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Genome wide measurement of DNA copy number changes in neuroblastoma: dissecting amplicons and mapping losses, gains and breakpoints

E. Michels[a] J. Vandesompele[a] J. Hoebeeck[a] B. Menten[a] K. De Preter[a] G. Laureys[b] N. Van Roy[a] F. Speleman[a]

[a]Center for Medical Genetics and
[b]Department of Pediatric Hematology and Oncology, Ghent University Hospital, Ghent (Belgium)

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Abstract. In the past few years high throughput methods for assessment of DNA copy number alterations have witnessed rapid progress. Both 'in house' developed BAC, cDNA, oligonucleotide and commercial arrays are now available and widely applied in the study of the human genome, particularly in the context of disease. Cancer cells are known to exhibit DNA losses, gains and amplifications affecting tumor suppressor genes and proto-oncogenes. Moreover, these patterns of genomic imbalances may be associated with particular tumor types or subtypes and may have prognostic value. Here we summarize recent array CGH findings in neuroblastoma, a pediatric tumor of the sympathetic nervous system. A total of 176 primary tumors and 53 cell lines have been analyzed on different platforms. Through these studies the genomic content and boundaries of deletions, gains and amplifications were characterized with unprecedented accuracy. Furthermore, in conjunction with cytogenetic findings, array CGH allows the mapping of breakpoints of unbalanced translocations at a very high resolution.
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Neuroblastoma (NB) is a pediatric tumor arising from primitive sympathetic nervous cells. The median age of diagnosis is two years and almost 90% of patients are diagnosed before the age of five years. Neuroblastomas have a remarkably variable clinical behavior ranging from spontaneous regression to widespread metastasis and fatal outcome (Brodeur, 2003). As a step towards more efficient treatment and better survival for children with aggressive NB, improved risk assessment and prognostic staging is needed. Also, insights into the genetic defects and altered molecular pathways governing NB oncogenesis are urgently required in order to design new, more efficient and less toxic therapeutic strategies.

DNA copy number alterations in the pre-FISH era

NB is one of the first tumors in which proto-oncogene amplification was discovered and used in therapy stratification (Alitalo et al., 1983; Schwab et al., 1983; Brodeur et al., 1984; Seeger et al., 1985; Schwab, 1999). Cytogenetically, aggressive NB tumors and cell lines derived from high-stage NB were shown to contain double minute chromatin bodies or homogeneously staining chromosome regions. Molecular analyses revealed that these unusual chromosomal alterations represented high-level amplification of a new pro-

Request reprints from Frank Speleman
Center for Medical Genetics
Ghent University Hospital, De Pintelaan 185
BE–9000 Ghent (Belgium)
telephone: +32 9 240 2451; fax: +32 9 240 6549
e-mail: Franki.Speleman@UGent.be

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to-oncogene with high homology to the previously discovered *MYC* gene. Hence, the amplified gene in NB was coined *MYCN* (Alitalo et al., 1983; Schwab et al., 1983). In addition to the high level amplification, these neuroblastoma cells often showed 1p-deletions or unbalanced translocations leading to distal 1p-loss (Brodeur et al., 1977; Maris et al., 2000). Further contribution of classical cytogenetics to the study of NB was restricted, especially by the difficulty of obtaining good metaphases, in particular in NB without *MYCN* amplification which roughly represents two thirds of all cases. Independent DNA content measurements and study of rare karyotypes indicated that a subset of these NB, in particular those with low stage, show near triploidy (Look et al., 1984; Kaneko et al., 1987). Further analyses of DNA copy number changes in NB were based on loss of heterozygosity studies, focussing on delineation of the critical region for 1p-deletions (Maris and Matthay, 1999; Bown, 2001; White et al., 2005) but also leading to the discovery of other important recurrent regions of loss at chromosome 3p, 11q and 14q (Takita et al., 1995; Hallstensson et al., 1997; Ejeskar et al., 1998; Guo et al., 1999; Theobald et al., 1999; Hoshi et al., 2000a, b; Plantaz et al., 2001; Thompson et al., 2001). Although these studies contributed to our insights in NB genetics, LOH analyses typically focused on one or a few specific regions and, at best, analyzed the entire genome at low resolution. Consequently, a genome-wide view of the detailed patterns of genomic imbalances associated with the different NB subtypes could not be described until the advent of chromosomal comparative genomic hybridisation (CGH).

From FISH, M-FISH and metaphase CGH to array CGH

The introduction of fluorescent in situ hybridisation (FISH) and chromosomal CGH analyses led to a number of remarkable findings in the analysis of genetic alterations in NB. The power of FISH was best illustrated by the finding of whole chromosome 17 and partial 17q gain as the most frequent recurrent chromosomal change in NB (Savelyeva et al., 1994; Van Roy et al., 1994; Vandesompele et al., 1998, 2001; Speleman and Bown, 2000). Only one cytogenetic study referred to chromosome 17 involvement in NB, all other previously conducted cytogenetic analysis overlooked the non-random involvement of chromosome 17 in NB (Gilbert et al., 1984; Brodeur and Fong, 1989). FISH studies including 24-colour M-FISH (Speicher et al., 1996) revealed gain of 17q in the majority of cell lines and high-stage tumors, most often as the result of unbalanced translocations (Van Roy et al., 2001; Schleiermacher et al., 2003). Although many partner chromosomes can be involved in these translocations, chromosome 1p and 11q seemed to be preferentially involved, leading to combined 17q gain and loss of putative tumor suppressor loci as a result of one single genetic event. It has been hypothesized that 17q gain contributes to NB oncogenesis due to copy number gain of one or more critical dosage sensitive genes (Speleman and Bown, 2000; De Preter et al., in preparation).

