

## Aurora kinase A induces *miR-17-92* cluster through regulation of E2F1 transcription factor

Shun He · Shangbin Yang · Guohua Deng · Mei Liu · Hongxia Zhu · Wei Zhang · Shuang Yan · Lanping Quan · Jinfeng Bai · Ningzhi Xu

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**Abstract** Aurora kinase A (AURKA) is an essential mitotic serine/threonine kinase and its abnormal expression is observed in many malignancies, yet the exact role for AURKA in tumorigenesis still remains elusive. Here, through a transcription factor array, we show that the transcription activity of E2F1 was increased by AURKA overexpression. Meanwhile, the E2F1 protein level was found to be upregulated and a correlation between AURKA and E2F1 expression was observed in cancer specimens. Further analysis revealed that AURKA increased E2F1 protein stability by inhibiting proteasome-dependent degradation of this protein. Additionally, a microRNA cluster, *miR-17-92*, was found to be upregulated upon AURKA overexpression, and this stimulation was largely repressed by E2F1 knockdown. Chromatin immunoprecipitation further demonstrated that AURKA enhanced E2F1 occupancy to the promoter of the *miR-17-92* cluster. These data reveal a novel link between AURKA and microRNAs via the regulation of E2F1, providing new clues for understanding the role of AURKA in tumorigenesis.

**Keywords** AURKA · *miR-17-92* · E2F1 · Transcription factor · Tumorigenesis

### Introduction

*AURKA* encodes an important member of the Aurora kinase family, an evolutionally conserved serine/threonine kinase that plays a crucial role in centrosome cycling during mitosis. Located on 20q13, a region frequently found to be amplified, *AURKA* amplification and overexpression have been observed in various types of tumor, including breast, colon, ovarian, bladder, hepatocellular, and pancreatic cancers [1–6]. The oncogenic activity of *AURKA* was first demonstrated by the ectopic expression of *AURKA* in rodent fibroblast cells, which led to full transformation of these cells [6]. Further investigations revealed that *AURKA* is involved in the regulation of a series of signaling molecules that have key roles in modulation of cell proliferation and apoptosis, such as p53 [7], BRCA1 [8], c-Myc [9], AKT [10], as well as NF $\kappa$ B pathways [11], indicating a complex network by which *AURKA* promotes cell transformation and tumorigenesis. However, despite the piles of literature, the exact mechanism by which *AURKA* contribute to tumor remains to be elucidated.

To further explore downstream events taking place after *AURKA* overexpression, we take advantage of a transcription factor array capable of monitoring the transcription activities of general transcription factors in the cells, and by this method, we identified a key transcription factor, E2F1, whose transactivity is upregulated in response to *AURKA* overexpression. We then further demonstrated that *AURKA* induces expression of a microRNA (miRNA) cluster, the *miR-17-92* cluster, through the regulation of the E2F1 transcription factor.

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S. He · S. Yang · M. Liu · H. Zhu · W. Zhang · S. Yan · L. Quan · J. Bai · N. Xu (✉)

Laboratory of Cell and Molecular Biology and State Key Laboratory of Molecular Oncology, Cancer Institute and Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 17 Panjiayuan Nanli, Chaoyang District, PO Box 2258, Beijing 100021, People's Republic of China  
e-mail: xningzhi@public.bta.net.cn

G. Deng

Department of Pathology, Cancer Institute and Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

## Materials and methods

### Tissue specimens

All specimens were collected from surgically excised tissues of breast cancer patients treated at the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China. The protocol was approved by the Institutional Review Board of the hospital.

### Plasmids, cell culture, siRNA, and transfection

The *AURKA* expression plasmid was constructed by cloning *AURKA* cDNA into the pcDNA4<sup>TM</sup>/TO/myc-His-B vector (Invitrogen) using the following primers: forward: 5'CGGG ATCCATGGACCGATTAAAGAAAAC3', reverse: 5'GCC TCGAGTAAGACTGTTTGCTAGC3'. *AURKA* kinase-dead (KD) mutant was created through K162M site-directed mutagenesis as described previously [12]. The 293TRES cell line was kindly provided by Professor Chen Quan and was cultured in DMEM, and the breast cancer cell lines, SK-BR-3 and MCF-7, as well as the esophageal cancer cell line EC9706, were cultured in RPMI 1640, supplemented with 10% fetal bovine serum, respectively. *AURKA* siRNA sequence: sense 5'AUGCCCUGUCUUACUGUC ATT3', antisense 5'UGACAGUAAGACAGGGCAUTT3'; *E2F1* siRNA sequence: sense 5'GCAUCCAGCUCAU GCCAATT3', antisense 5'UUGGCAAUGAGCUGGAUGC TT3'; the *luciferase gene* (GL2) siRNA sequence: sense 5'CGUACGCGAAUACUUCGATT3', antisense 5'UCGA AGUAUCCGCGUACGTT3'. All cell transfections were performed with Lipofectamine2000 (Invitrogen) according to the manufacturer's instruction. For stable cell-line establishment, cells were selected with Zeocin 24 h after transfection at a concentration of 100 µg/ml for 2 weeks.

