Nuclear AURKA acquires kinase-independent transactivating function to enhance breast cancer stem cell phenotype

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Centrosome-localized mitotic Aurora kinase A (AURKA) facilitates G2/M events. Here we show that AURKA translocates to the nucleus and causes distinct oncogenic properties in malignant cells by enhancing breast cancer stem cell (BCSC) phenotype. Unexpectedly, this function is independent of its kinase activity. Instead, AURKA preferentially interacts with heterogeneous nuclear ribonucleoprotein K (hnRNP K) in the nucleus and acts as a transcription factor in a complex that induces a shift in MYC promoter usage and activates the MYC promoter. Blocking AURKA nuclear localization inhibits this newly discovered transactivating function of AURKA, sensitizing resistant BCSC to kinase inhibition. These findings identify a previously unknown oncogenic property of the spatially deregulated AURKA in tumorigenesis and provide a potential therapeutic opportunity to overcome kinase inhibitor resistance.

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The oncogenic activation of kinases through mutation\(^1\) and amplification\(^2\) often leads to constitutively active kinase activity and cancer development\(^3\). This characteristic renders them addicted to constitutive kinase activation and thereby susceptible to kinase inhibition by small targeted molecules\(^4\). Despite the success of molecular targeted therapies that inhibit kinase activity in multiple cancers such as chronic myelogenous leukemia\(^5\) and lung cancer\(^6\), the development of resistance to kinase inhibition is inevitable, leading to cancer recurrence\(^7\). Missense mutations in the catalytic core of kinases account for the majority of clinically observed drug resistance cases\(^8\). Although new small molecule inhibitors can tolerate diverse mutations at the catalytic core, the blockade of kinase activation alone is often not sufficient to attain maximum therapeutic efficacy\(^9\).

Emerging evidence indicates that current therapeutic inhibitors do not effectively eliminate cancer stem cells (CSCs), thereby leading to drug resistance\(^10\). Several mechanisms of drug resistance have been proposed in CSCs, including tumour microenvironment nursing, CSCs\(^11\), metabolic pathway alterations\(^12\) and epigenetic alterations\(^13\). However, the mechanisms that underlie therapeutic kinase inhibitor resistance remain elusive and require further elucidation.

Activation of Aurora kinase A (AURKA) plays an essential role in the control of mitosis progression, centrosome maturation/ segregation and mitotic spindle function\(^14\). AURKA has attracted a great deal of interest as a potential therapeutic target due to its overexpression in cancers\(^14\). Inhibitors of Aurora kinases, such as MLN8237 and PHA-739358, have been developed\(^15\), but were found to be moderately effective in preclinical and clinical studies\(^15,16\). These data suggest that a kinase-independent mechanism contributes to inhibitor insensitivity. There is emerging evidence to suggest that AURKA also promotes cancer development through mechanisms independently of its kinase activity\(^17\). Moreover, AURKA localizes to structures other than the mitotic apparatus during interphase to regulate neurite elongation and ciliary resorption, suggesting that AURKA possesses functions beyond its kinase activity\(^18\), and that inhibition of Aurora kinase alone may not be sufficient to repress AURKA oncogenic functions.

Previous study shows that the tumour tissues display nuclear AURKA staining\(^19,20\), which predicts a poorer clinical outcome in ovarian cancer\(^20\). Conversely, cytoplasmic localized AURKA consistently fails to enhance the H-Ras-induced transformation in BALB/c 3T3 A31-1-1 cells\(^21\). These studies suggest an oncogenic role of nuclear AURKA that might be independent of its kinase activity. Here we demonstrate that AURKA displays a kinase-independent function in the nucleus to activate the transcriptional activity of primary mouse embryonic fibroblasts by overexpressing K-Ras (G12V mutant) or H-Ras (G12V mutant; Fig. 1c left panel) increased both cytoplastic and nuclear AURKA expression (Fig. 1c right panel). Importantly, the ratio of nuclear/cytoplastic AURKA was significantly increased in Ras-transformed cells compared with the wild-type (WT) counterpart (Fig. 1c right panel).

AURKA displays transcriptional activity. To determine the transcriptional activity of AURKA, we fused GAL4 DNA-binding domain (DBD) to AURKA and expressed it in 293T cells with a luciferase reporter containing GAL4 DNA-binding sites upstream of a TATA box-containing minimal promoter. Expression of AURKA (WT)-DBD protein increased reporter activities by nearly tenfold compared with the vector control (DBD only; Fig. 2a), suggesting that AURKA transactivates gene expression. Interestingly, the expression of the KA (kinase active) or KD (kinase dead) mutants of the AURKA-DBD protein also increased the reporter activity to levels comparable to that induced by AURKA (WT)-DBD (Fig. 2a). We further tested the
Figure 1 | Nuclear AURKA enhances breast cancer stem cell phenotype. (a) Primary cells were extracted from breast cancer tissues and adjacent normal breast tissues. The cytoplasmic and nuclear protein lysates representing an equal number of cells were subjected to immunoblot (IB) analysis. Representative IHC staining showing AURKA expression. Images were magnified with a × 4 or × 40 objective. Scale bar, 50 μm. (c) Mouse embryonic fibroblasts (MEFs) overexpressing K-Ras, H-Ras and the vector control (Vec) were analysed by IB for indicated antibodies (left panel). Cytoplasmic and nuclear lysates of WT (−/−), vector control (Vec−), K-Ras- and H-Ras-overexpressed MEFs were subjected to IB analysis (right panel). (d) MDA-MB-231, SUM149 or BT549 cells were treated with AURKA siRNAs for 96 h. Adherent cells were collected for IB analysis (left panel), CD24/CD44 staining and flow cytometry (right panel). (e) HA-tagged AURKA was overexpressed in MCF-10A, SUM149 and BT549 cells through lentivirus-mediated gene transfer. Puromycin (1 μg ml⁻¹) selected cells were collected for IB analysis (left panel), CD24/CD44 staining and flow cytometry (right panel). (f) Gene expression data acquired from a public database (GEO ID: GSE23541, the group of AURKA + Dox and AURKA-Dox) were subjected to GSEA using the gene expression signature that was downregulated in the CD24<sup>low</sup>/CD44<sup>high</sup>-sphere population. (g) MDA-MB-231 cells were co-transfected with shRNA vector (sh Control or shAURKA) and the overexpression vector (ER or AER) via lentivirus-mediated gene transfer. Puromycin (1 μg ml⁻¹) and blasticidin (2 μg ml⁻¹) double selected cells were subjected to CD24/CD44 staining and analysis via flow cytometry. (h) Cells derived from g were subjected to mammosphere culture assay (6 days, upper panel) and the secondary passaging (additional 6 days, lower panel). Scale bar, 100 μm. (i) Data from three independent experiments derived from g were used for quantitative analysis of mammosphere size (diameter, Φ) and mammosphere number. Left panel shows distribution pattern of mammosphere size from MDA-MB-231 (Kruskal–Wallis test followed by Dunn’s multiple comparison test, ***P<0.001). Right panel shows the number of mammospheres (Φ>60 μm). Bars represent the means ± s.e.m. of three independent experiments (analysis of variance (ANOVA) followed by least significant difference (LSD) test; *P<0.05, **P<0.01, ***P<0.001).
transactivation activity of AURKA (WT)-DBD in the presence of three different Aurora kinase inhibitors (Fig. 2b). In agreement, inhibition of AURKA kinase activity did not abolish its transactivating function. Together, these results suggest that AURKA possesses transactivation activity in a kinase-independent manner.

