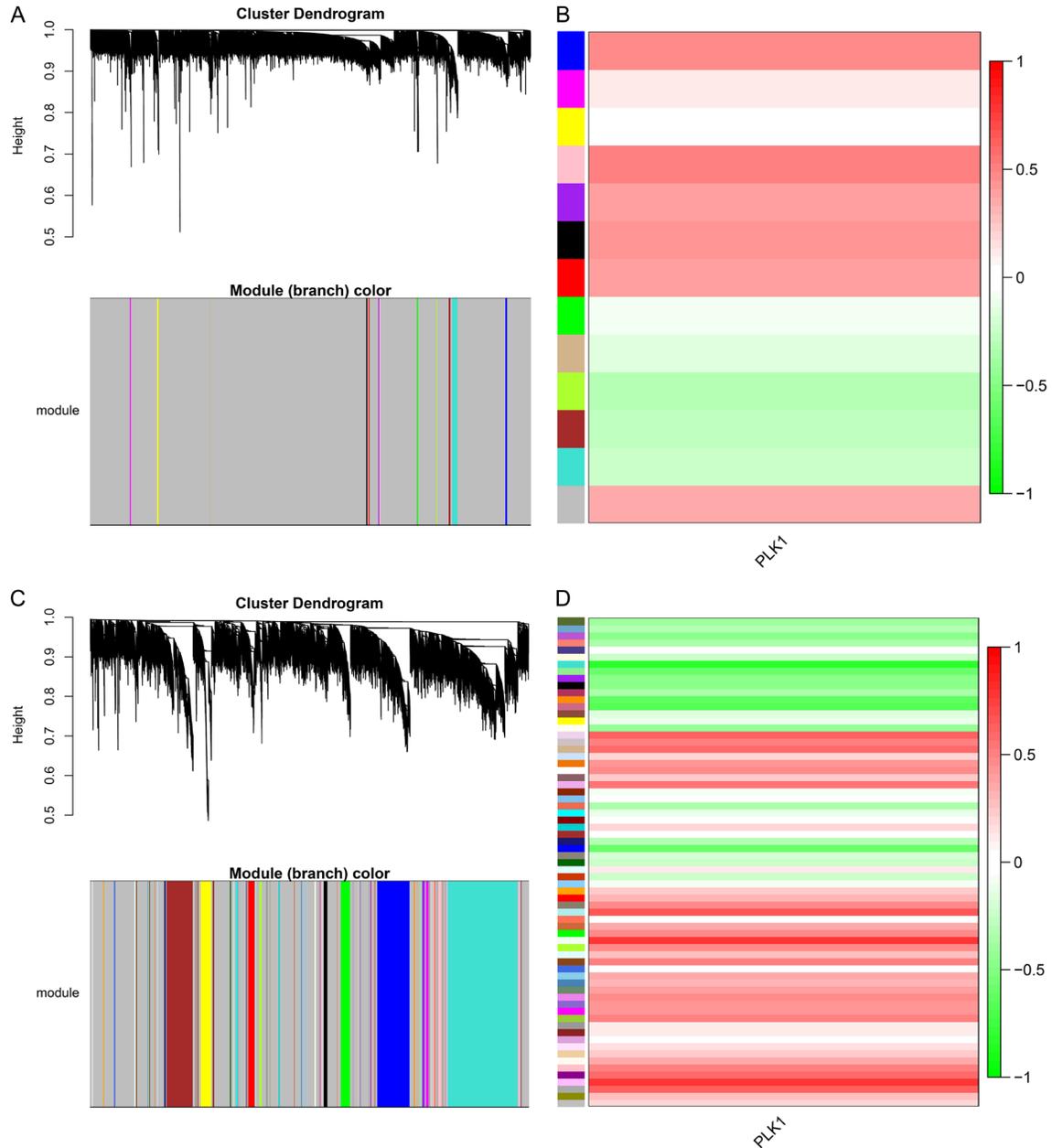


Figure 6. Weighted correlation network analysis (WGCNA). Genes were divided into several modules in merged Affymetrix chips (A) and GSE53819 database (B). The relationships between these modules and PLK1 were also explored through merged Affymetrix chips (C) and GSE53819 database (D), respectively.

PLK1 was upregulated in NPC tissues

We first examined the protein expression level of PLK1 in NPC with tissue microarray. The IHC results showed that PLK1 was markedly upregulated in NPC tissues compared with non-NPC tissues (Figure 2). When the ranked data were used to define the protein level of PLK1 in NPC, the apparently higher positive ratio could be observed. In NPC, 272 cases were PLK1 positive stained with a ratio of 93.2%; however, the

positive ratio was only 4.5% for the non-NPC controls ($P < 0.001$).

