**Table 2** Clinicopathological variables in hepatocellular carcinoma in relation to Aurora kinase B expression

| | Negative ($n = 42$) | Positive ($n = 25$) | $P$ |
|---|---|---|---|
| Recurrence | | | < 0·001† |
| No | 27 | 5 | |
| Yes, meeting criteria | 10 | 3 | |
| Yes, exceeding criteria | 5 | 17 | |
| Tumour number (primary) | | | 0·029† |
| 1–2 | 37 | 16 | |
| ≥ 3 | 5 | 9 | |
| Tumour size (primary) (cm)* | 4·3(0·5) | 7·1(1·1) | 0·009‡ |
| Histological differentiation (primary) | | | 0·084† |
| Good | 12 | 2 | |
| Moderate | 17 | 10 | |
| Poor | 13 | 13 | |
| Portal vein invasion (primary) | | | < 0·001† |
| No | 28 | 5 | |
| Yes | 14 | 20 | |
| Hepatic vein invasion (primary) | | | 0·045§ |
| No | 40 | 19 | |
| Yes | 2 | 6 | |
| Growth (primary) | | | 0·649† |
| Expansive | 28 | 18 | |
| Invasive | 14 | 7 | |
| Arterial invasion (primary) | | | 0·373§ |
| No | 42 | 24 | |
| Yes | 0 | 1 | |
| Capsule formation (primary) | | | 0·427† |
| No | 16 | 12 | |
| Yes | 26 | 13 | |
| Capsule formation with cancer cell infiltration | | | 0·774† |
| No | 22 | 14 | |
| Yes | 20 | 11 | |
| TNM stage (primary) | | | 0·003† |
| I | 5 | 0 | |
| II | 17 | 2 | |
| III | 13 | 10 | |
| IVA | 6 | 10 | |
| IVB | 1 | 3 | |
| α-Fetoprotein ($\times 10^4$ ng/ml)* | 1·9(1·8) | 2·6(1·2) | 0·816‡ |
| α-Fetoprotein lectin 3 (%) | | | |
| ≤ 10 | 14 | 6 | 0·419† |
| > 10 | 28 | 19 | |
| PIVKA-II ($\times 10^4$ mAU/ml)* | 2·2(1·8) | 6·0(4·8) | 0·393‡ |

*Values are mean(s.e.m.). TNM, tumour node metastasis; PIVKA, protein induced by vitamin K absence or antagonists; AU, arbitrary units. †$\chi^2$ test; ‡Student's $t$ test; §Fisher's exact test.

factor in both the six-factor model and the reduced three-factor model.

## Higher genomic instability in Aurora kinase B-positive cases

The extent of the genome affected was defined as the FGA, as represented by the clones on the array for the

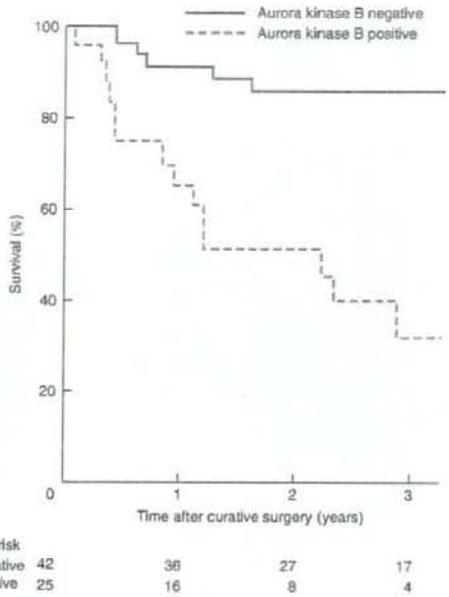


**Fig. 3** Cumulative survival curves of patients with primary hepatocellular carcinoma negative or positive for Aurora kinase B protein expression. $P < 0·001$ (log rank test)

