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Aurora B expression modulates paclitaxel response in non-small cell lung cancer

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Background: Taxanes are mitotic poisons widely used in the treatment of non-small cell lung cancer (NSCLC), however, little is known about potential molecular modulators of response to these compounds. Aurora B (AURKB) is a critical regulator of the mitotic spindle assembly, previously shown overexpressed in NSCLC. Here we investigated the hypothesis that AURKB expression modulates the efficacy of taxanes in NSCLC cells.

Methods: *AURKB* mRNA expression was determined by qPCR in 132 frozen NSCLC tissues and nine NSCLC cell lines. Aurora B expression was knocked down in cell lines using multiple shRNA constructs. Barasertib was used to specifically inhibit AURKB activity, determined by the level of H3S10 phosphorylation.

Results: Frequent *AURKB* mRNA upregulation was observed in NSCLC tissues ($P < 0.0001$), being more prominent in squamous carcinomas ($P < 0.0001$). Aurora B expression in cell lines strongly correlated with sensitivity to both docetaxel ($P = 0.004$) and paclitaxel ($P = 0.007$). Aurora B knockdown derivatives consistently showed a dose-dependent association between low-AURKB expression and resistance to paclitaxel. Specific chemical inhibition of Aurora B activity also demonstrated a strong dose-dependent efficiency in triggering paclitaxel resistance.

Conclusions: Aurora B activity is an important modulator of taxane response in NSCLC cells. This may lead to further insights into taxane sensitivity of NSCLC tumours.

Lung cancer is currently responsible for almost a quarter of cancer-related deaths in the developed countries. Non-small cell lung carcinoma (NSCLC) is the most frequent type with adenocarcinoma (AdC) and squamous cell carcinoma (SqCC) being the most common subtypes (Travis *et al*, 2013). Despite the recent advances in targeted therapies for lung cancer, including agents targeting EGFR, VEGF and ALK, the therapeutic regimes currently used for NSCLC treatment are largely still based on the use of organometallics and anti-mitotics (Camidge *et al*, 2014).

Taxanes are microtubule targeting agents (MTAs), which are used to treat a variety of human cancers (Del Mastro *et al*, 2013; Isonishi *et al*, 2013; Van Veldhuizen *et al*, 2003) including lung carcinomas (Maemondo *et al*, 2014). Paclitaxel and Docetaxel represent the most prominent members of the taxane family and have manifested significant activity, mainly used as part of complex chemotherapeutic regimens (Tsao *et al*, 2011; Oh *et al*, 2013) or less frequently as monotherapy (Chu *et al*, 2005; Herbst *et al*, 2010). The cytotoxic action of these compounds is mediated primarily through their binding

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of β tubulin monomers, leading to microtubule stabilisation, thus blocking their depolymerisation and subsequently triggering cell cycle arrest at the G2/M phase (Monzo *et al*, 1999). However, many other pathways may also be involved in modulating their therapeutic effect. Thus far, the range of relevant interactions is not clear and the mechanisms related to taxane resistance acquisition are largely unexplored, in spite of their long clinical use in cancer therapeutics (Murray *et al*, 2012; Che *et al*, 2013). Although an increasing number of targeted therapeutic agents have come into clinical use (Kris *et al*, 2014), it is estimated that taxanes will still be used in chemotherapy regimens for lung cancer treatment for the foreseeable future (Cagle and Chiriac, 2012). Therefore, the identification of relevant response predictors will highly benefit clinical practice by stratifying patients into suitable currently available schemes (Cottini and Lautenschlaeger, 2013).

A number of genes have been previously implicated in taxane resistance in NSCLC. Functional p53 was shown to induce sensitivity to docetaxel (Jinturkar *et al*, 2012) and paclitaxel (Guntur *et al*, 2010) in NSCLC cell lines. A phase-III randomised trial showed that KRAS mutations decrease response of NSCLC to therapy involving paclitaxel, carboplatin and erlotinib (Eberhard *et al*, 2005). It is reasonable to hypothesise that the expression of genes involved in mitotic spindle formation may be predictors of sensitivity to taxanes. Tubulin β III mutations have been associated with resistance to taxanes in NSCLC patients (Kaira *et al*, 2013) as well as cultured lung cancer cells (Kavallaris *et al*, 1999). However, the inclusion of *TUBB3* expression levels in the prediction model for docetaxel sensitivity shows no improvement while certain drug pump genes (MRP5 and MVP) and detoxification genes appear as better predictors (Glaysner *et al*, 2009). The CHFR gene has also been suggested as a potential predictor of response of NSCLC patients to first-line therapy with carboplatin and paclitaxel (Pillai *et al*, 2013). Nevertheless, the available information on taxane efficacy predictors does not provide conclusive evidence. Considering the role of Aurora kinases in spindle formation and the reported extent of their deregulation in cancer, one may hypothesise that Aurora-dependent modulation of taxane efficiency is probable.