The introduction of chromosomal CGH in the study of NB opened up new possibilities (Kallioniemi et al., 1992; du Manoir et al., 1993). CGH was rapidly recognized as a powerful method for detection of DNA copy number imbalances in tumors, in particular for those that were difficult to karyotype or for retrospective studies on frozen or formalin fixed samples. CGH indeed yielded a number of fundamental new insights in NB (Altura et al., 1997; Brinkschmidt et al., 1997, 1998; Lastowska et al., 1997; Plantaz et al., 1997, 2001; Van Gele et al., 1997; Van Roy et al., 1997; Vandesompele et al., 1998, 2001; Hirai et al., 1999; Breen et al., 2000; Cunsolo et al., 2000; Vettenranta et al., 2001; Iehara et al., 2002). Most importantly, this method provided a genome-wide overview of genomic imbalances occurring in these tumors and, for the first time, the association of all these alterations within tumors and subgroups could be analyzed. This series of investigations allowed us and others to categorize NB into three major subgroups: favorable near triploid NB with a recognizable pattern of predominantly numerical gains and losses (losses of chromosome 3, 4, 9, 11, 14 and gains of chromosomes 6, 7, 17 and 18) (subtype 1) and two subtypes of unfavorable NB with either 11q-loss without *MYCN* amplification (subtype 2A) or those with *MYCN* amplification and 1p-deletions (subtype 2B). The latter two subtypes both typically present with 17q gain. Multi-center studies revealed 17q gain as the strongest independent genetic prognostic marker in NB (Bown et al., 1999; Vandesompele et al., 2005).

Although CGH has been an important tool in the investigation of large genomic changes in NB, the technique also suffers from a number of limitations. Most importantly, this method is rather time consuming and, due to the use of chromosome preparations in the analysis, the resolution remains restricted at about ~10 Mb for single copy changes in most studies. Array CGH has overcome these two limitations and is now providing fast assays with a resolution at the kilobase level. Although the principle of array CGH had been reported as early as 1997 by Solinas-Toldo and colleagues (Solinas-Toldo et al., 1997), it has taken several years before this methodology became more widely used. Below, we review the results obtained with various array CGH platforms in the genetic study of NB.

Platforms for array CGH

In total, 11 array CGH studies on neuroblastoma have been performed using a variety of platforms including BAC, custom made cDNA and commercial oligonucleotide arrays (Beheshti et al., 2003; Mosse et al., 2003, 2005; Chen et al., 2004; De Preter et al., 2004, 2005; Scaruffi et al., 2004; Selzer et al., 2005; Stallings et al., 2006; Hoebeeck et al., in press; Michels et al., in preparation). A representative overview of the three types of neuroblastomas analysed by array CGH is shown in Fig. 1. Typically, the resolution of the arrays depends on the number and size of reporters that are tested. BAC arrays were initially developed in house by a number of pioneering groups (Solinas-Toldo et al., 1997; Snijders et al.,

