Amplifications of oncogene erbB-2 and chromosome 20q in breast cancer determined by differentially competitive polymerase chain reaction

Guoren Deng¹, Mei Yu¹, Ling-Chun Chen¹, Dan Moore¹, Wayne Kurisu¹, Anne Kallioniemi², Frederick M. Waldman², Colin Collins⁴ and Helene S. Smith¹
¹ Geraldine Brush Cancer Research Institute at California Pacific Medical Center, SF, CA 94115, USA; ² Div. of Molecular Cytometry, Dept. of Lab. Medicine, UCSF, SF, CA 94143, USA; ³ present address: Tampere University Hospital, P.O. Box 2000, FIN-33521 Tampere, Finland; ⁴ Lawrence Berkeley Laboratory, Berkeley, CA 94720, USA

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Summary

A new method of measuring gene copy number in small samples of DNA was used to measure amplification of the erbB-2 gene and of chromosome 20q in breast cancers. This method, termed 'differentially competitive polymerase chain reaction' (DC-PCR) combines the advantages of two other techniques for measuring amplification by PCR, namely differential PCR and competitive PCR. The DC-PCR methodology was evaluated for sensitivity and specificity by comparing amplification of erbB-2 measured by DC-PCR with that obtained by fluorescence in situ hybridization (FISH) for 42 cases or Southern blotting and/or slot blot analysis for 34 cases. There was over 90 percent concordance with both FISH and Southern blotting and/or slot blot analysis.

DC-PCR was used to further characterize the newly described amplicon at chromosome 20q. By analyzing DNA from 10 breast cancer cell lines at 7 different loci, we identified a potential common region of amplification of approximately 5 centimorgans at chromosome 20q13 bordered by loci D20S52 and RMC20C001-S1. One hundred and seventeen cases of primary breast cancer were evaluated for amplification at these two loci. Amplification at one or more loci, defined as > 1.5 fold higher copy number than that of normal DNA, was found in 25 cases (21%). Sixteen cases were amplified at only one of the two probes (12 cases for RMC20C001-S1 and 4 cases for D20S52), suggesting that the target gene lies between the two markers or that there are two independent target genes within a small chromosome region.

Introduction

Gene amplification is commonly found in breast cancers and is considered an important mechanism by which tumor cells increase expression of critical genes. Chromosomal regions which are known to be amplified in at least 10–30% of cases include 17q12 (erbB-2), 8q24 (myc), 11q13 (pradl/cyclin D), 8p12 (flg), and 10q24 (bek) [1–4]. Amplification of erbB-2 [5–9], pradl/cyclinD [4, 10–13], and myc [14–18] have been associated with poor prognosis as well as with other indicators of aggressive behavior. Three additional chromosome regions have been identified that show a high frequency of amplifica-
tion, 8q21–22, 17q22–24, and 20q13 [19], but the target genes for these regions have not yet been identified.

A major limitation to identifying new target genes and evaluating the pathobiological relevance of various amplicons in breast cancer is the need for a rapid and reproducible assay suitable for small samples of archival, paraffin-embedded tissues. In some cases, overexpression of the target gene can be measured immunohistochemically. However, when the target gene is unknown and/or if suitable antibodies are not available, molecular assays of gene amplification using the polymerase chain reaction (PCR) are an alternative approach.

Although PCR based methodologies have been used to measure gene copy number in tissues [20–22], there have been limitations to this approach. Because tumor DNA preparations frequently are contaminated with RNA and often show variable degrees of DNA degradation, the amount of DNA judged by optical density does not necessarily represent the amount of target DNA in the sample in vivo. To circumvent the uncertainty in amount of DNA added to a reaction, previous studies co-amplified a second gene of known copy number within the same PCR reaction as an internal standard (differential PCR). However, even this improvement is sub-optimal because PCR is problematic when more than one set of primers are amplified in the same sample. Additionally, differences in the initial amount of target and reference sequences between samples will be obscured when PCR reaches a plateau. A more recent approach, termed ‘competitive PCR’ [23–26] circumvents the problems of two primer sets being amplified in the same reaction and the plateau effect, but does not account for the difficulty in quantitating DNA from tumor samples. With competitive PCR, a standard is added to the PCR reaction consisting of fragments constructed so that they can be amplified with the same primers as the target gene. Under these conditions the ratio of target to standard after PCR is presumed to give the absolute amount of target sequence in that particular reaction. However, results cannot be compared to a second tissue sample unless both contain the same amount of total DNA, which is not possible for tumor DNA samples.

In order to use competitive PCR with tumor-derived DNA, we added a second PCR reaction using the same tumor DNA to amplify a second non-amplified gene [27]. The amplified fragment of the control gene is similar in size to the target fragment so that it should have the same degree of degradation as the target sequence. A second standard constructed so that it amplifies with the same primers as the control gene is added to the second PCR reaction. Under these conditions, the quotient of the ratio of target to its standard divided by the ratio of reaction control to its standard will accurately represent the relative copy number of the target gene in the cells. We have termed this modification ‘differentially competitive PCR’ (DC-PCR).

In this manuscript, the sensitivity and specificity of DC-PCR were evaluated by comparing copy number of erbB-2 measured by DC-PCR with that obtained by other methods of measuring amplification, namely fluorescence in situ hybridization (FISH), Southern blotting, and/or slot blot analysis. We then used DC-PCR to further characterize the newly described amplicon at chromosome 20q.

Many polymorphic markers on various chromosomes have been mapped by the Human Genome Project. These markers can be used as primers for DC-PCR to quantitate the number of gene copies in a given tumor. By examining different loci within a very short chromosomal region (e.g. chromosome 20q13) in many tumor samples, one can define the shortest commonly amplified region, thus defining the amplicon.

**Materials and methods**

**DNA from cell lines and primary breast cancer tissues**

Ten breast cancer cell lines (BT-474, SK-BR-3, MCF7, MDA-157, MDA-361, MDA-453, CAMA1, T47D, 600MPE and PU4475) were obtained from American Type Culture Collection (Rockville, MD). Primary breast carcinomas were collected from 117 patients, who had undergone surgery at California Pacific Medical Center and University of California, San Francisco. Normal skin samples