Aurora kinase B (AURKB) is a member of the Aurora kinase subfamily of conserved Serine/Threonine kinases, which also includes Aurora A and Aurora C. Deregulation of Aurora kinases leads to impairment of mitotic spindle checkpoints causing abnormal spindle assembly (Fu *et al*, 2007). Aurora B is encoded by the *AURKB* gene (17p13.1) and plays a key role during mitosis by regulating chromosomal alignment, segregation and cytokinesis, as the catalytic protein of the chromosomal passenger complex. Activation of Aurora B by INCENP is required for promoting transfer of the chromosomal passenger complex (INCENP–Survivin–Borealin) to the spindle midzone during mitotic exit (Xu *et al*, 2009). It is localised to the centromere and is required for kinetochore localisation and chromosome attachment to the mitotic spindle (Hauf *et al*, 2003), as well as establishing the spindle assembly checkpoint to correct anomalous chromosome-kinetochore microtubule attachment before the cell enters anaphase (Carmena *et al*, 2012).

AURKB is frequently overexpressed in NSCLC (Vischioni *et al*, 2006) and is associated with genetic instability (Smith *et al*, 2005), aneuploidy and poor patient prognosis (Takeshita *et al*, 2013). However, high Aurora B kinase expression has been associated with improved relapse-free survival in ovarian cancer patients on taxane-based therapy (Beussel *et al*, 2012). Currently, there is very limited information on the potential impact of Aurora B on taxane response in cancer cells. In this study, we confirmed the extent of *AURKB* deregulation in NSCLC and utilised *in vitro* approaches to investigate the hypothesis that

AURKB activity may modulate and thus be predictive of the sensitivity to taxanes in NSCLC.

METHODS

Primary NSCLC tumour samples. This study was undertaken in association with the Liverpool Lung Project. Appropriate Ethical approval has been acquired and all patients have provided written informed consent. 132 snap-frozen tumour samples were obtained at surgery and included in the study; 56 from AdC and 76 from SqCC. Pathological review of the sections used in this study confirmed inclusion of only specimens with >70% tumour cell content. In addition, 44 non-tumour paired surgical tissues (20 from AdC and 24 from SqCC patients) were available for analysis, taken from the distant edge of the lung resection. The clinicopathological characteristics of this cohort are provided in Supplementary Table 1. The median age of those patients was 67 (45–82); 56 of the patients were females and 77 were males.

Cell lines and growth conditions. All cell lines in this study were authenticated using the GenePrint 10 System (Promega, Southampton, UK) and analysis on a 3130 Genetic Analyzer (Life Technologies, Paisley, UK) and mycoplasma tested utilising the e-MycoTM plus Mycoplasma PCR Detection Kit (Intron Biotechnology, Gyeonggi-do, Korea). Nine NSCLC cell lines (A549, Calu3, CALU6, CRL5802, CORL23, H358, LUDLU1, SKLU1 and SK-MES1) were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/Ham's Nutrient Mixture F-12 (1:1) containing 5% foetal bovine serum (Sigma-Aldrich, Gillingham, UK). Non-tumourigenic immortalised human bronchial epithelial cells (HBEC3KT) along with their isogenic derivatives; p53 knockouts (HBEC3KT53), KRAS mutants (HBEC3KTR) and cells with both aberrations (HBEC3KTR53) were also employed in this study. Human bronchial epithelial cells were cultured in Keratinocyte-SFM medium supplemented with 50 $\mu\text{g ml}^{-1}$ bovine pituitary extract (BPE) and 5 ng ml^{-1} human recombinant epidermal growth factor (rEGF; Life Technologies). All cell lines were maintained at 37 °C, 5% CO₂.

Knockdown of *AURKB* by short hairpin RNA (shRNA). A549 and SK-MES1 cell lines were transfected with five different *AURKB*-targeting MISSION shRNA constructs (Sigma-Aldrich, clone numbers: TRCN0000000777, TRCN0000000776, TRCN0000000778, TRCN0000000779 and TRCN0000010547) along with a scrambled control construct, using Attractene Transfection Reagent (Qiagen, Manchester, UK). Stable clones were selected by exposure to Puromycin (4 nM) and knockdown efficiency was confirmed by qPCR and western blotting.

***AURKB* overexpression.** The *AURKB*-PCMV6-XL4 construct (Cat no. PCMV6XL4, SC117526 NM_004217 *AURKB*—OriGene, Rockville, MD, USA) was digested with PstI (New England Biolabs, Herts, UK) and ligated with a Blasticidin cassette-containing fragment, which was derived by SmaI digestion of the pCMV/Bsd plasmid (Catalogue no. V510-20—Life Technologies). Standard blunt end ligation was performed using T4 DNA ligase (New England Biolabs). One Shot TOP10 Chemically Competent *E. coli* cells (Invitrogen, Paisley, UK) were transformed and clones were selected for validation. The derived constructs were confirmed by dideoxyterminator sequencing (Life Technologies).

