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Aberrations of *ERBB2* and *TOP2A* genes in breast cancer

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ABSTRACT

Copy number changes in *TOP2A* have frequently been linked to *ERBB2* (*HER2*) amplified breast cancers. To study this relationship, copy number changes of *ERBB2* and *TOP2A* were investigated by fluorescence in situ hybridization (FISH) in two cell lines; one characterized by having amplification of both genes and the other by having amplification of *ERBB2* and deletion of *TOP2A*. The characteristics are compared to findings on paired *ERBB2* and *TOP2A* data from 649 patients with invasive breast cancer from a previously published biomarker study. The physical localization of FISH signals in metaphase spreads from cell lines showed that simultaneous amplification is not a simple co-amplification of a whole amplicon containing both genes. Most gene signals are translocated to abnormal marker chromosomes. *ERBB2* genes but not *TOP2A* genes are present in tandem amplicons, leading to a higher *ERBB2* ratio. This observation was confirmed by patient FISH data: among 276 (43% of all patients) abnormal tumors, 67% had different *ERBB2* and *TOP2A* status. *ERBB2* amplification with normal *TOP2A* status was found in 36% of the abnormal tumors (15% of all patients). Simultaneous amplification of both genes was found in 28% of the abnormal tumors (12% of all patients) while *TOP2A* deletion and *ERBB2* amplification was observed in 16% of the abnormal cases (8% of all patients). A small number of tumors had *TOP2A* amplification (4%) or deletion (6%) without simultaneous changes of the *ERBB2* gene. *ERBB2* deletion was also observed (5%) but only in tumors with simultaneous *TOP2A* deletion. The average gene/reference ratio was significantly different: 5.0 for *TOP2A* but 7.2 for *ERBB2* in the amplified tumors ($P < 0.01$). Amplification of the two genes may be caused by different mechanisms, leading to higher level of amplification for *ERBB2* compared to *TOP2A*. In the majority of breast cancer patients, simultaneous aberration of *ERBB2* and *TOP2A* is not explained by simple co-amplification.

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1. Introduction

Amplification of *ERBB2*, commonly known by the alias *HER2* or *HER-2/neu*, is observed in 18–20% of breast cancers and is used as a selection criterion for *HER2* targeted therapies (Wolff et al., 2007). *TOP2A*, encoding topoisomerase IIα, on 17q21 is located close to *ERBB2* on chromosome 17q12 and copy number changes of *TOP2A* may be associated with a higher

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sensitivity to topoisomerase poisons, e.g. anthracyclines (Di Leo et al., 2002; Knoop et al., 2005; O'Malley et al., 2009). The proximity of the two genes has lead to much debate on their use as predictive markers for anthracyclines (reviewed in Pritchard et al., 2008). *TOP2A* aberrations were initially reported in *ERBB2* amplified tumors (Järvinen et al., 1999, 2000) and although the genes were shown to be located in different amplicons (Kauraniemi et al., 2003), the proximity of the two genes has led to a conception of co-amplification of a whole *ERBB2* amplicon containing both genes (Harris et al., 2009; Slamon and Press, 2009). This conception is in contrast to other and more complex hypotheses regarding the underlying mechanisms of changes in copy number of particular genes or genomic regions during the cancerous process (Albertson et al., 2003; Tanaka and Yao, 2009). Although amplification of oncogenes and drug resistance genes were originally observed in cell lines harboring double minute chromosomes (DMs) and homogeneously staining regions (HSRs) (Alitalo et al., 1983), amplifications may also be viewed as distributed insertions, either single or in tandem (Albertson, 2006). The original studies on drug resistance in rodent cell lines (Stark et al., 1989) lead to the presumption that DMs/HSRs were the predominant mechanism, but this could not be confirmed when cytogenetic analyses were performed on tumor specimens and cell lines established from breast tumors (Albertson, 2006; Pandis et al., 1998). Many hypotheses have been proposed to explain how genes are amplified or how DMs or HSRs are generated (Singer et al., 2000). The "episome model" argues that circular molecules excised from chromosomes may play an important role in gene amplification (Pauletti et al., 1990). The most popular hypothesis is the breakage-fusion-bridge (BFB) cycle model (McClintock, 1941), but conclusive evidence is still lacking after more than 60 years (Albertson, 2006; Myllykangas and Knuutila, 2006; Tanaka and Yao, 2009). The BFB model has also been used to explain co-amplification of *ERBB2* and *TOP2A* (Jacobson et al., 2004), but *TOP2A* is located outside the *ERBB2* amplicon of 280 kb (Kauraniemi et al., 2003). More recently, the mechanisms behind amplification have regained interest and several new models have been proposed, e.g. the Hairpin-capped double strand breaks (Narayanan et al., 2006), the translocation-excision-deletion-amplification mechanism (Van Roy et al., 2006) and the "hot-spot" mechanism (Kuwahara et al., 2004).

