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Original Paper

EGFR amplification and lack of activating mutations in metaplastic breast carcinomas

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Abstract

Metaplastic breast carcinomas are reported to harbour epidermal growth factor receptor (EGFR) overexpression in up to 80% of the cases, but *EGFR* gene amplification is the underlying genetic mechanism in around one-third of these. In this study, *EGFR* gene amplification as defined by chromogenic *in situ* hybridization and protein overexpression was examined in a cohort of 47 metaplastic breast carcinomas. Furthermore, the presence of activating *EGFR* mutations in exons 18, 19, 20, and 21 was investigated. Thirty-two cases showed EGFR overexpression and of these, 11 (34%) harboured *EGFR* gene amplification. In addition, *EGFR* amplification showed a statistically significant association with EGFR overexpression ($p < 0.0094$) and was restricted to carcinomas with homologous metaplasia. Ten cases, five with and five without *EGFR* amplification, were subjected to microarray-based CGH, which demonstrated that *EGFR* copy number gain may occur by amplification of a discrete genomic region or by gains of the short arm of chromosome 7 with a breakpoint near the *EGFR* gene locus, the minimal region of amplification mapping to *EGFR, LANCL2*, and *SEC61G*. No activating *EGFR* mutations were identified, suggesting that this is unlikely to be a common alternative underlying genetic mechanism for EGFR expression in metaplastic breast carcinomas. Given that metaplastic breast carcinomas are resistant to conventional chemotherapy or hormone therapy regimens and that tumours with *EGFR* amplification are reported to be sensitive to EGFR tyrosine kinase inhibitors, these findings indicate that further studies are warranted to explore EGFR tyrosine kinase inhibitors as potential therapeutic agents for metaplastic breast carcinomas harbouring amplification of 7p11.2.

Keywords: breast cancer; chromogenic *in situ* hybridization; microarrays; gene mutation; immunohistochemistry

Introduction

The gene for the epidermal growth factor receptor (*EGFR*) maps to 7p11.2–p12 and comprises 28 exons [1], which encode a protein containing an extracellular ligand-binding domain, a transmembrane domain, and a tyrosine kinase domain [1]. EGFR was the first tyrosine kinase transmembrane receptor to be directly linked with human cancer [1]. In recent years, EGFR tyrosine kinase inhibitors have received FDA approval and are currently being tested in patients with lung, gastric, and breast cancer [2]. There appear to be distinct mechanisms for EGFR activation in different types of human neoplasms. *EGFR* gene amplification has been described in oligodendrogliomas [3], glioblastomas [4], lung carcinomas [2,5,6], gastric carcinomas [7], and, recently, breast carcinomas [8–11]. On the other hand, *EGFR* activating mutations have proven to be present in a subset of central nervous system tumours and lung cancer [1,5,12], but are remarkably rare in breast cancer cell lines and human breast cancer samples [8].

Metaplastic breast carcinomas (MBCs) account for up to 3.7% of all breast carcinomas. We and others have demonstrated that these neoplasms consistently harbour a basal/myoepithelial phenotype [13–19],

therefore suggesting that they may be part of the morphological spectrum of 'basal-like' breast carcinomas.

Our group and others have shown that MBCs consistently overexpress EGFR but usually lack HER2 overexpression and amplification [11,13,20,21]. In a preliminary study, we demonstrated that in 37% of EGFR 3+ MBCs, the underlying genetic mechanism for *EGFR* overexpression is gene amplification [11]. However, molecular mechanisms for EGFR overexpression in the majority of cases are yet to be identified.

The aim of this study was to investigate the prevalence of EGFR overexpression, *EGFR* gene amplification, and activating mutations in the tyrosine kinase domain of this gene in a cohort of 47 MBCs. In addition, we studied the *EGFR* amplicon in detail in ten cases by means of a comparative genomic hybridization (CGH) array with a 0.6 Mb resolution on chromosome 7 to determine whether these gains were specific to *EGFR* or whether they represented polysomy of chromosome 7.

Materials and methods

Case selection

Cases of MBC were retrieved from the pathology files of the Royal Marsden Hospital, London, UK; IPATIMUP, Porto, Portugal; Laboratório Salomão & Zoppi, São Paolo, Brazil; the Norwegian Radium Hospital, Montebello, Norway; and the Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. This project was approved by the Local Ethics Committees.