The mRNA expression level of PLK1 was validated when using public microarray datasets. Altogether, there were five microarray studies enrolled in the current study (Table 1), including four Affymetrix and one Agilent microarrays. The microarrays produced by Affymetrix were merged into one part. The batch effect was removed for further analysis (Figure 3). The dif-

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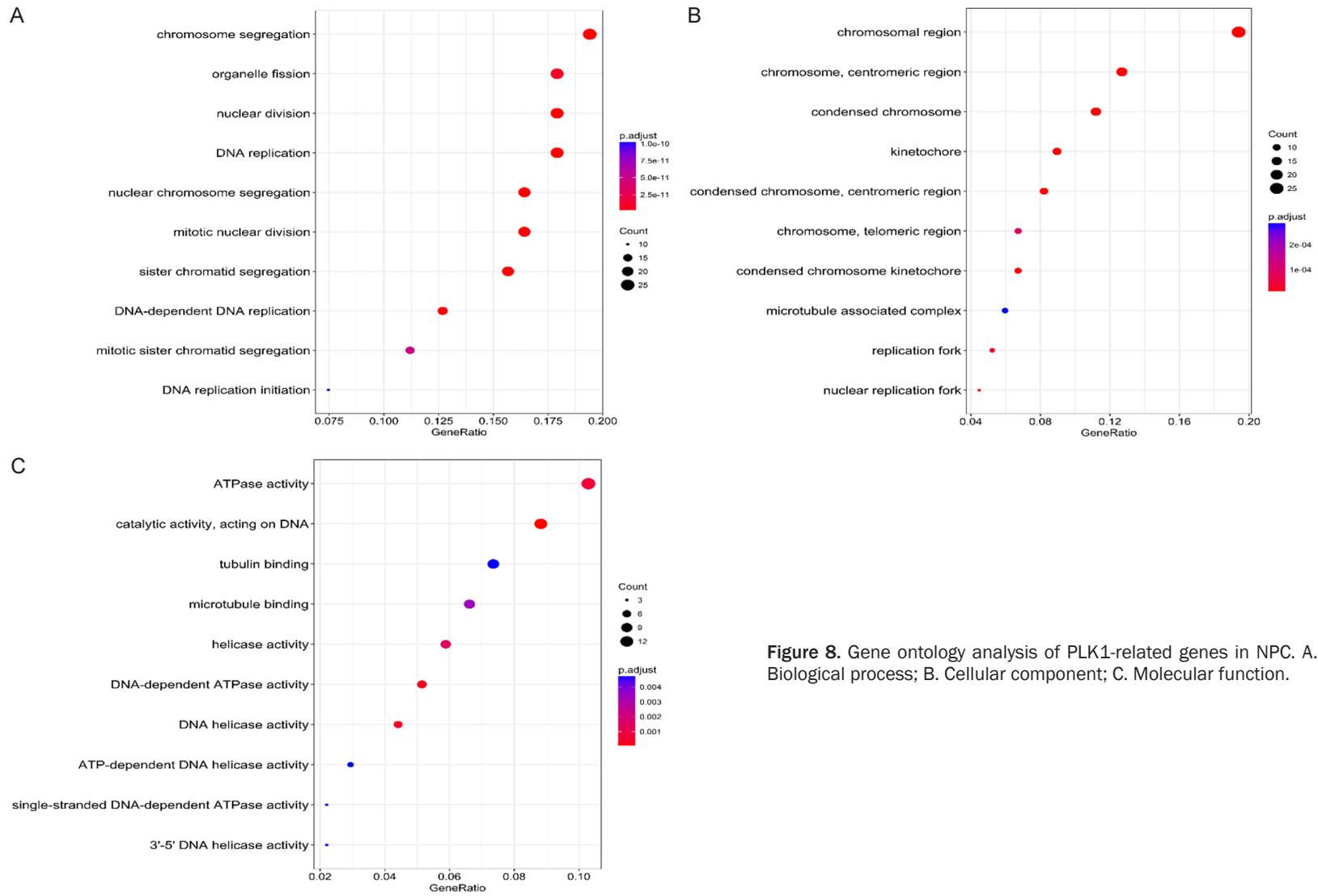


Figure 8. Gene ontology analysis of PLK1-related genes in NPC. A. Biological process; B. Cellular component; C. Molecular function.

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Table 2. Gene functional enrichment analysis of PLK1-related genes

