Aurora Kinase A is an Independent Predictor of Invasive Recurrence in Breast Ductal Carcinoma *in situ*

Islam M Miligy[a,b], Michael S Toss[a], Kylie L Gorringe[c,d], Ian O Ellis[a], Andrew R Green[a], Emad A Rakha[a]

[a]Nottingham Breast Cancer Research Centre, Division of Cancer and Stem Cells, School of Medicine, The University of Nottingham, Nottingham City Hospital, UK.

[b]Histopathology Department, Faculty of Medicine, Menoufia University, Egypt,

[c]Cancer Genomics Program, Peter MacCallum Cancer Centre, Melbourne,

[d]The Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia.

Short title: AURKA expression in DCIS

Correspondence:

Professor Emad A Rakha

Department of Histopathology

Nottingham University Hospital NHS Trust, City Hospital Campus

Hucknall Road, Nottingham, NG5 1PB, UK

Tel: (44) 0115-9691169, Fax: (44) 0115-9627768

Email: mszima@nottingham.ac.uk

Number of Tables: 2

Number of Figures: 4

Word count: 2895

Key words: DCIS, AURKA, poor prognosis, invasive recurrence

Abstract

Introduction: Aurora Kinase A (AURKA/ STK15) has a role in centrosome duplication and is a regulator of mitotic cell proliferation. It is over-expressed in breast cancer and other cancers, however; its role in ductal carcinoma *in situ* (DCIS) remains to be defined. This study aims to characterise AURKA protein expression in DCIS and evaluate its prognostic significance.

Methods: AURKA was assessed immunohistochemically in a large well-characterised cohort of DCIS (n=776 pure DCIS and 239 DCIS associated with invasive breast cancer (DCIS-mixed)) with long term follow-up data (median=105 months) and basic molecular characterisation.

Results: High AURKA expression was observed in 15% of DCIS cases and was associated with features of aggressiveness including larger tumour size, high nuclear grade, hormone receptor negativity, HER2 positivity and high Ki67 proliferation index. AURKA expression was higher in DCIS associated with invasive breast cancer than in pure DCIS (p<0.0001). In the DCIS-mixed cohort, the invasive component showed higher AURKA expression than the DCIS component (p<0.0001). Outcome analysis revealed that AURKA was a predictor of invasive recurrence (p=0.002).

Conclusion: High AURKA expression is associated with poor prognosis in DCIS and might be a potential marker to predict DCIS progression to invasive disease.

INTRODUCTION:

Although ductal carcinoma *in situ* (DCIS) is not life-threatening disease, it is a precursor of invasive breast cancer (IBC) with subsequent risks of distant metastasis and breast cancer-related mortality [1, 2]. A plethora of studies have investigated different potential risk factors predicting local recurrence or DCIS progression [3-5] however; predicting progression to invasive disease remains a challenge. As a consequence, large proportion of patients with DCIS are treated with extensive surgery including mastectomy or breast conserving surgery (BCS) with often frequent re-operations and/or radiotherapy [6, 7] which could be considered as over-treatment of precursor lesions. Therefore, distinguishing at diagnosis which DCIS that might be fatal is of great importance to reduce over- or under-treatment. Clinicopathological characteristics of DCIS such as lesion size, margin status, nuclear grade and presence of necrosis are useful predictors of DCIS behaviour [8, 9]. However, these do not discriminate between invasive and *in situ* recurrence, and the best combination of these variables and the improved performance using molecular markers remain to be defined [5]. Identification of novel markers that play a role in DCIS progression might aid our understanding of the disease biology and risk stratification.

Aurora kinase A (AURKA) belongs to the family of serine-threonine kinases that play an integral role in cell cycle regulation [10] by recruiting the cyclin B1/CDK1 complex and committing cells to mitosis [11]. It has a key role in centrosome duplication and is a critical regulator of mitotic cell proliferation playing essential roles in mitotic entry, centrosome maturation, mitotic spindle assembly, and chromosome segregation processes [12]. The *AURKA* gene is localised on chromosome segment 20q13, which is amplified in many human cancers [13-15]. Ectopic over-expression of the kinase induces chromosomal instability, centrosome anomalies, and tumorigenic transformation of human cells [16, 17]. AURKA represents a unique proto-oncogenic mitotic kinase that is involved in the genetic pathways underlying the two most commonly observed phenotypic alterations in human cancer cells: aneuploidy and centrosome aberrations [18]. This property is critically relevant for breast cancer as a disease driven by chromosome copy number alterations [19].