The aim of this study was to investigate the mechanism behind simultaneous amplification of *ERBB2* and *TOP2A*. Demonstration of the exact number and chromosomal localization of the genes by FISH require the use of metaphase spreads. Metaphase spreads are very difficult to obtain from patient samples, but the use of cell lines established from breast cancer specimens enables access to this information. As results obtained on cell lines can be affected by long-term culturing of the cells, findings always need to be confirmed on patient samples (Lacroix and Leclercq, 2004). In the present study, *ERBB2* and *TOP2A* signals were investigated simultaneously with the centromere 17 signals in breast cancer cell lines, both on metaphase spreads, in interphases and in cut sections of formalin fixed, paraffin-embedded (FFPE) tissue with the intention of gaining insight in the mechanism behind the gene amplification. Secondly, from a previously published biomarker study, results on *ERBB2* and *TOP2A* from 649 patients participating in the Danish Breast Cancer Cooperative Group (DBCG) trial 89D (Ejlertsen et al., 2007; Knoop et al., 2005; Nielsen et al., 2008) were used to verify the results obtained in cell lines.

2. Results

2.1. Cell line data

From cultures of four breast cancer cell lines, MDA-231, MDA-175, MDA-361 and SKBR3, metaphase spreads were prepared by standard cytogenetic techniques. The number of FISH signals and the localization of the signals were analyzed in 20 interphase nuclei. Karyotype analysis was based on a consensus of four to 15 metaphases from each cell line. The cells from three of the cell lines (MDA-231, MDA-361 and SKBR3) were also embedded in paraffin and cut sections from these paraffin blocks were used for the FISH analysis. These cell lines are well characterized and used as control cells for the HercepTest™ (K5204, Dako, Denmark).

The four breast cancer cell lines were screened for *TOP2A* aberrations (Table 1). Ratios found in metaphase spreads and in whole interphases nuclei showed a good correlation. The number of signals and the localization of the signals were analyzed in detail and illustrated in Figure 1. Normal ratios near 1.0 were found in two of the cell lines, MDA-231 and MDA-175. In one of these, the triploid cell line MDA-231, three copies of apparently normal chromosomes 17 were present (Figure 1A) each with a green CEN-17 signal and a red *TOP2A* signal. In the other, the tetraploid cell line MDA-175, four copies of *TOP2A* and four copies of CEN-17 were present, two of which appeared normal (not shown). Loss of *TOP2A* signals (*TOP2A* deletion) was found in the triploid cell line, MDA-361, as four CEN-17 bearing chromosomes were identified but only one of them had a *TOP2A* signal (Figure 1B). The tetraploid cell line, SKBR3, showed borderline amplification with predominantly 11 red *TOP2A* signals and six or seven green CEN-17 signals (Figure 1C). Estimated by visual inspection none of the chromosomes harboring signals had normal appearance and only four of the *TOP2A* signals were located in the expected distance from centromere 17.

Cut sections of paraffin-embedded cells were available from three of the cell lines and the *TOP2A* ratio was determined (Table 1). A linear correlation between the *TOP2A* ratio in truncated nuclei, whole interphases and metaphase spreads from the same cell line was demonstrated. The *TOP2A* ratio of SKBR3 metaphases was 1.7, but when counted in the truncated nuclei of the paraffin-embedded cells, SKBR3 had a ratio just above the cutoff of 2.0.

ERBB2 (*HER2*) gene status was investigated in the two cell lines, MDA-361 and SKBR3, with *TOP2A* aberrations. Figures 1C,D show the *TOP2A* and *ERBB2* signals, respectively, on metaphase spreads from SKBR3. Figure 2 is based on a detailed analysis of the signals in metaphase spreads of the cell lines and illustrates the marker chromosomes (M) harboring the red and green signals. None of the gene signals are located on a normal chromosome 17. The red gene signals can apparently be found on chromosomes with and without a CEN-17

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