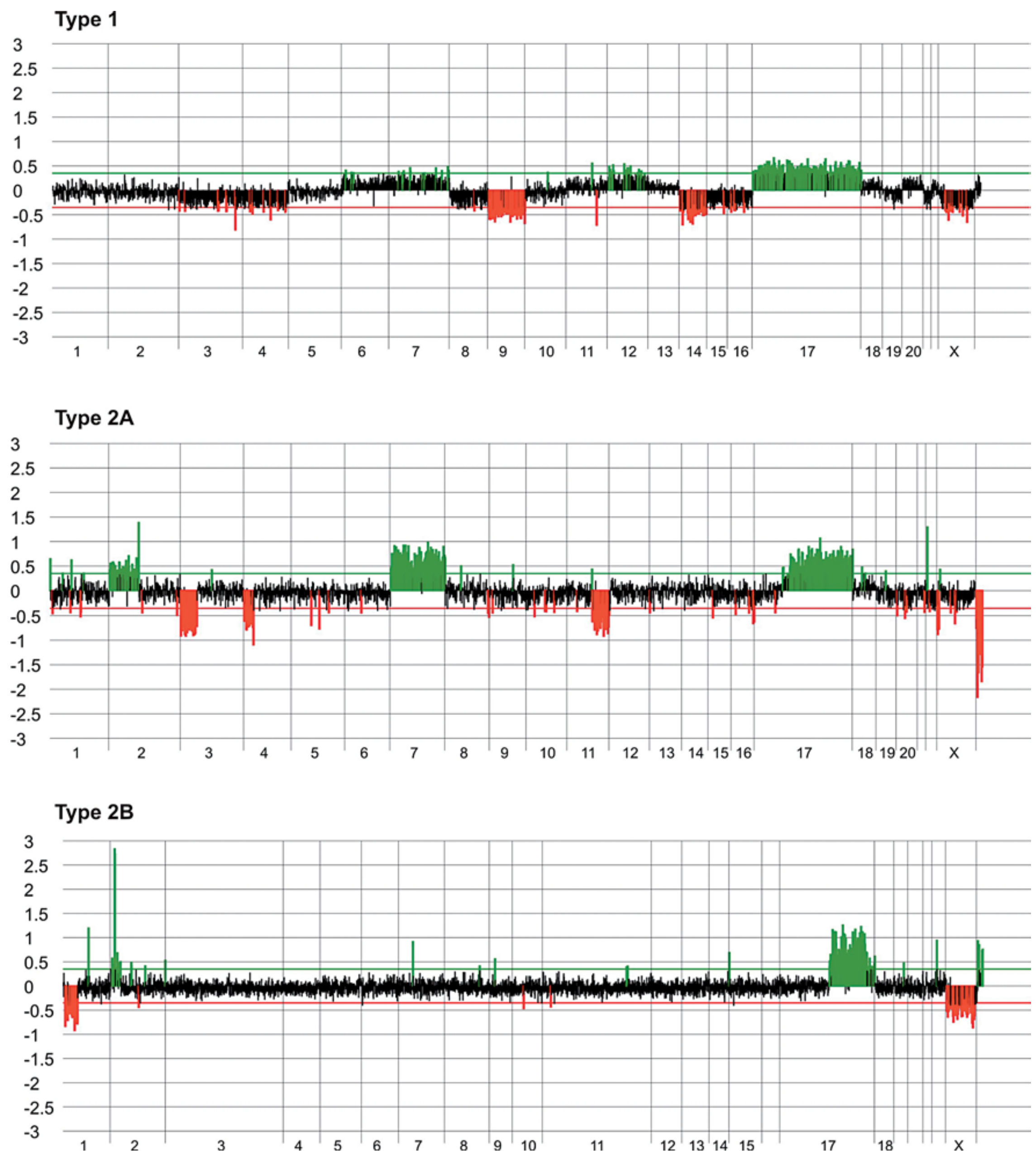

Fig. 1. ArrayCGH visualisation of representative neuroblastoma tumors, each belonging to a specific subtype (**1, 2A, 2B**). The X-axis represents the chromosomes, the Y-axis the ratio tumor versus control material.

2001; Cai et al., 2002; Wessendorf et al., 2002; Fiegler et al., 2003; Greshock et al., 2004). At the same time, oligonucleotide arrays were explored for DNA copy number change measurements (Lucito et al., 2003; Bignell et al., 2004).

The availability of a set of BAC clones with an average spacing of 1 Mb across the genome and further improvements in the protocols (Fiegler et al., 2003) as well as the production of reliable commercial platforms (Agilent, NimbleGen, Spectral Genomics, Affymetrix) have given a major boost to wide implementation of array CGH in the field of cancer genetics. The great interest in the potential of array CGH has led to further increase in resolution, e.g. by producing tiling path arrays for given chromosomal regions (Fix et al., 2004; Hoebeeck et al., in press; Michels et al., in preparation) as well as whole genome tiling path BAC arrays (Garnis et al., 2003; Ishkanian et al., 2004; van Duin et al., 2005).

Similarly, oligonucleotide arrays have been produced with up to 40,000 oligos or more (Agilent), SNP chips probing about 500,000 SNPs (Affymetrix) or NimbleGen chips with up to 385,000 features. The SNP chips offer the additional advantage of allele status determination, as illustrat-

