Commentary

c-myc, not her-2/neu, can predict the prognosis of breast cancer patients: how novel, how accurate, and how significant?
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Published: 24 April 2003

Breast Cancer Res 2003, 5:188-191 (DOI 10.1186/bcr606)
© 2003 BioMed Central Ltd (Print ISSN 1465-5411; Online ISSN 1465-542X)

See related Research article: http://breast-cancer-research.com/content/5/2/R30

Abstract

The predictive and prognostic implication of oncogene amplification in breast cancer has received great attention in the past two decades. her-2/neu and c-myc are two oncogenes that are frequently amplified and overexpressed in breast carcinomas. Despite the extensive data on these oncogenes, their prognostic and predictive impact on breast cancer patients remains controversial. Schlotter and colleagues have recently suggested that c-myc, and not her-2/neu, could predict the recurrence and mortality of patients with node-negative breast carcinomas. Regardless of the promising results, caution should be exercised in the interpretation of data from studies assessing gene amplification without in situ analysis. We address the novelty, accuracy and clinical significance of the study by Schlotter and colleagues.

Keywords: c-erbB-2, c-myc, her-2/neu, oncogene, polymerase chain reaction, prognosis

Introduction

Breast cancer encompasses a heterogeneous group of neoplasms, characterised by distinct morphological appearances, genetic alterations and biological behaviours. Comprehension of the molecular and genetic events affecting breast cancer development not only helps in addressing complex and relevant biological questions but also helps in gaining insights into the complexity of the disease. In breast cancer, a frequent mechanism of oncogene activation is gene amplification. Some of the most frequently amplified genes in these tumours are her-2/neu, c-myc and the genes encoding cyclin D1 and topoisomerase II-α. Recently, Schlotter and colleagues [1] provided new data about the role of c-myc and her-2/neu oncogenes as prognostic factors in breast cancer. In brief, the authors evaluated the amplification of c-myc and her-2/neu in a cohort of 181 patients with node-negative breast cancer by means of double differential polymerase chain reaction (ddPCR). c-myc and her-2/neu were amplified in 21.5% and 30.4% of the cases, respectively. Interestingly, it was shown by both univariate and multivariate analysis that c-myc, but not her-2/neu, was associated with poor disease-free survival (DFS). The authors also demonstrated that when different adjuvant therapies were included in the statistical analyses, neither c-myc nor her-2/neu could predict the DFS or overall survival (OS) in those patients who received chemotherapy and endocrine treatment. However, c-myc amplification was associated with shorter DFS and OS in patients who did not receive any sort of adjuvant therapy. The authors concluded that c-myc amplification seems to be a strong prognostic marker that might predict early recurrence for node-negative breast cancer patients.

How novel?

Associations between oncogene amplifications and prognosis of breast cancer patients have been described for more than 20 years. In 1987, Slamon and colleagues [2] described the association between her-2/neu amplification and DFS and OS of breast cancer patients. Since then, various studies have been published, most of them...
with conflicting results. Although it is clear that her-2/neu amplification in breast cancer is associated with high histological grade, lack of hormone receptors and a relative or absolute resistance to endocrine therapy, the data on the prognostic significance of her-2/neu are rather controversial and still remain unsettled [3–5].

The situation is not so different for c-myc [6,7]. The rates of c-myc amplification in breast cancer described so far are highly variable, ranging from 1% to 94.4% ([6] and references therein). A review of these studies has shown that c-myc amplification is more likely to be found in tumours of high histological grade, with positive lymph-node status and negative progesterone-receptor status and less likely to be observed in tumours from post-menopausal women [6]. Apart from the study of Schlotter and colleagues [1], all studies performed so far that have shown the independent prognostic significance of c-myc were retrospective [6,7]. In addition, other studies addressing the influence of c-myc on the response for therapeutic regimens in breast cancer are very scant.

The concept of concurrent amplification of multiple oncogenes in breast carcinomas is not novel. Cuny and colleagues [8] have demonstrated the importance of concomitant amplification of oncogenes in breast cancer: in a large cohort of breast carcinomas, her-2/neu and c-myc held no statistically significant association with disease outcome, whereas the concomitant amplification of both oncogenes proved to be associated with DFS or OS. Taken together, the above data and the present study show only that c-myc is a promising putative prognostic factor that should be validated in larger and more robust prospective studies.

**How accurate?**

Several studies have stressed that in situ methods are more reliable in detecting her-2/neu and c-myc amplification than those using molecular methods (Southern blot, slot-blot, dot-blot and PCR) on fresh or frozen tumour tissue macerates without the use of laser-capture microdissection [3,5,9,10]. In the study performed by Schlotter and colleagues (1), ddPCR was used to assess her-2/neu and c-myc amplification. This technique was first described for the analyses of her-2/neu amplification by Brandt and colleagues [11] in 1995 and for c-myc amplification by Beckmann and colleagues in 1999 [12]. Basically, in this method, DNA fragments of two different single-copy reference genes (manganese superoxide dismutase [SOD] and human β-globin [HBB]) and the target DNA fragment (her-2/neu or c-myc) are amplified simultaneously in one reaction tube. The her-2/neu or c-myc PCR product is bracketed by the SOD2 (90 base pairs) and HBB (252 base pairs) PCR fragments. Sequences are amplified exponentially in a thermocycler and the results can be assessed by (1) the analysis of band intensities of the PCR products in silver-stained polyacrylamide gels, which expresses the average gene copy number per cell (as described for her-2/neu), or (2) the analysis of the products by means of standardized laser-induced capillary electrophoresis.