All cases were initially reviewed by the contributing authors, who used additional immunohistochemical markers to corroborate the diagnosis. The cases were centrally reviewed by three of the authors (JSRF, FM, and FCS) on a multi-headed microscope and classified according to previously described criteria [11,13,22–28] into four groups: matrix-producing breast carcinomas [26]; spindle cell carcinomas [25]; carcinomas with heterologous elements [24,27]; and carcinomas with squamous differentiation [23,28].

Immunohistochemistry

EGFR overexpression was analysed immunohistochemically using the monoclonal antibody 31G7 (Zymed) essentially as previously described [11]. EGFR immunostaining was analysed by three of the authors (JSRF, FM, and FCS) on a multi-headed microscope according to the Herceptest® (Dako) scoring system. Results on EGFR overexpression in 23 cases included in the present study have been previously described [11].

Chromogenic *in situ* hybridization (CISH)

CISH was performed using Spot-Light amplification probes for *EGFR* (Zymed), according to the manufacturer's protocol and as previously described [11]. As the interpretation guidelines for the Spot-Light *EGFR* amplification probe have been previously validated [8], we did not use an α-satellite probe for chromosome 7. Signals were evaluated at $\times$400 and $\times$630 by three of the authors (FM, SC, and JSR-F) and at least 60 cells were counted for the presence of the *EGFR* probe. A given area was considered to be amplified for *EGFR* when more than 50% of the neoplastic cells harboured (i) more than 5 signals per nuclei or (ii) large gene copy clusters [8,11]. Results on *EGFR* amplification in 23 cases included in the present study have been previously described [11].

DNA extraction

Representative sections of each tumour were microdissected with a sterile needle under a stereo microscope to ensure a purity of at least 70% of neoplastic cells as previously described [29].

Mutation analysis

Exon-specific primers were designed and DNA was subjected to PCR amplification of exons 18, 19, 20, and 21 of the *EGFR* gene. Primer sequences are shown in Table 1. PCR reactions were performed in a final volume of 25 µl, with the following composition: 1$\times$ Buffer (Bioron, Germany); 1.25 mM $MgCl_2$ (Bioron, Germany) for exon 18 and 1.5 mM $MgCl_2$ for exons 19, 20, and 21; 200 µM dNTPs (Fermentas, USA); 0.3 µM of each primer (MWG Biotech, Germany); and 1 unit of Super Hot *Taq* Polymerase (Bioron, Germany). Thirty-six cycles of denaturation (96 °C), annealing (60 °C), and extension (72 °C) for 45 s each were carried out in a thermocycler (BioRad, Hercules, CA, USA).

PCR amplification was followed by SSCP analysis. PCR products were mixed with an equal volume of denaturing loading buffer (98% formamide, 10 mM EDTA, 1 mg/ml bromophenol blue, and xylene cyanol). After denaturing (95 °C for 10 min) and quenching on ice, the mixtures were loaded onto 0.8$\times$ MDE gel (Cambrex, Rockland, USA). Gels were run at 3 W, 20 °C for 20 h, silver-stained, and dried at 80 °C. All samples were analysed in duplicate. Samples with a SSCP pattern different from normal were directly sequenced (Seqlab, Germany and Institute of Cancer

Table 1. Primers used for mutation analyses of the *EGFR* gene

Exon		Primer sequence	PCR product size (bp)
Exon 18	Forward	TGGGCCATGTCTGGCACTGC	283
	Reverse	ACAGCTTGCAAGGACTCTGG	
Exon 19	Forward	TCACTGGGCAGCATGTGGCA	241
	Reverse	CAGCTGCCAGACATGAGAAA	
Exon 20	Forward	CCTTCTGGCCACCATGCGAA	295
	Reverse	CGCATGTGAGGATCCTGGCT	
Exon 21	Forward	ATTCGGATGCAGAGCTTCTT	265
	Reverse	CCTGGTGTCAGGAAAATGCT	

Research Sequencing Facility, UK) after purification using MicroSpinTM S-400 HR Columns (Amersham Biosciences, Piscataway, NJ, USA).