Category	ID	Description	P adjust	Q value	Gene ID	Count
Biological process	GO:0007059	Chromosome segregation	2.00E-15	1.76E-15	CENPA, CCNE1, DSCC1, PSRC1, CENPI, ERCC6L, MKI67, BIRC5, UBE2C, AURKB etc.	26
Biological process	GO:0048285	Organelle fission	7.70E-12	6.78E-12	MYBL2, CCNE1, DSCC1, PSRC1, MKI67, BIRC5, UBE2C, CDC25C, AURKB, KIF2C etc.	24
Biological process	GO:0000280	Nuclear division	7.61E-13	6.71E-13	MYBL2, CCNE1, DSCC1, PSRC1, MKI67, BIRC5, UBE2C, CDC25C, AURKB, KIF2C etc.	24
Cellular component	GO:0098687	Chromosomal region	9.60E-17	7.52E-17	CENPA, ORC1, H2AFX, DSCC1, CENPI, ERCC6L, MKI67, BIRC5, RECQL4, AURKB etc.	26
Cellular component	GO:0000775	Chromosome, centromeric region	4.70E-12	3.68E-12	CENPA, DSCC1, CENPI, ERCC6L, MKI67, BIRC5, AURKB, KIF2C, CDT1, OIP5 etc.	17
Cellular component	GO:0000793	Condensed chromosome	3.83E-09	3.00E-09	CENPA, ERCC6L, MKI67, BIRC5, AURKB, KIF2C, CDT1, CENPN, BRCA1, HJURP etc.	15
Molecular function	GO:0016887	ATPase activity	0.000675067	0.000585853	DSCC1, RECQL4, KIF2C, KIFC1, MCM4, KIF22, MCM7, DDX28, RAD51, KIF15 etc.	14
Molecular function	GO:0140097	Catalytic activity, acting on DNA	9.79E-06	8.50E-06	GIN52, RECQL4, POLD1, POLD2, EXO1, POLE2, MCM4, MCM7, RAD51, RUVBL2 etc.	12
Molecular function	GO:0015631	Tubulin binding	0.004665057	0.00404854	PSRC1, BIRC5, KIF2C, BRCA1, KIFC1, PPP5C, KIF22, SPAG5, KIF15, KIF18B	10
KEGG pathway	hsa04110	Cell cycle	2.08E-10	1.81E-10	ORC1, CCNE1, E2F1, CDC25C, CCNB2, PKMYT1, CDC25A, MCM4, ORC6, CCNA2 etc.	14
KEGG pathway	hsa04218	Cellular senescence	0.001407125	0.001223587	MYBL2, CCNE1, E2F1, CCNB2, CDC25A, FOXM1, CCNA2, CDK1	8
KEGG pathway	hsa04114	Oocyte meiosis	0.001592885	0.001385117	CCNE1, CDC25C, CCNB2, PKMYT1, CDC20, PPP2R5D, CDK1	7
Disease ontology	DOID:2174	Ocular cancer	0.042790542	0.038146891	H2AFX, CCNE1, E2F1, MKI67, BIRC5, CCNA2, CDK1	7
Disease ontology	DOID:0060116	Sensory system cancer	0.042790542	0.038146891	H2AFX, CCNE1, E2F1, MKI67, BIRC5, CCNA2, CDK1	7
Disease ontology	DOID:4645	Retinal cancer	0.045348899	0.040427614	H2AFX, CCNE1, E2F1, BIRC5, CCNA2, CDK1	6

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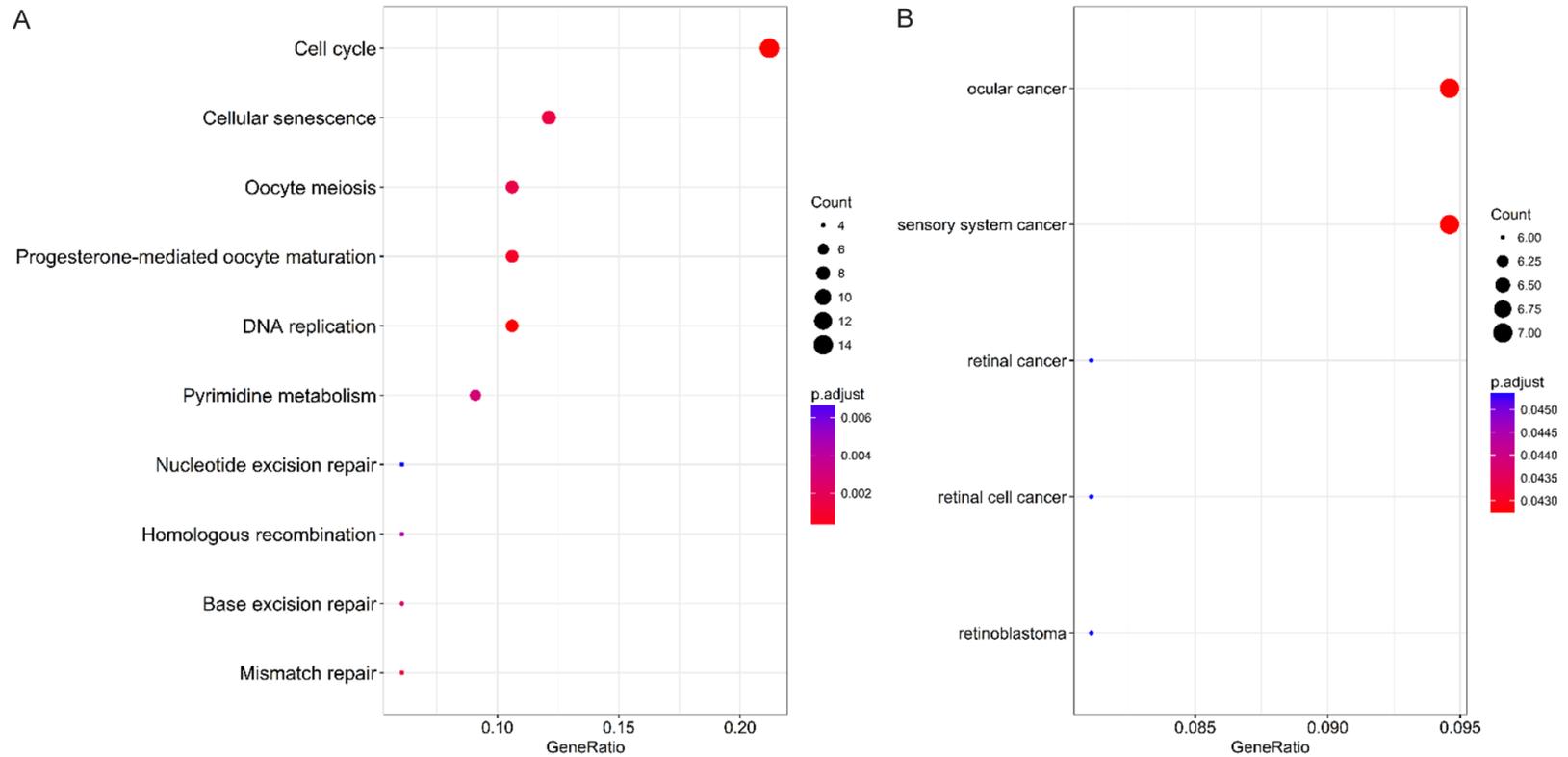


