Cellular Oncology 31 (2009) 31–39
DOI 10.3233/CLO-2009-0457
IOS Press

Genomic profiling by array comparative genomic hybridization reveals novel DNA copy number changes in breast phyllodes tumours

Arno Kuijper [a], Antoine M. Snijders [b,c], Els M.J.J. Berns [d], Vibeke Kuenen-Boumeester [e], Elsken van der Wall [f], Donna G. Albertson [b,c,g] and Paul J. van Diest [a,*]

[a] *Department of Pathology, University Medical Centre Utrecht, Utrecht, The Netherlands*
[b] *Cancer Research Institute, University of California San Francisco, San Francisco, CA, USA*
[c] *Comprehensive Cancer Centre, University of California San Francisco, San Francisco, CA, USA*
[d] *Department of Medical Oncology, Erasmus MC, Rotterdam, The Netherlands*
[e] *Department of Pathology, Erasmus MC, Rotterdam, The Netherlands*
[f] *Division of Internal Medicine and Dermatology, University Medical Centre Utrecht, Utrecht, The Netherlands*
[g] *Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, USA*

Abstract. Breast phyllodes tumour (PT) is a rare fibroepithelial tumour. The genetic alterations contributing to its tumorigenesis are largely unknown. To identify genomic regions involved in pathogenesis and progression of PTs we obtained genome-wide copy number profiles by array comparative genomic hybridization (CGH).

DNA was isolated from fresh-frozen tissue samples. 11 PTs and 3 fibroadenomas, a frequently occurring fibroepithelial breast tumour, were analyzed. Arrays composed of 2464 genomic clones were used, providing a resolution of ∼1.4 Mb across the genome. Each clone contains at least one STS for linkage to the human genome sequence.

No copy number changes were detected in fibroadenomas. On the other hand, 10 of 11 PT (91%) showed DNA copy number alterations. The mean number of chromosomal events in PT was 5.5 (range 0–16) per case. A mean of 2.0 gains (range 0–10) and 3.0 losses (range 0–9) was seen per case of PT. Three cases showed amplifications. DNA copy number change was not related to PT grade. We observed recurrent loss on chromosome 1q, 4p, 10, 13q, 15q, 16, 17p, 19 and X. Recurrent copy number gain was seen on 1q, 2p, 3q, 7p, 8q, 16q, 20.

In this study we used array CGH for genomic profiling of fibroepithelial breast tumours. Whereas most PT showed chromosomal instability, fibroadenomas lacked copy number changes. Some copy number aberrations had not previously been associated with PT. Several well-known cancer related genes, such as *TP53* and members of the *Cadherin*, reside within the recurrent regions of copy number alteration. Since copy number change was found in all benign PT, genomic instability may be an early event in PT genesis.

Keywords: Breast, phyllodes tumour, fibroadenoma, array CGH

1. Introduction

Phyllodes tumour (PT) is a biphasic tumour of the breast, i.e. composed of an epithelial and a stromal component. With an incidence of 2.1 per 1 million women per year [1], PT is a rare tumour. Based on several characteristics of the overgrowing stroma, PTs are graded as benign, borderline or malignant [2]. Depending on the grade of the primary tumour, local recurrences can be seen in 8–65% of cases [3]. Moreover, up to 25% of malignant cases metastasize, mostly to the lung [2].

Several biological factors have been implicated in

*Corresponding author: Paul J. van Diest, MD, PhD, Professor of Pathology, Department of Pathology, University Medical Centre Utrecht, P.O. Box 85500, 3508 GA, Utrecht, The Netherlands. Tel.: +31 30 250 6565; Fax: +31 30 254 4990; E-mail: P.J.vanDiest@umcutrecht.nl.

the pathogenesis and progression of PT. With higher tumour grade, PTs show overexpression of p53 [4,5], EGFR [6] and HIF-1α [7]. Furthermore, p53 accumulation was found to predict prognosis [4]. Little is known about the genetic alterations contributing to PT genesis, however. Conventional cytogenetic studies using short term culture and G-banding failed to detect recurrent PT-specific aberrations [8,9]. Using CGH, a technique which screens the whole genome for DNA copy number alterations, Lu et al. found recurrent gain of 1q and loss of 3p in PT. In addition, gain of 1q was shown to be predictive of clinical behaviour [10]. Another group narrowed the minimal overlapping region down to 1q21–q23, but was unable to confirm its relation to clinical behaviour [11].

Recently, CGH was refined by the introduction of microarray based CGH (array CGH) [12,13]. In contrast to chromosome based CGH, which uses metaphase chromosomes as hybridization targets, array CGH uses genomic clones (BAC, P1, cosmid or cDNAs) or oligonucleotides as targets. The genomic resolution of chromosome based CGH is approximately 10–20 Mb, whereas the resolution of array CGH is mainly dependent on the genomic distance between the arrayed DNA elements and the size of the elements [12]. Besides this superior resolution, array CGH allows direct mapping of alterations to the human genome sequence [12,14]. These properties facilitate the identification of candidate oncogenes and tumour suppressors participating in tumour formation and progression [15]. Acquiring genomic profiles of PT by array CGH may identify chromosomal alterations involved in PT tumorigenesis. Further, comparing different tumour grades may reveal genomic regions and genes contributing to progression of PT towards a malignant phenotype.