CALU3 cell line was transfected with pCMV6-XL4-*AURKB*/Bsd recombinant plasmid using Attractene Transfection Reagent (Qiagen). Stable transfectant clones were selected in the presence of 5 $\mu\text{g ml}^{-1}$ Blasticidin (Sigma-Aldrich). mRNA overexpression of *AURKB* was confirmed by qPCR.

Taxane and AZD1152 (Barasertib) exposure experiments. Depending on the growth rate of each cell line, 5×10^4 to 8×10^4 cells were seeded in each well of flat-bottomed 48-well plates in six

replicates and cultured in 500 μ l of medium. After overnight incubation, the medium was replaced with media containing a range of Paclitaxel (Sigma-Aldrich) or Docetaxel (FLUKA) concentrations (0–35 nM) and/or Barasertib (Selleck Chemicals, Munich, Germany) (0–3.2 nM). Cells were incubated for 72 h with replenishment of medium with drug at 36 h. Toxicity was measured by MTT assay. Briefly, 200 μ l of fresh medium containing 0.75 mg ml⁻¹ 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich) was added and incubated at 37 °C for 3 h. Cells were lysed using 0.04 M HCl in isopropanol and the optical density (OD) was measured at 590 nm with 630 nm as reference in a GENios plate reader (Tecan, Männedorf, Switzerland).

DNA and RNA extraction, reverse transcription and qPCR.

Deoxyribonucleic acid was extracted from cell lines and primary lung tumours using the DNeasy Blood and Tissue Kit (Qiagen). Total RNA was extracted using Direct-zol RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA). Quality and quantity of DNA and RNA were determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific). In total, 200 ng RNA was reverse transcribed using the high capacity cDNA Reverse Transcription Kit (Life Technologies). A pre-designed FAM-labelled Taqman expression assay (Hs00945858_g1—Life Technologies) was employed to analyse *AURKB* mRNA expression, with a VIC-labelled *ACTB* Taqman assay (4326315E—Life Technologies) serving as endogenous control. Real-time PCR assays were performed in triplicate using a StepOnePlus Real-Time PCR System (Life Technologies). HBEC3KT RNA was used as calibrator for calculating delta-delta cycle thresholds ($\Delta\Delta$ Ct) and relative quantification values.

Western blotting. Cultured cells were lysed in SDS-based cell lysis buffer containing protease/phosphatase inhibitors and whole cell lysates were sonicated 10 times for 5–15 s with 10 s intervening pauses (AC 100 V 50/60 Hz). Protein concentrations were determined using the BCA assay (Thermo Scientific) on a NanoDrop 2000 Spectrophotometer (Thermo Scientific). Ten to fifty microgram of total protein was reduced by NuPAGE Reducing Agent (Life Technologies) and incubated at 70 °C for 10 min.

Samples were run in NuPAGE Novex Bis-Tris Gels utilising NuPAGE Antioxidant containing NuPAGE MES Running Buffer (Life Technologies). Electroblotting on PVDF membranes was undertaken by iBlot Dry Blotting System (Life Technologies). The iBind Western System (Life Technologies) was employed for application of blocking, primary and secondary antibodies (at dilution 1:1000) and washing steps. Mouse monoclonal primary antibodies to total Histone H3 (phospho S10; ab14955) and β -actin (ab8226; Abcam) were used, while IRDye 800CW Goat Anti-Mouse (LI 925–32210) antibody (Odyssey – LI-COR Biosciences) served as secondary. The immune complexes were detected using Odyssey CLx Infrared Imaging System (Odyssey – LI-COR Biosciences).

Statistical analysis. All statistical analyses were performed using IBM SPSS statistical software version 22.0 (Armonk, NY: IBM Corp) unless otherwise stated. Covariates were assessed for compliance to normal distribution graphically using histograms and by analytical methods using Kolmogorov–Smirnov/Shapiro–Wilk tests. In the absence of normality, Mann–Whitney, Wilcoxon rank or Kruskal–Wallis was employed to assess the difference in *AURKB* mRNA expression and response to taxanes. Kaplan–Meier method was employed for survival analysis and statistical differences between groups were examined with Log-rank test. The IC₅₀ values were calculated using Prism 5 (GraphPad) in comparison to untreated cells at time 0.