We next determined whether AURKA contains transactivating domains. We identified three putative nine amino acid transactivation domains (9aaTAD) in AURKA (Fig. 2c) and established their respective hydrophobicity profiles (Supplementary Fig. 2a blue line). To determine the transactivating activity of each putative 9aaTAD, the hydrophobic residues at position 6 and/or 7 were substituted with hydrophilic amino acids (Supplementary Fig. 2a red line). Mutation of the second (M2, A234Q/L244S) but not the first (M1, L222S) or the third (M3, L363A/L364S) 9aaTAD reduced the transactivating activity of AURKA (Fig. 2d), indicating that the second 9aaTAD is responsible for the transactivating activity of AURKA.

**Nuclear AURKA activates MYC transcription.** To identify the downstream transcriptional targets of AURKA, RNA-sequencing analysis was performed on paired messenger RNA samples isolated from AURKA-overexpressed MCF-10A cells and control.
Expression of a fraction of all identified genes was validated in MCF-10A and MDA-MB-231 cells (Supplementary Fig. 2b,c). These differentially expressed genes were then subjected to STRING analysis\(^3\). STRING analysis indicated that the oncogene MYC connected with a portion of AURKA-modulated genes with high confidence (>0.7; Fig. 2e and Supplementary Table 2) and might function as a hub molecule. Owing to the role of c-Myc in regulating diverse cellular functions\(^3\), we speculated that MYC might be a critical downstream target of AURKA that mediates the BCSC-related function.

We next examined whether AURKA regulates MYC gene expression at the transcriptional level. MYC mRNA was downregulated on AURKA depletion by siRNA and enhanced by the overexpression of AURKA in MDA-MB-231 cells (Supplementary Fig. 2d,e). Interestingly, WT AURKA, as well as KA and KD mutants, upregulated MYC mRNA expression at comparable levels (Supplementary Fig. 2e). Similar observation was made in BTS549 and SUM149 cells (Supplementary Fig. 2f-i). Although AURKA depletion decreased the basal level of c-Myc, the half-life of c-Myc was not affected (Supplementary Fig. 2j). Furthermore, we found that AURKA expression induced the activity of the MYC promoter (from −2,269 to +516; Supplementary Fig. 2k,l), which was more likely to be dependent on the nuclear localization of AURKA (Fig. 2f,g) than its kinase activity (Supplementary Fig. 2i).

Nuclear AURKA induces a shift in MYC promoter usage. The human MYC gene mainly transcripts from its P1 and P2 promoters\(^3\). Owing to the role of AURKA in BCSC, we first examined the relative utilization of P1 and P2 in BCSC and non-BCSC. S1 nuclear protection assay showed that the P1/P2 ratio was higher in the sphere, which is enriched in BCSC, compared with cells from adherent culture (Fig. 2l). Furthermore, CD24\(^{low}\) or side population cells also displayed higher P1/P2 ratio compared with CD24\(^{high}\) or non-side population cells (Fig. 2m and Supplementary Fig. 2n). We next examined whether nuclear AURKA regulates the differential utilization of P1 and P2 promoters. As shown in Fig. 2n, nuclear, but not cytoplasmic, AURKA increased the P1/P2 ratio, inducing a shift in MYC promoter usage. Consistently, mutation of the second (M2, A234Q/L244S) AURKA transactivation domain (TAD) abolished the AURKA-induced shift in MYC promoter usage (Fig. 2o).

**AURKA and hnRNP K efficiently interact in the nucleus.** We next identified the proteins that were critical for AURKA-promoted activation of MYC promoter. We determined the proteins interacting with AURKA by SILAC\(^7\). We searched for proteins that interacted with AURKA and are also promoter regulatory proteins of MYC (Supplementary Table 3)\(^3\). We identified two heterogeneous nuclear ribonucleoproteins, hnRNP A1 and hnRNP K (Fig. 3a). Co-expression of AURKA and hnRNP K significantly increased MYC promoter activities compared with either alone, whereas co-expression of AURKA and hnRNP A1 did not show similar result (Supplementary Fig. 3a–d). As expected, there was no significant difference in the activation of the MYC promoter between KA and KD forms of AURKA (Supplementary Fig. 3e–g). We thus chose hnRNP K for further studies.

The interaction between AURKA and hnRNP K was validated via co-immunoprecipitation (co-IP) of ectopically expressed (Supplementary Fig. 3h,i) and endogenous (Supplementary Fig. 3j) proteins. This binding was not affected by mutation of S379 site in hnRNP K\(^3\) (Supplementary Fig. 3k). We further performed glutathione S-transferase (GST) pull-down assays with truncated forms of both proteins (Supplementary Fig. 3l,m). Results showed that hnRNP K KI domain interacts with AURKA 283–333 domain (Supplementary Fig. 3n,o). Moreover, molecular dynamics simulation revealed 35 combinations shown high interaction possibilities between AURKA and hnRNP K. Notably, the amino acids within AURKA 283–333 region accounted for 74.3% of these combinations (Fig. 3b). The amino acids responsible for the AURKA and hnRNP K interaction (red colour) displayed spatial proximity (Fig. 3c).

We next examined the location where AURKA and hnRNP K interact with each other. Although AURKA and hnRNP K were expressed in both the cytoplasm and the nucleus, their interaction was observed predominantly in the nucleus (Fig. 3d). The interaction between AURKA and hnRNP K in the nucleus was