2. Material and methods

2.1. Tissue

Fresh frozen tissue samples, which were used anonymously [16], were retrieved from the tissue banks of our hospitals. 11 PT, 10 primary tumours and one recurrence, were studied. Microscopically, benign PT may resemble fibroadenoma, a frequently occurring benign fibroepithelial breast tumour. We therefore analyzed three fibroadenomas as controls. Two 4 μm sandwich H&E cryosections were examined to identify normal tissue and for grading of the tumour. PT was graded according to the criteria of Moffat et al. [2]. In brief, based on the degree of stromal overgrowth, margin infiltration, stromal cellularity, stromal atypia and number of mitosis, tumours were graded as benign, borderline or malignant. Mitotic figures were counted using established criteria in ten consecutive fields at $400\times$ magnification [17]. The percentage stroma and epithelium was estimated for each case. Blocks containing areas of normal tissue were trimmed. Ten μm sections were cut for DNA isolation. DNA extraction was performed using affinity columns (QIAmp Tissue Kit, QIAgen Inc.) with modifications to the manufacturer's protocol [18].

2.2. Array CGH

Array CGH was carried out as described previously [14] using arrays of 2464 BAC clones printed in triplicate (HumArray 2.0). Approximately 300 ng of tumour and reference DNA were labelled by random priming (BioPrime DNA labelling, Gibco BRL) to incorporate Cy3 dCTP and Cy5 dCTP (Amersham Pharmacia Biotech). Images were acquired with a custom build CCD camera system [19] resulting in 16 bit 1024×1024 pixel DAPI, Cy3 and Cy5 images. Imaging processing was performed using two custom programs, SPOT and SPROC software packages (http://www.jainlab.org/downloads.html) [20]. The normalized $\log_2$ transformed fluorescence ratio generated for each spot is associated with a BAC clone mapped on the July 2003 freeze of the draft sequence of the human genome (http://genome.ucsc.edu). Fluorescence ratios of clones for which only one of the triplicate values remained after SPROC analysis or for which the standard deviation was >0.2 were rejected from further analysis.

DNA copy number profiles were visually inspected and DNA copy number gains or losses were called when two interpreters (AK and AMS) independently identified a genomic region as either gained or lossed. Low-level single BAC clone alterations were not counted as real events. An amplification was defined by a single clone or group of clones with a normalized $\log_2$ transformed fluorescence ratio of 1.0 or larger, with the graph showing a peak rather than a plateau. Multiple gains, losses and amplifications on a chromosome arm were counted as separate events.

3. Results

Array CGH was performed on 5 benign, one borderline and 5 malignant tumours. A mean of 253 (range 153–479) clones per hybridization were rejected from final analysis. Not surprisingly, as stromal overgrowth is a diagnostic criterion, the stroma was the dominant component in PT, comprising more than 75% of the tumour (mean 87%, range 78–99%). Ten of 11 PT (91%) showed chromosomal aberrations, whereas fibroadenomas displayed no copy number changes at all. Even low-level single BAC clone alterations were absent in fibroadenomas. The mean number of chromosomal events, i.e. the sum of all gains, losses and amplifications, was 5.5 (range 0–16) per case of PT. A mean of 2.0 gains (range 0–10) and 3.0 losses (range 0–9) was seen per case of PT. The relation between tumour grade and DNA copy number changes was not significant. Surprisingly, case 1, a benign PT, harboured the highest number of genomic alterations and may therefore responsible for the lack of correlation between grade and copy number change. We considered misclassification, but after reevaluation benign grade still remained appropriate for this case.

Detailed information on copy number alterations per case is displayed in Table 1. A schematic overview of the distribution of chromosomal aberrations per case is given in Fig. 1. We observed recurrent losses on chromosome 1q, 4p, 10, 13q, 15q, 16, 17p, 19 and X. Recurrent copy number gains were seen on 1q, 2p, 3q, 7p, 8q, 16q, 20.

Amplifications were seen infrequently (3 of 11 PT). One malignant PT (case 7, Table 1) displayed a complex cluster of 3 amplicons on chromosome 5q (Fig. 2). The proximal amplicon, located at 5q11.2, harbours interleukin 6 signal transducer isoform 1 (*IL6ST)* and *MAP3K1*. *PIK3R1* maps to the second amplicon at 5q12, whereas no known cancer-related genes map to the distal amplicon at 5q13. A primary PT (case 9, Table 1) and its corresponding recurrence (case 10, Table 1) shared an amplification at 22q11.22. Genes mapping to this amplicon include *PPM1F*, *TOP3B*, *MAPK1*, *PIK4CA* and *PRAME.*

Losses at 16q were observed in 5 of 11 PT. One PT showed loss of the 16q-arm and two PTs showed loss of the entire chromosome 16, whereas two other tumours showed focal loss at 16q. Combining these results revealed two minimal regions of deletion; one at 16q22–23 (4.4 Mb), the other distally at 16q24-tel (6.8 Mb). Recurrent copy number loss was found at chromosome 13q (3/11 PT), overlapping at 13q13–q14.3 (12.2 Mb). Loss at chromosome 19 was detected in 3 of 11 PT. Interestingly, losses at 19q never included the *CCNE1* region, which remained at normal copy number. Further, loss at chromosome 17p, overlapping at 17p12–p13 (14.9 Mb), was found in 2 of 11 PT.

DNA copy number gains were detected on chromosome 7p (2/11 PT) and chromosome 8q (2/11 PT). In previous reports gain at 1q was a prominent alteration [10,11,21]. In our work, two (18%) PT showed gain of the complete 1q arm.