**Table 3** Logistic regression models for prediction of recurrence exceeding the Milan criteria

| Features of primary HCC | Coefficient | Odds ratio | $P$ |
|---|---|---|---|
| Six-factor model | | | |
| Expression of Aurora kinase B (no *versus* yes) | 2·18 | 8·84 (1·21, 64·56) | 0·031 |
| No. of tumours (1–2 *versus* ≥ 3) | 1·61 | 4·98 (0·35, 70·19) | 0·234 |
| Tumour size (≤ 5 *versus* > 5 cm) | 0·67 | 1·95 (0·19, 19·9) | 0·571 |
| Portal vein invasion (no *versus* yes) | 1·78 | 5·95 (0·36, 97·85) | 0·212 |
| Hepatic vein invasion (no *versus* yes) | −0·41 | 0·66 (0·03, 15·13) | 0·797 |
| TNM stage (I/II/III *versus* IV) | 1·19 | 3·29 (0·26, 41·12) | 0·356 |
| Three-factor model* | | | |
| Expression of Aurora kinase B (no *versus* yes) | 2·17 | 8·77 (1·57, 49·78) | 0·009 |
| Tumour size (≤ 5 *versus* > 5 cm) | 0·81 | 2·25 (0·29, 20·29) | 0·471 |
| Portal vein invasion (no *versus* yes) | 0·95 | 2·58 (0·35, 18·88) | 0·349 |

Values in parentheses are 95 per cent confidence intervals. *Three factors selected by stepwise variable selection. HCC, hepatocellular carcinoma; TNM, tumour node metastasis.

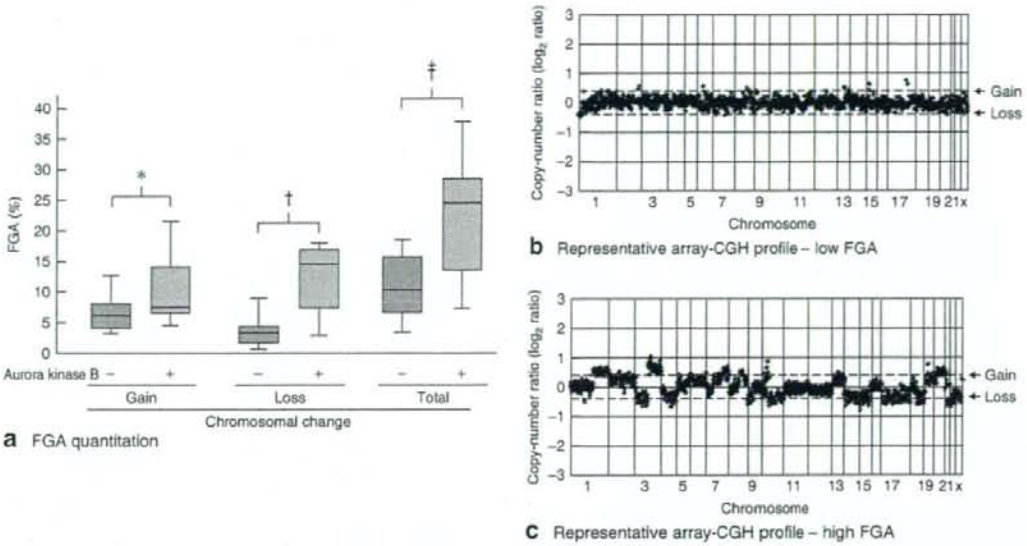


**Fig. 4** **a** Fraction of genome altered (FGA) determined by array-based comparative genomic hybridization (array-CGH) in ten primary hepatocellular carcinomas (HCCs) without and nine with Aurora kinase B immunoreactivity. Horizontal bars within boxes, boxes and error bars represent median, interquartile range and range respectively. *$P = 0{\cdot}221$, †$P = 0{\cdot}009$, ‡$P = 0{\cdot}011$ (Mann–Whitney $U$ test). **b,c** Representative array-CGH profiles from samples demonstrating **b** low FGA (3·4 per cent) or **c** high FGA (30·5 per cent) which showed negative and positive Aurora kinase B immunoreactivity respectively. Profiles are copy-number alterations relative to normal genomic DNA ordered by chromosome, with each spot representing one of the duplicated clones. The thresholds for determining chromosomal gain and loss were defined as $\log_2$ ratio more than + 0·4 and less than − 0·4 respectively[15]

19 primary HCCs in which high-quality DNA, suitable for an array-CGH analysis, were available. Nine tumours with Aurora kinase B immunoreactivity had a median FGA of 7·3, 14·4 and 24·5 per cent in gained, lost and total alterations respectively, whereas the ten without Aurora kinase B immunoreactivity had a median FGA of 5·8, 3·2 and 10·1 per cent respectively (*Fig. 4*). The FGA values for lost and total alterations in the Aurora kinase B-positive cases were significantly higher than those in the negative cases, suggesting that greater Aurora kinase B expression may contribute to the genomic instability in HCC.