### Transcription factor array

Cells were grown to 70–80% confluence and induced with doxycycline for 24 h in medium with 0.5% serum. Nuclear extract was isolated using the Nuclear Extraction Kit (Panomics), and the array hybridization was performed using TranSignal<sup>TM</sup> protein/DNA arrays (Panomics) according to the manufacturer's instructions as described previously [13].

### Electrophoresis mobility shift assay (EMSA)

EMSA was performed using the DIG Gel Shift Kit (Roche Applied Science) according to the manufacturer's protocol. Briefly, nuclear extracts were prepared from 293TRES-*AURKA* and 293TRES-Vector cells treated with or without doxycycline for 24 h. Two micrograms of nuclear

extracts was incubated with the previously reported digoxigenin (DIG)-labeled E2F-binding oligonucleotide 5'ATTTAAGTTTCGCGCCCTTCTCAA3' [14]. The DNA/protein complex was then separated on a nondenaturing 6% polyacrylamide gel and then blotted by electro-blotting to a positively charged nylon membrane. The DIG-labeled DNA fragments were visualized by an enzyme immunoassay using anti-DIG-AP and the chemiluminescent substrates.

### Reporter assays

Cells were cotransfected in 24-well plates with pGL3-6 × E2F-luciferase and pRL-SV40 as well as the indicated plasmids. Firefly and Renilla luciferase activities were measured consecutively by using the Dual Luciferase Assay (Promega) 24 h after transfection.

### RNA extraction and real-time PCR

Total RNA was extracted with TRIZOL (Invitrogen) and reverse-transcribed to cDNA using MMLV Reverse Transcriptase (Promega). The quantitative real-time PCR of each sample was performed in triplicate on an ABI Prism 7000 (Applied Biosystems, CA) with SYBR Green PCR core reagents (Applied Biosystems, CA) according to the manufacturer's protocol.  $\beta$ -actin was used as a reference gene. The relative transcript levels in the treated sample compared to the control sample were determined as fold changes. Primers: *miR-17-92* cluster: Forward: 5'CTG TCGCCCAATCAAACCTG3', Reverse: 5'GTCACAATCC CCACCAAAC3';  $\beta$ -actin: Forward: 5'GGCGGCACCACC ATGTACCCT3', Reverse: 5'AGGGGCCGGACTCGTCA TACT3'.

### Chromatin immunoprecipitation (ChIP)

ChIP assay was conducted with ChIP-IT<sup>TM</sup> Chromatin Immunoprecipitation Kits & Shearing Kits (Active Motif) according to the manufacturer's protocol. The antibodies for immunoprecipitation include an anti-E2F1 (sc-193x, Santa Cruz) antibody or mouse IgG (Active Motif). Primers specific for putative E2F-binding sites upstream of the *miR-17-92* cluster were reported previously [15]: P1 Forward: 5'GAAGAAGGAGGTGCTCCTGA3'; Reverse: 5'GCCAGCTGCATTTAGTAAGA3'; P2 Forward: 5'ATTC ACCACATGGTCTCTTC3'; Reverse: 5'GCCTGCGCTTT ACTACGAC3'.

### Immunoblotting and immunohistochemistry

Standard procedures were used for protein preparation and immunoblotting. The antibodies used are listed as below:

anti-AURKA (IAK1, BD Biosciences), anti-ACTIN (Sigma-Aldrich), anti-laminB (Santa Cruz, sc-6217), anti-TFIID (Santa Cruz, sc-204). Immunohistochemistry was done as described previously. Specimens were incubated with anti-AURKA (Abcam, Ab1287; 1:700) and anti-E2F1 (Santa Cruz, sc-251; 1:150) at 4°C overnight following the antigen retrieval in pH9.0 EDTA buffer in an autoclave for 2 min. All immunostaining experiments were assessed by an experienced pathologist. The protein expression within the cancer tissue was evaluated and categorized according to the percentage and intensity of positive cells (–, +, ++, and +++). Tumors were then further grouped into weak (– and +) and strong (++ and +++) expression of each protein.