4. Discussion

In the present study, genomic profiling by array CGH revealed chromosomal instability in the majority of PT (91%). PT grade and copy number alterations were not related. In line with previous studies [21–23], not a single chromosomal copy number alteration was found in 3 fibroadenomas. The differences in clinical behaviour between fibroadenoma and PT therefore seem to be reflected at the genomic level. We here describe novel regions of altered DNA copy number in PT, some of which mapped to regions harbouring well known oncogenes or tumour suppressor genes.

Since both fibroadenomas and PT are biphasic tumours, a separate analysis of epithelium and stroma would be preferable. However, since the epithelium is mostly one-layered (like normal breast epithelium), harvesting sufficient DNA for array CGH is very difficult. In addition, genomic amplification techniques such as DOP-PCR need further optimization. In the present study we, therefore, analyzed each tumour as a whole. Since array CGH reliably detects copy number change against a background of up to 50% contaminating cells [24], previous studies allowed a maximum of 25% contaminating cells [25,26]. Because the epithelial component comprises less than 25% of the whole tumour, our results reflect copy number changes of the stroma, which usually is the progressive component of PT and therefore most interesting to study. Further refinement of amplification techniques may make whole genome screening of the epithelial component feasible.

Ultimately, the effect of copy number change at the gene expression level determines the impact of individual chromosomal alterations on tumour progression. This relation between gene dosage and expression is obvious in oncogenic activation by DNA sequence amplification, such as *HER2*. Chromosomal amplifications were rare and were found exclusively

Table 1
Summary of DNA copy number changes in fibroepithelial breast tumours

Case	Lesion	Gains	Losses	Amplifications
1	Be	2p11.2–p13 (9 Mb)	7q11.1–q21.1 (12 Mb)	–
		7p14–p15.2 (5.5 Mb)	10	
		7p11.2–p13 (9 Mb)	13q12.1–q14.3 (31.2 Mb)	
		8q24.1–q24.2 (8.1 Mb)	15q11.2–q21.3 (30 Mb)	
		12p11.2-ptel (30.7 Mb)	16q22-qtel (14.2 Mb)	
		15q23-qtel (33.2 Mb)	17p11.2-ptel (19.5 Mb)	
		16q12.1–q22 (16.4 Mb)		
		18p12–p11.21 (2.4 Mb)		
		18q		
		20		
2	Be	1q	–	–
		16		
3	Be	–	19p13.12-ptel (14.3 Mb)	–
			19q13.2–q13.4 (14.8 Mb)	
4	Be	–	19p	–
			19q12-qtel (29 Mb)	
5	Be	1	–	–
6	Bo	20	6	–
7	Ma	3q23-qtel (61.3 Mb)	5q11.2–q32 (88.4 Mb)	5q11.2
		5q34 (1.4 Mb)	5q34–q35 (7.6 Mb)	5q13.1
		8q	8p11.2–p23.1 (37.6 Mb)	5q14.1
			16q21–q23 (8.9 Mb)	
			16q24-qtel (5.3 Mb)	
			17p12–p13 (14.8 Mb)	
			19p13.1-ptel (21 Mb)	
			19q13.1-qtel (21 Mb)	
			21q22.1–q22.2 (6.6 Mb)	
8	Ma	–	–	–
9	Ma	–	1q41-qtel (17.4 Mb)	22q11.2
			4p11–p16.2 (39.1 Mb)	
			13q14.2–q32 (50.4 Mb)	
			16	
			X	
10	Ma	–	1q42-qtel (17.4 Mb)	22q11.2
			4p13-ptel (38.1 Mb)	
			13q13-qtel (72.5 Mb)	
			16	
			22q13.2-qtel (10.2 Mb)	
			X	
11	Ma	2	9p21 (5.8 Mb)	–
		3q	10	
		7p	16q	
		7q21.11–q21.3 (18.9 Mb)		
		7q31.1-qtel (51.7 Mb)		
12	Fa	–	–	–
13	Fa	–	–	–
14	Fa	–	–	–

Be: benign PT; Bo: borderline PT; Ma: malignant PT; FA: fibroadenoma. Size of gains and losses are displayed in parentheses when not comprising a complete arm.