## Discussion

Although surgical procedures and perioperative care for patients with HCC have improved markedly, tumour recurrence remains a major obstacle to better survival. Repeat hepatectomy and salvage transplantation have both been proposed as treatment options for recurrent tumours meeting the Milan criteria[18,19]. Indeed, hepatic resection is performed as an initial treatment, followed by salvage transplantation when recurrence develops and/or liver function has deteriorated. Correct prediction of the type of recurrence at the initial operation would be useful in the selection of optimal adjuvant therapy[3,9].

In the present study a microarray technique was used to determine whether prediction of aggressive recurrence is possible, and several cytokinesis-associated genes were found to be highly upregulated in the primary HCC, resulting in recurrence exceeding the criteria. Of these, Aurora kinase B was identified as the most significant predictor of such recurrence. Univariable analysis revealed that Aurora kinase B protein expression was significantly related to aggressive recurrence and to prognosis, and additional multivariable analyses confirmed that Aurora kinase B protein was the only statistically significant predictor of such recurrence.

Aurora kinase B is a chromosomal passenger serine/threonine protein kinase that regulates accurate chromosomal segregation, cytokinesis, protein localization to the centromere and kinetochore, correct microtubule–kinetochore attachments, and regulation

of the mitotic checkpoint[20]. It localizes first to the chromosomes during the prophase and then to the inner centromere region between the sister chromatids during the prometaphase and metaphase. During the prometaphase, Aurora kinase B is responsible for the correct localization and stabilization of the centromeric proteins, including Borealin, Bub1, the inner centromeric protein and survivin/baculoviral inhibitor of apoptosis repeat-containing 5 (BIRC5)[21]. Interestingly, the present microarray analysis revealed that other chromosome passenger proteins, including Bub1 and survivin/BIRC5, were also significantly overexpressed in primary HCCs with an aggressive pattern of recurrence (*Appendix*, available as supplementary material online at http://www.bjs.co.uk). One of the essential functions of Aurora kinase B is in mitotic spindle attachment. Because of its vital role in correcting chromosome–spindle attachment, deregulated expression of Aurora kinase B can be expected to impair chromosome segregation and mitotic progression, leading to aneuploidy.

Overexpression of Aurora kinase B has been reported in a variety of malignant cancers[22,23]. Several studies have used xenograft models of localized tumour formation in mice injected with cells that overexpress Aurora kinase B to demonstrate that such overexpression induces oncogenic transformation[23,24]. In these studies, oncogenic transformation appeared to be mediated by aneuploidy, and to be a consequence of Aurora kinase B overexpression in relation to genomic instability. Similarly, in the present study genomic instability was closely associated with Aurora kinase B overexpression in HCC. Future investigations should focus on the direct ability of genomic instability to progress malignant phenotypes of cancer cells, as well as on the identification of 'stem cell-ness' in the genetics[8,25].

Aurora serine/threonine kinases have recently received increasing attention as eligible targets for molecular cancer therapy[26]. Aurora kinase B inhibition results in abnormal kinetochore–microtubule attachments, failure to achieve chromosomal biorientation and failure of cytokinesis. These *in vitro* effects were greater in transformed cells than in either non-transformed or non-dividing cells[27]. Thus, targeting the Aurora kinases may achieve *in vivo* selectivity for cancer. Given their preclinical antitumour activity and potential for tumour selectivity, small-molecule pan inhibitors and even selective inhibitors of Aurora kinase B have been developed, such as MK0457/VX-680[28] and AZD1152[29]. Clinical trials of these and other Aurora kinase inhibitors are ongoing. The recent finding of an indispensable requirement for Aurora kinase B in polyploid cells[30] indicates that ploidy-specific lethality might represent the basis for a strategy to identify new cancer therapies.

This small study has identified Aurora kinase B as a predictor of the aggressive recurrence of HCC based on a genome-wide microarray study. Patients identified as being at high risk of aggressive recurrence should be followed up closely. This approach to the stratification of patients with HCC after surgical resection might provide an avenue to more rational clinical trials of postoperative adjuvant therapy using Aurora kinase inhibitors[31].

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