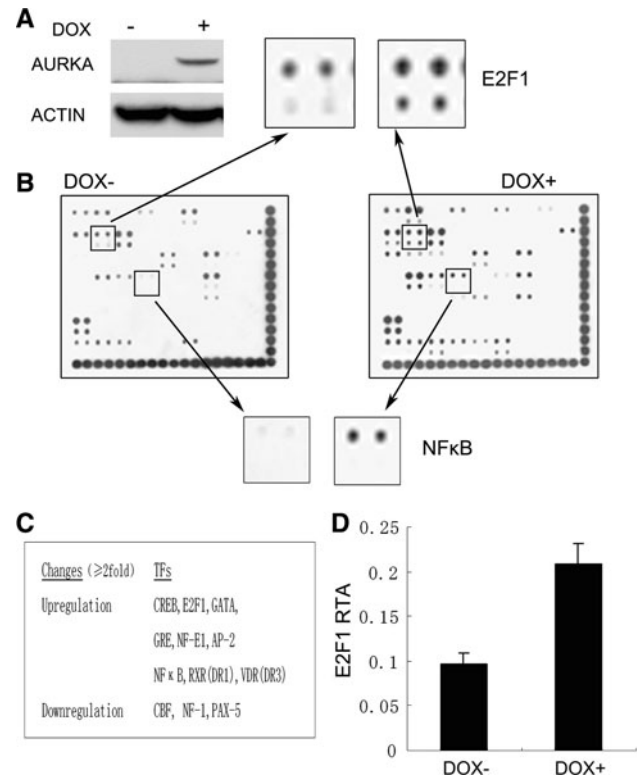
### Statistical analysis

SPSS 15.0 for Windows (SPSS Inc.) was used for statistical analysis. Correlation between the AURKA expression levels and E2F1 expression profiles on a per-case basis was analyzed by Spearman's rank test. Values of  $p < 0.01$  were considered statistically significant.

## Results

### The profiling of transcription factors that responded to AURKA overexpression

To get a better understanding of downstream events of abnormal AURKA expression in tumorigenesis, a transcription factor array that simultaneously profiles the activities of 54 transcription factors was employed to screen for the potential transcription factors that respond to AURKA induction and thus may be involved in AURKA-elicited tumorigenesis. An inducible cell line 293TRES–AURKA in which exogenous AURKA was efficiently induced by doxycycline (DOX) was generated (Fig. 1a). The nuclear extracts of the cells cultured in the presence or absence of DOX were subjected to the transcription factor array assay. As shown in Fig. 1b, c, several transcription factors significantly responded to AURKA expression ( $\geq$ two-fold). The activities of nine transcription factors, including some general factors like CREB, GRE, and GATA were elevated, while three out of 54 had their transcription activities decreased. The changes of these global transcription factors might, to a certain extent, reflect the intensive impact of AURKA overexpression on the cell transcriptome and the cellular activities. A key transcription factor, NF $\kappa$ B was also shown to be upregulated after AURKA induction, consistent with a previous report in which NF $\kappa$ B pathway was discovered to be activated by AURKA [11], demonstrating the reliability of the transcription array.



**Fig. 1** Transcription factor activity profiling of 293TRES cells with high and low AURKA expression. **a** Immunoblotting analysis of AURKA expression in untreated and doxycycline (DOX)-treated 293TRES–AURKA cells. Actin was also probed to act as a loading control. **b** Transcription factor arrays hybridized with nuclear extracts from untreated (DOX–) or DOX-treated (DOX+) 293TRES–AURKA cells. Magnified panels show the spots representing E2F1 and NF $\kappa$ B transcription factors that were upregulated upon AURKA overexpression. **c** The summary of the transcription factors with significant changes (either upregulation or downregulation  $\geq$ two-fold) in their transcription activities upon AURKA induction. **d** Relative transcription activities (RTA) of E2F1 in DOX– and DOX+ cells after being normalized to respective internal controls. The data were depicted as mean  $\pm$  SD ( $n = 4$ )

To our surprise, after being normalized to respective internal control, the spots representing the E2F1 transcription factor in DOX-treated cells also showed about a two-fold increase when compared to those of the DOX-untreated samples (Fig. 1b, d), suggesting an elevation of E2F1 transcription activity. A number of studies have shown that E2F1 plays an important role in the regulation of both cell proliferation and apoptosis [16, 17]. The active form of E2F1 controls expression of various genes required for DNA replication, as well as further cell-cycle progression [18, 19]. The particular status of E2F1 prompted us to investigate the potential role of AURKA in the regulation of E2F1.