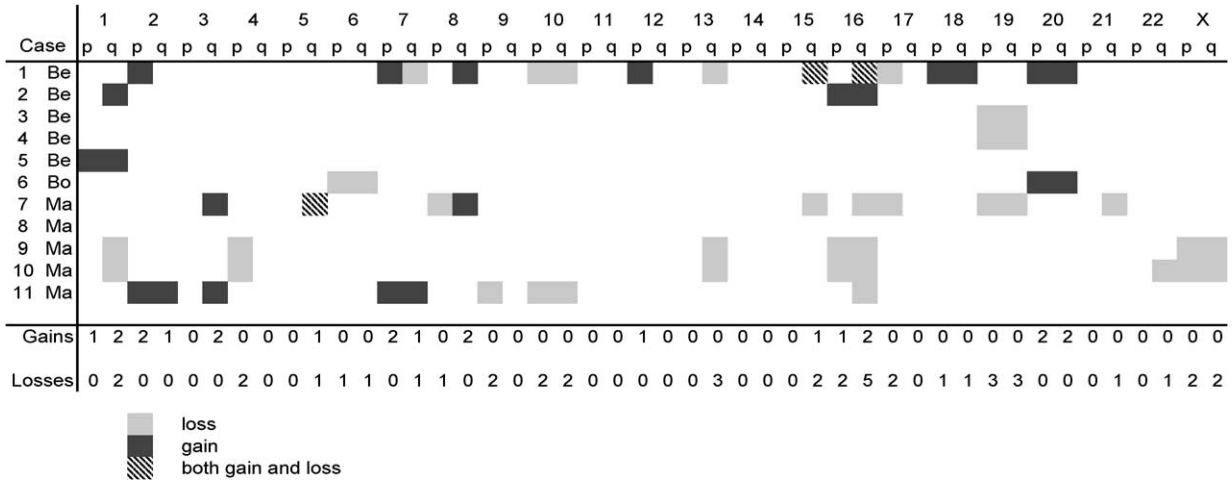

Fig. 1. Schematic overview of affected chromosomal regions in 11 phyllodes tumours of the breast. Amplifications are not shown here. Be: benign grade phyllodes tumour; Bo: borderline; Ma: malignant.

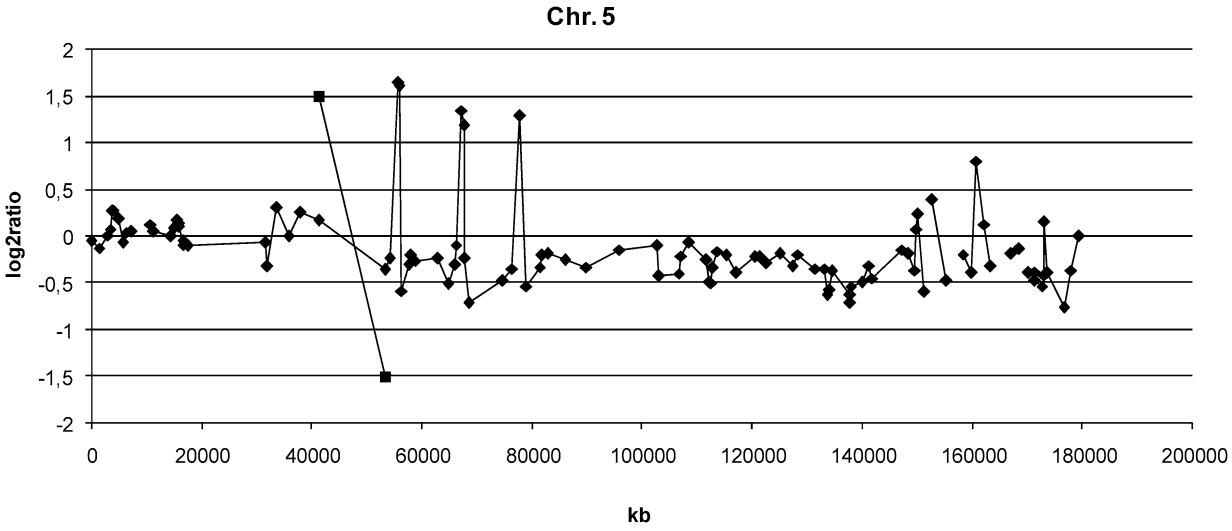

Fig. 2. Although amplifications are rare in PT, this malignant PT showed 3 amplifications on proximal 5q. Shown are the normalized $\log_2$ ratios of clones on chromosome 5 ordered by position in the genome. Copy number loss was seen in between the amplifications. The distal peaks on 5q were counted as a gain. The vertical bar represents the location of the centromere.

in malignant PTs, in keeping with the general notion that amplifications are more prevalent at advanced tumour stage [27]. One malignant PT displayed a complex cluster of three amplicons with similar amplitudes on 5q (Fig. 2), suggesting that these regions are co-amplified and physically present in one amplicon. The fact that no known cancer related genes are present in the third amplicon can thus be explained. The mechanisms behind the formation of these complex amplicons remain elusive, though. Two previous studies found no amplifications in PT [10,11]. Very recently, Jones et al. analyzed 40 PTs by array CGH and detected 5 amplifications in borderline and malignant grade tumours [21]. All this emphasizes that high-level amplifications are infrequent. On the other hand, low-level copy number changes were frequently found. The relevance of these alterations has been disputed for a long time. The influence of modest copy number change on gene expression was recently demonstrated in genome-wide experiments [28,29]. Further, low-level copy number gain of *PIK3CA* results in its increased expression [30,31]. Therefore, the low-level changes described here might result in a survival advantage to the cell. We included low-level changes

composed of multiple altered BAC clones, but chose to exclude single BAC clone changes. Although these alterations may reflect genetic instability and may be counted as real events [32,33], single clone changes may be a result of clone mismapping or inadequate hybridization. Some changes may have been underestimated by this approach (such as 8q and 13q alterations), but we feel we have focused on the biologically most relevant changes for the tumour.

Two previous studies used chromosomal CGH to obtain genomic profiles of PT [10,11]. On the whole, we found more chromosomal events per case with array CGH. Further, we added novel regions of DNA copy number change (e.g. loss at chromosome 19) to those of presumed importance in tumorigenesis of PT. The resolution and sensitivity of array CGH enabled us to pick up small regions of altered DNA copy number, which in chromosome CGH may be masked by averaging effects. All studies, including the present, are hampered by suboptimal sample size due the low incidence of PT and may, therefore, be liable to sampling bias. Previously, we detected a positive relation between tumour grade and cell cycle deregulation in the stroma of PT [4]. Deregulation of the cell cycle machinery may lead to loss of genetic integrity and accumulation of chromosomal alterations at higher grade. In the study by Jones et al. grade and genomic instability were indeed related [21]. Like Jee et al. [11], we did not find a significant relation between copy number changes and tumour grade, however. We found copy number alterations in all benign PTs, suggesting that genomic instability is an early event in PT tumorigenesis. The presence of unbalanced chromosomal aberrations in benign grade tumours may be surprising. Benign PTs, however, have the capacity to recur and progress to higher grade. The genomic imbalances in these "benign" tumours may reflect these characteristics. In some respects "benign" grade is, therefore, somewhat misleading. "True benign" fibroadenomas did not show any alterations, demonstrating that genomic instability is not a general feature of fibroepithelial tumours.