Direct sequencing

All cases with abnormal migration patterns on SSCP analysis and an additional 16 randomly selected cases were subjected to direct sequencing. Direct sequencing was performed using the dideoxy chain termination method and Big Dye technology (Applied Biosystems, Foster City, CA, USA), using AmpliTaq Gold™ DNA Polymerase. The primers were the same as those used for SSCP analysis. Cycling conditions were as follows: 94 °C for 10 min; 41 cycles each of 30 s at 94 °C, 30 s at 55 °C, and 1.5 min at 72 °C; followed by 7 min at 72 °C ending at 4 °C.

The products were run on a 3% agarose gel and the DNA was extracted using the BIO 101 gene clean II kit (QBiogene, Cambridge, UK) and then run on an ABI 3100 or ABI 3700 sequencer (AB Applied Biosystems). The results were analysed using 3100 data collection software. Sequencing was performed twice for each sample to rule out the possibility of PCR fidelity artefacts and was carried out in both directions.

Microarray-based comparative genomic hybridization (CGH)

Ten cases, five with and five without known *EGFR* amplifications (as defined by CISH), were subjected to microarray-based CGH using the 4.6K 1.1.2 Breakthrough Breast Cancer microarray-CGH platform. This platform comprises approximately 4200 bacterial artificial chromosomes (BACs) spaced at approximately 1 Mb intervals throughout the genome. BAC clones were spotted in triplicate onto Corning GAPSII-coated glass slides (Corning, NY, USA). Labelling, hybridization, and washes were carried out essentially as previously described [29]. Arrays were scanned with a GenePix 4000A scanner (Axon Instruments, Inc, Union City, CA, USA); fluorescence data were processed with GenePix 4.1 image analysis software (Axon Instruments, Inc).

Data analysis

The $\log_2$ ratios were normalized for spatial and intensity-dependent biases using a two-dimensional Loess local regression. The median of BAC clone replicate spots was calculated after exclusion of excessively flagged clones (>70% of samples). The median $\log_2$ ratio for each clone was averaged across the 'dye-swaps'. This left a final data set of 3664 clones with unambiguous mapping information according to the May 2004 build of the human genome (hg17), of which 272 clones map to chromosome 7, conferring a resolution of approximately 0.6 Mb. Data were smoothed using a local polynomial adaptive weights smoothing (aws) procedure for regression problems with additive errors [30]. A categorical analysis was applied to the BACs after classifying them as representing gain, loss, or no change according to their smoothed $\log_2$ ratio values. Smoothed $\log_2$ ratio values below -0.09 were categorized as losses; those above 0.09 as gains; and those in between as unchanged. Data processing and analysis were carried out in R 2.0.1 (http://www.r-project.org/) and BioConductor 1.5 (http://www.bioconductor.org/), making extensive use of modified versions of the packages, in particular aCGH, marray, and aws [29].

Statistical analysis

The Statview software package was used for all calculations. Correlations between categorical variables were performed using the chi-square test and Fisher's exact test. Correlations between continuous and categorical variables were performed with analysis of variance (ANOVA).

Follow-up information was available for 38 out of 47 patients, ranging from 5.5 to 124.3 months (median = 36.9 months; mean = 51.9 months). Disease-free and overall survival was expressed as the number of months from diagnosis to the occurrence of an event (local recurrence/metastasis and disease-related death, respectively). Cumulative survival probabilities were calculated using the Kaplan–Meier method. Differences between survival rates were tested with the log-rank test. All tests were two-tailed, with a confidence interval of 95%.

Results

EGFR overexpression (2+/3+) was observed in 32 of 47 cases (Table 2). A significant correlation between EGFR overexpression and type of metaplastic elements was observed. Carcinomas with homologous differentiation (ie spindle and squamous differentiation) were more frequently positive for EGFR than carcinomas with heterologous differentiation ($p < 0.0239$, Fisher's exact test).

EGFR amplification was found in 11 of 47 cases (23%). All *EGFR* amplified cases were either spindle cell carcinomas or carcinomas with squamous differentiation (Figure 1). A statistically significant association between *EGFR* gene amplification and type of metaplastic elements was found: 31% of carcinomas with homologous metaplasia versus 0% of carcinomas with heterologous elements ($p < 0.0457$, Fisher's exact test).