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**Figure 2** | Nuclear AURKA activates MYC transcription and induces a shift in MYC promoter usage. (a) DBD/AURKA-DBD, DBD-reporter and pRL-TK were co-transfected into 293T for 24 h. Immunoblotting (IB; left panel) and dual-luciferase reporter (right panel) analysis were performed. (b) DBD/AURKA-DBD, DBD-reporter and pRL-TK were co-transfected into 293T cells. After 12 h, cells were treated with VX-680, MLN8237 or Aurora-A inhibitor I for 12 h. IB (left panel) and dual-luciferase reporter assay (right panel) were performed. (c) Diagram showing potential TADs in AURKA. (d) DBD/AURKA-DBD, DBD-reporter and pRL-TK were co-transfected into 293T cells for 24 h. IB (left panel) and dual-luciferase reporter assay (right panel) were performed. (e) STRING analysis showing connections among AURKA-regulated genes. (f) ER/AER, MYC promoter/basic reporter (P0) and pRL-TK were co-transfected into MDA-MB-231 cells. After 6 h, AURKA nuclear translocation was induced by treating cells with 200 nM OHT for 18 h. Dual-luciferase reporter assay was performed. (g) Treatment was similar to d, except for using MYC promoter reporter instead of DBD reporter. EB (left panel) and a dual-luciferase reporter (right panel) analysis were performed. (h) Treatment was similar to d, except for using MYC promoter reporter instead of DBD reporter. EB (left panel) and a dual-luciferase reporter (right panel) analysis were performed. (i) AURKA or empty vector was co-transfected into MDA-MB-231 cells with truncated MYC promoter (MYC) or basic reporter (Vec) along with pRL-TK. After 24 h, dual-luciferase reporter assays were performed. (j) MDA-MB-231 cells were subjected for ChIP analysis of MYC promoter occupancy. Results were normalized with the input. (k) VX-680-treated MDA-MB-231 cells (48 h) were subjected for ChIP analysis of MYC promoter occupancy. (l) Cells were cultured in suspended or adherent condition with same medium for 7 days. S1 nuclear protection assay (SNPA) was performed to examine MYC P1 and P2 transcripts. Control gene, β-2-microglobulin (B2M), mRNA in parallel identical samples were also determined. (m) BCSC and non-BCSC populations were isolated according to CD24 expression. SNPA was performed. (n) ER/AER plasmids were transfected into 293T cells. After 6 h, AURKA nuclear translocation was induced by treating cells with 200 nM OHT for 18 h. SNPA was performed. (o) DBD/AURKA-DBD were transfected into 293T cells for 24 h. SNPA was performed. Bars represent the means ± s.e.m. of three independent experiments (analysis of variance (ANOVA) followed by least significant difference (LSD) test; *P<0.05, **P<0.01, ***P<0.001).
Figure 3 | AURKA and hnRNP K efficiently interact in the nucleus. (a) AURKA-interacting proteins were identified using SILAC assay (red area). MYC promoter-regulating proteins were previously reported (blue area). Proteins presented in both categories were selected for further analysis. (b) Thirty-five combinations of the amino acid derived from AURKA and hnRNP K with high probabilities of interactions were used to compile a dot plot. The amino acids in orange box were located in the nucleotide 283–333 region of the AURKA sequence. (c) The simulated interaction diagram of AURKA and hnRNP K. Kl domain was shown. (d) Nuclear/cytoplasmic protein fractions of MDA-MB-231 cells were subjected to IP and immunoblotting (IB) using antibodies as indicated. (e) hnRNP K-ECFP- and AURKA-EYFP-co-transfected 293T cells were subjected to FRET efficiency analysis. ROI1 and ROI2 were selected for the analysis in the cytoplasmic and nuclear regions, respectively. Enhanced cyan fluorescent protein (ECFP)- and enhanced yellow fluorescent protein (EYFP)-co-transfected cells were used as negative controls. Scale bar, 50 μm. (f) Twenty micrograms of WT or NLS deletion mutant hnRNPK were co-transfected with Flag-AURKA-enhanced green fluorescent protein (EGFP) into 293T cells for 24 h. Cells were then subjected to IP and IB using antibodies as indicated. (g) Twenty micrograms of WT or NLS deletion mutant hnRNPK were co-transfected with Flag-AURKA-EGFP in 293T for 24 h. Cytoplasmic and nuclear proteins were separated and subjected to IP and IB using antibodies as indicated. (h) Twenty micrograms of AER was co-transfected with Flag-tagged hnRNPK into 293T cells. After 6 h, AURKA nuclear translocation was induced by treatment with 200 nM OHT for 18 h. Cytoplasmic and nuclear proteins were then separated and subjected to IP and IB using antibodies as indicated. (i) Samples 1–3 were subjected to IHC staining of AURKA. Scale bar: 100 μm. (j) The lysates of samples 1–3 were subjected to IB using antibodies as indicated. (k) The lysates of samples 1–3 were subjected to IP and IB using antibodies as indicated. Bars represent the means ± s.e.m. of three independent experiments (analysis of variance (ANOVA) followed by least significant difference (LSD) test; *P<0.05, **P<0.01, ***P<0.001).

Further supported by fluorescence resonance energy transfer (FRET) assay (Fig. 3e), hnRNPK contains two nuclear localization signals (NLSs), amino acids 21–37 and amino acids 323–361 (ref. 39). We next examined whether deletion of these two NLSs might affect its interaction with AURKA. Deletion of two NLS (labelled as Del-NLS) partially relocated hnRNPK to the cytoplasm (Supplementary Fig. 3p), indicating that other way might allow hnRNPK to enter the nucleus in addition to these specific NLSs. Co-IP showed that deletion of two NLSs weakened the interaction between AURKA and hnRNPK (Fig. 3f). We also extracted the cytoplasmic and nuclear lysates from cells over-expressing the WT hnRNPK or Del-NLS mutant for co-IP...
Figure 4 | hnRNP K is required for AURKA to activate MYC transcription and enhance BCSC phenotype. (a) Chromatin from MDA-MB-231 cells was extracted for ChIP and re-ChIP analysis. Results were normalized by input. (b) MDA-MB-231 cells were transfected with siRNA against negative control (NC), AURKA or hnRNPK for 48 h. ChIP assays were performed. (c) MDA-MB-231 cells overexpressing HA-tagged AURKA were transfected with hnRNPK or NC siRNA. After 24 h, cells were transfected with MYC promoter reporter (MYC) or basic reporter (Vec) along with pRL-TK plasmids for another 24 h. Dual-luciferase reporter assay was performed. (d) Mutated reporter along with pRL-TK were co-transfected with AURKA or/and hnRNPK plasmids for 24 h. Dual-luciferase reporter assay was performed. (e) 293T cells overexpressing HA-AURKA were transfected with hnRNPK or NC siRNA for 48 h. S1 nucleosome protection assay (SNPA) was performed to monitor MYC P1 and P2 transcripts. (f) Structure of chimeric gene consists of firefly luciferase and MYC promoter (-226/+211) (upper panel). Lower panel shows probes used for SNPA. ‘CT’ represented CT element. The mutant chimeric gene was mutated at hnRNPK K-binding site. (g) Chimeric gene or its mutant were co-transfected with AURKA and pRL-TK plasmids into 293T cells for 24 h. SNPA was performed using the probes shown in f. In parallel, a fraction of these cells was evaluated for Renilla luciferase activity, which reflects the transfection efficiency. (h,i,j) 293T cells were transfected with hnRNPK siRNA for 24 h. Cells were then co-transfected with WT or NLS-deleted hnRNPK, AURKA and MYC promoter or basic reporter along with pRL-TK for another 24 h. Transfected cells were harvested for immunoblotting (IB) (h), dual-luciferase reporter (i) and real-time PCR (j) analysis. (k) MCF-10A cells overexpressing HA-AURKA were transfected with hnRNPK or NC siRNA. After 48 h, cells were collected to analyse CD24/CD44. (l) hnRNPK K was knocked down in AURKA-overexpressing or control 10A-K-Ras (G12V) cells. Cells were sorted according to CD24 expression. CD24\(^\text{\textsuperscript{low}}\) population was used to perform limiting dilution assays. Bar represented the means ± s.e.m. of three independent experiments (analysis of variance (ANOVA) followed by least significant difference (LSD) test; \(P<0.05\); \(PP<0.01\), ***\(P<0.001\).
hnRNP K is required for AURKA to enhance BCSC phenotype.