Overall, loss at 16q was the most frequent chromosomal aberration found in our study. Two distinct regions of copy number loss were identified. The proximal overlapping region harbours *CDH1* (E-cadherin), *CDH3*, *CTCF*, *NQO1* and *TERF2*. Cancer related genes mapping to the distal region of overlap include *CDH15* (M cadherin), *CDK10*, *FANCA* and *GAS8*. Losses at 16q were described by Jones et al. as well [21]. Several members of the cadherin family may be involved in 16q loss. Although loss of 16q and E-cadherin expression play major roles in the pathogenesis of lobular breast cancer [34], E-cadherin seems to be of minor relevance in PT or fibroadenomas [35]. Cadherin-family members 3 and 15 also map to these regions of loss. The role of the cadherin family in fibroepithelial tumours is unclear, but loss of cellular cohesion may reflect an early step towards the capacity to disseminate.

We firstly describe recurrent losses at chromosome 19 in PT. Candidate tumour suppressor genes mapping to the areas of copy number loss include *XRCC1* and *BAX* (Bcl-2 associated X protein). *BAX* is a pro-apoptotic gene functioning as a tumour-suppressor gene [36]. Reduced levels of BAX mRNA have been described in invasive breast cancer as compared to normal breast tissue [37] and reduced immunostaining has been associated with shorter times to tumour progression and overall survival [38]. In a previous work BAX expression was found in three of 19 PTs, whereas it was absent in normal mammary stroma [5]. This suggests that in fibroepithelial tumours baseline BAX expression differs from that in epithelial malignancies and that expression of BAX is the pathological state. Furthermore, losses at 19q did not seem to include the *CCNE1* region, suggesting the presence of negative selection pressures against loss of this region. All this implicates other chromosome 19 genes as more likely candidates in PT development.

Whole arm gain of 1q was the most common alteration in previous studies [10,11,21]. The incidence was lower in our group, being present in two benign tumours (18%). In the largest study, 1q gain was found exclusively in borderline and malignant tumours [21], while others, like us, showed it in benign tumors as well [10,11]. Lu et al. demonstrated that gain of 1q was predictive of clinical behavior [10], but others could not confirm this [11]. These differences are most likely related to intratumoural heterogeneity [21] and/or selection bias related to the low incidence of PT. Still, 1q gain is a common alteration in PT and may be an important event in PT progression. Many cancer related genes reside on 1q, including *MUC1*, *HDGF*, *MDM4*, *WNT3A* and *AKT3*, but the contribution of the individual genes to PT genesis is currently unknown.

Additional data on tumour suppressor and oncogene expression to which we can relate our current findings is sparse. Recently, we demonstrated that TP53 overexpression is related to tumour grade and predicts prognosis, hereby establishing the importance of the TP53-axis in PT [4]. Mutations in *TP53* have been described as well [5,39]. Not surprisingly therefore, we

found a region of common loss at 17p12–p13, which contains *TP53*. In addition, a recent study described copy number loss at 17p13 in 8 of 40 tumours [21]. However, 17p13 loss was not accompanied by TP53 immunostaining or mutation, suggesting that 17p13 loss may not involve *TP53* or alternatively reflect loss of only one allele, with deletion of the other allele with further disease progression [21]. Interestingly, recurrent losses at 16q may involve the TP53 stabilizing gene *NQO1*. Lack of NQO1, leading to increased proteasomal degradation of TP53 [40], may disable the TP53 pathway as well. Previously, we found that loss of RB1 expression was not related to grade or survival [4]. However, immunoquantification was problematic due to marked intratumoural heterogeneity. In the present study, we found loss at 13q13–q14.3 (12.2 Mb) containing *RB1* in three tumours, including one malignant PT and its recurrence. In addition, isolated BAC clone loss was seen in two tumours. Losses at 13q were described previously as well [10,11,21]. These findings may indicate a more prominent role for *RB1* loss than suggested by immunohistochemistry. cMYC expression has been demonstrated in the stroma of PT [41]. Gain at 8q24.1–q24.2 (9.1 Mb) was found in two tumours, with *MYC* situated in the minimal region of overlap. In addition, one tumour showed isolated BAC clone loss here. Lu et al. previously described gain at this region [10]. Although copy number gain may play a role in cMYC expression, it has been demonstrated that this is not the major mechanism of cMYC expression in PT since a minority of tumours showed additional copies of *MYC* as determined by FISH [41]. In this light, recurrent losses at 16q22–q23 which contain the candidate tumour suppressor gene *CTCF* are interesting. CTCF was found to be a transcriptional repressor of *MYC* [42]. Deletions at its corresponding locus have been commonly observed in breast and prostate cancer [43]. Loss of *CTCF* copy number may therefore influence cMYC expression levels.

