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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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

ddPCR = double differential polymerase chain reaction; DFS = disease-free survival; HBB = human β-globin; OS = overall survival.

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 tumours 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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