A significant direct correlation between *EGFR* amplification and overexpression was found ($p = 0.0094$, Fisher's exact test). Eleven amplified cases showed EGFR overexpression, but EGFR overexpression was more pervasive. In fact, amplification was the underlying mechanism of EGFR overexpression in 34% (11 of 32) of the cases. No association between EGFR overexpression or amplification and age, tumour size, lympho-vascular invasion and lymph node metastasis was found (for all, $p > 0.05$).

Table 2. Summary of correlations between *EGFR* gene copy numbers and overexpression and clinico-pathological findings

	EGFR IHC			*EGFR* CISH		
Parameter	**0/1+**	**2+/3+**	***p* value**	**No Amp**	**Amp**	***p* value**
Age			0.2124*			0.0933*
≤50	5	18		8	15	
>50	10	14		3	21	
Size			0.5159*			0.4508*
T1/T2	9	19		23	5	
T3/T4	6	8		10	4	
Type of metaplasia			0.0227*			0.0457*
Homologous ($n = 36$)	8	28		25	11	
Heterologous ($n = 11$)	7	4		11	0	
Histological type			0.0316*			0.1701*
Spcc ($n = 13$)	2	11		10	3	
Squamous ($n = 23$)	6	17		15	8	
Matrix ($n = 8$)	6	2		8	0	
Heterologous ($n = 3$)	1	2		3	0	
Lympho-vascular invasion‡			0.4717*			0.4318†
Absent	3	13		14	2	
Present	8	15		17	6	
Lymph node metastasis§			0.9999*			0.2152*
Absent	7	16		3	20	
Present	5	9		5	9	

* Fisher's exact test.
† Chi-square test.
‡ Information on lympho-vascular invasion was not available in eight cases.
§ Information on lymph node metastasis was not available in ten cases.
CISH = chromogenic *in situ* hybridization; heterologous = carcinoma with heterologous metaplasia; IHC = immunohistochemistry; matrix = matrix-producing breast cancer; SpCC = spindle cell carcinoma; squamous = carcinoma with squamous metaplasia.