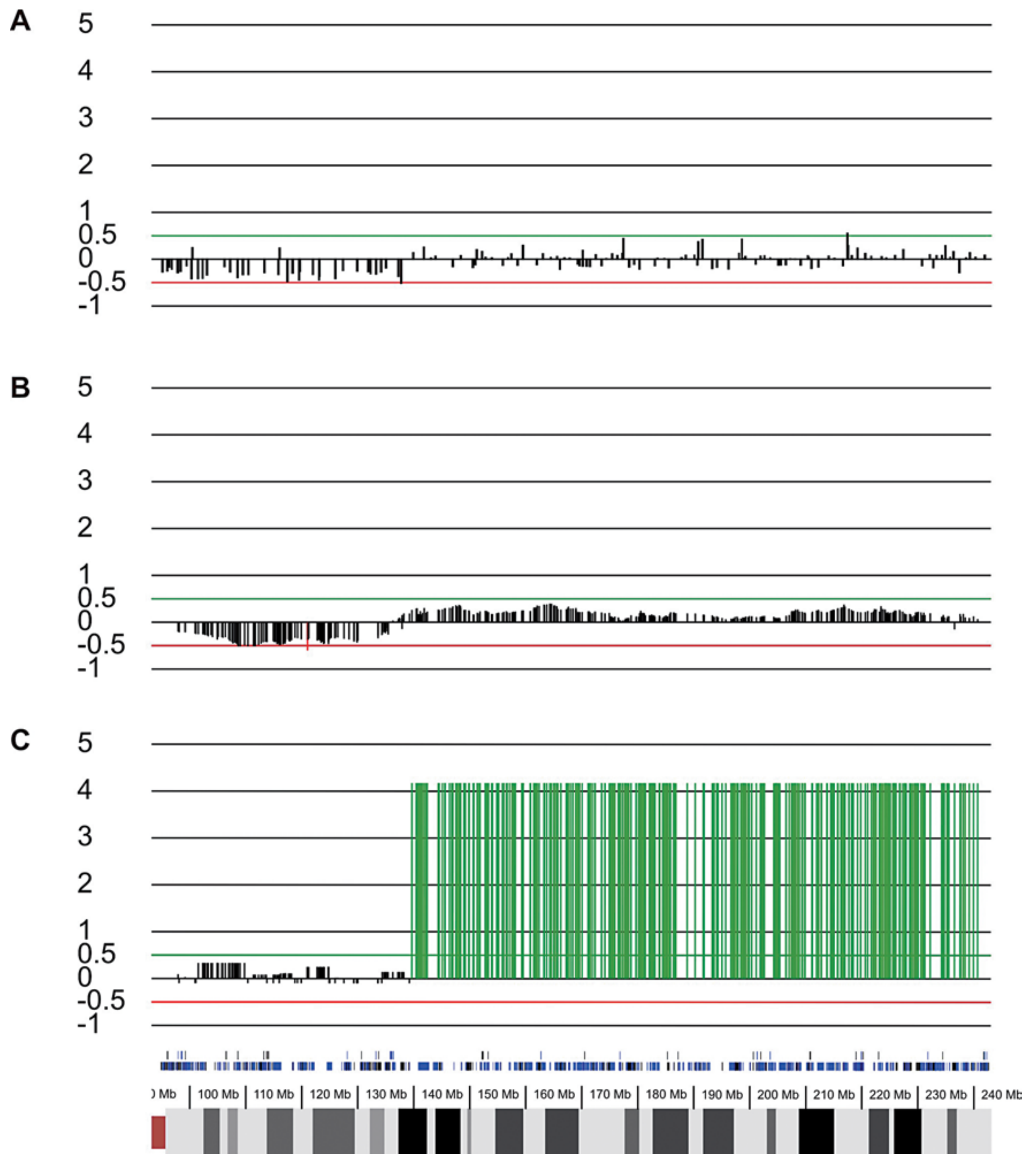

Fig. 2. Comparison of arrayCGH platforms focused on chromosome 2q in NB cell line SJNB-12. Part **A** depicts arrayCGH with a 1-Mb BAC set. Parts **B** and **C** represent Affymetrix SNP data, assessed for DNA copy number changes (**B**) or allele status determination (loss of heterozygosity) (**C**) (LOH-value out of Affymetrix GeneChip Chromosome Copy Number Analysis Tool was divided by 20 for rescaling reasons).

ed for chromosome 2 in NB cell line SJNB-12 (Fig. 2). This allows simultaneous copy number measurement, and detection of extended regions of homozygosity, even without copy number change (Bignell et al., 2004; Zhao et al., 2004). As a disadvantage, oligonucleotide and cDNA arrays typically produce more noise than BAC arrays (presumably due to the limited size of the reporter). Interpretation of copy number status for a given locus therefore requires combination of several adjacent reporters. Using the approach of a sliding window, good results can be produced, but at the cost of resolution. However, thanks to the recent production of (ultra) high-density oligonucleotide arrays, sensitivity remains very high even when 10–50 reporters have to be combined for copy number analysis at a given region. This is probably best illustrated by the current Affymetrix Gene Chip Mapping 500K Array set (3–5 kb) and NimbleGen chips that offer a standard 6 kilobase resolution but can even go up to an amazing resolution of 10 basepairs.

Dissection of amplicons

In total 53 NB cell lines and 176 tumors have been investigated by several groups (Beheshti et al., 2003; Chen et al., 2004; De Preter et al., 2004, 2005; Scaruffi et al., 2004; Mosse et al., 2005; Selzer et al., 2005; Stallings et al., 2006; Hoebeeck et al., in press; Michels et al., in preparation). See also the Wellcome Trust Sanger Institute Cancer Genome Project web site (http://www.sanger.ac.uk/genetics/CGP). With respect to amplicon dissection, the *MYCN* locus, amplified in most of these cell lines, has been investigated in great detail in previous studies (Manohar et al., 1995; Kuroda et al., 1996; Reiter and Brodeur, 1996, 1998). Recent array CGH investigations focused on detailed dissection of complex *MYCN* amplicons as well as rare but putatively important amplicons involving other loci than those on the short arm of chromosome 2 (De Preter et al., 2004).

NB cell line IMR-32 contains a known complex non-contiguous amplicon including *MYCN* (Shiloh et al., 1985; Van Roy et al., 1997; Jones et al., 2000; Spieker et al., 2001) and was included in several array CGH studies (Beheshti et al., 2003; Chen et al., 2004; Mosse et al., 2005; Michels et al., in preparation). These revealed, in addition to *MYCN* and adjacent genes, also involvement of non-contiguous segments from the chromosome 2 short arm. In addition to our standard BAC array analysis we also studied this cell line with 10K SNP chips and Agilent Human Genome CGH MicroArray 44A oligonucleotide chips. Furthermore, IMR-32 was included in a PCR based subtractive cloning procedure in our laboratory (De Preter et al., 2004). Deposition of the subtracted cDNA clones on a custom microarray and hybridization with IMR-32 DNA, resulted in the identification of clones that were overexpressed due to gene amplification. Using this approach, amplification of all previously reported amplified genes in this cell line was detected. Furthermore, four additional clones were found to be amplified, including the *ANTXR1* gene on 2p13.1, two anonymous transcripts, and a fusion transcript resulting from 2p13.3 and 2p24.3 juxtaposed sequences. Table 1 and Fig. 3 summarize the results of all studies on the 2p amplicon in IMR-32 (Beheshti et al., 2003; Chen et al., 2004; De Preter et al., 2004; Mosse et al., 2005; Michels et al., in preparation). Clearly, array CGH is well suited for amplicon dissection but it is of interest that the combined subtractive cloning and array CGH yielded complementary data. Involvement of other regions of 2p in *MYCN* amplicon formation was also detected in a few other cell lines and primary tumors (Beheshti et al., 2003; Chen et al., 2004; Mosse et al., 2005).