3

AURKA is expressed at elevated levels in IBC [15, 20], colorectal [18], ovary [21] and gastric carcinomas [22] and is associated with poor prognosis [23]. It is one of the proliferation genes included within the gene panel of the Oncotype DX prognostic assay for both DCIS and IBC [24-27]. Moreover, AURKA is differentially expressed between normal breast tissue and IBC [28], however the role of AURKA in DCIS has yet to be established. In this study, we aim to assess the pattern of AURKA protein expression and its prognostic significance in a large well-annotated DCIS series. In addition to further characterise the prognostic significance of *AURKA* at the transcriptomic level, a large cohort of invasive breast cancer (n=1980) with long term follow-up was used as surrogate for DCIS.

METHODS

Study cohort

A well characterised annotated cohort of DCIS including pure DCIS (n=776) and DCIS mixed with IBC (DCIS-Mixed) (n=239) diagnosed between 1990 to 2012 at Nottingham City Hospital, Nottingham, United Kingdom was used as previously described [29]. Patients' demographic data, histopathological characteristics, management including post-operative radiotherapy and development of local recurrence were collected. Patients were presented as asymptomatic screen detected (52.2%) or symptomatic palpable lesions (47.8%). Along the study period, patients were managed either by mastectomy (51.9%) or BCS (48.1%) with or without radiotherapy (29.6% and 70.4% respectively).

Local recurrence free survival (LRFS) was defined as the time (in months) between six months after the first DCIS surgery and occurrence of ipsilateral local recurrence (either as DCIS or IBC). Cases undergoing re-excision within the first six months due to close surgical margins or presence of residual disease were not considered as recurrence. Patients who developed contralateral disease following DCIS diagnosis were censored at the time of development of the contralateral cancer. Within a median follow up period of 105 months (range 6-240), 83 patients (11%) developed local ipsilateral recurrence including invasive (53/83; 64%) or DCIS (30/83; 36%). Eight recurrence events developed after management with BCS followed by adjuvant radiotherapy while the majority of the recurrences occurred after BCS alone. Patients who developed contralateral

4

disease following DCIS diagnosis were censored at the time of diagnosis of the contralateral cancer.

Additionally, data on ER, PR, HER2 and Ki67 [29] was available and included. Classification of DCIS was done according to the molecular classes defined according to St. Gallen International Expert Consensus [30]. These classes are: i) Luminal A (ER and/or PR positive, HER2 negative and Ki67 <14%); ii) Luminal B/HER2- (ER and/or PR positive, HER2 negative and Ki67 ≥14%); iii) Luminal B/HER2+ (ER and/or PR positive, HER2 positive); iv) HER2+/ER- (non-luminal) (ER and PR negative and HER2 positive); and v) Triple Negative, (ER, PR and HER2 negative). For ER and PR, a 1% cut-off value was used to dichotomise cases into positive and negative [31]. HER2 status was considered negative if the immunohistochemical score was 0 or 1+, equivocal if the score was 2+, and positive if the score was 3+ [32]. For HER2 2+ cases, HER2 gene amplification was detected using Chromogenic in situ Hybridization (CISH) and confirmed gene amplification was defined as six or more signals per nucleus or when clusters (clumps of aggregated green signals) were identified in the cell nuclei in more than 50% of tumour cells [33]. Ki67 high proliferation index was considered when more than or equal to 14% positively stained tumour cell nuclei were detected [34].

Tissue Microarrays and Immunohistochemistry

Tissue microarrays were prepared from representative DCIS lesions of the pure cases and from DCIS and invasive tumours from the mixed cases as previously described [29]. In addition, a set of whole tissue sections from ten cases containing DCIS and invasive tumours were assessed to evaluate heterogeneity and the pattern of AURKA expression in malignant breast lesions and adjacent stroma and normal tissue.

Primary antibody specificity for rabbit polyclonal AURKA antibody (Abcam; UK) was validated using Western blotting on whole cell lysates of MCF-7, SKBr3 and MDA-MB-231 human breast cancer cell lines (obtained from the American Type Culture Collection; Rockville, MD, USA). AURKA antibody was used at a dilution of 1:500 which showed a single specific band at the predicted size of 44 kDa.

Expression of AURKA protein was assessed by immunohistochemistry using the Novocastra Novolink polymer detection system (Code: RE7280-K, Leica, Newcastle, UK). 4 μm tissue microarray and full-face sections were stained with the AURKA antibody (1:200) incubated for 24 hours. 3,30-Diaminobenzidine tetrahydrochloride (Novolink DAB substrate buffer) was used as a chromogenic substance. Sections were counterstained with haematoxylin. Positive staining controls (human tonsil) were included while a negative control was achieved by omitting the application of the primary antibody.