### AURKA increased E2F1 transcription activity

Electrophoresis mobility shift assay (EMSA) and reporter assays were then performed to confirm the array results. As

shown in Fig. 2a, it is demonstrated by EMSA that induction of exogenous AURKA greatly enhanced the DNA-binding ability of E2F1. In contrast, there was little change in 293TREX-Vector cells in which the endogenous AURKA expression was unaffected by DOX treatment. AURKA overexpression in 293TREX-AURKA cells also led to increased transcription of luciferase reporter genes linked to the E2F1-binding sites (Fig. 2b), further suggesting a role of AURKA in the elevation of E2F1 transcription activity. To rule out the possibility of cell type-specific effects, EC9706, an esophageal cancer cell line, was also used to perform the transient transfection with AURKA expression plasmids. As shown in Fig. 2c, the reporter activity was upregulated by ectopic AURKA in a dose-dependent manner, while the kinase-dead (KD) mutant form of AURKA exhibited little effects, confirming that AURKA regulates E2F1 transcription activity, as well as suggesting that the kinase activity is critical for this regulation.

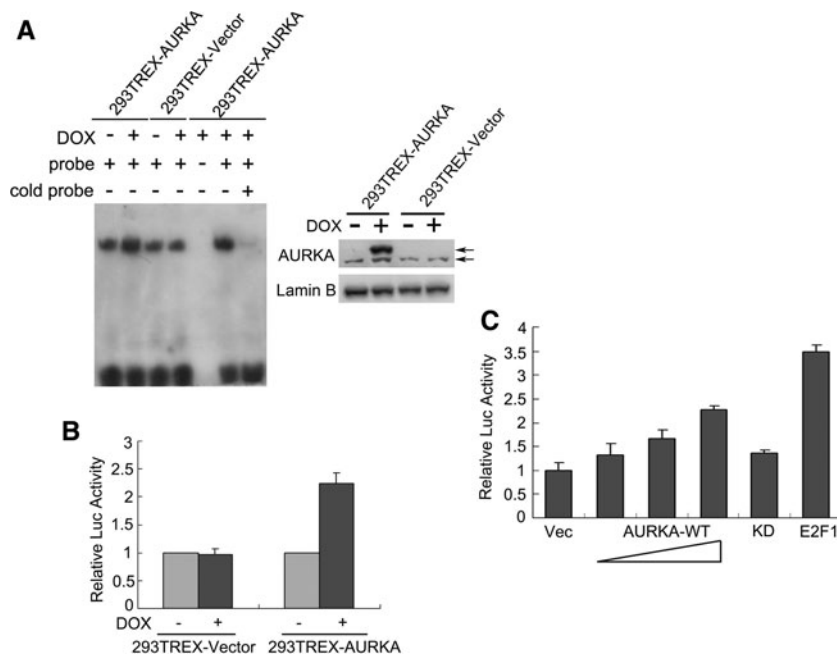
### AURKA upregulated E2F1 protein expression

To reason how AURKA increased transactivity of E2F1, we examined E2F1 mRNA and protein levels. Surprisingly, the E2F1 mRNA level was not affected by AURKA

overexpression (Fig. 3a). However, both the induction of AURKA in 293TREX-AURKA cells and the transient transfection of AURKA in SK-BR-3 breast cancer cells resulted in a similar increase of E2F1 on the protein level. Besides, in MCF-7 cells previously reported to harbor AURKA overexpression [20], knockdown of AURKA through RNAi efficiently reduced E2F1 protein (Fig. 3b), further confirming the modulation of E2F1 protein by AURKA. To verify the clinical relevance of the *in vitro* results regarding AURKA regulation over E2F1 protein, 100 samples from patients with primary breast tumors were examined by immunohistochemical staining. Examples of weak and strong staining for both AURKA and E2F1 are shown in Fig. 3c and the correlation between each paired immunohistochemical scores was analyzed with Spearman's rank test. The result showed that there was a positive correlation between AURKA and E2F1 expression ( $p = 0.003$ ; Table 1), further supporting a role for AURKA in the regulation of E2F1.