Figure 9. Kyoto Encyclopedia of Genes and Genomes (KEGG) and disease ontology (DO) analysis of PLK1-related genes. A. KEGG analysis revealed that “cell cycle” was the most significant pathway; B. Several relevant DO terms of PLK1-related genes.

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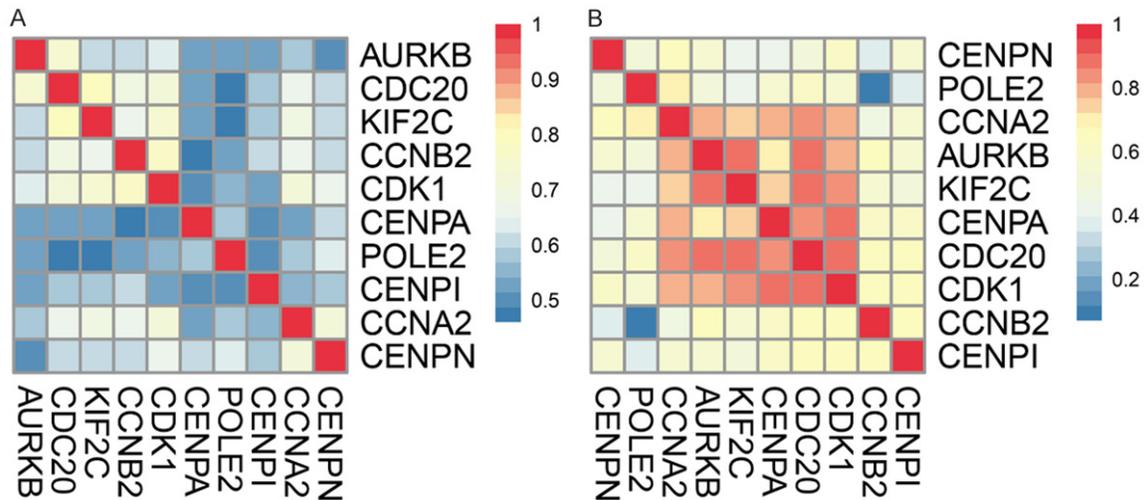


Figure 10. Correlations of the top 10 hub PLK1-related genes. (A) Relationships in merged Affymetrix chips dataset and (B) GSE53819 dataset.

including tissue microarray, IHC, gene chip, and meta-analyses. Interestingly, both PLK1 mRNA and protein levels were observed to be apparently up-regulated in NPC tissues. The silico approaches indicated that PLK1 may exert its function in a cell cycle by influencing certain key genes. Since PLK1 inhibitors have great potential to be clinically applied for cancer treatment, PLK1 could be a promising target for precision treatment of NPC too.

PLK1 has been well documented to be over-expressed in different malignant tumor cells, which have generally high mitotic ratios [19]. The oncogenic role of PLK1 has been clarified in multiple types of cancers, including non-small lung cancer [20, 21], pancreatic cancer [22, 23], esophageal squamous cell carcinoma [24], hepatocellular carcinoma [25], bladder cancer [26], gastric cancer [14, 27], and etc. However, so far only one group has studied the expression level of PLK1 in NPC tissue. In that study, forty cases of NPC were collected and stained with PLK1 by IHC. The positive ratio of PLK1 protein was 70% [9]. But no non-NPC nasopharyngeal tissues were included in that study as controls. In our current study, the PLK1 levels from both tissue microarray and gene chips were observed to clearly have an up-regulating trend as compared to the non-cancerous controls. This has also been supported by our subsequent meta-analysis, where the AUC of sROC reached 0.87 based on 393 cases of NPC. This finding suggests that PLK1 may also play a vital role in the tumorigenesis and development of

NPC as a potential oncogene. However, our conclusions still need to be validated by research using larger cohorts.