RESULTS

***AURKB* mRNA is overexpressed in NSCLC tissues and cell lines.** mRNA expression profiling of *AURKB* in snap-frozen tissues demonstrated significant overexpression of the gene transcript in tumour tissue compared to the adjacent normal tissues (Mann–Whitney test, $P < 0.0001$, Figure 1a). This overexpression was more profound in SqCC than AdCs (Mann–Whitney test, $P < 0.0001$, Figure 1b). In addition, *AURKB*

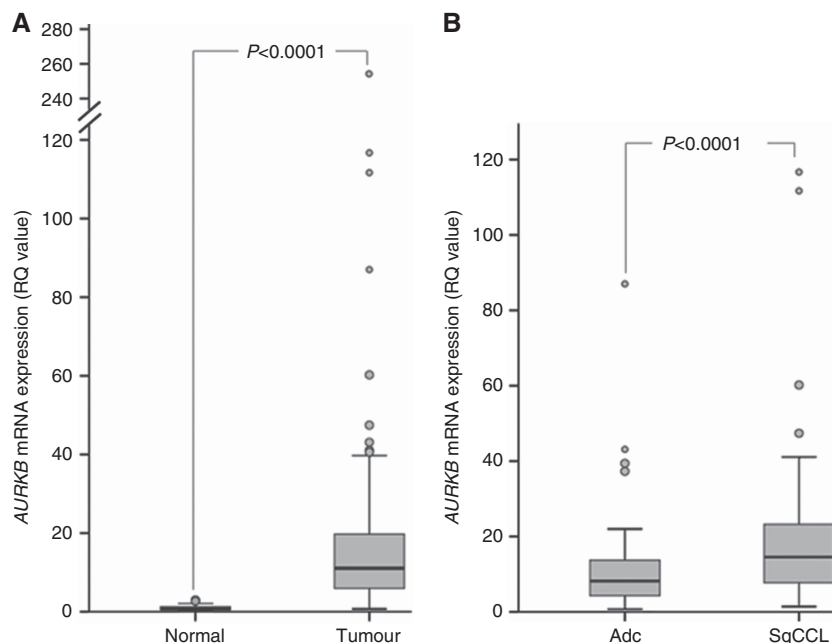


Figure 1. *AURKB* mRNA expression in primary NSCLC. Boxplots demonstrating *AURKB* mRNA expression in primary lung tumours compared to adjacent normal lung tissues (A) and in squamous cell carcinomas (SqCC) compared to adenocarcinomas (AdC) (B). P values are derived from Mann–Whitney tests. Relative quantification was calculated using RNA from the non-tumourigenic cell line HBEC3KT as a calibrator. Larger circles represent outlier values ($> 1.5 \times$ interquartile range); smaller circles represent extreme values ($> 3 \times$ interquartile range).

expression was more elevated in higher pathological T stages (T1 vs T2, $P = 0.012$, Supplementary Figure 1), however, this finding has to be treated with caution as the great majority of tumours in this sample set fall into the pT2 stage group ($n = 101$) while pT1 and pT3/4 group comprised 19 and 12 patients, respectively. This bias reflects the stage distribution among the operable NSCLC cases in the UK and therefore our access to the relevant tissue. *AURKB* upregulation did not demonstrate a significant impact on overall survival (OS) in this set, although a non-significant trend was demonstrated in adenocarcinoma patients ($P = 0.079$, Supplementary Figure 2). Survival analysis using the top and bottom terciles instead of the median did not result in a significant relationship, most probably due to the significant reduction of numbers of patients in these groups. This weak correlation prompted us to check the available data in the public domain. In there (<http://kmplot.com/analysis/index.php?p=service&cancer=lung>) when selecting all NSCLC, *AURKB* expression significantly reduces survival ($P < 10^{-16}$). However when selecting only the NSCLC group subjected to chemotherapy this correlation is not true ($P = 0.3$). No further associations were found between *AURKB* mRNA expression and age, gender, clinical stage or nodal status in our patient set.

AURKB mRNA expression profiling was also undertaken in a panel of lung cancer cell lines, along with immortalised normal human bronchial epithelial cells (HBEC3KT and its isogenic p53 knockout and *KRAS* mutant derivatives). *AURKB* expression was variable among the lung cancer cell lines, however, it was markedly higher in comparison to that of non-tumourigenic HBECs (Figure 2). It is of note that among HBECs *AURKB* mRNA expression was higher in the p53 knockout derivatives (HBEC3KTp53 and HBEC3KTRp53) while a borderline reduction was seen in *KRAS* mutants (HBEC3KTR).

Modulation of NSCLC cells resistance to taxanes by *AURKB* expression. The IC₅₀ values for paclitaxel and docetaxel toxicity among the available lung cell lines were determined following treatment with a range of concentrations (1–35 nM) of the two drugs (Supplementary Table 2). IC₅₀s for docetaxel were

consistently lower than those of paclitaxel with one exception (SKLU1 cells). Interestingly, mRNA expression of *AURKB* in NSCLC cell lines inversely correlated with resistance to both docetaxel (Spearman's test, $\rho = -0.850$, $P = 0.004$) and paclitaxel ($\rho = -0.817$, $P = 0.007$, Supplementary Table 2). We explored the hypothesis this effect simply being a correlation between taxane resistance and doubling time (Supplementary Table 3). However, our results did not demonstrate any correlation between doubling time with either *AURKB* expression (Spearman's test, $\rho = -0.533$, $P = 0.139$) or resistance to paclitaxel ($\rho = 0.333$, $P = 0.381$) or docetaxel ($\rho = 0.500$, $P = 0.170$, Supplementary Table 2).