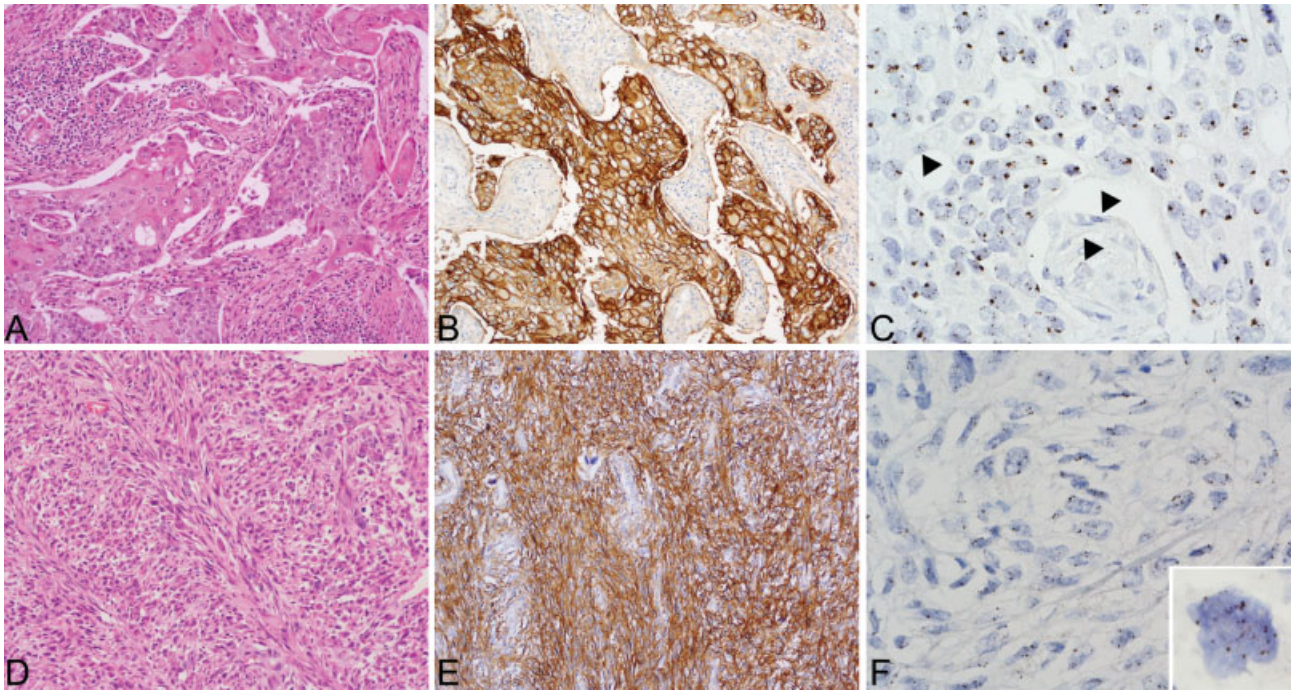

Figure 1. Carcinoma with squamous metaplasia (A — H&E) showing 3+ immunoreactivity for EGFR (B — DAB/Harris's haematoxylin) and *EGFR* gene amplification in the form of large clusters of signals (C — CISH DAB/Harris's haematoxylin). Note the presence of stromal cells with one to two signals per nucleus (arrowheads). Spindle cell carcinoma composed of sheets of spindle-shaped cells, with marked nuclear pleomorphism (D — H&E), displaying EGFR overexpression (3+) (E — DAB/Harris's haematoxylin) and gene amplification (F — CISH DAB/Harris's haematoxylin). Inset: note the presence of more than ten signals (*EGFR* gene copies), sometimes arranged in small clusters, in the nucleus of a pleomorphic neoplastic cell

Table 3. Univariate disease-free survival (DFS) and overall survival (OS) of 47 patients with metaplastic breast carcinomas

	Disease-free survival			Overall Survival		
Parameter	**Mean DFS (months)**	**SE (months)**	***p* value***	**Mean OS (months)**	**SE (months)**	***p* value***
Age			0.1577			0.1274
≤50	75.5	10.7		89.4	11.0	
>50	51.3	11.5		60.2	11.6	
Size			0.0035			0.0108
T1/T2	82.9	10.1		92.2	9.03	
T3/T4	32.9	10.1		49.7	15.82	
Type of metaplasia			0.5608			0.5901
Homologous ($n = 36$)	60.9	9.4		75.3	9.85	
Heterologous ($n = 11$)	61.2	10.9		93.1	14.59	
Histological type			0.7336			0.8172
SpCC ($n = 13$)	46.9	10.6		64.0	12.7	
Squamous ($n = 23$)	75.7	14.6		85.0	13.5	
Matrix ($n = 8$)	55.2	12.8		84.9	18.8	
Heterologous ($n = 3$)	37.3	0.0		37.3	0.0	
Lympho-vascular invasion			0.0213			0.0012
Absent	89.6	11.93		109.9	7.73	
Present	43.6	8.63		54.6	9.90	
Lymph node metastasis			0.0007			0.0006
Absent	85.5	10.1		98.8	8.61	
Present	33.0	9.9		45.0	13.42	
EGFR IHC			0.3722			0.1943
Negative	69.9	11.23		100.0	13.10	
Positive	58.5	9.91		68.4	9.65	
EGFR CISH			0.0676			0.1047
No amplification	70.4	9.17		84.9	9.31	
Amplification	34.6	11.23		49.2	15.22	

* *p* values were calculated by the log-rank test.
Heterologous = carcinoma with heterologous metaplasia; matrix = matrix-producing breast cancer; SpCC = spindle cell carcinoma; squamous = carcinoma with squamous metaplasia.

Univariate survival analysis (Table 3) demonstrated that size, lympho-vascular invasion, and lymph node metastasis were significant predictors of disease-free and overall survival. Tumours with EGFR overexpression and/or amplification showed a shorter disease-free and overall survival; however, these associations failed to reach statistically significant levels (Table 3).

Array CGH

In two cases (M4 and M6) with known amplifications as defined by CISH, the peak of the amplicon on 7p was restricted to 7p11.2, encompassing a genomic region of 0.9 Mb (Figure 2), which was covered by BACs RP11-1013E24, RP11-81B20, RP11-14K11, RP11-97P11, RP11-34J24, RP11-29K01, and RP11-251I15. In three further cases, copy number gains of 7p11.2-tel were observed. Interestingly, in these cases the breakpoints were close to the *EGFR* locus (Figure 2). M4 showed a high-level gain of the *EGFR* locus, but the shoulders of the amplicon comprised the chromosome 7 centromere. Microarray-based CGH of the five cases without *EGFR* amplification as defined by CISH confirmed the lack of 7p genomic aberrations.