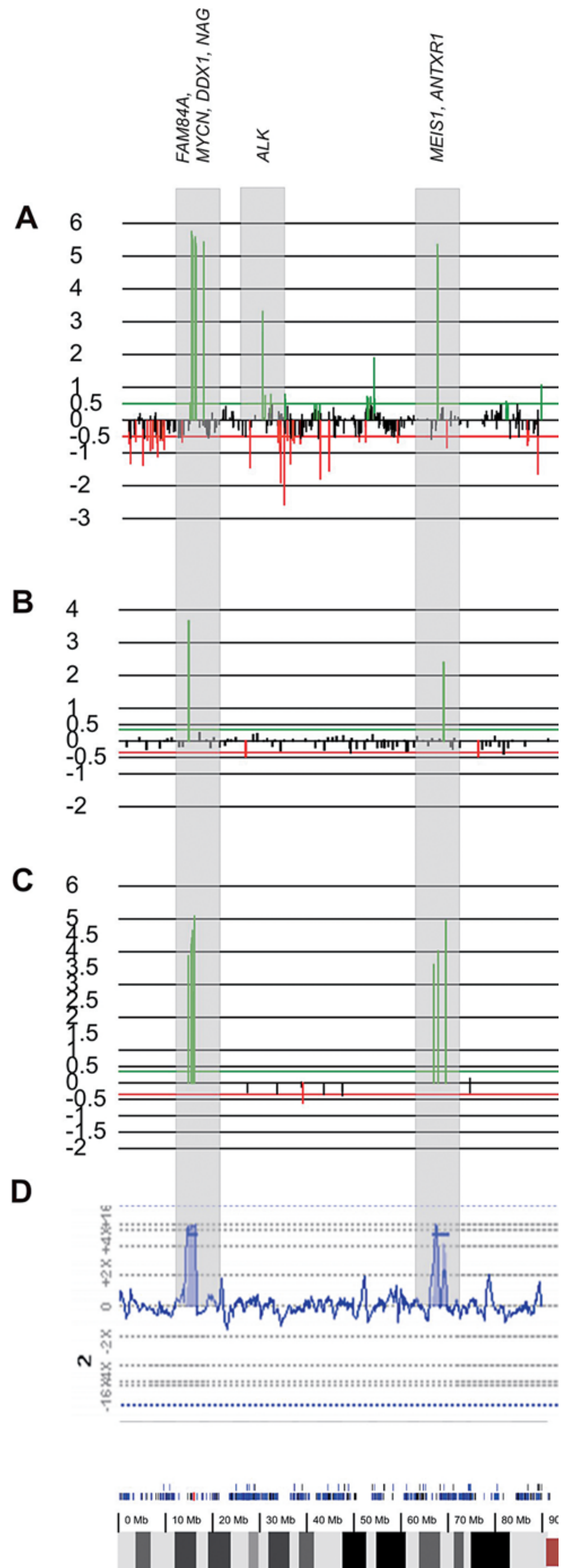

Fig. 3. Dissection of the 2p amplicons on IMR32 by the use of different platforms. (**A**) = Affymetrix 10K SNP chip, (**B**) = BAC array CGH, (**C**) = combined subtractive cDNA cloning and BAC array CGH and (**D**) = Agilent Human Genome CGH MicroArray 44A oligonucleotide chips.

Table 1. Summary of all studies on the 2p amplicon in NB cell line IMR-32

Methodology	Publication	*MEIS1* 2p14–p13	*ANTXR1* 2p13.1	*FAM84A* 2p24.3	*NAG* 2p24	*DDX1* 2p24	*MYCN* 2p24.1	*ALK* 2p23
CGH on cDNA microarrays	Beheshti et al. (2003)	×		×	×		×	
Combined subtractive cDNA cloning and BAC array CGH	De Preter et al. (2004)	×	×	×	×	×	×	
CGH on cDNA microarrays	Chen et al. (2004)	×	×	×	×	×	×	
BAC arrayCGH	Michels et al. (in prep.)	×	×	×	×	×	×	
Agilent	Michels et al. (in prep.)	×	×	×	×	×	×	
Affymetrix SNP chip	Michels et al. (in prep.)	×		×	×	×	×	×

Table 2. Dissection of amplicons in NB cell line NGP

Methodology	Publication	*TSPAN31* 12q13.3	*CDK4* 12q14	*AVIL* 12q14.1	*CTDSP2* 12q13–q15	*MDM2* 12q14.3–q15	*CNOT2* 12q15	*FOXN4* 12q24.11	*ANAPC5* 12q24.31	*RSN* 12q24.3	*KNTC1* 12q24.31
Hybridization of total genomic DNA to cDNA microarrays	Heiskanen et al. (2000)	×	×		×	×					
Array CGH	Michels et al. (in prep.)					×	×			×	×
Affymetrix SNP chip	Michels et al. (in prep.)	×	×	×		×	×	×	×	×	×

NB cell line NGP contains, in addition to the *MYCN* amplicon, also a second non-contiguous complex amplicon consisting of 12q14 and 12q24 sequences (Corvi et al., 1995; Van Roy et al., 1995). This cell line and its amplified sequences were investigated in several array CGH studies (Heiskanen et al., 2000; Chen et al., 2004; Mosse et al., 2005), including a BAC and SNP array analysis in our series of investigations (De Preter et al., 2004; Michels et al., in preparation). These analyses revealed a number of previously not recognized genes implicated in the amplicon formation on 12q in NB cell line NGP; i.e. *CNOT2* at 12q15, *FOXN4* at 12q24.11 and *KNTC1* at 12q24.31. The amplification status of all above-mentioned genes was validated and confirmed by FISH analysis (Table 2). Interestingly, positional expression mapping yielded evidence for a subset of primary NB with 12q amplification (Su et al., 2004).