We next evaluated whether nuclear AURKA-enhanced BCSC phenotype was mediated by hnRNP K. Depletion of hnRNP K by siRNA suppressed the CD24low/CD44high population to the same extent observed in MCF-10A and SUM149 cells in the presence or the absence of AURKA overexpression (Fig. 4k and Supplementary Fig. 4g). We therefore propose that the ability of AURKA to promote BCSC population depends on hnRNP K. To validate this, we expressed c-Myc at physiological levels from an exogenous WT or mutated MYC promoter (Mut CT) and then monitored mammosphere formation in these cells. A slightly increased expression of c-Myc (Supplementary Fig. 4h, comparing lanes 4 and 7 with lane 1) indicated that exogenous AURKA and hnRNP K were expressed at physiological levels. MDA-MB-231 cells harbouring exogenous WT, but not mutant, MYC promoter expressed higher levels of c-Myc when AURKA or/and hnRNP K were also expressed (Supplementary Fig. 4i, comparing lane 5 with 4, lane 8 with 7 and lane 11 with 10). The size and number of spheres also indicated that only cells harbouring WT MYC promoter showed enhanced sphere formation in response to the expression of exogenous AURKA or/and hnRNP K (Supplementary Fig. 4j). Cells used in mammosphere formation assays were then subjected to adherent culture. These cells displayed similar proliferation kinetics (Supplementary Fig. 4j), indicating that the above-mentioned effects were probably linked to self-renewal.

Limiting dilution assays were performed in nonobese diabetic/severe combined immunodeficient mice, to analyse the potential role of hnRNP K in regulating nuclear AURKA-promoted BCSC property. The tumorigenicity of cells overexpressing AURKA was examined in the context of hnRNP K downregulation. The CD24low population failed to generate tumours over a 3-month period with injection of <1 × 10^5 cells (Supplementary Fig. 4k). In contrast, tumour growth was observed in the CD24low population when cell number was <1 × 10^5 and became more apparent when AURKA was overexpressed (Fig. 4l), suggesting that AURKA plays a role in tumour initiation. The effect of AURKA on promoting tumour initiation was abolished by knocking down hnRNP K (Fig. 4l), further supporting the role of hnRNP K in AURKA-mediated BCSC property. The CD24low cells were isolated and subjected to adherent culture, to evaluate their proliferation potential. Overexpression of AURKA marginally increased proliferation rate relative to vector control, whereas downregulation of hnRNP K abolished the growth-promoting effects of AURKA and slightly decreased the proliferation relative to the vector control (Supplementary Fig. 4l). These results indicate that the dramatic reduction in tumorigenicity induced by knocking down hnRNP K was not caused by the mild
proliferation attenuation that we observed in the adherent culture.

**AURKA NLS is required to enhance BCSC phenotype.** We also asked whether AURKA contains an NLS. We found that deletion of the amino acids 333–383 in AURKA blocked its nuclear localization (Fig. 5a and Supplementary Fig. 5a), indicating that this region contains NLS. These data also suggest that the NES of AURKA may reside in amino acids 1–333. We fused the putative NLS (amino acids 333–383 amino acid) to DsRed. Cells expressing DsRed alone demonstrated a nearly homogeneous distribution in 293T cells (Fig. 5b left panel), whereas DsRed-333–383 fusion protein was partially induced to localize to the nucleus (Fig. 5b right panel), indicating that this region of AURKA possesses nuclear localizing function. hnRNP K overexpression, inhibition of AURKA kinase activity or deletion of NLS of hnRNP K did not significantly affect the subcellular distribution of AURKA (Supplementary Fig. 5b–d).

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**Figure Descriptions**

- **AURKA NLS and EGFP Fusions**: Images showing the localization of AURKA NLS and EGFP fusions in 293T cells.
- **DsRed and AURKA(333–383)-DsRed**: Images showing the localization of DsRed and AURKA(333–383)-DsRed in 293T cells.
- **AURKA and GAPDH Western Blots**: Images showing the expression levels of AURKA and GAPDH.
- **CD24 Low/CD44 High and ER-LS-AUR333**: Images showing the expression levels of CD24 Low/CD44 High and ER-LS-AUR333 in different conditions.
- **Number of Spheres per 500 Cells**: Bar graphs showing the number of spheres per 500 cells under different conditions.
- **55 kDa and 35 kDa Proteins**: Images showing the expression levels of 55 kDa and 35 kDa proteins.

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**Supplementary Figures**

- Supplementary Fig. 5a: Western Blots showing the expression levels of AURKA NLS and EGFP fusions.
- Supplementary Fig. 5b–d: Bar graphs showing the number of spheres per 500 cells under different conditions.

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**Supplementary Tables**

- Table showing the expression levels of CD24 Low/CD44 High and ER-LS-AUR333 in different conditions.
Truncated forms of AURKA (1–383 and 1–333) were used to perform rescue assay, to examine the role of nuclear AURKA in regulating BCSC phenotype. Although both the 1–383 and 1–333 regions rescued the proliferation in adherent culture (Supplementary Fig. 5e), only 1–383 regions of AURKA rescued BCSC phenotype (Fig. 5c–e). To confirm this, amino acids 1–333 of AURKA were fused with ER via the SV40 T-antigen NLS, to generate fusion protein labelled as ER-NLS-AUR333 (Fig. 5f upper panel). In the absence of OHT, ER-NLS-AUR333 predominantly localized to the cytoplasm. However, following incubation with OHT, a fraction of ER-NLS-AUR333 proteins translocated to the nucleus (Supplementary Fig. 5f). We then investigated whether ER-NLS-AUR333 would reconstitute BCSC expansion following endogenous AURKA depletion by short hairpin RNA (shRNA; Supplementary Fig. 5g). Only nuclear-localized ER-NLS-AUR333 rescued the CD24low/CD44high population (Fig. 5f lower panel and Supplementary Fig. 5h) and the ability to form spheres following depletion of endogenous AURKA (Fig. 5g). Furthermore, we found that the ability to form spheroids in secondary passage was retained only when the ER-NLS-AUR333 was expressed in the nucleus (Fig. 5g). The proliferation of the cells used to perform mammosphere culture could be rescued by ER-NLS-AUR333 in adherent culture regardless of its cellular localization, indicating that the above-mentioned effects were more probably linked to self-renewal (Supplementary Fig. 5i).