The importance of the EGFR pathway in PT progression has recently been reported [6,44]. Overexpression of EGFR was found more frequently at higher grade and was related to both amplification of the CA repeat in intron 1 of *EGFR* and to *EGFR* whole gene amplification. Intron 1 CA repeat amplification was present in 42% of cases, whereas whole gene amplification was detected in 16% of tumours. Comparable to the latter, we found gain at 7p with the minimal overlapping region (9.2 Mb) containing *EGFR* in 2 of 11 tumours (18%). Caveolin-1 and eps15 are involved in EGFR signalling and turnover and their expression was found to be related to intron 1 CA repeat amplification [44]. This may be indicative of an important molecular pathway driving PT progression. We found no copy number alterations at the corresponding loci of caveolin-1 and eps15, suggesting that mechanisms other than gene dosage are responsible for increased expression of these genes.

In conclusion, fibroadenomas lacked copy number alterations, whereas chromosomal instability was found in all but one PT. Copy number change was observed in all PT grades, suggesting that genomic instability is an early event in PT genesis. Several areas of recurrent copy number change harbour well-known oncogenes or tumour suppressor genes. With the development of arrays with overlapping clones and contiguous coverage of the genome [45], it will become possible to pin-point the most relevant changes even further.

Acknowledgements

We thank members of the UCSF Comprehensive Cancer Centre Microarray Shared Resource, Greg Hamilton, Anthony Lam and Facility Manager, Randy Davis for providing BAC arrays and Jane Fridlyand of the Department of Epidemiology and Biostatistics for her help with analysis of the data. This work was supported by NIH grants CA90421 and CA101359 and by the First Aegon International Scholarship in Oncology.

References

[1] L. Bernstein, D. Deapen and R.K. Koss, The descriptive epidemiology of malignant cystosarcoma phyllodes tumour of the breast, *Cancer* **71** (1993), 3020–3024.

[2] C. Moffat, S. Pinder, A. Dixon, R. Blamey and I. Ellis, Phyllodes tumours of the breast: a clinicopathologic review of thirty-two cases, *Histopathology* **27** (1995), 205–218.

[3] R.J. Barth Jr., Histologic features predict local recurrence after breast conserving therapy of phyllodes tumours, *Breast Cancer Res. Treat.* **57** (1999), 291–295.

[4] A. Kuijper, R.A.I. de Vos, J.H. Lagendijk, E. van der Wall and P.J. van Diest, Progressive deregulation of the cell cycle with higher tumour grade in the stroma of breast phyllodes tumours, *Am. J. Clin. Pathol.* **123** (2005), 690–698.

[5] V. Kuenen-Boumeester, S.C. Henzen-Logmans, M.M. Timmermans, I.L. van Staveren, A. van Geel, H.J. Peterse, J. Bonnema and E.M. Berns, Altered expression of p53 and its regulated proteins in phyllodes tumours of the breast, *J. Pathol.* **189** (1999), 169–175.

[6] C. Kersting, A. Kuijper, H. Schmidt, J. Packeisen, C. Leidtke, N. Tidow, C. Gustmann, B. Hinrichs, J. Tio, W. Boecker, P.J. van Diest, B. Brandt and H. Buerger, Amplifications of the epidermal growth factor receptor gene (EGFR) are common in phyllodes tumours of the breast and are associated with tumour progression, *Lab. Invest.* **86** (2006), 54–61.

[7] A. Kuijper, P. van de Groep, E. van der Wall and P.J. van Diest, Role and prognostic relevance of HIF-1α and its downstream targets in fibroepithelial tumours of the breast, *Breast Cancer Res.* **7** (2005), R808–R818.

[8] C.U. Dietrich, N. Pandis, G. Bardi, M.R. Teixeira, T. Soukhikh, C. Petersson, J.A. Andersen and S. Heim, Karyotypic changes in phyllodes tumours of the breast, *Cancer Genet. Cytogenet.* **76** (1994), 200–206.

[9] C.U. Dietrich, N. Pandis, H. Rizou, C. Petersson, G. Bardi, H. Qvist, N. Apostolikas, P.J. Bohler, J.A. Andersen, I. Idvall, F. Mitelman and S. Heim, Cytogenetic findings in phyllodes tumours of the breast: karyotypic complexity differentiates between malignant and benign tumours, *Hum. Pathol.* **28** (1997), 1379–1382.

[10] Y.J. Lu, S. Birdsall, P. Osin, B. Gusterson and J. Shipley, Phyllodes tumours of the breast analyzed by comparative genomic hybridization and association of increased 1q copy number with stromal overgrowth and recurrence, *Gen. Chromosom. Cancer* **20** (1995), 275–281.

[11] K.J. Jee, G. Gong, S. Hyun Ahn, J. Mi Park and S. Knuutila, Gain in 1q is a common abnormality in phyllodes tumours of the breast, *Anal. Cell. Pathol.* **25** (2003), 89–93.

[12] D. Pinkel, R. Segraves, D. Sudar, S. Clark, I. Poole, D. Kowbel, C. Collins, W.L. Kuo, C. Chen, Y. Zhai, S.H. Dairkee, B.M. Ljung, J.W. Gray and D.G. Albertson, High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays, *Nat. Genet.* **20** (1998), 207–211.

[13] S. Solinas-Toldo, S. Lampel, S. Stilgenbauer, J. Nickolenko, A. Benner, H. Dohner, T. Cremer and P. Lichter, Matrix-based comparative genomic hybridization: biochips to creen for genomic imbalances, *Gen. Chromosom. Cancer* **20** (1997), 399–407.