Assessment of AURKA expression

The percentage of nuclear AURKA staining was estimated in pure DCIS and mixed cases. Cores containing <15% of tumour epithelial cells were excluded from assessment. All cases were scored blinded to clinicopathological and outcome data. For the mixed cohort, each component; DCIS and invasive, was scored separately. For dichotomisation of protein expression, cut-off points were defined according to the calculated results from X-tile bioinformatics software (Yale University, version 3.6.1) [35, 36] with corrected p value and relative risk against local recurrence free survival. High AURKA expression was considered when more than 60% of tumour cells showed staining.

Analysis of *AURKA* mRNA expression in invasive breast cancer:

To confirm the prognostic significance of AURKA in breast cancer and given the deficiency of data on the transcriptomic profiles of DCIS, AURKA normalised mRNA expression was evaluated as a potential prognostic marker using the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) dataset that comprises 1980 tumours of invasive breast cancer with comprehensive molecular characterisation and long-term follow-up [37].

Statistical analysis

Statistical analyses were performed using SPSS v26 (Chicago, IL, USA) for Windows. Student's t test and analysis of variance (ANOVA) were used to correlate between AURKA mRNA level as a continuous variable and other clinicopathological parameters in METABRIC data. Association with AURKA mRNA expression and breast cancer specific survival was

performed after dichotomisation of expression into high and low groups based on the median value.

Association between AURKA expression and clinicopathological parameters in pure DCIS was performed using Chi-square for categorised data, and Mann-Whitney and Kruskal-Wallis tests for continuous variables. Wilcoxon signed rank test was used to compare the expression of AURKA between DCIS component and the invasive component within the mixed cases. Survival rates were determined using the Kaplan–Meier method and compared by the log-rank test. Multivariate analysis using Cox proportional hazard regression model determined the influence of AURKA expression, when adjusted to other variables, for all local recurrences (either DCIS or invasive breast cancer) and invasive recurrences. All tests were 2-tailed and a p value of less than 0.05 was considered as statistically significant.

RESULTS

Frequency and localisation pattern of AURKA

Assessment of ten whole tissue sections revealed nuclear expression of AURKA in a homogenous distribution pattern confirming the validity of using tissue microarrays to assess its expression. Epithelial cells in the adjacent normal breast terminal duct-lobular units showed negative or very weak cytoplasmic staining of AURKA "shown in Figure 1".

After exclusion of uninformative cases (i.e., lost cores, folded tissue during processing and cores containing scanty tumour cells), a total of 632 pure DCIS and 217 cases associated with mixed DCIS and invasive components were assessed. AURKA expression showed a unimodal distribution with a median percentage of 30% positive nuclei in pure DCIS, 45% in the DCIS component of mixed cases and 65% in invasive component (range 0-100%). Within the pure DCIS cohort, high AURKA expression was observed in 15% of cases which was significantly lower than that in the DCIS component of the mixed cases (29% with high expression, p=0.004) and the invasive component (55% with high expression, p<0.0001). A statistically significant difference in AURKA expression was also observed between tumour cells of DCIS and corresponding invasive components of the mixed cases (p<0.0001) "shown in Figure 2".

Association of AURKA with clinicopathological parameters in pure DCIS

In BCS treated patients, high AURKA expression was significantly associated with poor prognostic factors including large tumour size, high nuclear grade, negative hormone receptor expression, HER2 positivity and high Ki67 proliferation index (Table 1). On the other hand, low AURKA expression was significantly associated with factors of good prognosis with highly significant association with luminal A subtype according to the molecular classification of breast cancer.

Analysis of continuous data of AURKA expression showed similar results (Supplementary Table 1).

Association between AURKA expression and Patient Outcome

In univariate analysis, with over 20 years follow-up "shown in Figure 3", high AURKA expression within DCIS treated with BCS only without adjuvant radiotherapy showed a significantly shorter ipsilateral LRFS for all recurrences including *in situ* and invasive disease (Hazard Ratio (HR)=2.7, 95% CI=1.6-4.6 and p<0.0001). However, this association was not maintained in patients who received adjuvant radiotherapy (p>0.05). When the subtype of recurrence was considered, high AURKA expression was associated with recurrence as invasive disease (HR=2.9, 95% CI=1.5-5.8 and p=0.002). It is also shown that the probability of recurrence in cases with high AURKA expression was significantly lower compared to cases with lower expression over 20- and 10-years' time. However, this was not statistically significant over 3- and 5-years' time, "shown in Supplementary Figure 1 and Supplementary Table 2".

Multivariate survival analysis showed that AURKA expression is a predictor of local recurrence in patients treated with BCS independent of other well-established prognostic factors including patient age, tumour size, tumour grade, comedo necrosis, surgical margin width, molecular classes and adjuvant radiotherapy use (HR=6.9, 95% CI=2.7-10.0, p<0.0001). AURKA was also shown to be an independent predictor of invasive recurrence (HR=3.9, 95% CI=1.7-9.1, p=0.001) (shown in Table 2, and Figure 4).