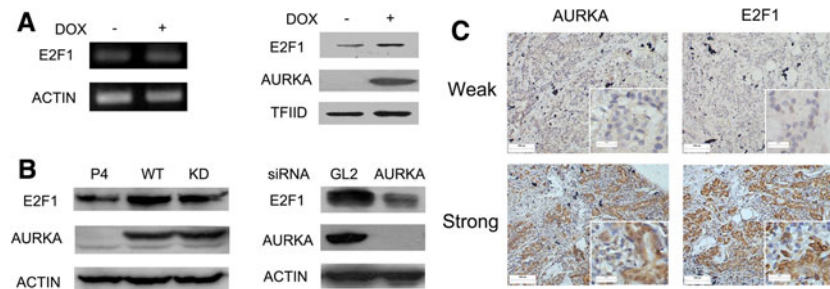
### AURKA alleviated E2F1 proteasome-dependent degradation

Since E2F1 was unchanged at the mRNA level, we next consider whether the degradation rate of E2F1 was altered



**Fig. 2** AURKA increased E2F1 transcription activity. **a** E2F1 DNA-binding ability was examined by electrophoresis mobility shift assay (EMSA) in nuclear extracts of 293TREX-AURKA cells. The lysates of 293TREX-Vector cells were examined as well to exclude the unspecific effects of DOX. The extracts of DOX-treated 293TREX-AURKA cells were also incubated either without probes or with probes in combination with excess cold probes to indicate the specificity of the probes. *Right panel* shows equal amounts of nuclear extracts were used in EMSA for the two cell lines, respectively. The *upper* and the *lower arrows* indicate exogenous and endogenous

AURKA, respectively. **b, c** Reporter gene analysis with the pGL3-6 × E2F-luciferase plasmids. 293TREX-AURKA cells treated with or without DOX (**b**) and EC9706 cells transfected with 50 ng Vec, 50/100/200 ng AURKA-WT, 50 ng AURKA-KD and 50 ng E2F1 expression plasmids (**c**) were assayed for E2F-dependent luciferase activities. Firefly/Renilla luciferase ratios were used to calculate fold induction. Vec, pcDNA4 vector; KD, the kinase-dead mutant form of AURKA. All experiments were performed in triplicate and are depicted as mean ± SD



**Fig. 3** AURKA upregulated E2F1 protein expression. **a** E2F1 mRNA and protein levels in 293TRES-AURKA cells treated with/without DOX for 24 h. **b** E2F1 protein levels in SK-BR-3 cells transiently transfected with AURKA plasmids (*left*) and in MCF7 cells treated with AURKA siRNA for 48 h (*right*). P4, pcDNA4 vector; WT and KD, the wild-type and kinase-dead mutant form of AURKA,

respectively; GL2 siRNA was used as a negative control for AURKA siRNA. **c** Immunohistochemical analysis of AURKA and E2F1 expression in breast cancer samples. Representative photographs of weak and strong staining for AURKA and E2F1 are shown. The original magnification:  $\times 100$  (pictures, size bars 100  $\mu\text{m}$ ), and  $\times 400$  (insets, size bars 20  $\mu\text{m}$ )

**Table 1** Summary of AURKA and E2F1 expression in breast cancers

AURKA staining	E2F1 staining		Total (%)
	Weak (%)	Strong (%)	
Weak	50 (50)	18 (18)	68 (68)
Strong	14 (14)	18 (18)	32 (32)
Total	64 (64)	36 (36)	100 (100)

The level of AURKA and E2F1 expression were determined in 100 surgical specimens of primary breast cancer, as shown in Fig. 3c. The correlation was analyzed using the Spearman's rank correlation test,  $p = 0.003$