**Reducing nuclear AURKA sensitizes AKI-resistant BCSC.**

Endogenous AURKA was downregulated by shRNA (expressing red fluorescent protein (RFP)) in AER-expressing MDA-MB-231 cells. The resulting RFP-positive cells separated by fluorescence-activated cell sorting (FACS) were subjected to immunoblotting analysis (Fig. 6a red rectangle) and assayed for their ability to establish tumours in nude mice. Blocking AURKA nuclear localization (in the absence of OHT, group 2) attenuated tumour growth in xenograft model (Fig. 6b). Results from these experiments also established that the combination of AURKA inhibition (VX-680) and blocking AURKA nuclear localization had an additive tumour growth suppression effect (Fig. 6b, group 4). IHC staining of subcutaneous tumour sections confirmed that OHT regulated the subcellular localization of AURKA (Fig. 6c upper panel; image 2/4 compared with image 1/3), and that VX-680 suppressed AURKA phosphorylation at T288 (Fig. 6c lower panel; image 3/4 compared with image 1/2).

Next, we analysed the BCSC phenotype of cells isolated from the xenografts. The CD24low/CD44high population was somewhat decreased when either the kinase activity or nuclear translocation of AURKA was blocked and even more substantially when both were inhibited (Fig. 6d). Sphere-formation assays also revealed similar synergistic anti-BCSC effects (first and second) when both AURKA kinase activity and nuclear translocation were blocked (Fig. 6e,f). These cells also displayed similar proliferative potential in adherent culture (Supplementary Fig. 6a), indicating that the effects observed in mammosphere culture were more likely to be specifically linked to self-renewal.

We further evaluated the effects of Aurora kinase inhibition on the oncogenic function of nuclear or cytoplasmic AURKA. Immunofluorescent staining showed that NES- and NLS-fused AURKA localized to the cytoplasm and the nucleus, respectively (Supplementary Fig. 6b). The kinase activity of these fusion proteins could be effectively inhibited by MLN8237 (Supplementary Fig. 6c). AURKA kinase inhibitor significantly suppressed the CD24low/CD44high population in cells expressing AURKA-NES (Supplementary Fig. 6d,e) but not in cells expressing AURKA-NLS (Supplementary Fig. 6d,e). These results suggest that the oncogenic function of cytoplasmic AURKA relies predominantly on its kinase function, and that nuclear AURKA possesses kinase-independent activity, which is not repressed by the AURKA kinase inhibitor.

**Clinical relevance of nuclear AURKA expression.**

To further substantiate the positive regulatory role of nuclear AURKA and hnRNP K in MYC expression and promotion of BCSC phenotype, we isolated primary cells from human breast cancer tissues. The purity of the enriched epithelial cells from these tissues was >85% in ten samples (Supplementary Fig. 7a). A correlation analysis was performed for the expression of nuclear/cytoplasmic AURKA, c-Myc, hnRNP K and the ratio of the CD24low/CD44high population (Fig. 7b). Nuclear but not cytoplasmic AURKA expression was positively correlated with the percentage of the CD24low/CD44high population and the expression of nuclear c-Myc (Fig. 7c). We then performed IHC staining of AURKA, CD24, c-Myc and hnRNP K, to determine their correlations. The cutoff values used to distinguish between high and low expression were determined by a receiver operating characteristic plot (Supplementary Fig. 7b–d). Nuclear AURKA expression was positively correlated with c-Myc expression and negatively correlated with CD24 expression (Fig. 7d). A higher nuclear AURKA expression indicated a poorer prognosis for patients (Supplementary Fig. 7e). Nuclear AURKAhigh/c-Mychigh expression predicted an inferior overall survival when compared with AURKAhigh/c-Myclow, AURKAlow/c-Mychigh or AURKAlow/c-Myclow expression (Fig. 7e). MYC is frequently amplified in various cancers40. To confirm the correlation between AURKA and MYC, we re-evaluated their correlations in samples with normal MYC copy number. We found that 53 of 59 samples showed normal MYC copy number. We then subjected these samples to IHC staining of AURKA and c-Myc, and studied 31 samples with both of IHC staining of AURKA and c-Myc. Nuclear AURKA expression was still positively correlated with c-Myc expression (Supplementary Fig. 7f). In summary, these clinical data support our findings that nuclear AURKA is important for the regulation of MYC expression and thus the oncogenic properties of BCSC.

**Discussion**

AURKA is known for its function in cell cycle, where it acts in the cytoplasm during prophase of mitosis to organize the centrosomes. Here we establish a new molecular role for AURKA by demonstrating that nuclear AURKA promotes the expansion of BCSC. Mechanistically, nuclear AURKA acts as a transactivating factor to activate the expression of MYC from P1 promoter via its interaction with hnRNP K. The activation of MYC by AURKA is dependent on the nuclear localization of AURKA rather than its kinase activity. We further demonstrate that amino acids 333–383 are responsible for nuclear translocation of AURKA. Finally, we show that blocking AURKA nuclear localization sensitizes resistant BSCCs to AURKA kinase-targeted therapy.