Yet another nice illustration of the resolving power of array CGH but also of the importance of including metaphase FISH data, which allow interpretation of array CGH data in the context of the genomic position of the aberrant clones, was obtained through the study of the SJNB-12 NB cell line. This cell line is unusual as it is the only NB cell line with amplified sequences in the absence of *MYCN* amplification. Initial CGH and FISH studies showed amplification of sequences derived from 16q22.2→q22.3 including the *ATBF1* transcription factor (Van Roy et al., 2001). Literature data (Boon et al., 2001) demonstrating *MYC* amplification in this cell line prompted us to perform dual-colour FISH with a *MYC* and *ATBF1* clone. This showed amplification of *MYC* and *ATBF1* on double minutes. The detection of a reciprocal t(8;16) with breakpoints in the chromosomal regions containing *ATBF1* and *MYC* suggested that this translocation might have triggered or accompanied amplicon formation in these tumor cells. A combination of FISH, SNP chip and BAC array CGH allowed us to map the amplified sequences, amplicon borders and a total of nine breakpoints that were implicated in a complex process of translocation-excision-deletion-amplification mechanism leading to nonsyntenic co-amplification of *MYC* and *ATBF1* (Van Roy et al., 2006).

Our array CGH screening of 75 primary NB tumors and 29 NB cell lines also revealed amplicons on 11q in five samples, which were confirmed with q-PCR at the genomic level (Michels et al., in preparation). All 11q amplicons were located at chromosomal band 11q13 containing the *CCND1* gene in keeping with previous findings of Molenaar et al. (2003).

Fix et al. (2004) analysed 1p-amplicons located at 1p34.2 and 1p36.3, respectively, found in two neuroblastomas by chromosomal CGH. Array CGH on a medium-resolution genomic array containing 178 PACs/BACs from 1p and subsequent high-resolution arrays containing contigs of overlapping PACs/BACs from the amplified regions, enabled precise mapping and delineation of both amplicons. The 1p34.2 amplicon appeared as a homogeneous amplification unit, whereas the 1p36.3 amplicon exhibited a more complex structure, with two non-contiguous, highly amplified regions and several moderately amplified units.

Genome-wide assessment of chromosomal gains and losses

In total 176 primary tumors and 53 cell lines have been tested by array CGH for chromosomal gains and losses. This approach has several advantages as compared to previous loss of heterozygosity studies using polymorphic markers. First, a genome wide appraisal for gains and losses is obtained in one single experiment by array CGH whereas LOH analysis requires a rather labour-intensive series of PCRs, even when multiplexing is performed. Second, the resolution that is obtained for mapping deletion borders is superior to LOH, in particular when high density oligonucleotide arrays or BAC tiling path arrays are used. Finally, although LOH studies have the advantage of detecting uniparental disomies, interpretation of allelic imbalance in hyperdiploid tumors or losses in tumors with substantial infiltrating normal cells can be problematic. Recent studies, as well as our analyses on a number of cell lines have shown that SNP arrays could be instrumental in the detection of losses and in particular regions of uniparental disomy. In leukemia, a screening of 64 AML cases with normal karyotypes indicated as much as 20% of samples with disomy for particular chromosomal segments (Raghavan et al., 2005). Subsequent analysis for selected genes in these regions showed the presence of mutations e.g. in *RUNX1,* indicating that loss of the normal homologue and reduplication of the chromosome containing the mutated allele contributed to tumor formation (Fitzgibbon et al., 2005). Such an extensive analysis has not been performed in NB and it remains to be determined whether this mechanism of oncogenesis is also implicated in neuroblastoma. For chromosome 2 in SJNB-12, uniparental disomy has been observed (Fig. 2).

In view of the frequent occurrence of 1p and 11q deletions, previous studies using conventional techniques on a large series of tumors have allowed a detailed delineation of the critical regions of loss. The recent array CGH studies have also contributed to the detection and delineation of losses at other chromosomal regions. We have focused specifically on 3p deletions in NB using a chromosome 3p BAC tiling path array. This allowed us to detect small interstitial deletions in NB cell lines as well as a number of critically important distal deletions in tumors which defined in total three putative SROs. Interestingly, the two most convincing SROs were located within regions that are frequently affected in common tumors such as lung and breast cancers (Hoebeeck et al., in press). Array CGH analysis was also used in an effort to fine map the region for 11q deletion in NB. In a previous study, functional evidence for a neuroblastoma suppressor gene on chromosome 11 was obtained through microcell mediated chromosome transfer, indicated by differentiation of neuroblastoma cells with loss of distal 11q upon introduction of chromosome 11 (Bader et al., 1991). Interestingly, some of these microcell hybrid clones were shown to harbor deletions in the transferred chromosome 11. This model system was further exploited as a means to identify candidate tumor suppressor or differentiation genes located on chromosome 11. To this purpose, array CGH was performed to evaluate the chromosome 11 status in the hybrids and allowed the delineation of three putative regions that could harbor the responsible differentiation gene on chromosome 11: 11q25, 11p15.1→p13 and 11p15.3 (De Preter et al., 2005).