Most recently, the subversive tumor-suppressive role of PLK1 has been reported in a subtype of breast cancer, even though PLK1 has been verified to be up-regulated in most of the breast cancers [28-30]. PLK1 up-regulation was found to lead to aberrant chromosome segregation and cytokinesis, which in turn generates polyploid cells with suppressed proliferative potential. In animal models, the up-regulation of PLK1 stops the progress of a subtype of breast cancer induced by KRAS and HER2 with an augmented rate of chromosome instability. Surprisingly, PLK1 up-regulation was also related to a more favorable outcome in this special subtype of breast cancer [28]. Similarly, PLK1 has also been reported to have tumor-suppressive potential in APC-truncated colon cancer cells [31]. Hence, despite the acknowledged therapeutic benefits of suppressing PLK1 due to its role in cancer cell cycles, PLK1 up-regulation may also have tumor-suppressive capacities through other mechanisms. As for the function of PLK1 in NPC, PLK1 knock-down was found to decrease Cdc25c levels in NPC cell lines in vitro and in vivo, which led to the inhibition of cell growth in C666-1 cells, as well as G2/M arrest, increased DNA double-strand breaks, apoptosis, and caspase activation. However, the effect of PLK1 in different subtypes of NPC still needs to be tested.

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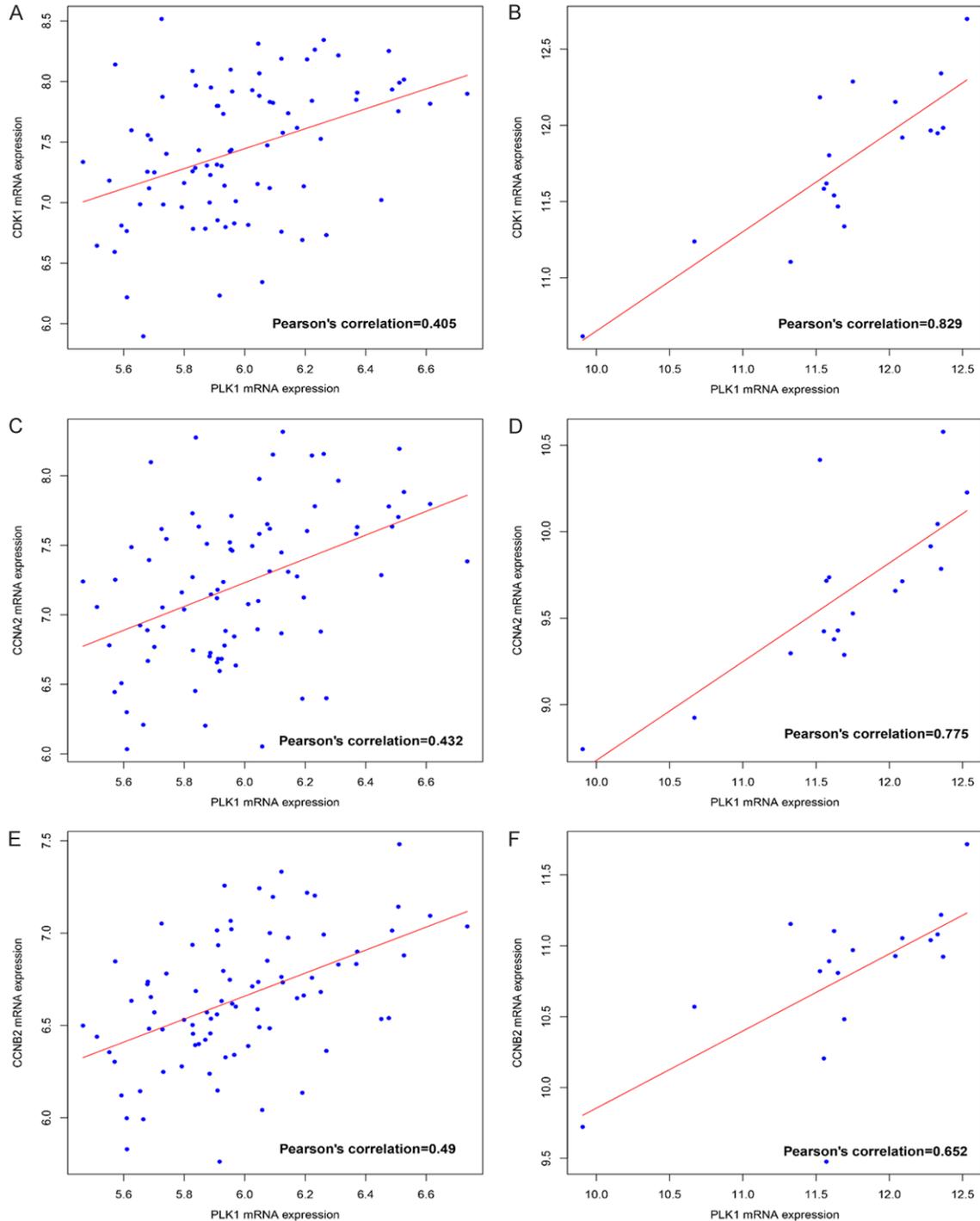


Figure 11. Correlation analyses were performed between PLK1 and the top 3 hub genes. (A) Relationship between PLK1 and CDK1 in merged Affymetrix data and (B) GSE53819 dataset. (C) Relationship between PLK1 and CCNA2 in merged Affymetrix data and (D) GSE53819 dataset. (E) Relationship between PLK1 and CCNB2 in merged Affymetrix data and (F) GSE53819 dataset.