When AURKA expression was combined with the other determinants of DCIS risk described by the VNPI [38], expression of AURKA was associated with outcome in all VNPI risk groups. Inclusion of AURKA into VNPI affected the HR of prediction of outcome from 2.9 (95% CI 1.7-4.9) to 3.3 (95% CI 1.4-6.7).

To validate the prognostic importance of *AURKA* in IBC, the METABRIC cohort was used to assess the correlation between AURKA mRNA levels and clinicopathological variables and outcome. High AURKA mRNA level was associated with young patient age (p=0.001), high histological grade (*p*=0.034), negative hormone receptor expression and HER2-positive phenotype (*p*<0.0001), in addition to worse outcome in terms of shorter breast cancer specific survival (HR=1.4, 95% CI=1.1-1.6, *p*<0.0001) (shown in Supplementary Table 3 and Supplementary Figure 2).

DISCUSSION

The treatment of DCIS remains a challenge, as the clinicopathological features of the disease do not reliably stratify patients into distinct risk groups to guide treatment decisions [39]. For this reason, some studies have attempted to risk stratify DCIS based on genetic and molecular factors including the *Oncotype* DX® DCIS score [40, 41, 26, 42, 43]. Although this score minimises the proportion of patients undergoing radiotherapy, the assay is relatively expensive and showed some inconsistent results [44, 45]. Therefore, there is a pressing need to identify robust and cost-effective biomarker(s) to predict outcome for DCIS patients. AURKA was a candidate to study and immunohistochemical detection of the protein is a robust and relatively inexpensive method that may be able to assist with DCIS prognostication. Indeed, the HR we observed for the combination of VNPI and AURKA for any local recurrence after BCS without postoperative radiotherapy (HR=3.3) is strikingly similar to that obtained for the Oncotype DX score in a recent analysis (HR=1.95 and 2.48 in two cohorts) [7].

This study showed that high expression of AURKA was associated with factors of poor prognosis including high nuclear grade, negative hormone receptor expression, HER2 positivity and high proliferative activity of tumours expressed as high Ki67 LI. This goes

9

in line with other studies [46, 47, 20]. This is explained by the proliferation driving properties of AURKA that regulates the transition of cells from the G2 to M phase [46].

The current study showed that high AURKA expression is a poor prognostic factor for DCIS which is independent of other clinicopathological factors. These findings were similar for all recurrence events (DCIS or invasive) and also when the analysis was restricted to invasive recurrences only. These findings suggest that AURKA is a promising marker for introduction of a new high-risk DCIS group in addition to identification of patients with low risk for whom radiotherapy could be avoided.

Increased copy number of AURKA is associated with progression from a colonic polyp to invasive malignancy and is one of the most common copy number alterations in cancer. This finding reveals that it has a role not only in tumour migration and invasion but also in tumour development [48, 49]. Similarly, although 20q gain is common in DCIS (~25%)[50], it has also been observed as an IBC-specific event in mixed DCIS, suggesting an association with invasiveness in breast cancer [51]. In contrast to the study of 37 breast cancer patients by Hoque et al., who reported that loss of AURKA expression correlates with the transition from in situ to invasive carcinoma of the breast, AURKA is differentially expressed between normal breast tissue and invasive breast [52, 28]. Herein, we show that AURKA expression is most prevalent in invasive tumour cells, in line with [25], followed by a lower level in DCIS coexisting with invasive carcinoma and much lower in pure DCIS. These findings corroborate the role of AURKA in DCIS progression. Moreover, using the METABRIC series, we have shown a significant association between aggressive behaviour of IBC and higher levels of AURKA mRNA. These observations support our hypothesis that AURKA is a promising candidate biomarker that requires further functional studies to decipher its role in DCIS behaviour.

There are a number of plausible biological mechanisms to explain the involvement of AURKA in breast carcinogenesis and cancer progression. AURKA over-expression in fibroblasts and breast epithelial cells results in centrosome amplification and aneuploidy, suggesting it plays an important role in malignant transformation [53]. It is expressed in

multiple carcinomas including breast cancer [54-56, 22] and this over-expression is associated with centrosome amplification and DNA instability [57]. The non-mitotic function of AURKA is implicated in breast cancer progression and resistance to chemotherapy agents through epithelial to mesenchymal transition and the acquisition of stem cell-like characteristics [58, 59]. Breast cancer cells with these stem cell-like properties have consequently been associated with tumour progression and onset of distant metastasis [60, 61].