Chromosome breakpoint analysis

Apart from the delineation of breakpoints of deleted regions and amplicons, array CGH analysis also allowed us to pinpoint the breakpoints of unbalanced translocations that result in partial gains and losses. In the absence of karyotypes, the nature of the chromosomal rearrangement leading to these gains and/or losses is difficult to determine. For cell lines however, karyotypes and (M)-FISH data are available, thus facilitating the interpretation of the array CGH findings. As mentioned above, gain of 17q represents the most frequent alteration in high-stage NB and typically results from unbalanced rearrangements with involvement of various partner chromosomes. Given the frequency and prognostic importance of 17q gain in NB, we performed array CGH with a chromosome 17 tiling path with an average resolution of 130 kb on a total of 28 NB cell lines and 69 primary tumors (Vandesompele et al., in preparation). One of the primary aims was to define a small critical region of gain in order to facilitate the identification of the genes contributing to NB pathogenesis. In total, twenty-one different chromosome 17 breakpoint regions were identified at the BAC level. The most proximal and distal breakpoints mapped at position 28.58 Mb (17q11.2) and 44.3 Mb (17q21.32), respectively and no small interstitial gains were found (Ensembl version 36). Given the fact that we did not find more distal 17q breakpoints in this series, we hypothesize that one or more critical genes sensitive to a gene dosage effect contributing to neuroblastoma oncogenesis could be located just telomeric to the region harboring the most distally located breakpoint at 17q21.32. Data-mining of gene expression profiles from primary NB tumors for genes located within this presumed critical 1–2 Mb segment allowed the identification of a number of interesting candidate genes (De Preter et al., in preparation; Vandesompele et al., in preparation). Also, we performed an innovative data-mining approach using the L2L (list-to-lists) concept of microarray data analysis which allowed us to compare our expression data of fetal neuroblasts and primary NB with published microarray gene lists (http://medgen.UGent.be/NBGS/) (Pattyn et al., in preparation). This provided additional genes of possible interest in NB development.

Breakpoints can be analyzed at an even higher resolution up to 50 bp to 10 kb. This was illustrated by recent papers by Selzer et al. (2005) and Stallings et al. (2006) who used fine-tiling oligonucleotide array CGH in the study of, amongst others, 17q breakpoints in NB cell lines and primary NB tumors.

Future challenges for array CGH in NB

The results summarized in this review clearly illustrate the analytical power of array CGH methods in the study of genomic imbalances in NB. Array CGH offers a rapid and detailed genome-wide picture of amplifications, deletions and gains and, in conjunction with cytogenetic data, allows high-resolution mapping of unbalanced translocation breakpoints. An increasing number of studies are now focusing on the combined analysis of gene expression and DNA copy number alteration profiling data, as also illustrated in this review. This approach will add more power to the data-mining efforts of the large expression data sets, e.g. for matching genomic subclasses of tumors with particular sets of differentially expressed genes and for focusing on altered gene expression in regions affected by genomic alterations in order to facilitate identification of candidate tumor suppressor genes in the deleted regions or proto-oncogenes in the gained or amplified chromosomal segments.

At the individual patient level, particular patterns of genomic imbalances may have important prognostic predictive value and, therefore, array CGH profiling might become an important aspect of the genetic diagnostic workup of certain tumors. Currently, the widespread use of this methodology is prevented by the high costs of the procedure but it is anticipated that due to further developments and the huge potential market for these products, array CGH will become a standard procedure for genome-wide detection of chromosomal imbalances.

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References

Alitalo K, Schwab M, Lin CC, Varmus HE, Bishop JM: Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene *(c-myc)* in malignant neuroendocrine cells from a human colon carcinoma. Proc Natl Acad Sci USA 80: 1707–1711 (1983).

Altura RA, Maris JM, Li H, Boyett JM, Brodeur GM, Look AT: Novel regions of chromosomal loss in familial neuroblastoma by comparative genomic hybridization. Genes Chromosomes Cancer 19:176–184 (1997).

Bader SA, Fasching C, Brodeur GM, Stanbridge EJ: Dissociation of suppression of tumorigenicity and differentiation in vitro effected by transfer of single human chromosomes into human neuroblastoma cells. Cell Growth Differ 2:245–255 (1991).

Beheshti B, Braude I, Marrano P, Thorner P, Zielenska M, Squire JA: Chromosomal localization of DNA amplifications in neuroblastoma tumors using cDNA microarray comparative genomic hybridization. Neoplasia 5:53–62 (2003).

Bignell GR, Huang J, Greshock J, Watt S, Butler A, et al: High-resolution analysis of DNA copy number using oligonucleotide microarrays. Genome Res 14:287–295 (2004).

Boon K, Caron HN, van Asperen R, Valentijn L, Hermus MC, et al: N-myc enhances the expression of a large set of genes functioning in ribosome biogenesis and protein synthesis. EMBO J 20:1383–1393 (2001).

Bown N: Neuroblastoma tumour genetics: clinical and biological aspects. J Clin Pathol 54:897–910 (2001).

Bown N, Cotterill S, Lastowska M, O'Neill S, Pearson AD, et al: Gain of chromosome arm 17q and adverse outcome in patients with neuroblastoma. N Engl J Med 340:1954–1961 (1999).

Breen CJ, O'Meara A, McDermott M, Mullarkey M, Stallings RL: Coordinate deletion of chromosome 3p and 11q in neuroblastoma detected by comparative genomic hybridization. Cancer Genet Cytogenet 120:44–49 (2000).