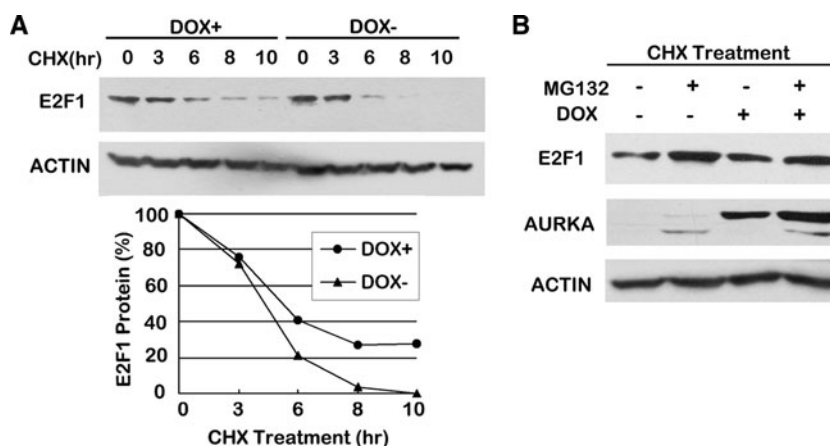
by AURKA overexpression. To address this question, 293TRES-AURKA cells were exposed to cycloheximide (CHX), an inhibitor of protein synthesis, in time-course experiments. We estimated the relative amounts of E2F1 in cells treated with CHX for 3, 6, 8, and 10 h and found consistently higher levels of E2F1 in cells with AURKA induction than in cells without AURKA overexpression (Fig. 4a). Treatment with CHX for 6 h decreased E2F1 level by approximately 80% in the DOX- cells but only less than 60% in the DOX+ cells; after 10 h treatment, E2F1 expression was almost undetectable in DOX- cells while there were still about 20% of the original E2F1 level remaining in DOX+ cells, suggesting an impaired protein degradation rate of E2F1 when AURKA is overexpressed.

As it has long been recognized that the turnover of E2F1 protein is through the ubiquitin-mediated proteasome pathway [21], we thus assess the effect of proteasome inhibition on E2F1 degradation in cells with/without DOX treatment. As shown in Fig. 4b, in the presence of CHX, treatment with proteasome inhibitor MG132 unsurprisingly resulted in an accumulation of E2F1 in both DOX- and DOX+ cells; however, the degree of restoration between the two samples varied significantly: cells overexpressing AURKA alleviated E2F1 degradation when exposed to CHX alone, thus generating only a little increase of E2F1

protein level when MG132 was added, while a more abrupt elevation of E2F1 was observed upon proteasome inhibition in the cells without AURKA induction. These data combined together indicate a role for AURKA in increasing the stability of E2F1 by inhibition of proteasome-dependent degradation of this protein.

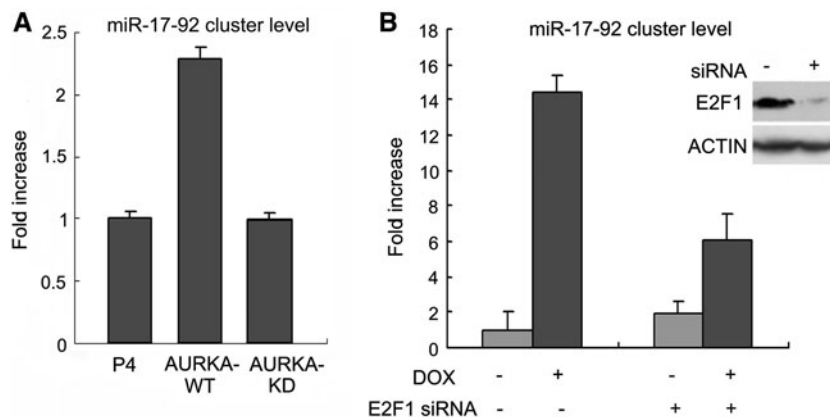
#### AURKA induced *miR-17-92* cluster expression