In order to further explore the possible modulation of taxane response by *AURKB*, we investigated the resistance of lung cancer cell lines to paclitaxel by (a) knocking down *AURKB* expression and (b) chemically inhibiting its activity. Successful *AURKB* knock down clones were derived from A549 and SK-MES1 cells using different shRNA constructs. Knockdown efficiency was assessed by qPCR and western blot (Figure 3). The clones, as expected, demonstrated variable knock down efficiency. When exposing these clones to paclitaxel it was apparent that response to the drug inversely correlated to the level of *AURKB* mRNA expression in a dose-dependent manner. This was true for both clones derived from A549 and four clones derived from SK-MES1, while scrambled shRNA clones did not demonstrate altered response to paclitaxel when compared to the parental cells (Figure 3).

Additional support to the knock down experiments came from overexpressing *AURKB* in Calu3 cell line. A stable overexpressing clone derived from these cells demonstrated higher sensitivity to paclitaxel (Supplementary Figure 6).

Selective inhibition of *AURKB* desensitises NSCLC cells to paclitaxel. In order to gain additional supporting evidence, we undertook selective inhibition of Aurora B using a highly specific Aurora B inhibitor (Barasertib). After experimentally determining the IC₅₀s of Barasertib in A549, SK-MES1 and SKLU1 as 0.9 1.2 and 2.3 nM, respectively, we simultaneously exposed cells to paclitaxel and a range of Barasertib concentrations. *AURKB*

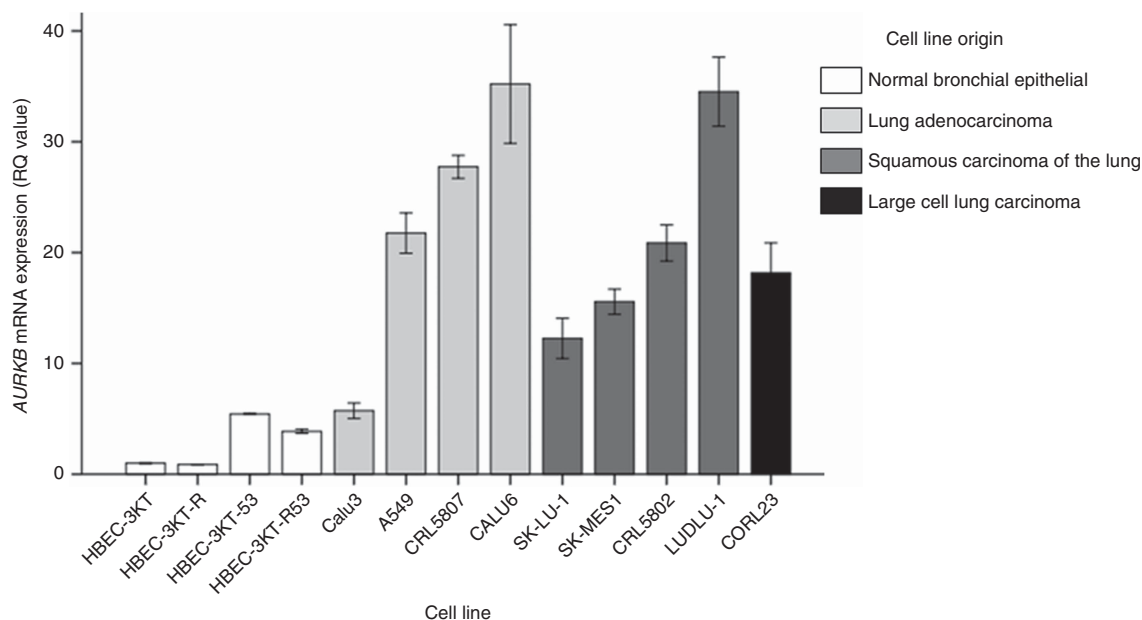


Figure 2. *AURKB* mRNA expression in human bronchial epithelial cells (HBEC) and lung cancer cell lines. Bars represent mean values from six biological repeats and error bars represent standard error of the mean. All NSCLC cell lines show a higher expression than the non-tumourigenic immortalised bronchial cells (HBEC3KT). Also, an increased *AURKB* expression is shown in the isogenic p53 derivatives (3KT-53 and 3KT-R53) of the latter.