Transcripts initiated from two tandem promoters, P1 and P2, account for the majority of MYC expression. In normal proliferating cells, the ratio of P1/P2 transcripts varies between 1:10 and 1:5 (ref. 36). In some cases, the relative utilization of P1 and P2 promoter is changed, inducing a higher ratio of P1 to P2 transcripts. In Burkitt’s lymphoma, the MYC locus (8q24) is commonly juxtaposed through chromosomal translocation to one of the immunoglobulin loci IgH (14q32), IgK (2p12) or Igλ (22q11)41. These translocations cause P1 to be more active than P2. In addition, the shift in MYC P1/P2 promoter utilization is also found during the cell cycle activation of lymphocytes42 and the postnatal cerebellar development43. Interestingly, we also...
Figure 6 | Blocking AURKA nuclear translocation enhances the anticancer effects of Aurora kinase inhibitor. (a) MDA-MB-231 cells expressing AURKA-ER were infected with lentiviral expressing AURKA shRNA or control. The cells were sorted for RFP (+) and the lysates were examined via immunoblotting (IB). (b) MDA-MB-231 cells sorted from a (red rectangle) were inoculated subcutaneously in nude mice. Mice treated with OHT were then administered the indicated treatments via intraperitoneal injection. Data were presented as the means ± s.e.m.; n = 5 per group. The statistical significance between tumour volumes was calculated using one-way analysis of variance (ANOVA) with Dunnett’s multiple comparison tests (group1 versus group4). See also Supplementary Methods. (c) IHC staining was performed on tumours harvested at the end of the animal experiment (b). Scale bar, 100 μm. (d) Flow cytometry for the expression of CD44 and CD24 on single cells from tumour xenografts. (e) Mammosphere formation by single-cell suspensions derived from tumour xenografts (left panel). The mammosphere were used to perform a secondary passage (right panel). (f) Quantification of the diameters of the spheres from e. The upper panel shows the number of spheres of 60 μm mammospheres. Statistical comparison was performed (lower panel) using the Kruskal–Wallis test followed by Dunn’s multiple comparison test (**P<0.001). Data are presented as the means ± s.e.m. of three independent experiments (analysis of variance (ANOVA) followed by least significant difference (LSD) test; *P<0.05; **P<0.01; ***P<0.001).
found that the shift in MYC P1/P2 promoter utilization also occurs in BCSC (Fig. 2l and Supplementary Fig. 2n), suggesting that the relative utilization of MYC P1 and P2 could be a hallmark for BCSC. Our data indicate that AURKA binds to MYC to induce a shift in MYC P1/P2 promoter utilization (Fig. 2j,n). The CT element locates at 100–150 bp 5′ of the P1 promoter and contains five, in part, imperfect repeats of the sequence 5′-CCCTCCCC-3′ (refs 44,45). The integrity of CT element is essential for P1 promoter activation and is important for maintaining maximal P2 activity45. Various factors bind
specifically to CT element, to modulate MYC promoter activity. For example, Sp1 binds to the duplex form, hnRNP K to C-rich single strands, and CNBP to G-rich single strands. Our data show that mutation of CT element abolished AURKA-induced P1 promoter activation (Fig. 4d,g). Consistently, downregulation of hnRNP K suppressed the interaction between AURKA and CT element, and abolished the AURKA-induced shift in MYC P1/P2 promoter utilization (Fig. 4b,e). Although we show that AURKA promotes MYC expression by acting as a transactivating factor in a kinase-independent manner, there are multiple routes through which AURKA could activate MYC expression. AURKA may interact with the WNT/β-catenin pathway. Overexpression of AURKA increases the phosphorylation of GSK-3β at Ser 9 and the nuclear β-catenin levels, which enhances β-catenin/TCF transcription activity and the transcription of its downstream target genes including CCND1 and MYC. p53 controls the expression of multiple cell cycle genes such as MYC. AURKA may also regulate cell cycle activation or MYC expression by modulating p53 function. Our previous study showed that inhibition of AURKA kinase activity by AKI603 induced the downregulation of c-Myc protein level. We considered that the downregulation of c-Myc by Aurora kinase inhibitor is caused by the secondary effects of growth arrest. To exclude the impact of growth arrest, we conducted kinase activity inhibition in cells synchronized in M phase, to evaluate c-Myc expression. Our data showed that the expression of c-Myc is not reduced by Aurora kinase inhibitor VX-680 in synchronized cells, whereas the expression of c-Myc was reduced in unsynchronized cells after VX-680 treatment (Supplementary Fig. 7g).

AURKA is critical for mitosis because of its role in regulating centromere function and microtubule dynamics. This AURKA function is probably dependent on its cytoplasmic activity due to the cytoplasmic localization of its substrates in the centrosome and microtubule. Recent studies show that mitotic function of AURKA is critical for regulating stem cell function. AURKA participates in the control of cell fate determination through modulating the asymmetric distribution of cell fate determinants and the orientation of the mitotic spindle.

Consistent with previous study, our data show that inhibition of AURKA kinase activity reduces the BCSC population (Fig. 6d). The suppression of BCSC through inhibition of AURKA kinase activity might be mainly due to impairing the function of cytoplasmic AURKA. Accordingly, we found that inhibition of AURKA kinase activity greatly reduced the CD24low/CD44high BCSC population in cells expressing NES-fused AURKA, but not in cells expressing NES-fused AURKA (Supplementary Fig. 6d,e). These results suggest that the oncogenic function of cytoplasmic AURKA relies predominantly on its kinase function, and that nuclear AURKA possesses kinase-independent activity, which is not repressed by the AURKA kinase inhibitor.

Although inhibiting the kinase activity of AURKA induced anti-BCSC effects, a significant proportion of BCSC is refractory to the kinase activity inhibition (Fig. 6d). Targeting the transactivating function by preventing AURKA translocation to the nucleus significantly enhanced the ability of AURKA kinase to inhibit the expansion of BCSC (Fig. 6e,f). Previous studies have identified several mutations of AURKA in cancer, such as F311 and V57I. Interestingly, the low-kinase-activity AURKA mutant (F311/I57) can induce higher levels of genomic instability and increase esophageal cancer risk compared with other kinase-dependent mutations such as (I31/V57)34. These findings are consistent with our results that indicated AURKA may promote cancer development via mechanisms that are independent of its kinase activity. In addition, other studies have indicated that AURKA stabilizes N-Myc independently of its kinase activity17, and that N-Myc can be destabilized using the Aurora kinase inhibitor, MLN8237, which disrupts the interaction between N-Myc and AURKA. Although these previous studies suggest a strategy to overcome drug resistance to kinase-activity inhibition by disrupting protein–protein interactions, our new findings strongly support an alternative therapeutic avenue by blocking the nuclear localization of AURKA.

In summary, our study demonstrates that the spatial deregulation of AURKA confers a previously unknown oncogenic function to AURKA that is not sensitive to conventional kinase inhibitory strategies and contributes to drug resistance. These new findings further advance our understanding of the mechanism of drug insensitivity and provide a novel strategy to overcome drug resistance (Fig. 7f).

Methods

Chemicals and antibodies. VX-680, MLN8237 and Aurora A Inhibitor I were purchased from Selleck Chemicals. OHT, Nocodazole and puromycin were purchased from Sigma-Aldrich. The following primary antibodies were used: AURKA (rabbit; Millipore, 1:4,000, catalogue number 07-648), AURKA (mouse; Abcam, 1:4,000, catalogue number ab13824), phospho-AURKA (T288; Cell Signaling, 1:1,000, catalogue number 3079), haemagglutinin (HA) tag (Sigma-Aldrich, 1:4,000, catalogue number SAB4300603), FLAG tag (Sigma-Aldrich, 1:4,000, catalogue number F1804), glyceraldehyde 3-phosphate dehydrogenase (Thermo Fisher Scientific, 1:4,000, catalogue number AM4300), β-actin (Cell Signaling, 1:4,000, catalogue number 4967), c-Myc (Santa Cruz, 1:2,000, catalogue number sc-764), Histone H3 (Cell Signaling, 1:2,000, catalogue number 4499), hnRNP K (Cell Signaling, 1:4,000, catalogue number 4675), enhanced green fluorescent protein (Cell Signaling, 1:4,000, catalogue number 2555), GST (Cell Signaling, 1:4,000, catalogue number 2624s), Erk1/2 (Cell Signaling, 1:2,000, catalogue number 9102), phospho-Erk1/2 (Cell Signaling, 1:2,000, catalogue number 9101), Rac (Cell Signaling, 1:1,000, catalogue number 3965), phospho-p53 (Cell Signaling, 1:1,000, catalogue number 3965), phospho-Histone H3 (Cell Signaling, 1:1,000, catalogue number 9701).