Considering the possible molecular mechanisms of PLK1 in NPC cells, we performed WGCNA based on different microarray data to collect the co-expressed genes and relative sig-

naling pathways of PLK1 in NPC. Not surprisingly, the co-expressed genes were enriched in pathways closely related to cell cycles. Especially the well-known cell cycle genes CDK1,

CCNA2 and CCNB2, are all positively related to the PLK1. CDK1 and PLK1 have been shown to support mitotic nuclear pore complexes disintegration by hyperphosphorylation of Nup98 together [32, 33]. However, the exact molecular mechanism of PLK1 in the modulation of NPC cell cycle still requires further investigation.

The potential to target PLK1 as a tool to treat cancers has been noted. Different ways to inhibit PLK1 have been tested, including using antibodies, RNA interference, or small molecule inhibitors [19, 34, 35]. For instance, BI2536, a potent and selective inhibitor of PLK1, has been verified to gain a synergistic effect on stomach cancer cells when combined with cisplatin [36]. The combination of the PI3K/Akt pathway suppression and PLK1 inhibition was also able to improve the chemosensitivity of pancreatic cancer cells to gemcitabine treatment [37]. Besides, PLK1 inhibitor BI-6727 could cause an increase in the radiosensitivity of NSCLC cells [38]. The similar role of PLK1 in the radiosensitivity of esophageal squamous cell carcinoma cells has also been reported [39]. While radiation therapy is currently the primary standard of treatment for NPC, regrettably the rates of failure range from 7% up to 58% [40, 41]. Targeting PLK1 may have the potential to enhance the efficiency of radiation therapy in NPC. However, the effect of PLK1 inhibition may have in assisting radiosensitivity needs to be tested in the future.

Based on our findings, more research must be conducted to address the shortcomings of our study. First, a larger cohort is needed to confirm the clinical role and prognostic value of PLK1 in NPC patients. Second, the biological effects of PLK1 inhibition and over-expression need to be verified in more NPC cell lines that cover different subtypes of NPC. Third, the therapeutic potential of PLK1 inhibition, alone or with other treating methods, must be carried out.

In conclusion, both PLK1 mRNA and protein levels were observed to be evidently up-regulated in NPC tissues, which suggest its potential oncogenic role in the tumorigenesis and development of NPC. Since several PLK1 inhibitors have been developed, PLK1 inhibitors may have important therapeutic potential in clinic application for NPC patients.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

- [1] You B, Shan Y, Bao L, Chen J, Yang L, Zhang Q, Zhang W, Zhang Z, Zhang J, Shi S and You Y. The biology and function of extracellular vesicles in nasopharyngeal carcinoma (Review). *Int J Oncol* 2018; 52: 38-46.
- [2] Sun J, Huang Z, Hu Z and Sun R. Benefits of local tumor excision and pharyngectomy on the survival of nasopharyngeal carcinoma patients: a retrospective observational study based on SEER database. *J Transl Med* 2017; 15: 116.
- [3] Guo H, Huang S, Li S, Yu H, Wu S and Zhou X. Prognostic significance of the long noncoding RNAs in nasopharyngeal carcinoma: a systematic review and meta-analysis. *Cancer Manag Res* 2018; 10: 1763-1779.
- [4] Wang J, Deng L, Huang J, Cai R, Zhu X, Liu F, Wang Q, Zhang J and Zheng Y. High expression of Fibronectin 1 suppresses apoptosis through the NF-kappaB pathway and is associated with migration in nasopharyngeal carcinoma. *Am J Transl Res* 2017; 9: 4502-4511.
- [5] Cheng N and Guo Y. Long noncoding RNA NEAT1 promotes nasopharyngeal carcinoma progression through regulation of miR-124/NF-kappaB pathway. *Onco Targets Ther* 2017; 10: 5843-5853.