The minimal region of overlap in the five samples with *EGFR* gene copy number gain mapped to 7p11.2, spanning a region of 0.9 Mb (between 54.4 and 55.3 Mb on 7p). Interestingly, this region corresponded to the peaks observed in cases M4 and M6, and encompasses three known genes, the Sec61 gamma subunit (*SEC61G*), *EGFR*, and LanC lantibiotic synthetase component C-like 2 (*LANCL2*) (Figure 2).

Mutation analysis

Activating mutations in the tyrosine kinase domain (exons 18, 19, 20, and 21) of *EGFR* were not identified in 47 MBCs, either by SSCP or by direct sequencing. Intronic and silent mutations found in this series are summarized in Table 4. The frequency of polymorphisms at codon 787 CAG to CAA (Gln/Gln) in MBCs was 61.7% (29/47) and at codon 836 CGT to CGC (Arg/Arg) 6.4% (3/47). No statistically significant correlation between the gene sequencing findings (intronic mutations, silent mutations, and gene polymorphisms) and clinico-pathological characteristics or *EGFR* amplification or overexpression was found (data not shown).

Discussion

In previous studies, we and others have demonstrated that MBCs are part of the spectrum of basal-like breast carcinomas [11,13,14,18,19,31,32]. One

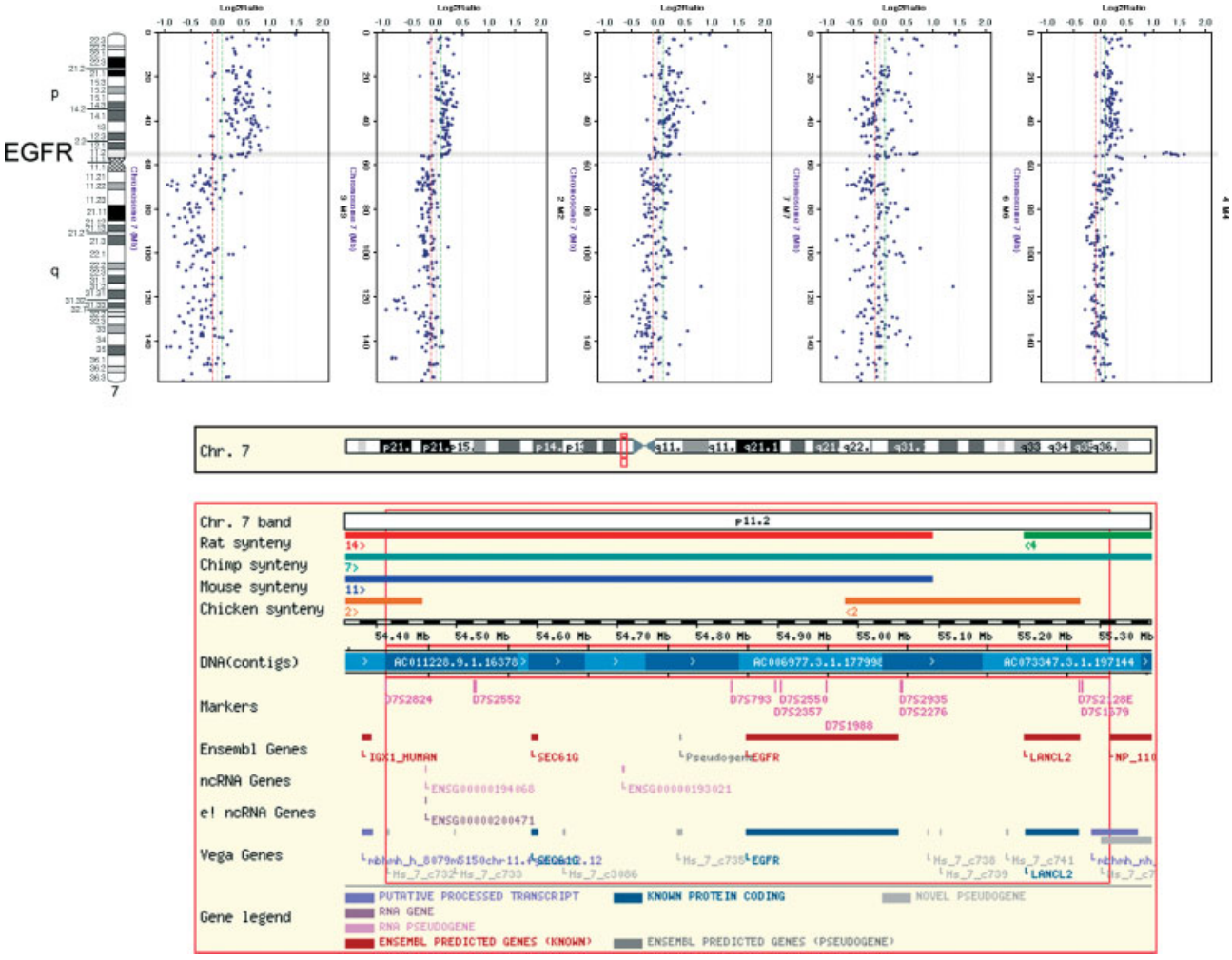

Figure 2. Ideogram and microarray CGH chromosome plots of chromosome 7, where the minimal region of amplification is highlighted in grey. Log_2 ratios are plotted on the *x* axis against each clone according to genomic location on the *y* axis. The centromere is represented by a horizontal dotted line. Vertical dashed lines correspond to log_2 ratios of 0.09 (green) and −0.09 (red). For details of genes mapping to the minimal region of amplification, see http://www.ensembl.org

Table 4. Summary of *EGFR* exon 18, 19, 20, and 21 sequencing results

Histological type	EGFR IHC	*EGFR* CISH	Mutation
SpCC	3+	Not amplified	Intron 18, IVS18 + 19G > A
Squamous	3+	Not amplified	Exon 18, 2178G > A (V716V) Exon 19, 2274A > G (E758E)
SpCC	3+	Amplified	Intron 18, IVS18 + 19G > A
SpCC	3+	Amplified	Exon 18, GAG > GAA (E690E)
Heterologous	3+	Not amplified	Intron 18, IVS18 + 19G > A
Squamous	3+	Not amplified	Exon 18, 2027A > G (E699E)

CISH = chromogenic *in situ* hybridization; heterologous = carcinoma with heterologous elements; IHC = immunohistochemistry; SpCC = spindle cell carcinoma; squamous = carcinoma with squamous metaplasia.