Cell lines. Human breast cancer cell lines (BT549, MDA-MB-231, MCF-10A and Sk-br-3), mouse embryonic fibroblasts and 293T cells were obtained from the...
American Type Culture Collection (ATCC). These cell lines were authenticated at ATCC before purchase by their standard short tandem repeat DNA-typing methodology. SUM149 was kindly provided by Professor Zhimin Shao at ATCC before purchase. These cell lines were authenticated using 1 μg ml⁻¹ paromycin for 48 h, followed by FACS sorting according to the RFP signal intensity. Female nude mice (4–6 weeks of age) were injected subcutaneously into the right flank with 2 × 10³ cells containing 30% Matrigel. At the time of cell injection, OHT (100 μg per 100 μl in peanut oil) was delivered via intraperitoneal injection every other day. When the tumors reached ~150 mm³ in volume, the mice were randomly separated into four groups and treated with OHT + polyethylene glycol 300 (PEG), OHT (removed) + PEG, OHT + VX-680 or oil (OHT removed) + VX-680, respectively, via intraperitoneal injection. VX-680 was prepared in a vehicle of 50% PEG in PBS and was administered at a dose of 40 mg kg⁻¹. The tumour volumes were measured using calipers to measure the (length) × (shortest (diameter)) every other day and were calculated according to the standard formula (length × diameter²/2 × 0.523). Next, the tumour samples were harvested from killed mice and were processed for single-cell suspension preparation and IHC analysis.

Flow cytometric analysis. Anti-Human/Mouse CD44 FITC (eBioscience) and Anti-Human CD24 PerCP-eFluor 710 (eBioscience), Anti-Human CD24 PE (eBioscience) and Anti-Human CD326 (EpCAM) PerCP-eFluor 710 (eBioscience) were used for flow cytometric analysis. Cells were harvested by trypsinization and washed once in cold PBS. Next, cells were counted and resuspended in cold PBS at a concentration of 1 × 10⁶ cells per 100 μl. Staining was performed by incubating 100 μl cells with 5 μl antibody on ice for 30 min.

Cell sorting. For flow cytometric cell sorting, the cells were dissociated into single-cell suspensions, followed by labelling with Anti-Human CD24 PerCP-eFluor 710 (eBioscience), the labelled cells were sorted into a 96-well plate. The protein-expressed cells were physically sorted by using a FACS flow cytometer (Beckman). The enrichment was confirmed by flow cytometric analysis.

For side population sorting, cells were resuspended in 37°C DMEM medium containing 2% fetal bovine serum at a density of 1 × 10⁶ cells per ml. Cells were incubated with 5 μg ml⁻¹ Hoechst 33342 (Sigma) in the presence of 50 μM verapamil (Sigma) for 1.5 h at 37°C in darkness with intermittent shaking. Next, cells were washed twice with PBS and resuspended in PBS for flow cytometric sorting. Cell sorting was performed on a FACS flow cytometer (Beckman).

Lentivirus preparation and transfection. Lentivirus was produced in 293T cells using the second-generation packaging system plasmids psPAX2 (Addgene) and pMD2.G (Addgene). One 10-cm culture dish containing 5 × 10⁶ 293T cells was transduced with Lipofectamine 2000 (Life) with 12 μg lentiviral vector, 9 μg psPAX2 and 3 μg pMD2.G. Supernatants were collected every 24 h between 24 and 72 h after transfection, pulled together and concentrated via ultracentrifugation, and the viral titre was determined by serial dilutions. The multiplicity of infection during transfection was 5.

Mammosphere culture. Single-cell suspension was obtained by trypsinization and sorting through a 40-μm sieve and single-cell suspension was examined via microscopy. Single cells were plated at a density 1 × 10³ cells per ml in ultralow attachment six-well plates (Corning). The cells were cultured in medium consisting of DMEM/F12 (Gibco), supplemented with B27 (Life Technologies), 20 ng ml⁻¹ epidermal growth factor (Sigma-Aldrich), 20 ng ml⁻¹ basic fibroblast growth factor (BD Biosciences) and 4 μg ml⁻¹ heparin (Sigma-Aldrich) for 9–12 days. For evaluation, the mammospheres were photographed under an inverted microscope (× 100/100x, Olympus). The mammosphere diameters were analysed using Image pro plus 5.0 software (Media Cybernetics).

Cytoplastic/nuclear protein extraction. Cytoplasmic extracts were prepared by resuspending the cell pellets in Buffer I, which contained 25 mM HEPES pH 7.9, 5 mM KCl, 0.5 mM MgCl₂ and 1 mM dithiothreitol (DTT) for 5 min. Next, an equal volume of Buffer II containing 25 mM HEPES pH 7.9, 5 mM KCl, 0.5 mM MgCl₂, 1 mM DTT and 0.4% (v/v) NP40 supplemented with protease and phosphatase inhibitors was added and the samples were incubated with rotation for 4°C for 15 min. The lysates were centrifuged for 5 min at 4°C at 2,500 r.p.m. in a microfuge. Next, the supernatants were transferred to new Eppendorf tubes. The pellets were resuspended once with Buffer II and the wash buffer twice with Buffer I and the wash buffer once with Buffer II. The cytoplasmic protein. The lysates were centrifuged again for 5 min at 4°C at 10,000g, to remove the residual nuclei. Next, the supernatants were transferred to new Eppendorf tubes.

To obtain nuclear extracts, the pellets from the cytoplasmic extraction were treated with Buffer III containing 25 mM HEPES pH 7.9, 400 mM NaCl, 10% sucrose or dextrose, 0.05% NP-40 and 1 mM DTT supplemented with protease and phosphatase inhibitors. After rotating for 1 h at 4°C, the lysates were centrifuged for 10 min at 4°C, at 10,000g. Supernatants after this spin contained the nuclear protein preparation.

Dual-luciferase reporter assays. Luciferase activity was measured using the Dual-Luciferase Reporter Assay system (Promega) according to the manufacturer's instructions. Growth media were removed and cells were washed with PBS. Passive lysis buffer (Promega) 500 μl per well was added with gentle rocking for 15 min at
RT. Ten microlitres of lysate were transferred in black 96-well plate (Thermo). Firefly and Renilla luciferase activity were assayed sequentially to the cell lysate in each well. For each luciferase reading, there would be a 2-4 pre-measurement delay after injector dispensing assay reagents into each well, followed by a 10-s measurement time. Transcriptional activity was calculated as the ratio of firefly luciferase activity (reporter) to Renilla luciferase activity (control).