inhibition was confirmed by measuring phosphorylation of histone 3 serine 10 (H3S10), which is on one of its prime substrates (Supplementary Figure 3). In addition, we confirmed that Barasertib exposure does not alter the mRNA expression of AURKB (Supplementary Figure 3). Barasertib-mediated AURKB inhibition clearly demonstrated a dose-dependent effect on paclitaxel efficiency; increased Barasertib concentrations resulted in increased resistance to paclitaxel in all three cell lines (Figure 4). We subsequently confirmed the Barasertib-mediated resistance to paclitaxel in the remaining NSCLC cell lines included in this study (LUDLU1, CRL5807, CRL5802, CORL23, CALU6 and CALU3) as well as all the AURKB knockdown derivatives from SKMES and A549 (Supplementary Figure 4 and 5). It is of note that in the AURKB knockdown clones the Barasertib effect, while still visible,

is reduced in comparison to the non-AURKB engineered NSCLC cell lines, as expected.

DISCUSSION

Aurora B kinase is an important contributor to the mitotic spindle assembly and its overexpression in human cancer has been frequently reported (Sorrentino *et al*, 2005; Lin *et al*, 2010; Pohl *et al*, 2011), therefore attracting significant interest in both cancer biology and therapeutics. In this study, we hypothesised that Aurora B activity may be implicated in modulating cellular response to taxanes, due to its role in mitotic spindle function, which is the target of these compounds. mRNA profiling of our

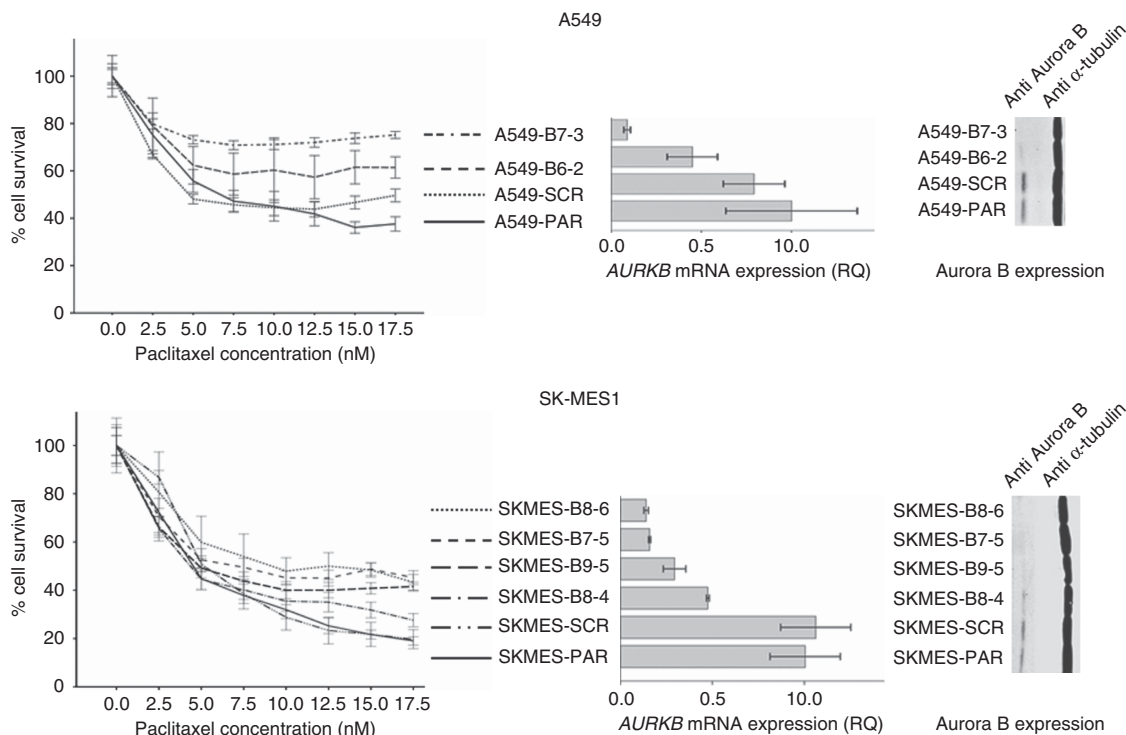


Figure 3. Paclitaxel response of lung cancer cell lines A549 and SK-MES1 and their AURKB knock down derivative clones in relation to AURKB mRNA and protein expression. Error bars in both the line graphs and the expression bar charts represent 95% CI. PAR, parental; SCR, scrambled; -Bx-y, knockdown clones where x is the shRNA construct and y is the clone number from this transfection. It is of note that the different knockdown efficiencies in the clones are associated with inverse impact on paclitaxel sensitivity.