To study the functional consequences of E2F1 transcription activity elevation induced by AURKA, we examined the expression of the validated E2F1 target genes. The miRNA cluster *miR-17-92* is an oncogenic polycistronic cluster of miRNAs that were previously identified to be regulated by E2F transcription factors [15, 22]. The oncogenic activity of the *miR-17-92* cluster has been extensively studied recently [23]. We then tried to figure out whether AURKA is functionally linked to *miR-17-92* cluster. As shown in Fig. 5a, transient expression of AURKA plasmids increased the expression of *miR-17-92* cluster in SK-BR-3 breast cancer cells, while the KD mutant form of AURKA failed to induce the *miR-17-92* cluster level, indicating the importance of kinase activity of AURKA in the regulation of *miR-17-92* cluster. Furthermore, in 293TRES-AURKA cells, AURKA expression induced a more dramatic elevation of the *miR-17-92* expression level, reaching to almost 15-fold of that in the DOX-untreated cells (Fig. 5b). However, when E2F1 was knocked down by siRNA, the induction of *miR-17-92* by AURKA was greatly repressed to an extent as low as threefold. These results taken together demonstrated that AURKA was able to induce *miR-17-92* cluster expression and E2F1 might contribute a large part to this modulation. The remaining induction caused by AURKA independent of E2F1 might probably be mediated by additional factors. Another oncoprotein, c-Myc, is likely to be the player, as Yang et al. [9] showed that c-Myc was upregulated by AURKA while O'Donnell et al. [24]



**Fig. 4** AURKA alleviated E2F1 proteasome-dependent degradation. **a** Lysates of 293TRES–AURKA cells treated with or without DOX were collected at the indicated time points after addition of CHX and subjected to immunoblotting. The amounts of E2F1 were quantified by densitometry and normalized to the corresponding actin expression. The degradation rate was shown by comparing the amounts of

normalized E2F1 at each time point with the original levels (0 h) in DOX+ and DOX– cells, respectively. **b** Lysates of 293TRES–AURKA cells treated with or without DOX were collected 8 h after addition of CHX alone or in combination with MG132 and subjected to immunoblotting



**Fig. 5** AURKA-induced *miR-17-92* cluster expression. **a** Real-time PCR analysis of the *miR-17-92* cluster expression in SK-BR-3 cells transiently transfected with the indicated plasmids. All experiments were performed in triplicate and are expressed as mean  $\pm$  SD. **b** Real-time PCR analysis of the *miR-17-92* cluster expression in

293TRES–AURKA cells treated with E2F1 siRNA for 48 h in the absence or presence of DOX treatment. All experiments were performed in triplicate and are expressed as mean  $\pm$  SD. The efficiency of E2F1 siRNA was tested by immunoblotting with GL2 siRNA as a negative control

reported that *miR-17-92* cluster was also regulated by c-Myc. Further work is obviously required to validate this hypothesis.

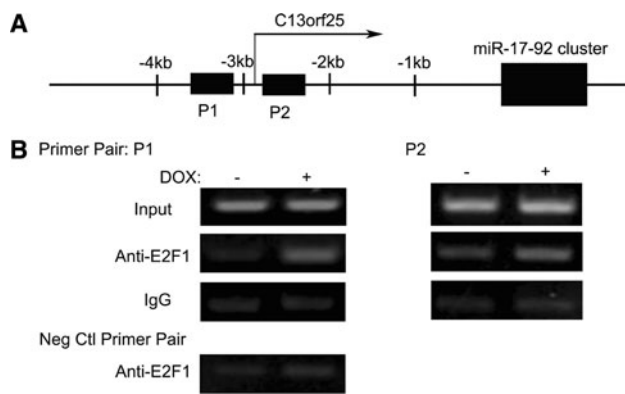
AURKA enhanced *in vivo* binding of E2F1 to the promoter of the *miR-17-92* cluster

To confirm that AURKA-mediated regulation of the *miR-17-92* cluster is indeed directly through E2F1, chromatin immunoprecipitation assay was performed using two amplicons, which were previously reported to overlap two different E2F-binding sites at *miR-17-92* cluster promoter [15, shown in Fig. 6a]. As shown in Fig. 6b, with the DNA templates pulled down by an anti-E2F1 antibody, much stronger PCR amplifications were observed for both of the

two amplicons in the DOX-treated cells than those in the control samples, while the unspecific immunoprecipitation with mouse IgG generated no obvious difference between the two samples, demonstrating that the *in vivo*-enhanced association of the endogenous E2F1 with the *miR-17-92* promoter occurred upon AURKA overexpression. Therefore, the induction of the *miR-17-92* cluster by AURKA may at least be in part through increasing the binding of E2F1 to the promoter.

