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High-resolution cDNA microarray-based comparative genomic hybridization analysis in neuroblastoma

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Abstract

Neuroblastoma (NB) is one of the most common pediatric solid tumors and displays a broad variety of genomic alterations. Array-based comparative genomic hybridization (A-CGH) is a novel technology enabling the high-resolution detection of DNA copy number aberrations. In this article, we outline features of this new technology and approaches of data analysis. We focus on stage specific DNA copy number variations in neuroblastoma detected by cDNA array-based comparative genomic hybridization (A-CGH). We also discuss hypothetic evolutionary models of neuroblastoma progression that can be derived from A-CGH data.
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Keywords: Neuroblastoma; Array CGH; Microarray; Tumor evolution; DNA copy number; Genomic alteration; Amplification; *MYCN*

1. Introduction

Neuroblastoma (NB) is a tumor derived from primitive cells of the sympathetic nervous system and is one of the most common pediatric solid tumors. The disease is characterized by diverse clinical behaviors ranging from spontaneous regression to rapid malignant progression [1]. Many important factors such as stage, age and ploidy have been identified to be associated with the biological and clinical heterogeneity of NB tumors. This diverse biological behavior makes NB a paradigm for the investigation of genomic alterations and associating it with clinical outcome. In neuroblastoma, genomic alterations have been investigated by cytogenetic, and molecular methods including spectral karyotyping and metaphase comparative genomic hybridization (M-CGH) [2–6]. Here, we focus on DNA copy number alterations in NB detected by the recently developed cDNA array-based comparative genomic hybridization (A-CGH). This review will describe and illustrate this new technology and its application in NB, and will discuss novel analysis approaches currently used in DNA array CGH profiling that reduces noise and increases sensitivity to detect genomic alterations at high resolution.

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2. Array-CGH

Comparative genomic hybridization (CGH) was developed as a molecular cytogenetic technique to compensate for the difficulties presented by conventional cytogenetics and fluorescence in situ hybridization (FISH) analysis; it does not require culturing of tumor cells in vitro and has substantially increased genome-wide information on unbalanced chromosomal changes [7]. Many novel and non-random genomic alterations in NB have been detected using metaphase CGH (M-CGH) analyses [3,8,9], however, this technique is hampered by a limited resolution of 10–20 Mb. To overcome this, array-based CGH (A-CGH) on BAC, cDNA or oligonucleotides microarray has been developed and can detect genomic alterations with higher resolution [10–15] which is determined by the distribution and spacing of the clones along the genome. Relative copy number is measured at these specific loci by hybridization of fluorescently labeled test and reference DNA as in conventional M-CGH [11,13]. Since, the clones used on the array contain sequence tags, their positions are accurately known relative to the genome sequence, and genes mapping within regions of copy number alteration can be readily identified. Arrays comprised of large insert genomic clones such as BACs provide reliable copy number measurements on individual clones, since they generally have a higher signal to noise ratio. Both small and large DNA arrays have been made consisting of ~2500 BAC clones represent at an average interval of ~1.4 Mb [12] to large tiling-array of 32,433 overlapping BAC clones covering the entire genome [16]. The latter increases the ability of BAC A-CGH to identify genetic alterations and their boundaries throughout the genome in much higher resolution. The limitations of BAC A-CGH, include the fact that preparation and spotting of BAC DNA is labor intensive and can be very expensive [17], and that each clone may represent more than one gene and it is not possible to do parallel gene expression and copy number changes using the same arrays.

A-CGH on cDNA arrays have the distinct advantage of providing a direct method to detect individual gene copy number changes in unbalanced chromosomal rearrangements [13]. The second significant advantage is that it allows us to investigate the direct effects of genomic changes over gene expression level by using the same microarray for both ACGH and gene expression analysis [18,19]. However, because of lower signal to noise ratios the detection of lower copy number changes requires additional methods such as calculating the running average of multiple clones along the genome, where typically 5–10 clones are used. It is also frequently necessary to discard measurements on a substantial fraction of clones because they do not provide adequate signals. Thus, the actual genomic resolution of the boundaries of single copy changes and the ability to detect focal single copy changes is considerably less than implied by the average genomic spacing between the clones on the array.

More recently, oligonucleotide-based arrays have been used for DNA copy number analysis [20–22]. The studies clearly showed the ability to detect high-level amplifications and to determine the boundaries of high copy number portions of the genome. Single copy changes could be detected in different resolutions depending on the type of oligo-arrays and the techniques of hybridization. However, like with cDNA arrays, detection of single copy changes requires running average with a corresponding reduction in genomic resolution. Oligo-based microarrays hold the potential of enhanced design flexibility and full-genome representation of probes capable of accurately reporting single-copy number changes.

3. Amplicon mapping by cDNA array CGH in NB

Conventional CGH profiling of NBs has identified many genomic aberrations, although the limited resolution has precluded a precise localization of sequences of interest within amplicons. Application of cDNA A-CGH in NB allows genome-wide identification of amplified genes [14,23,24]. In a recent study, we performed A-CGH for 12 NB cell lines and 32 NB primary tumor samples using microarrays containing 42,000 cDNA clones (~24,000 UniGenes) [14]. We found that only two chromosomes (2p and 12q) showed amplifications and all in the *MYCN* amplified samples (Fig. 1A and B). Focusing on 2p (Fig. 1A), we found six independent non-contiguous amplicons (10.4–69.4 Mb). For the *MYCN* amplicon, the largest contiguous region was 1.7 Mb and bounded by *NAG*