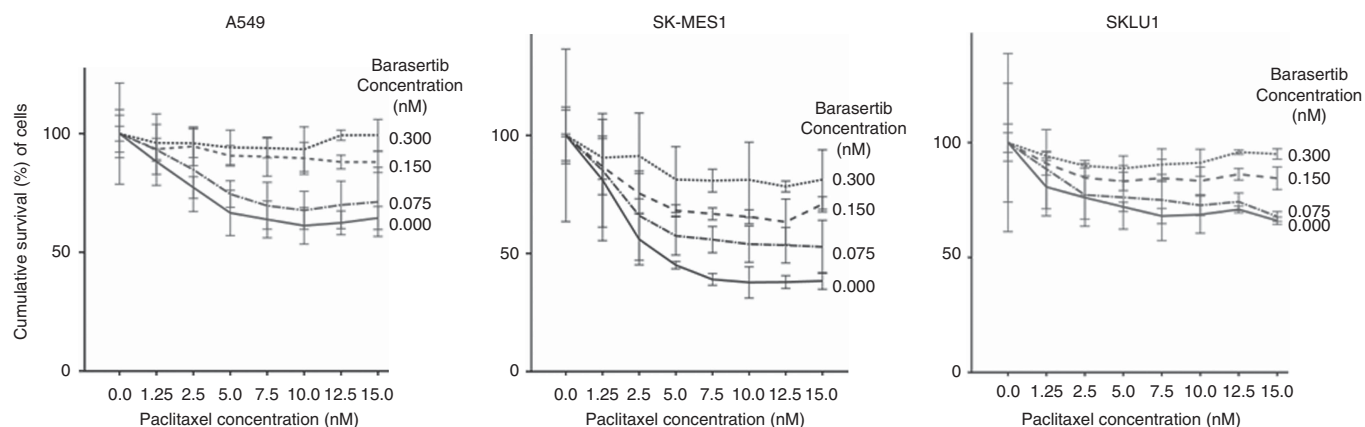


Figure 4. Sensitivity of lung cancer cell lines to paclitaxel in the presence of the highly selective Aurora B inhibitor (Barasertib). Error bars represent 95% CI. A clear quantitative effect is demonstrated where increasing concentrations of Barasertib, and therefore, inhibition of AURKB activity, is associated with increased resistance to paclitaxel.

NSCLC patient cohort confirmed the extensive overexpression of this gene, as previously reported (Takeshita *et al*, 2013; Vischioni *et al*, 2006; Smith *et al*, 2005). *AURKB* overexpression seems to be the main abnormality related with this gene in NSCLC. The relevant entries in COSMIC database (<http://cancer.sanger.ac.uk/cosmic>) report low number of point mutations (5 out of 1985, 0.2%), copy number variations (4 out of 843, 0.4%) and frequent overexpression (139 out of 1008, 13.8%). In addition to the frequency difference in histological NSCLC types, *AURKB* overexpression appeared to be weakly associated with higher pathological stages. This observation may indicate that *AURKB* expression deregulation is a progressive phenomenon; however, one has to replicate this finding in additional cohorts. Nevertheless, investigation of the data available in TCGA study (<http://www.cbioportal.org/index.do>) do not show any pT stage related differences in lung adenocarcinoma or tumour dimensions for squamous lung carcinomas (pT stage was not available in the query in this histology).

The molecular triggers behind *AURKB* overexpression in NSCLC are not yet clear. We have investigated a possible relationship between *AURKB* and *KRAS/TP53* status in the studied cancer cell lines, however no such correlation was obtained. This is not surprising, as tumour cell lines are highly abnormal and diverse at the genetic and epigenetic levels, therefore many confounding factors will influence *AURKB* expression. An interesting observation came from our HBEC isogenic lines (Figure 2), where the addition of mutant *KRAS* actually significantly reduces *AURKB* expression, either in the presence or absence of *TP53*. This is in contrast to a single report available (Dos Santos *et al*, 2016) which demonstrates upregulation of *AURKB* by *KRAS* in a different immortalised lung epithelial cell line. Therefore it is not yet clear whether *KRAS* has a positive or negative impact on *AURKB* expression. In contrast, *TP53* knockdown is clearly associated with increased *AURKB* levels. While no evidence for direct transcriptional inhibition of *AURKB* by *p53* exists, a possible mechanism is through *c-myc*. *p53* represses, through induction of *miR-145 c-myc* (Sachdeva *et al*, 2009), which is known to induce expression of both *AURKA* and *AURKB* (den Hollander *et al*, 2010).

AURKB mRNA overexpression alone did not correlate with overall survival, in agreement with two previous studies (Smith *et al*, 2005; Perumal *et al*, 2012), although the latter has suggested that *AURKB* expression can be part of a prognostic multi-gene signature. In contrast, immunohistochemical analysis of *AURKB* protein expression was reported to correlate with poor prognosis (Vischioni *et al*, 2006; Takeshita *et al*, 2013) suggesting that stability and/or correct localisation of the protein may also be important.

