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Using gene chips to count DNA copy numbers

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Abstract

The use of cDNA microarrays for the study and mapping of gene amplifications and deletions on a whole-genome scale provides a comparison of DNA copy numbers in normal and tumor cells at unprecedented resolution.

Significance and context

DNA microarrays are now being used frequently to measure changes in mRNA expression. This is one of the few papers in which microarrays have been used for the significantly more challenging task of detecting changes in gene copy number in genomic DNA. The degree of difficulty is increased for two main reasons: firstly, mRNA represents about only 2% of the total genomic DNA in mammalian cells, so the DNA samples are 50-fold more complex. Secondly, changes in DNA copy number often fall into a smaller dynamic range (between zero and ten copies per cell) than changes in mRNA (that is to say, expression differences), which can vary 10,000 fold between different cell types. The authors use a moving average of fluorescence ratios to achieve the sensitivity and specificity required to identify small changes in DNA copy number.

Key results

Using a series of human cell lines which contain between one and five copies of the X chromosome, Pollack *et al*. are able to 'calibrate' the system, and show that it is possible to detect gene copy number changes of this magnitude. They are further able to confirm (at higher resolution than previously possible) known gene amplifications in a breast cancer cell line (BT474). They also identify previously unknown areas of gene amplification and deletion in BT474, in other breast tumor cell lines, and in a primary breast tumor sample. This method, therefore, has clear applications in identifying potential oncogenes (increased copy number), and tumor suppressor genes (decreased copy number), in a variety of cancers.

Methodological innovations

Pollack *et al*. use the 'standard' methods, developed in their own laboratory, for making and using microarrays. Generously, all relevant methods as well as analysis software are freely available from the Brown lab web sites (listed below). The major methodological innovation in this paper is in the analysis of the data. Pollack *et al*. find that, at the single-gene level, each array element gives only 85% sensitivity and 85% specificity for detection of a deletion. This translates to a 15% false positive and 15% false negative rate (an enormous problem when analyzing thousands of genes). They overcome this problem by using a moving average of fluorescence ratios, calculated for sets of three genes known to be adjacent along the chromosome. They find that this increases the sensitivity and specificity of deletion detection to around 98%.

Links

Further details can be found in the Supplementary data to *Nat Genet* **23**: 41-46 to this paper. The DNA labeling protocol can be accessed through the Patrick Brown's laboratoryhomepage. The MicroArray analysis software used is freely available to academic and non-profit institutions.

Conclusions

This method of analysis is ideal for locating relatively large chromosomal deletions (for example, those detected in the breast tumor cell lines). This method would be less optimal for finding smaller deletions (for example, loss of a single copy of only one gene), although it would be possible using a purpose-built array containing multiple, exon-specific probes for each gene.

Reporter's comments

This paper opens up new possibilities for detecting gene copy number changes in complex genomes such as those of mammals. As well as the demonstrated and exciting application in cancer research, the technologies described in this paper are also likely to be useful in understanding certain human congenital abnormalities. They may also prove useful in identifying phenotype-causing mutations in mouse and rat gene deletion libraries.

Table of links

Nature Genetics

Supplementary data to *Nat Genet* **23**: 41-46

DNA labeling protocol

Patrick Brown's laboratory

MicroArray analysis software

References

1. Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO: Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet. 1999, 23: 41-46. 1061-4036