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- [6] Ma J, Xuan SH, Li Y, Zhang ZP and Li XH. Role of the TGFbeta/PDCD4/AP-1 signaling pathway in nasopharyngeal carcinoma and its relationship to prognosis. *Cell Physiol Biochem* 2017; 43: 1392-1401.
- [7] Li X, Liu F, Lin B, Luo H, Liu M, Wu J, Li C, Li R, Zhang X, Zhou K and Ren D. miR150 inhibits proliferation and tumorigenicity via retarding G1/S phase transition in nasopharyngeal carcinoma. *Int J Oncol* 2017; [Epub ahead of print].
- [8] Shakil S, Baig MH, Tabrez S, Rizvi SM, Zaidi SK, Ashraf GM, Ansari SA, Khan AA, Al-Qahtani MH, Abuzenadah AM and Chaudhary AG. Molecular and enoinformatics perspectives of targeting Polo-like kinase 1 in cancer therapy. *Semin Cancer Biol* 2017; [Epub ahead of print].
- [9] Shi W, Alajez NM, Bastianutto C, Hui AB, Mocanu JD, Ito E, Busson P, Lo KW, Ng R, Waldron J, O'Sullivan B and Liu FF. Significance of Plk1 regulation by miR-100 in human nasopharyngeal cancer. *Int J Cancer* 2010; 126: 2036-2048.
- [10] Gao L, Zhong JC, Huang WT, Dang YW, Kang M and Chen G. Integrative analysis of BSG expression in NPC through immunohistochemistry and public high-throughput gene expression data. *Am J Transl Res* 2017; 9: 4574-4592.
- [11] Zhang Y, Huang JC, Cai KT, Yu XB, Chen YR, Pan WY, He ZL, Lv J, Feng ZB and Chen G. Long noncoding RNA HOTTIP promotes hepatocellular carcinoma tumorigenesis and development: a comprehensive investigation based on bioinformatics, qRTPCR and metaanalysis of 393 cases. *Int J Oncol* 2017; 51: 1705-1721.
- [12] Yang X, Pang YY, He RQ, Lin P, Cen JM, Yang H, Ma J and Chen G. Diagnostic value of strand-specific miRNA-101-3p and miRNA-101-5p for hepatocellular carcinoma and a bioinformatic analysis of their possible mechanism of action. *FEBS Open Bio* 2018; 8: 64-84.
- [13] Gao L, Li SH, Tian YX, Zhu QQ, Chen G, Pang YY and Hu XH. Role of downregulated miR-133a-3p expression in bladder cancer: a bioinformatics study. *Onco Targets Ther* 2017; 10: 3667-3683.
- [14] Lin P, Xiong DD, Dang YW, Yang H, He Y, Wen DY, Qin XG and Chen G. The anticipating value of PLK1 for diagnosis, progress and prognosis and its prospective mechanism in gastric cancer: a comprehensive investigation based on high-throughput data and immunohistochemical validation. *Oncotarget* 2017; 8: 92497-92521.
- [15] He R, Gao L, Ma J, Peng Z, Zhou S, Yang L, Feng Z, Dang Y and Chen G. The essential role of MTDH in the progression of HCC: a study with immunohistochemistry, TCGA, meta-analysis and in vitro investigation. *Am J Transl Res* 2017; 9: 1561-1579.
- [16] Langfelder P and Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 2008; 9: 559.
- [17] Yu G, Wang LG, Han Y and He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 2012; 16: 284-287.
- [18] Liang L, Zeng JH, Wang JY, He RQ, Ma J, Chen G, Cai XY and Hu XH. Down-regulation of miR-26a-5p in hepatocellular carcinoma: a qRT-PCR and bioinformatics study. *Pathol Res Pract* 2017; 213: 1494-1509.
- [19] Fu Z and Wen D. The emerging role of polo-like kinase 1 in epithelial-mesenchymal transition and tumor metastasis. *Cancers (Basel)* 2017; 9.
- [20] Tang Q, Zhang H, Kong M, Mao X and Cao X. Hub genes and key pathways of non-small lung cancer identified using bioinformatics. *Oncol Lett* 2018; 16: 2344-2354.
- [21] Van den Bossche J, Deben C, Op de Beeck K, Deschoolmeester V, Hermans C, De Pauw I, Jacobs J, Van Schil P, Vermorken JB, Pauwels P, Peeters M, Lardon F and Wouters A. Towards prognostic profiling of non-small cell lung cancer: new perspectives on the relevance of polo-like kinase 1 expression, the TP53 mutation status and hypoxia. *J Cancer* 2017; 8: 1441-1452.
- [22] Chen B, Zhu A, Tian L, Xin Y, Liu X, Peng Y, Zhang J, Miao Y and Wei J. miR23a suppresses pancreatic cancer cell progression by inhibiting PLK1 expression. *Mol Med Rep* 2018; 18: 105-112.
- [23] Huang WJ, Wang Y, Liu S, Yang J, Guo SX, Wang L, Wang H and Fan YF. Silencing circular RNA hsa_circ_0000977 suppresses pancreatic ductal adenocarcinoma progression by stimulating miR-874-3p and inhibiting PLK1 expression. *Cancer Lett* 2018; 422: 70-80.
- [24] Liu TT, Yang KX, Yu J, Cao YY, Ren JS, Hao JJ, Pan BQ, Ma S, Yang LY, Cai Y, Wang MR and Zhang Y. Co-targeting PLK1 and mTOR induces synergistic inhibitory effects against esophageal squamous cell carcinoma. *J Mol Med (Berl)* 2018; 96: 807-817.
- [25] Lin P, Wen DY, Dang YW, He Y, Yang H and Chen G. Comprehensive and integrative analysis reveals the diagnostic, clinicopathological and prognostic significance of polo-like kinase 1 in hepatocellular carcinoma. *Cell Physiol Biochem* 2018; 47: 925-947.
- [26] Zhang Z, Zhang G, Gao Z, Li S, Li Z, Bi J, Liu X, Li Z and Kong C. Comprehensive analysis of differentially expressed genes associated with PLK1 in bladder cancer. *BMC Cancer* 2017; 17: 861.
- [27] Lian G, Li L, Ye F, Wang D, Liu J, Shi Y, Jing C, Suo J, Zhang DY and Chen M. The screening and analysis of protein signatures and signal-