## Discussion

Recent studies unveiling substrates of AURKA have helped to elucidate the physiological functions of AURKA



**Fig. 6** AURKA-enhanced in vivo binding of E2F1 to the promoter of the *miR-17-92* cluster. **a** Schematic representation of the promoter of the *miR-17-92* cluster. The transcription start site of C13orf25 and the primer pairs (P1 and P2) flanking the putative E2F1-binding sites are indicated. **b** PCR analysis of E2F1 chromatin immunoprecipitates. Chromatins prepared from 293T cells treated with DOX or not were subjected to immunoprecipitation with either an anti-E2F1 antibody or mouse IgG. Associated DNA was detected by PCR with two primer pairs: P1 (flanking E2F-binding site 1) and P2 (flanking E2F-binding site 2). Input DNA of the two samples were subjected to PCR to ensure that equal amounts of total DNA were used in the immunoprecipitation. A negative control primer pair flanking a genomic DNA sequence that should not be bound by E2F1 was also used to exclude the possibility that the variances are due to contamination of different amounts of genomic DNA through unspecific immunoprecipitation

in centrosome cycling and cell mitosis, yet how this kinase promotes tumorigenesis still needs to be further explored. Concerning the importance of AURKA in chromosome segregation, it's often tempting to speculate that centrosome aberrancy arising from abnormal AURKA might provide a route to aneuploidy and thereby contribute to tumor development. However, recent findings showed that some mitosis-related proteins are also involved in regulation of transcription either directly or through transcription factors so as to aid the pathogenesis of cancer. Securin, a mitotic protein that keeps separase from promoting sister chromatids separation before the onset of anaphase, is found to be a global transcription factor which is able to activate c-Myc, FGF2, and cyclinD3 in different cellular processes [25]. PLK1 is another important kinase that controls centrosome maturation. Interestingly, its expression is reported to repress p53 transcription activity but to activate the heat shock factor 1 (HSF1) transcription factor [26]. Similarly, AURKA has been reported to regulate two important transcription pathways in different cancer cells. Briassouli et al. [11] demonstrated that AURKA overexpression in HeLa cells resulted in phosphorylation of I $\kappa$ B $\alpha$ , which is critical for activation of the NF $\kappa$ B transcription pathway. Darr et al. [27] found that AURKA deregulation relieved  $\beta$ -catenin/TCF4 transcription pathway by increasing the Ser-9 phosphorylation of GSK3 $\beta$  in

gastric cancer cells. Here, through transcription array profiles, we show that another general transcription factor E2F1 is also under the regulation of AURKA. AURKA overexpression inhibited the turnover of the E2F1 protein, and the accumulation further resulted in upregulation of E2F1 transcription activity. It is of note that the induction of E2F1 is not that dramatic (Figs. 1b, 3b, 4b), yet this may not be unexpected, as the overexpression and hyper-activation of E2F1 can lead to cell death rather than proliferation through p53-dependent apoptosis [17]. Importantly, the moderate accumulation of E2F1 we show here is sufficient to upregulate its transcription activity and elevate the expression of the downstream target *miR-17-92* cluster, and therefore E2F1 may function in this manner to play a role in AURKA-induced tumorigenesis. This discrepancy may be explained by the "fine-tuning" theory that only the precisely appropriate oncogenic signals, no more and no less, are required for a cell on its way to transformation. There is currently no evidence for us to show how AURKA exactly functions to inhibit E2F1 degradation. However, there are several possibilities. Possibly, AURKA overexpression might induce phosphorylation and inactivation of some ligase in ubiquitin system, thus leading to impairment of E2F1 degradation. Alternatively, E2F1 stability was reported to be increased through phosphorylation at the ser-31 site by ataxia telangiectasia mutated (ATM) or ATM- and Rad3-related (ATR) kinase [28]. It is therefore interesting to explore whether AURKA has a role in this modulation.

Our data for the first time provide evidence that miRNAs are involved in the network of AURKA. Recent studies have uncovered the involvement of some miRNAs in the development of cancer. The amplification of the *miR-17-92* cluster is seen in large-B cell lymphoma and in other malignant lymphomas, while miRNAs from this cluster are discovered to be overexpressed in several human solid tumors [29]. As a result, miRNAs from the *miR-17-92* cluster are thought to be oncogenic miRNAs or "oncomirs". Our discovery of AURKA regulation over the *miR-17-92* cluster might therefore provide an additional mechanism for the role of AURKA in malignant transformation besides mitotic control. As miRNAs function through the RNA interference to regulate gene expression, it would be of great interest to explore which genes are targeted by miRNAs from the *miR-17-92* cluster in this cascade. This knowledge would nevertheless have implications for further understanding the role of AURKA in tumorigenesis.

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