ChIP and re-ChIP assays. ChIP was performed using Chip-IT Express Chromatin Immunoprecipitation Kits (Active Motif) according to the manufacturer’s protocol. Briefly, 1 × 10^6 cells, which were treated with the conditions described in the figure legends or Supplementary Figure legends, were fixed with 1% formaldehyde for 10 min at RT. Next, the cells were washed twice with PBS at 4°C, collected and resuspended in ice-cold lysis buffer and lysed on ice for 30 min. The cells were homogenized on ice, to aid in nuclei release. Cells were sonicated five times for 5 s at 25% (Fisher Sonic, Diatom). The chromatin (25 μl) was immunoprecipitated for 12 h with 2 μg of specific antibodies against AURKA (Millipore), hnRNP K (Cell Signaling) or IgG (rabbit, Santa Cruz) and Protein G magnetic beads (25 μl). Beads were then washed sequentially for 5 min with the following buffers: ChIP Buffer I for one time and ChIP Buffer II for two times. The immunocomplexes were eluted with 50 μl elution buffer AM2. The supernatants were reverse cross-linked by heating at 65°C for 12 h, treated with 1 μl RNaseA at RT for 30 min and digested with 2 μl proteinase K at 37°C for 1 h. DNA was obtained by phenol and phenol/chloroform extractions. The human MYC promoter-specific primers used for PCR were 5'-GTCAAGAGTCTGTTCA CGG-3' and 5'-TGGGCTCTTCTCCCGGG-3' (reverse). The primer targeting a gene exon was used as a negative control in these experiments. MYC exon-specific primers were 5'-GGATATTGGTGAAGAGAATTGGCAGGC-3' (forward) and 5'-GATGAAGGTCTGTCGTGCCGC-3' (reverse). ChIP assays were performed as described using the Re-ChIP-IT kit (Active Motif). Briefly, the precipitated chromatin from the first ChIP reaction was eluted by 100 μl diluted Re-Chip-IT elution buffer at RT for 30 min. Next, the eluted precipitate was desalted by the desalting column provided in the kit. The second ChIP was performed with 25 μl Protein G magnetic beads, 90 μl desalted chromatin and 2 μg second antibody. Next, the second precipitate was washed, eluted and reverse cross-linked by the first ChIP. DNA was obtained by phenol/chloroform extractions, and subjected to real-time PCR evaluation.

IHC and scoring. Antigen retrieval was performed by heating the sample in EDTA buffer (pH 8.0) in a microwave oven for 15 min. The slides were stained for 25 min at RT. EnVision Detection Systems (Dako) was used to detect antigen expression. The IHC score was quantified as the H-score, which has been validated for breast cancer IHC staining. The images were acquired using a Nuance EX magnetic beads (25 μl). Beads were then washed sequentially for 5 min with the following buffers: ChIP Buffer I for one time and ChIP Buffer II for two times. The immunocomplexes were eluted with 50 μl elution buffer AM2. The supernatants were reverse cross-linked by heating at 65°C for 12 h, treated with 1 μl RNaseA at RT for 30 min and digested with 2 μl proteinase K at 37°C for 1 h. DNA was obtained by phenol and phenol/chloroform extractions. The human MYC promoter-specific primers used for PCR were 5'-GTCAAGAGTCTGTTCA CGG-3' and 5'-TGGGCTCTTCTCCCGGG-3' (reverse). The primer targeting a gene exon was used as a negative control in these experiments. MYC exon-specific primers were 5'-GGATATTGGTGAAGAGAATTGGCAGGC-3' (forward) and 5'-GATGAAGGTCTGTCGTGCCGC-3' (reverse). ChIP assays were performed as described using the Re-ChIP-IT kit (Active Motif). Briefly, the precipitated chromatin from the first ChIP reaction was eluted by 100 μl diluted Re-Chip-IT elution buffer at RT for 30 min. Next, the eluted precipitate was desalted by the desalting column provided in the kit. The second ChIP was performed with 25 μl Protein G magnetic beads, 90 μl desalted chromatin and 2 μg second antibody. Next, the second precipitate was washed, eluted and reverse cross-linked by the first ChIP. DNA was obtained by phenol/chloroform extractions, and subjected to real-time PCR evaluation.

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Bioinformatics analysis. For analysis using the Gene Expression Omnibus data (accession code: GSE23541), the probes with detection P-values < 0.01 in all samples were excluded from the data set before analyses. If multiple probes corresponded to the same gene, the expression values of these probes were averaged. GSEA were performed using GSEA v2.0.13 software (http://www.broad.mit.edu/gsea) with 1,000 data permutations. The gene expression signature were acquired from public research and GSEA website. Statistical significance was evaluated by means of false discovery rate (≤0.25) and P-value calculation (P < 0.05).

For STRING (http://string-db.org/) analysis, differential expression genes were subjected to non-redundant and its corresponding interaction number, we saved the residue pairs if the interaction occurs more than 1,000 times. These saved residue pairs were defined as the interface residues between these two proteins.

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residue at leucine, mass at 3.0188, mass tolerance at 0.01 Da, minimum threshold at 0.05. The level of significance was set at P<0.05.

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Acknowledgements

This work was supported by the National Basic Research Program of China (973 Program; No. 2012CB867000 to Q.L.). National Natural Science Foundation of China (No. 81130040 to Q.L.), Innovative Research Team in University of Ministry of Education of China (No. IRT13049) and Pandeng Scholar of Liaoning. We thank all members of Liu laboratory for their critical comments and technical support. We thank Dr Keith W. Kelley and Hua Huang for the critical comments to our paper. We thank Ms Jennifer I. Hu for editing our manuscript. We thank Professor Martin Piskacek for the valuable suggestions about AURKA transactivating function. We thank Dr Mingbo Zhang for his technical support in the study of molecular dynamics simulation.

Author contributions

F.Z. participated in design, acquisition, analysis and interpretation of data in the whole project, and made critical revision in the manuscript. C.Y. participated in the animal experiment about blocking AURKA nuclear localization, mouse embryonic fibroblast transformation study and the study of AURKA TAD function. G.L. designed the study of molecular dynamics simulation and performed data analysis. B.H. and W.C. participated in the study of FRET. X.W. and W.Q. participated in the collection of breast cancer molecular dynamics simulation and performed data analysis. W.Y.-F.L. made critical comments about the TAD value. We thank Zhang and the late editor Xue for invaluable suggestions about AURKA tranactivating function. We thank Dr Mingbo Zhang for his technical support in the study of molecular dynamics simulation.

Additional information

Accession code: GSE57931.

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Zheng, F. et al. Nuclear AURKA acquires kinase-independent transactivating function to enhance breast cancer stem cell phenotype. Nat. Commun. 7:10180 doi: 10.1038/ncomms10180 (2016).