[14] A.M. Snijders, N. Nowak, R. Segraves, S. Blackwood, N. Brown, J. Conroy, G. Hamilton, A.K. Hindle, B. Huey, K. Kimura, S. Law, K. Myambo, J. Palmer, B. Ylstra, J.P. Yue, J.W. Gray, A.N. Jain, D. Pinkel and D.G. Albertson, Assembly of microarrays for genome-wide measurement of DNA copy number, *Nat. Genet.* **29** (2001), 263–264.

[15] D.G. Albertson, B. Ylstra, R. Segraves, C. Collins, S.H. Dairkee, D. Kowbel, W.L. Kuo, J.W. Gray and D. Pinkel, Quantative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene, *Nat. Genet.* **25** (2000), 144–146.

[16] P.J. van Diest, J.P.A. Baak, P. Matze-Cok, E.C. Wisse-Brekelmans, C.M. van Galen, P.H. Kurver, S.M. Bellot, J. Fijnheer, L.H. van Gorp and W.S. Kwee, Reproducibility of mitosis counting in 2469 breast cancer specimens: results from the Multicenter Morphometric Mammary Carcinoma Project, *Hum. Pathol.* **23** (1992), 603–607.

[17] P.J. van Diest, For and against: No consent should be needed for using leftover body material for scientific purposes, *BMJ* **325** (2002), 648–651.

[18] M.M. Weiss, M.A.J.A. Hermsen, G.A. Meijer, N.C.T. van Grieken, J.P. Baak, E.J. Kuipers and P.J. van Diest, Comparative genomic hybridization, *Mol. Pathol.* **52** (1999), 243–251.

[19] G. Hamilton, N. Brown, V. Oseroff, B. Huey, R. Segraves, D. Sudar, J. Kumler, D. Albertson and D. Pinkel, A large field CCD system for quantitative imaging of microarrays, *Nucleic Acids Res.* **34** (2006), e58.

[20] A.N. Jain, T.A. Tokuyasu, A.M. Snijders, R. Segraves, D.G. Albertson and D. Pinkel, Fully automatic quantification of microarray data, *Genome Res.* **12** (2002), 325–332.

[21] A.M. Jones, R. Mitter, R. Springall, T. Graham, E. Winter, C. Gillett, A.M. Hanby, The Phyllodes Tumour Consortium, I.P.M. Tomlinson and E.J. Sawyer, A comprehensive genetic profile of phyllodes tumours of the breast detects important mutations, intra-tumoral genetic heterogeneity and new genetic changes on recurrence, *J. Pathol.* **214** (2008), 533–544.

[22] T. Ried, K.E. Just, H. Holtgreve-Grez, S. du Manoir, M.R. Speicher, E. Schrock, C. Latham, H. Blegen, A. Zetterberg, T. Cremer and G. Auer, Comparative genomic hybridization of formalin-fixed, paraffin-embedded breast tumours reveals different patterns of chromosomal gains and losses in fibroadenomas and diploid and aneuploid carcinomas, *Cancer Res.* **55** (1995), 5415–5423.

[23] L.R. Cavalli, D.A. Cornelio, R.S. Lima, C.A. Urban, J.D. Rone, I.J. Cavalli and B.R. Haddad, Lack of DNA copy number alterations revealed with comparative genomic hybridization in fibroadenomas of the breast, *Cancer Genet. Cytogenet.* **153** (2004), 173–176.

[24] D.G. Albertson, Profiling breast cancer by array CGH, *Breast Cancer Res. Treat.* **78** (2003), 289–298.

[25] M.M. Weiss, E.J. Kuipers, C. Postma, A.M. Snijders, I. Siccama, D. Pinkel, J. Westerga, S.G. Meuwissen, D.G. Albertson and G.A. Meijer, Genomic profiling of gastric cancer predicts lymph node status and survival, *Oncogene* **22** (2003), 1872–1879.

[26] M.M. Weiss, E.J. Kuipers, C. Postma, A.M. Snijders, M. Stolte, M. Vieth, D. Pinkel, S.G. Meuwissen, D.G. Albertson and G.A. Meijer, Genome wide array comparative genomic hybridisation analysis of premalignant lesions of the stomach, *Mol. Pathol.* **56** (2003), 293–298.

[27] T. Ried, K. Heselmeyer-Haddad, H. Blegen, E. Schrock and G. Auer, Genomic changes defining the genesis, progression, and malignancy potential in solid human tumours: a phenotype/genotype correlation, *Gen. Chromosom. Cancer* **25** (1999), 195–204.

[28] E. Hyman, P. Kauraniemi, S. Hautaniemi, M. Wolf, S. Mousses, E. Rozenblum, M. Ringner, G. Sauter, O. Monni, A. Elkahloun, O.P. Kallioniemi and A. Kallioniemi, Impact of DNA amplification on gene expression patterns in breast cancer, *Cancer Res.* **62** (2002), 6240–6245.

[29] J.L. Phillips, S.W. Hayward, Y. Wang, J. Vasselli, C. Pavlovich, H. Padilla-Nash, J.R. Pezullo, M. Ghadimi, G.D. Grossfeld, A. Rivera, M. Linehan, G.R. Cunha and T. Ried, The consequences of chromosomal aneuploidy on gene expression profiles in a cell line model for prostate carcinogenesis, *Cancer Res.* **61** (2001), 8143–8149.