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- ing associated with chemoresistance based on protein pathway array technology in gastric cancer. *Oncol Rep* 2018; 39: 307-315.
- [28] de Carcer G, Venkateswaran SV, Salgueiro L, El Bakkali A, Somogyi K, Rowald K, Montanes P, Sanclemente M, Escobar B, de Martino A, McGranahan N, Malumbres M and Sotillo R. Plk1 overexpression induces chromosomal instability and suppresses tumor development. *Nat Commun* 2018; 9: 3012.
- [29] Jeong SB, Im JH, Yoon JH, Bui QT, Lim SC, Song JM, Shim Y, Yun J, Hong J and Kang KW. Essential role of polo-like kinase 1 (Plk1) oncogene in tumor growth and metastasis of tamoxifen-resistant breast cancer. *Mol Cancer Ther* 2018; 17: 825-837.
- [30] Saatci O, Borgoni S, Akbulut O, Durmus S, Raza U, Eyupoglu E, Alkan C, Akyol A, Kutuk O, Wiemann S and Sahin O. Targeting PLK1 overcomes T-DM1 resistance via CDK1-dependent phosphorylation and inactivation of Bcl-2/xL in HER2-positive breast cancer. *Oncogene* 2018; 37: 2251-2269.
- [31] Raab M, Sanhaji M, Matthess Y, Horlin A, Lorenz I, Dotsch C, Habbe N, Waidmann O, Kurunci-Csacsco E, Firestein R, Becker S and Strebhardt K. PLK1 has tumor-suppressive potential in APC-truncated colon cancer cells. *Nat Commun* 2018; 9: 1106.
- [32] Linder MI, Kohler M, Boersema P, Weberruss M, Wandke C, Marino J, Ashiono C, Picotti P, Antonin W and Kutay U. Mitotic disassembly of nuclear pore complexes involves CDK1- and PLK1-mediated phosphorylation of key interconnecting nucleoporins. *Dev Cell* 2017; 43: 141-156, e147.
- [33] de Castro IJ, Gil RS, Ligamari L, Di Giacinto ML and Vagnarelli P. CDK1 and PLK1 coordinate the disassembly and reassembly of the nuclear envelope in vertebrate mitosis. *Oncotarget* 2018; 9: 7763-7773.
- [34] Hattori Y, Kikuchi T, Ozaki KI and Onishi H. Evaluation of in vitro and in vivo therapeutic antitumor efficacy of transduction of polo-like kinase 1 and heat shock transcription factor 1 small interfering RNA. *Exp Ther Med* 2017; 14: 4300-4306.
- [35] Noor A, Umelo IA, Kronenberger P, Giron P, De Vlieghe E, De Wever O, Teugels E and De Greve J. Targeting Polo-like kinase 1 and TRAIL enhances apoptosis in non-small cell lung cancer. *Oncotarget* 2018; 9: 28731-28744.
- [36] Lian G, Li L, Shi Y, Jing C, Liu J, Guo X, Zhang Q, Dai T, Ye F, Wang Y and Chen M. BI2536, a potent and selective inhibitor of polo-like kinase 1, in combination with cisplatin exerts synergistic effects on gastric cancer cells. *Int J Oncol* 2018; 52: 804-814.
- [37] Mao Y, Xi L, Li Q, Wang S, Cai Z, Zhang X and Yu C. Combination of PI3K/Akt pathway inhibition and Plk1 depletion can enhance chemosensitivity to gemcitabine in pancreatic carcinoma. *Transl Oncol* 2018; 11: 852-863.
- [38] Yao D, Gu P, Wang Y, Luo W, Chi H, Ge J and Qian Y. Inhibiting polo-like kinase 1 enhances radiosensitization via modulating DNA repair proteins in non-small-cell lung cancer. *Biochem Cell Biol* 2018; 96: 317-325.
- [39] Gu MM, Li M, Gao D, Liu LH, Lang Y, Yang SM, Ou H, Huang B, Zhou PK and Shang ZF. The vanillin derivative 6-bromine-5-hydroxy-4-methoxybenzaldehyde induces aberrant mitotic progression and enhances radio-sensitivity accompanying suppression the expression of PLK1 in esophageal squamous cell carcinoma. *Toxicol Appl Pharmacol* 2018; 348: 76-84.
- [40] Shuang H, Feng J, Caineng C, Qifeng J, Tin J, Yuanyuan C and Xiaozhong C. The value of radical radiotherapy in the primary tumor of newly diagnosed oligo-metastatic nasopharyngeal carcinoma patients. *Clin Transl Oncol* 2018; [Epub ahead of print].
- [41] Yan H, Cao X and Wang J. Application of intensity-modulated radiation therapy in the treatment of nasopharyngeal carcinoma. *Oncol Lett* 2017; 14: 7773-7776.