In addition, nuclear AURKA acts as a trans-activating factor to promote the expression of MYC. The activation of MYC by AURKA is dependent on the nuclear localisation of AURKA rather than its kinase activity [62]. Nuclear AURKA may also promote the expansion of breast cancer stem cells [63]. There is emerging evidence to suggest that AURKA also promotes cancer development through mechanisms independently of its kinase activity [64].

CONCLUSION

AURKA might have a potential role in DCIS aggressiveness through its oncogenic activity and its regulatory role in cellular division and proliferation. Additional functional studies are highly recommended to unravel the role of AURKA in regulating DCIS behaviour. AURKA might also be a useful prognostic indicator especially as a predictor of invasive recurrence.

ADDITIONAL INFORMATION

Statement of Ethics
Former written informed consent was obtained from all subjects included in this study to use their tissue materials in research. This work obtained ethics approval by the North West – Greater Manchester Central Research Ethics Committee under the title; Nottingham Health Science Biobank (NHSB), reference number 15/NW/0685. All samples and data were used fully anonymised. The research was carried out following Helsinki declaration of using human tissue in research.

Conflict of Interest Statement
The authors declare no conflict of interest.

Funding Statement

None

Author Contributions Statement

Conception and design: Islam M Miligy and Emad A Rakha

Cases collection and IHC staining and scoring: Islam M Miligy

Collection and assembly of data: Islam M Miligy, Michael S Toss and Kylie L Gorringe

Data analysis and interpretation: Islam M Miligy, Michael S Toss and Kylie L Gorringe

Manuscript writing: Islam M Miligy, Michael S Toss, Kylie L Gorringe, Ian O Ellis, Andrew R Green and Emad A Rakha

All authors contributed to writing the manuscript and approved the final version.

Data Availability Statement

The authors confirm the data that has been used in this work is available on reasonable request.

REFERENCES:

1. Bartlett JM, Nofech-Moses S, Rakovitch E. Ductal carcinoma in situ of the breast: can biomarkers improve current management? Clinical chemistry. 2014 Jan;60(1):60-7.
2. Visser LL, Elshof LE, Schaapveld M, van de Vijver K, Groen EJ, Almekinders MM, et al. Clinicopathological Risk Factors for an Invasive Breast Cancer Recurrence after Ductal Carcinoma In Situ-A Nested Case-Control Study. Clinical cancer research : an official journal of the American Association for Cancer Research. 2018 Aug 1;24(15):3593-601.
3. Lari SA, Kuerer HM. Biological Markers in DCIS and Risk of Breast Recurrence: A Systematic Review. Journal of Cancer. 2011;2:232-61.
4. Yi M, Meric-Bernstam F, Kuerer HM, Mittendorf EA, Bedrosian I, Lucci A, et al. Evaluation of a breast cancer nomogram for predicting risk of ipsilateral breast tumor recurrences in patients with ductal carcinoma in situ after local excision. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012 Feb 20;30(6):600-7.
5. Rakovitch E, Nofech-Mozes S, Narod SA, Hanna W, Thiruchelvam D, Saskin R, et al. Can we select individuals with low risk ductal carcinoma in situ (DCIS)? A population-based outcomes analysis. Breast cancer research and treatment. 2013 Apr;138(2):581-90.
6. Correa C, McGale P, Taylor C, Wang Y, Clarke M, Davies C, et al. Overview of the randomized trials of radiotherapy in ductal carcinoma in situ of the breast. Journal of the National Cancer Institute Monographs. 2010;2010(41):162-77.
7. Rakovitch E, Gray R, Baehner FL, Sutradhar R, Crager M, Gu S, et al. Refined estimates of local recurrence risks by DCIS score adjusting for clinicopathological features: a combined analysis of ECOG-ACRIN E5194 and Ontario DCIS cohort studies. Breast cancer research and treatment. 2018 Jun;169(2):359-69.
8. Collins LC, Achacoso N, Haque R, Nekhlyudov L, Fletcher SW, Quesenberry CP, Jr., et al. Risk factors for non-invasive and invasive local recurrence in patients with ductal carcinoma in situ. Breast cancer research and treatment. 2013 Jun;139(2):453-60.
9. Elshof LE, Schaapveld M, Schmidt MK, Rutgers EJ, van Leeuwen FE, Wesseling J. Subsequent risk of ipsilateral and contralateral invasive breast cancer after treatment for ductal carcinoma in situ: incidence and the effect of radiotherapy in a population-based cohort of 10,090 women. Breast cancer research and treatment. 2016 Oct;159(3):553-63.
10. Marumoto T, Zhang D, Saya H. Aurora-A - a guardian of poles. Nature reviews Cancer. 2005 Jan;5(1):42-50.
11. Portier N, Audhya A, Maddox PS, Green RA, Dammermann A, Desai A, et al. A microtubule-independent role for centrosomes and aurora a in nuclear envelope breakdown. Developmental cell. 2007 Apr;12(4):515-29.
12. Wang X, Zhou YX, Qiao W, Tominaga Y, Ouchi M, Ouchi T, et al. Overexpression of aurora kinase A in mouse mammary epithelium induces genetic instability preceding mammary tumor formation. Oncogene. 2006 Nov 16;25(54):7148-58.
13. Eterno V, Zambelli A, Villani L, Tuscano A, Manera S, Spitaleri A, et al. AurkA controls self-renewal of breast cancer-initiating cells promoting wnt3a stabilization through suppression of miR-128. Scientific reports. 2016 Jun 24;6:28436.
14. Chen C, Song G, Xiang J, Zhang H, Zhao S, Zhan Y. AURKA promotes cancer metastasis by regulating epithelial-mesenchymal transition and cancer stem cell properties in hepatocellular carcinoma. Biochemical and biophysical research communications. 2017 Apr 29;486(2):514-20.
15. Liao Y, Liao Y, Li J, Li J, Fan Y, Xu B. Polymorphisms in AURKA and AURKB are associated with the survival of triple-negative breast cancer patients treated with taxane-based adjuvant chemotherapy. Cancer management and research. 2018;10:3801-08.
16. Bischoff JR, Anderson L, Zhu Y, Mossie K, Ng L, Souza B, et al. A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancers. The EMBO journal. 1998 Jun 1;17(11):3052-65.

17. Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, et al. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat Genet. 1998 Oct;20(2):189-93.
18. Zhang C, Fang Z, Xiong Y, Li J, Liu L, Li M, et al. Copy number increase of aurora kinase A in colorectal cancers: a correlation with tumor progression. Acta biochimica et biophysica Sinica. 2010 Nov;42(11):834-8.
19. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. Nat Genet. 2013 Oct;45(10):1127-33.
20. Lykkesfeldt AE, Iversen BR, Jensen MB, Ejlertsen B, Giobbie-Hurder A, Reiter BE, et al. Aurora kinase A as a possible marker for endocrine resistance in early estrogen receptor positive breast cancer. Acta oncologica. 2018 Jan;57(1):67-73.
21. Gritsko TM, Coppola D, Paciga JE, Yang L, Sun M, Shelley SA, et al. Activation and overexpression of centrosome kinase BTAK/Aurora-A in human ovarian cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2003 Apr;9(4):1420-6.
22. Zhou X, Wang P, Zhao H. The Association Between AURKA Gene rs2273535 Polymorphism and Gastric Cancer Risk in a Chinese Population. Frontiers in physiology. 2018;9:1124.
23. Casorzo L, Dell'Aglio C, Sarotto I, Risio M. Aurora kinase A gene copy number is associated with the malignant transformation of colorectal adenomas but not with the serrated neoplasia progression. Human pathology. 2015 Mar;46(3):411-8.
24. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. The New England journal of medicine. 2004 Dec 30;351(27):2817-26.
25. Ingoldsby H, Webber M, Wall D, Scarrott C, Newell J, Callagy G. Prediction of Oncotype DX and TAILORx risk categories using histopathological and immunohistochemical markers by classification and regression tree (CART) analysis. Breast. 2013 Oct;22(5):879-86.
26. Solin LJ, Gray R, Baehner FL, Butler SM, Hughes LL, Yoshizawa C, et al. A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ of the breast. J Natl Cancer Inst. 2013 May 15;105(10):701-10.
27. Ahmed W, Malik MFA, Saeed M, Haq F. Copy number profiling of Oncotype DX genes reveals association with survival of breast cancer patients. Molecular biology reports. 2018 Sep 17.
28. Moelans CB, de Wegers RA, Monsuurs HN, Maess AH, van Diest PJ. Molecular differences between ductal carcinoma in situ and adjacent invasive breast carcinoma: a multiplex ligation-dependent probe amplification study. Cellular oncology (Dordrecht). 2011 Oct;34(5):475-82.
29. Miligy IM, Gorringe KL, Toss MS, Al-Kawaz AA, Simpson P, Diez-Rodriguez M, et al. Thioredoxin-interacting protein is an independent risk stratifier for breast ductal carcinoma in situ. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2018 Jun 28.
30. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2011 Aug;22(8):1736-47.
31. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010b Jul;134(7):e48-72.
32. Rakha EA, Pinder SE, Bartlett JM, Ibrahim M, Starczynski J, Carder PJ, et al. Updated UK Recommendations for HER2 assessment in breast cancer. Journal of clinical pathology. 2015 Feb;68(2):93-9.
33. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013 Nov 1;31(31):3997-4013.

34. Fulawka L, Halon A. Ki-67 evaluation in breast cancer: The daily diagnostic practice. Indian journal of pathology & microbiology. 2017 Apr-Jun;60(2):177-84.

35. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. Clinical cancer research : an official journal of the American Association for Cancer Research. 2004 Nov 01;10(21):7252-9.

36. Dolled-Filhart M, McCabe A, Giltnane J, Cregger M, Camp RL, Rimm DL. Quantitative in situ analysis of beta-catenin expression in breast cancer shows decreased expression is associated with poor outcome. Cancer research. 2006 May 15;66(10):5487-94.

37. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature. 2012 Apr 18;486(7403):346-52.

38. Boland GP, Chan KC, Knox WF, Roberts SA, Bundred NJ. Value of the Van Nuys Prognostic Index in prediction of recurrence of ductal carcinoma in situ after breast-conserving surgery. The British journal of surgery. 2003 Apr;90(4):426-32.

39. Allegra CJ, Aberle DR, Ganschow P, Hahn SM, Lee CN, Millon-Underwood S, et al. National Institutes of Health State-of-the-Science Conference statement: Diagnosis and Management of Ductal Carcinoma In Situ September 22-24, 2009. J Natl Cancer Inst. 2010 Feb 03;102(3):161-9.

40. Gauthier ML, Berman HK, Miller C, Kozakeiwicz K, Chew K, Moore D, et al. Abrogated response to cellular stress identifies DCIS associated with subsequent tumor events and defines basal-like breast tumors. Cancer cell. 2007 Nov;12(5):479-91.

41. Zhou W, Jirstrom K, Johansson C, Amini RM, Blomqvist C, Agbaje O, et al. Long-term survival of women with basal-like ductal carcinoma in situ of the breast: a population-based cohort study. BMC cancer. 2010;10:653.

42. Bartova M, Ondrias F, Muy-Kheng T, Kastner M, Singer C, Pohlodek K. COX-2, p16 and Ki67 expression in DCIS, microinvasive and early invasive breast carcinoma with extensive intraductal component. Bratislavske lekarske listy. 2014;115(7):445-51.

43. Perez AA, Balabram D, Rocha RM, da Silva Souza A, Gobbi H. Co-Expression of p16, Ki67 and COX-2 Is Associated with Basal Phenotype in High-Grade Ductal Carcinoma In Situ of the Breast. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 2015 Jun;63(6):408-16.

44. Knopfelmacher A, Fox J, Lo Y, Shapiro N, Fineberg S. Correlation of histopathologic features of ductal carcinoma in situ of the breast with the oncotype DX DCIS score. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2015 Sep;28(9):1167-73.

45. Raldow AC, Sher D, Chen AB, Recht A, Punglia RS. Cost Effectiveness of the Oncotype DX DCIS Score for Guiding Treatment of Patients With Ductal Carcinoma In Situ. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016 Sep 12.

46. Siggelkow W, Boehm D, Gebhard S, Battista M, Sicking I, Lebrecht A, et al. Expression of aurora kinase A is associated with metastasis-free survival in node-negative breast cancer patients. BMC cancer. 2012 Nov 27;12:562.

47. Cirak Y, Furuncuoglu Y, Yapicier O, Aksu A, Cubukcu E. Aurora A overexpression in breast cancer patients induces taxane resistance and results in worse prognosis. J buon. 2015 Nov-Dec;20(6):1414-9.

48. Carvalho B, Postma C, Mongera S, Hopmans E, Diskin S, van de Wiel MA, et al. Multiple putative oncogenes at the chromosome 20q amplicon contribute to colorectal adenoma to carcinoma progression. Gut. 2009 Jan;58(1):79-89.

49. Nikonova AS, Astsaturov I, Serebriiskii IG, Dunbrack RL, Jr., Golemis EA. Aurora A kinase (AURKA) in normal and pathological cell division. Cellular and molecular life sciences : CMLS. 2013 Feb;70(4):661-87.

50. Rane SU, Mirza H, Grigoriadis A, Pinder SE. Selection and evolution in the genomic landscape of copy number alterations in ductal carcinoma in situ (DCIS) and its progression to invasive carcinoma of ductal/no special type: a meta-analysis. Breast cancer research and treatment. 2015 Aug;153(1):101-21.

51. Johnson CE, Gorringe KL, Thompson ER, Opeskin K, Boyle SE, Wang Y, et al. Identification of copy number alterations associated with the progression of DCIS

to invasive ductal carcinoma. Breast cancer research and treatment. 2012 Jun;133(3):889-98.

52. Moelans CB, de Weger RA, Monsuur HN, Vijzelaar R, van Diest PJ. Molecular profiling of invasive breast cancer by multiplex ligation-dependent probe amplification-based copy number analysis of tumor suppressor and oncogenes. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2010 Jul;23(7):1029-39.