Despite the claimed reliability, sensitivity and rapidity of ddPCR for the quantification of gene dosages [11,12], this method cannot differentiate gains of chromosome 8q or 17q from c-myc or her-2/neu amplification, respectively; nor can it address the heterogeneity of gene amplification in a given neoplasm. c-myc amplification heterogeneity is quite marked in different neoplastic populations of a given breast carcinoma, even in those populations microdissected from the same slide [13]. It therefore seems reasonable that, ideally, ddPCR would require microdissected samples composed of representative populations of a given tumour; otherwise the sensitivity of the method would be significantly reduced. It should be noted that in recent years ddPCR has been replaced by real-time PCR. Real-time PCR has proved more robust than ddPCR and can also be performed on DNA extracted from formalin-fixed paraffin-embedded tissue sections [14,15].

Several lines of evidence support the proposal that aneuploidy is a very frequent event in breast carcinomas and it seems clear that breast carcinomas of different histological grades harbour distinct chromosomal gains and losses as detected by in situ hybridisation and comparative genomic hybridisation coupled with laser-capture microdissection [16,17]. Chromosomes 8q and 17q are among the most frequent chromosomal gains in breast carcinomas, mainly in those of high histological grades [17]. Interestingly, these are the chromosomal arms in which c-myc and her-2/neu, respectively, are located. It should be stressed that the issue on aneuploidy becomes even more complex if the results published by Rummukainen and colleagues [18] are taken into account; in brief, these authors, using FISH (fluorescence in situ hybridisation) with probes for chromosome 8 centromere and c-myc, demonstrated that c-myc is strongly associated with tumour aneuploidy. Moreover, Janocko and colleagues [19] demonstrated that the amplification of her-2/neu and c-myc is significantly more frequent in aneuploid neoplasms.

We do not dispute that the results published by Schlotter and colleagues [1] are very promising and clearly merit further study. However, it seems clear that their results are not definitive because of (1) intratumoural heterogeneity, (2) putative contamination with stromal cells (in the study by Schlotter and colleagues it is not clear whether the lesions were grossly or microscopically dissected, nor was the proportion of neoplastic to stromal cells available) and (3) the fact that the authors failed to validate their ddPCR results with in situ analysis with cosmid probes for the
target genes and centrosomic probes for chromosomes 8 and 17.

How significant?
As stressed above, breast carcinomas are highly heterogeneous, and different histological types show distinct oncogenetic pathways [16,17,20–23]. It has been demonstrated by in situ methods, when tumors are stratified according to histological type, that lobular carcinomas rarely harbour her-2/neu or c-myc amplification, whereas this event is significantly more frequent in non-lobular breast carcinomas [19]. Also, apart from the clinical pathological factors discussed above, some studies have shown that c-myc amplification is associated with high histological grade, proliferation index and apoptosis rate [6,7,11,18,24]. These findings are not surprising in view of the multifunctional nature of this oncogene, with pivotal roles in proliferation, differentiation and cell death [6,25]. The study by Schlotter and colleagues [1] failed to compare c-myc amplification with the pathological features of the tumours included in their series. In addition, in multivariate analysis, the histological type, progesterone receptor status, proliferation and apoptosis indices were not included. Moreover, the authors failed to evaluate critically the influence of radiotherapy on the DFS and OS of patients with and without c-myc and her-2/neu amplification.

Another confounding factor that seems to have been overlooked is the presence and extension of carcinoma in situ, which might be admixed with the invasive component and might differ from the invasive component regarding the amplification status of c-myc and her-2/neu. A word of caution should be voiced because the PCR analysis of tumour samples is often based on the assumption that all the material present in a given 'tumour section' represents tumour cells. This holds true only when the sections have been adequately microdissected. It should be emphasised that the inherent proclivity of PCR technology for contamination problems, coupled with its very high sensitivity and incapacity to determine whether the amplification is present in invasive or in situ components, merits a careful interpretation of the results of Schlotter and colleagues [1].

Conclusion
The prognostic and predictive significance of her-2/neu and c-myc amplification in breast carcinomas still constitutes one of the thorniest fields in breast cancer research. The use of genome-wide methods, such as array-based comparative genomic hybridisation, coupled with robust quantitative methods for the analysis of gene amplification [14,15] could, in the near future, allow the study of several (if not all known) oncogenes at once. For the time being, the study by Schlotter and colleagues [1], despite its methodological drawbacks, has provided a promising starting point for more comprehensive approaches.

Competing interests
None declared.

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