of the defining features of basal-like breast cancer [13,33] is EGFR overexpression, which is found in up to approximately 60% of basal-like breast carcinomas, as defined by expression profiling analysis [31,33]. MBCs frequently show EGFR overexpression at both the immunohistochemical and the mRNA levels [11,20,21].

In the present study, we have shown that amplification of *EGFR* is the underlying genetic mechanism in 34% of MBCs with EGFR overexpression. Of note, amplifications were significantly more frequently observed in the group of tumours with squamous or spindle cell metaplasia. These findings suggest that MBCs with spindle or squamous metaplasia may be part of the same entity. In fact, this group was historically classified under the term 'metaplastic carcinoma with homologous elements' [27]. In addition, it is well known that foci of squamous metaplasia are frequently found in spindle cell carcinomas and spindle cell metaplasia is not rare in breast carcinomas with squamous metaplasia [24,25,28,34,35]. Furthermore, in other anatomical sites, spindle cell carcinomas are considered variants of squamous cell carcinomas [36].

Microarray-based CGH revealed the complex nature of the 7p11.2 amplicon. Interestingly, the minimal region of amplification encompasses only three genes: *SEC61G, LANCL2*, and *EGFR*. *SEC61G* encodes the gamma-subunit protein of the Sec61 complex, which is part of the protein translocation apparatus of the endoplasmic reticulum (ER) membrane [37]; there is

currently no evidence to support an oncogenic role for this gene. The *LANCL2* gene encodes the lanthionine synthetase component C (LanC)-like protein 2, also known as testis adriamycin sensitivity protein, which is a member of the eukaryotic LanC-like protein family. This gene is co-amplified and overexpressed with EGFR in 20% of all glioblastomas [38]. Although there is no evidence to suggest that *LANCL2* may be an oncogene candidate, this gene is reported to play a role in increasing cellular sensitivity to adriamycin by decreasing the expression of P-glycoprotein in cell line models [39]. On the other hand, there are several lines of evidence to suggest that *EGFR* is the most likely oncogene candidate in this amplicon, given that all cases with *EGFR* amplification showed overexpression and that its oncogenic properties have been extensively characterized in different tumour types [1].