[30] R. Redon, D. Muller, K. Caulee, K. Wanherdrick, J. Abecassis and S. du Manoir, A simple specific pattern of chromosomal aberrations at early stages of head and neck sqaumous carcino-

mas: PIK3CA but not p63 gene as a target of 3q26-qter gains, *Cancer Res.* **61** (2001), 4122–4129.

[31] L. Shayesteh, Y. Lu, L. Kuo, R. Baldocchi, T. Godfrey, C. Collins, D. Pinkel, D. Powell, G.B. Mills and J.W. Gray, PIK3CA is implicated as an oncogene in ovarian cancer, *Nat. Genet.* **21** (1999), 64–65.

[32] J.A. Veltman, J. Fridlyand, S. Pejavar, A.B. Olshen, J.E. Korkola, S. DeVries, P. Carroll, W.L. Kuo, D. Pinkel, D. Albertson, C. Cordon-Cardo, A.N. Jain and F.M. Waldman, Array-based comparative genomic hybridization for genome-wide screening of DNA copy number in bladder tumours, *Cancer Res.* **63** (2003), 2872–2880.

[33] H. van Dekken, P.L. Paris, D.G. Albertson, J.C. Alers, A. Andaya, D. Kowbel, T.H. van der Kwast, D. Pinkel, F.H. Schroder, K.J. Vissers, M.F. Wildhagen and C. Collins, Evaluation of genetic patterns in different tumour areas of intermediate-grade prostatic adenocarcinomas by high-resolution genomic array analysis, *Gen. Chromosom. Cancer* **39** (2004), 249–256.

[34] J.E. Etzel, S. Devries, K. Chew, C. Florendo, A. Molinaro, B.M. Ljung and F.M. Waldman, Loss of chromosome 16q in lobular carcinoma *in situ*, *Hum. Pathol.* **32** (2001), 292–296.

[35] A.C. Han, A.P. Soler, K.A. Knudsen and H. Salazar, Distinct cadherin profiles in special variant carcinomas and other tumours of the breast, *Hum. Pathol.* **30** (1999), 1035–1039.

[36] C. Yin, S.J. Korsmeyer and T. van Dyke, Bax suppresses tumorigenesis and stimulates apoptosis *in vivo*, *Nature* **385** (1997), 637–640.

[37] Z. Gatalica, S. Finkelstein, E. Lucio, O. Tawfik, J. Palazzo, B. Hightower and E. Eyzaguirre, p53 protein expression and gene mutation in phyllodes tumors of the breast, *Pathol. Res. Pract.* **197** (2001), 183–187.

[38] R.C. Bargou, C. Wagener, K. Bommert, M.Y. Mapara, P.T. Daniel, W. Arnold, M. Dietel, H. Guski, H. Feller, H.D. Royer and B. Dorken, Overexpression of the death-promoting gene bax-alpha which is downregulated in breast cancer restores sensitivity to different apoptotic stimuli and reduces tumour growth in SCID mice, *J. Clin. Invest.* **97** (1996), 2651–2659.

[39] S. Krajewski, C. Blomqvist, K.Franssila, M. Krajewska, V.M. Wasenius, E. Niskanen, S. Nordling and J.C. Reed, Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma, *Cancer Res.* **55** (1995), 4471–4478.

[40] G. Asher, J. Lotem, B. Cohen, L. Sachs and Y. Shaul, Regulation of p53 stability and p53-dependent apoptosis by NADH quinoneoxidoreductase 1, *Proc. Natl. Acad. Sci. USA* **98** (2001), 1188–1193.

[41] E.J. Sawyer, R. Poulsom, F.T. Hunt, R. Jeffery, G. Elia, I.O. Ellis, P. Ellis, I.P.M. Tomlinson and A.M. Hanby, Malignant phyllodes tumours show stromal overexpression of c-myc and c-kit, *J. Pathol.* **200** (2003), 59–64.

[42] G.N. Filippova, S. Fagerlie, E.M. Klenova, C. Myers, Y. Dehner, G. Goodwin, P.E. Neiman, S.J. Collins and V.V. Lobanenkov, An exceptionally conserved transcriptional repressor, CTCF, employs different combinations of zinc fingers to bind diverged promoter sequences of avian and mammalian c-myc oncogenes, *Mol. Cell. Biol.* **16** (1996), 2802–2813.

[43] G.N. Filippova, A. Lindblom, L.J. Meincke, E.M. Klenova, P.E. Neiman, S.J. Collins, N.A. Doggett and V.V. Lobanenkov, A widely expressed transcription factor with multiple DNA sequence specificity, CTCF, is localized at chromosome segment 16q22.1 within one of the smallest regions of overlap for common deletions in breast and prostate cancers, *Gen. Chromosom. Cancer* **22** (1998), 26–36.

[44] K. Agelopoulos, C. Kersting, E. Korshing, H. Schmidt, A. Kuijper, C. August, P. Wulfing, J. Tio, W. Boecker, P.J. van Diest, B. Brandt and H. Buerger, *EGFR* amplification specific gene expression in phyllodes tumours of the breast, *Cell. Oncol.* **29** (2007), 443–451.

[45] A.S. Ishkanian, C.A. Malloff, S.K. Watson, R.J. DeLeeuw, B. Chi, B.P. Coe, A. Snijders, D.G. Albertson, D. Pinkel, M.A. Marra, V. Ling, C. MacAulay and W.L. Lam, A tiling resolution DNA microarray with complete coverage of the human genome, *Nat. Genet.* **36** (2004), 299–303.