AURKB mRNA expression was higher in all lung cancer cell lines tested in comparison to HBECs, as has been previously described (Hayama *et al*, 2007). The most significant observation of this study is the inverse correlation found in NSCLC cell lines between *AURKB* mRNA expression and sensitivity to both tested taxanes, with low levels of *AURKB* being associated with taxane resistance. The hypothesis that this change in resistance might be simply the reflection of a reduction in doubling times was disproved, as no such correlation was demonstrated. In order to verify the inverse correlation found between the endogenous *AURKB* levels and taxane resistance, we knocked down *AURKB* mRNA expression in two lung cancer cell lines. This knockdown consistently triggered a marked increased resistance to paclitaxel. Moreover, it was observed that different knock down efficiency in each clone led to a different level of resistance to paclitaxel, showing for the first time a quantitative effect between expression of this gene and modulation of resistance to a taxane in human cancer cells.

In order to exclude possible off-target effects of genetic manipulation, we used a highly selective *AURKB* inhibitor

(Barasertib or AZD1152), which was reported to have a 3700-fold higher specificity for *AURKB* inhibition in comparison to *AURKA* (Yang *et al*, 2007; Walsby *et al*, 2008). This experiment also demonstrated a clear dose-dependent increase of resistance to paclitaxel with increasing concentrations of Barasertib in all cell lines tested. Barasertib effect, as hypothesised, was moderated when tested in *AURKB* knockdown clones, due to the already reduced availability of *AURKB* protein in these clones. This data is in contrast to a previous report suggesting that Aurora B inhibition sensitises cells to antimetabolic agents (Tanaka *et al*, 2007). However, that study utilised a generic kinase inhibitor AT9283, which inhibits Aurora A and other kinases, such as *Jak2*, *Jak3* and *Abl* kinase as well as Aurora B (Curry *et al*, 2009). It is well known that Aurora A inhibition enhances chemosensitivity to paclitaxel in pancreatic cancer cells (Lin *et al*, 2012), to docetaxel in human oesophageal squamous cell carcinoma (Isham *et al*, 2013) and ovarian cancer cells (Scharer *et al*, 2008), therefore this 4. sensitisation can be most probably attributed to *AURKA* inhibition. The association of high Aurora B kinase expression sensitivity to taxane-based therapy has been also shown for ovarian cancer (Beussel *et al*, 2012). In addition, miRNA-dependent knockdown of *AURKB* also results in resistance to taxanes in an *in vivo* breast cancer model (Winsel *et al*, 2014). Therefore the mechanism behind resistance to paclitaxel therapy due to *AURKB* mitotic checkpoint abrogation is not limited to NSCLC and appears to be getting well established.

Possible mechanisms to explain the effect of Aurora B on taxane efficiency may be drawn from a number of previous observations. Paclitaxel acts by perturbation of mitotic spindle assembly and activating the spindle assembly checkpoint (Zachos *et al*, 2007; Hung *et al*, 2008). This leads to a cell death response triggered through *p53*-independent apoptotic pathways that depend upon maintaining mitotic arrest (Huang *et al*, 2009). If mitotic slippage occurs, evasion of apoptosis is allowed, mainly due to the stabilisation of anti-apoptotic proteins (Wertz *et al*, 2011). The use of Aurora B inhibitors (Ditchfield, 2003; Hauf *et al*, 2003), (Vader *et al*, 2007; Girdler *et al*, 2008), deactivation of Aurora B (Wang *et al*, 2008) or depletion of Aurora B activating proteins (Vader *et al*, 2007; Petsalaki *et al*, 2011) have shown to relieve mitotic arrest caused by paclitaxel. Taken together, these studies provide further evidence for the central role of Aurora B function for spindle checkpoint activation and cell death in response to low-dose paclitaxel and further support our findings.

Concerning the evidence so far, *AURKB* expression presents with a potential paradox: Its overexpression reduces survival in the chemotherapy naïve patients but appears to have a beneficial effect in patients treated with taxane regimens. The latter may draw additional support from the loss of the very strong correlation between *AURKB* and NSCLC patient survival available online and mentioned in the results section, when limiting the question in the chemotherapy recipient group. Of course 'chemotherapy' term in there is inclusive of every scheme used but nevertheless raises the question for investigating *AURKB* involvement in the resistance modulation of other common chemotherapeutics.

The present study clearly demonstrated a role for Aurora B in the response of NSCLC cells to paclitaxel and provided unique evidence for a dose-dependent association. Given the large extent of *AURKB* deregulation in NSCLC, these findings suggest that assessing the levels of *AURKB* protein in surgical samples and/or biopsy material could assist in the clinical decision tree for managing patients with NSCLC and have potential for development as a predictive biomarker. Unfortunately, chemotherapy data were not available for our tissue cohort, thus we could not investigate any correlation between *AURKB* expression and response to taxanes in this clinical set. Our findings, however, highlight the need for conducting such studies in large cohorts of NSCLC patients. Moreover, it may advise against the use of

AURKB inhibitors in regimens involving taxanes and this should be further investigated in clinical studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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