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TITLE: Identification of Genomic Determinants of Response of Metastatic Disease to Taxol and Adriamycin

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Identification of Genomic Determinants of Response of Metastatic Disease to Taxol and Adriamycin

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INTRODUCTION: Human breast cancer genesis and progression is caused by the aberrant function of genes that positively and negatively regulate aspects of cell proliferation, apoptosis, genome stability, angiogenesis, invasion and metastasis(7). Discovery and functional assessment of these genes is critical for understanding the biology of cancer, and for clinical applications including early cancer detection and improved prediction of cancer risk, disease course and response to therapy. Although many different events can cause aberrant gene function, chromosomal aberrations resulting in changes in gene dosage or structure play important roles. Interestingly, there is remarkable variability in the degree to which tumor genomes are aberrant at the chromosomal level. Some tumors have few chromosomal aberrations, while in others there may be dozens. Furthermore, the aberration spectrum typically differs substantially among clinically similar tumors. It is likely that many of these aberrations are accumulated by chance during the proliferation of cells with substantial genome instability and do not contribute to substantially to the tumor phenotype. However, some specific aberrations occur frequently and very likely do affect function. Extensive catalogues of recurrent abnormalities in a wide range of solid tumors have been compiled from cytogenetic(2) and CGH studies(3). These analyses show that tumors that arise in different anatomical sites differ significantly in recurrent aberration composition, as do histologically distinct tumors that arise in the same anatomic location(4-6). The spectrum of aberrations also varies with the genetic makeup of the patient. For example, recurrent aberrations in tumors that arise in individuals with BRCA1 mutations differ from those in tumors from BRCA2 carriers and from those in tumors that arise spontaneously(7). The influence of genetic background on tumorigenesis and recurrent aberration spectrum also is clear in analyses of murine tumor models(8, 9).

Experimentally, chromosomal aberrations are important because they are distinctive and can be readily detected and mapped using an increasing number of complementary technologies. Thus, their discovery and genomic localization provides important clues about the locations of genes that are important in breast cancer etiology. We hypothesize that analyses of breast tumors using array CGH techniques developed by us will reveal genomic changes that are associated with novel genes that influence cancer susceptibility and/or that predict response to therapy. Thus, our long-term plan is to apply array CGH to analysis tumors from patients treated with Herceptin to identify patients that are most likely to respond and to tumors from patients at high familial risk of developing breast cancer but not carriers of BRCA1 or BRCA2 mutations in order to facilitate additional cancer susceptibility gene.
Most pathological specimens that are now available from Herceptin treated and familial cancer patients are formalin-fixed and paraffin-embedded prior to histological evaluation. Unfortunately, extraction of high quality DNA suitable for array CGH from such tissues has been difficult, presumably because of damage and DNA-protein cross-linking that occurs during the fixation and embedding(10, 11). Some of the parameters that influence the degradation of DNA at the stage of tissue fixation are known, for example, the age of specimens, the fixative, and the duration of fixation(12, 13). In practice, these parameters are highly variable between hospitals and institutions. Thus, the goal of this project was optimize techniques for nucleic acid extraction from diverse paraffin-blocks to obtain DNA for the DNA microarray analysis. Paraffin blocks assessed during these studies were from the UCSF Cancer Center, the NSABP and several laboratories participating in an International Breast Cancer Susceptibility Gene Identification Consortium.

Tissue sections were cut at 30 μm, collected on clean untreated glass slides, deparaffinized twice with xylene and rehydrated with ethanol (95%, 75%, and 50%) and water followed by light staining with 0.1% methyl green. Five μm sections were cut before and after the 30 μm sections, deparaffinized and stained with hematoxylin and eosin and used for histological control to identify the area of interests. A pathologist selected areas of specific histological interest on the stained sections. Microdissection was performed under dissecting microscope with a surgical scalpel blade. The procedure consisted of the removal of unwanted (nontumorous) cells by gentle scraping and the collection of microdissected area of interests into 1.5 ml eppendorf tube for DNA extraction. We evaluated several methods reported in the literature to improve the quality and quantity of DNA extraction from paraffin-block including thorough de-waxing of the paraffin and digestion of the deparaffinized tissue with proteinase K. In addition, we tested several approaches to reverse DNA-protein cross-linking.

Overall, we analyzed forty-two paraffin embedded samples. Five of the cases were "large" blocks so that we were able to apply all extraction protocols in parallel. In general, the DNA extraction protocol using the Promega "Wizard Genomic DNA Extraction Kit" modified with (a) EDTA added in the lysis buffer to prevent degradation and (b) extended proteinase K digestion produced the most reliable results. On the other hand, pretreatment with "target antigen retrieval" using citrate buffer plus heat at 95C as recommended for immunohistochemistry produced degraded DNA. Lowering the temperature to 80C and the duration of heating to 20 min yielded higher quality DNA but the approach was still inferior to the "Promega" method.
We were able to obtain DNA that was adequate for CGH analysis in 28 cases. Figure 1 shows a typical array CGH profile for one of these cases. We were not able to extract DNA of adequate quality from 14 cases using any of the extraction protocols.

In general, DNA extraction was straightforward using the Promega Wizard kit when the samples were fixed and embedded in academic laboratories where fixation and embedding are rigorously controlled. However, we were much less successful in analyzing samples from European community hospitals where the preparation quality may have been less carefully controlled. This lack of success was sufficiently high that we were forced to abandon our efforts to apply array CGH to familial breast cancer samples collected from European community hospitals.

**KEY RESEARCH ACCOMPLISHMENTS:**

- We evaluated several DNA extraction protocols in an effort to find one that would be generally applicable to formal fixed, paraffin embedded samples from diverse sources.
- We found that our procedure based on the use of the Promega Wizard kit was satisfactory for samples prepared in academic laboratories where preparation quality control was high.
REPORTABLE OUTCOMES:

None

CONCLUSIONS:

Fixation and paraffin embedding procedures used to archive breast tumor samples dramatically influence subsequent efforts to extract DNA for array CGH analyses. Rigorous quality control of the fixation procedure is particularly critical. Extraction of high quality DNA is straightforward from properly prepared samples and impossible (at least using the methods evaluated in this study) from samples that have been improperly prepared. Guidelines for preparation of formal fixed, paraffin embedded samples are needed to insure the utility of these samples in future research projects.

REFERENCES:


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