53. Goepfert TM, Adigun YE, Zhong L, Gay J, Medina D, Brinkley WR. Centrosome amplification and overexpression of aurora A are early events in rat mammary carcinogenesis. Cancer research. 2002 Jul 15;62(14):4115-22.

54. Staff S, Isola J, Jumppanen M, Tanner M. Aurora-A gene is frequently amplified in basal-like breast cancer. Oncology reports. 2010 Feb;23(2):307-12.

55. Beltran H, Oromendia C, Danila DC, Montgomery B, Hoimes C, Szmulewitz RZ, et al. A phase II trial of the aurora kinase A inhibitor alisertib for patients with castration resistant and neuroendocrine prostate cancer: efficacy and biomarkers. Clinical cancer research : an official journal of the American Association for Cancer Research. 2018 Sep 19.

56. Li T, Chen Y, Zhang J, Liu S. LncRNA TUG1 promotes cells proliferation and inhibits cells apoptosis through regulating AURKA in epithelial ovarian cancer cells. Medicine. 2018 Sep;97(36):e12131.

57. Chou CH, Yang NK, Liu TY, Tai SK, Hsu DS, Chen YW, et al. Chromosome instability modulated by BMI1-AURKA signaling drives progression in head and neck cancer. Cancer research. 2013 Jan 15;73(2):953-66.

58. Regan JL, Sourisseau T, Soady K, Kendrick H, McCarthy A, Tang C, et al. Aurora A kinase regulates mammary epithelial cell fate by determining mitotic spindle orientation in a Notch-dependent manner. Cell reports. 2013 Jul 11;4(1):110-23.

59. D'Assoro AB, Liu T, Quatraro C, Amato A, Opyrchal M, Leontovich A, et al. The mitotic kinase Aurora--a promotes distant metastases by inducing epithelial-to-mesenchymal transition in ERalpha(+) breast cancer cells. Oncogene. 2014 Jan 30;33(5):599-610.

60. Giancotti FG. Mechanisms governing metastatic dormancy and reactivation. Cell. 2013 Nov 7;155(4):750-64.

61. Piva M, Domenici G, Iriondo O, Rabano M, Simoes BM, Comaills V, et al. Sox2 promotes tamoxifen resistance in breast cancer cells. EMBO molecular medicine. 2014 Jan;6(1):66-79.

62. Zheng FM, Long ZJ, Hou ZJ, Luo Y, Xu LZ, Xia JL, et al. A novel small molecule aurora kinase inhibitor attenuates breast tumor-initiating cells and overcomes drug resistance. Molecular cancer therapeutics. 2014 Aug;13(8):1991-2003.

63. Zheng F, Yue C, Li G, He B, Cheng W, Wang X, et al. Nuclear AURKA acquires kinase-independent transactivating function to enhance breast cancer stem cell phenotype. Nature communications. 2016 Jan 19;7:10180.

64. Otto T, Horn S, Brockmann M, Eilers U, Schuttrumpf L, Popov N, et al. Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. Cancer cell. 2009 Jan 6;15(1):67-78.

Figure Legends

Fig. 1. a: Normal breast ducto-lobular unit (x40) shows negative staining of AURKA; Fig. 1. b: Negative AURKA expression (x20) in a pure DCIS case; Fig. 1. c: Positive expression of AURKA (x20) in a pure DCIS case; Fig. 1. c: Expression of AURKA in a mixed case (x20) showing strong staining in the invasive component as well as the DCIS component.

Fig. 2: Violin plot showing differences of AURKA expression (percentage of positive nuclei) between pure DCIS and DCIS-mixed cases. The central line represents 95% confidence interval, and the central dot is the median.

Fig. 3. a: Kaplan Meier curves show that high expression of AURKA is associated with shorter local recurrence free survival (either invasive or DCIS) in breast conserving surgery (BCS) treated patients without adjuvant radiotherapy. Fig. 3. b: High expression also showed an association with shorter local recurrence free survival as invasive disease in the same cohort.

Fig. 4. a: Forest plots showing the hazard ratio of the different clinicopathological parameters and ipsilateral tumour recurrence for patients treated with breast conserving surgery based on the multivariate analysis results for all recurrences whether DCIS or invasive disease, and for invasive recurrence only Fig. 4. b.

Supplementary Fig. 1: Kaplan Meier curves showed that the probability of recurrence in cases with high AURKA expression was significantly lower compared to cases with lower expression over 20- and 10-years' time A & B). However, this was not statistically significant over 3- and 5-years' time (C & D).

Supplementary Fig. 2: Association between AURKA mRNA level and outcome in terms of breast cancer specific survival (BCSS) in the METABRIC series. The cohort was split into high and low mRNA expression based on the median (7.35).