As EGFR overexpression is more prevalent than *EGFR* gene amplification, we sought to investigate whether activating *EGFR* gene mutations would constitute an alternative mechanism for EGFR overexpression. In fact, activating *EGFR* mutations have been reported to correlate with EGFR overexpression in human tumours [40]. We could not identify activating mutations in the tyrosine kinase domain of 47 MBCs. Our results are in agreement with previous studies demonstrating the lack of *EGFR* activating mutations in breast cancer [8]. In contrast, Weber *et al* [41] found *EGFR* gene mutations in neoplastic cells of six of 72 breast carcinomas. Surprisingly, in that study, mutations were found in both stromal and neoplastic cells [41]. These differences may be explained by the different histological types analysed and gene sequencing approaches used. Furthermore, in the present study, only the *EGFR* tyrosine kinase domain was analysed. Although exons 18–21 are the hot-spot region for *EGFR* gain-of-function mutations [1,5,12], activating mutations in other domains of the gene cannot be excluded.

The mechanism for EGFR overexpression in the majority of MBCs remains to be identified. It is likely that, in the majority of cases, EGFR up-regulation happens at the transcriptional level [42]. Given that this gene is consistently expressed in normal myoepithelial cells [43] and tumours with basal and/or myoepithelial differentiation [13,33,44,45], one could argue that EGFR overexpression in MBCs would constitute only maintenance of a myoepithelial phenotype or would be part of a transcriptomic programme of myoepithelial/'basal-like' differentiation. In fact, there is evidence to suggest that EGFR expression may be regulated at the transcriptional level [46,47]. Recent *in vitro* studies with human mammary epithelial cells (HMECs) have demonstrated that up-regulation of the transcription factor Y-box-binding protein 1 induces EGFR overexpression and ligand-independent activation of the EGFR pathway [46]. However, the prevalence of this mechanism in human breast cancer is yet to be accurately determined.

The intron 1 CA repeat amplification, which has been reported in ~6% of invasive breast cancer [42], has also been postulated as an alternative genetic mechanism that may lead to EGFR overexpression. Although intron 1 CA repeat amplification does not always correlate with amplification of the whole *EGFR* gene [42], as defined by fluorescent *in situ* hybridization, it shows a significant correlation with EGFR overexpression and may be the underlying genetic mechanism driving EGFR expression in approximately 19% of the cases [42]. Another mechanism would be the presence of a type III EGF deletion-mutant receptor, known as EGFRvIII, which is characterized by the deletion of exons 2–7 in the *EGFR* mRNA, leading to deletion of 801 bp within the extracellular domain of the *EGFR* gene and causing an in-frame truncation of the normal EGFR protein [48,49]. EGFRvIII is constitutively activated and is reported to induce increased colony formation, anchorage-dependent and -independent growth, and greater tumourigenicity when transfected into MCF-7 cells [49]. The presence of EGFRvIII in breast cancer was originally thought to be very common (78%) [50]; however, more recent and better-designed studies have failed to identify this EGFR variant in breast cancers and breast cancer cell lines [51]. Further studies testing these alternative mechanisms as the drivers of EGFR overexpression in metaplastic breast cancer are warranted.

Given that (i) MBCs are reported not to respond to conventional chemo- and hormone therapy, (ii) tumours with *EGFR* amplifications are reported to respond to tyrosine kinase inhibitors [6,52,53], and (iii) a subset of basal-like breast carcinomas (metaplastic breast carcinomas) harbour these amplifications [11], studies addressing the efficacy of EGFR tyrosine kinase inhibitors in patients with *EGFR* amplification are warranted. As *EGFR* activating mutations in the tyrosine kinase domain are remarkably rare in breast carcinomas [8,41], our findings also suggest that *EGFR* gene copy number and other indicators of EGFR therapy effectiveness [12,53–57], rather than *EGFR* mutation analysis, should be assessed in larger cohorts of basal-like tumours to define patient eligibility for inclusion in clinical trials assessing the efficacy of EGFR tyrosine kinase inhibitors in breast cancer.

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