Brinkschmidt C, Christiansen H, Terpe HJ, Simon R, Boecker W, et al: Comparative genomic hybridization (CGH) analysis of neuroblastomas – an important methodological approach in paediatric tumour pathology. J Pathol 181: 394–400 (1997).

Brinkschmidt C, Poremba C, Christiansen H, Simon R, Schafer KL, et al: Comparative genomic hybridization and telomerase activity analysis identify two biologically different groups of 4s neuroblastomas. Br J Cancer 77:2223–2229 (1998).

Brodeur GM: Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer 3:203–216 (2003).

Brodeur GM, Fong CT: Molecular biology and genetics of human neuroblastoma. Cancer Genet Cytogenet 41:153–174 (1989).

Brodeur GM, Sekhon G, Goldstein MN: Chromosomal aberrations in human neuroblastomas. Cancer 40:2256–2263 (1977).

Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM: Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 224:1121–1124 (1984).

Cai WW, Mao JH, Chow CW, Damani S, Balmain A, Bradley A: Genome-wide detection of chromosomal imbalances in tumors using BAC microarrays. Nat Biotechnol 20:393–396 (2002).

Chen QR, Bilke S, Wei JS, Whiteford CC, Cenacchi N, et al: cDNA array-CGH profiling identifies genomic alterations specific to stage and *MYCN*-amplification in neuroblastoma. BMC Genomics 5:70 (2004).

Corvi R, Savelyeva L, Breit S, Wenzel A, Handgretinger R, et al: Non-syntenic amplification of *MDM2* and *MYCN* in human neuroblastoma. Oncogene 10:1081–1086 (1995).

Cunsolo CL, Bicocchi MP, Petti AR, Tonini GP: Numerical and structural aberrations in advanced neuroblastoma tumours by CGH analysis; survival correlates with chromosome 17 status. Br J Cancer 83:1295–1300 (2000).

De Preter K, Pattyn F, Berx G, Strumane K, Menten B, et al: Combined subtractive cDNA cloning and array CGH: an efficient approach for identification of overexpressed genes in DNA amplicons. BMC Genomics 5:11 (2004).

De Preter K, Vandesompele J, Menten B, Carr P, Fiegler H, et al: Positional and functional mapping of a neuroblastoma differentiation gene on chromosome 11. BMC Genomics 6:11 (2005).

du Manoir S, Speicher MR, Joos S, Schrock E, Popp S, et al: Detection of complete and partial chromosome gains and losses by comparative genomic in situ hybridization. Hum Genet 90: 590–610 (1993).

Ejeskar K, Aburatani H, Abrahamsson J, Kogner P, Martinsson T: Loss of heterozygosity of 3p markers in neuroblastoma tumours implicate a tumour-suppressor locus distal to the *FHIT* gene. Br J Cancer 77:1787–1791 (1998).

Fiegler H, Carr P, Douglas EJ, Burford DC, Hunt S, et al: DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. Genes Chromosomes Cancer 36:361–374 (2003).

Fitzgibbon J, Smith LL, Raghavan M, Smith ML, Debernardi S, et al: Association between acquired uniparental disomy and homozygous gene mutation in acute myeloid leukemias. Cancer Res 65:9152–9154 (2005).

Fix A, Peter M, Pierron G, Aurias A, Delattre O, Janoueix-Lerosey I: High-resolution mapping of amplicons of the short arm of chromosome 1 in two neuroblastoma tumors by microarray-based comparative genomic hybridization. Genes Chromosomes Cancer 40:266–270 (2004).

Garnis C, Baldwin C, Zhang L, Rosin MP, Lam WL: Use of complete coverage array comparative genomic hybridization to define copy number alterations on chromosome 3p in oral squamous cell carcinomas. Cancer Res 63:8582–8585 (2003).

Gilbert F, Feder M, Balaban G, Brangman D, Lurie DK, et al: Human neuroblastomas and abnormalities of chromosomes 1 and 17. Cancer Res 44:5444–5449 (1984).

Greshock J, Naylor TL, Margolin A, Diskin S, Cleaver SH, et al: 1-Mb resolution array-based comparative genomic hybridization using a BAC clone set optimized for cancer gene analysis. Genome Res 14:179–187 (2004).

Guo C, White PS, Weiss MJ, Hogarty MD, Thompson PM, et al: Allelic deletion at 11q23 is common in *MYCN* single copy neuroblastomas. Oncogene 18:4948–4957 (1999).

Hallstensson K, Thulin S, Aburatani H, Hippo Y, Martinsson T: Representational difference analysis and loss of heterozygosity studies detect 3p deletions in neuroblastoma. Eur J Cancer 33:1966–1970 (1997).

Heiskanen MA, Bittner ML, Chen Y, Khan J, Adler KE, et al: Detection of gene amplification by genomic hybridization to cDNA microarrays. Cancer Res 60:799–802 (2000).

Hirai M, Yoshida S, Kashiwagi H, Kawamura T, Ishikawa T, et al: 1q23 gain is associated with progressive neuroblastoma resistant to aggressive treatment. Genes Chromosomes Cancer 25:261–269 (1999).

Hoebeeck J, Michels E, Menten B, Van Roy N, Eggert A, et al: High resolution deletion breakpoint mapping using tiling-path BAC arrays defines two small distinct critical regions at 3p21–p22 in neuroblastoma. Int J Cancer in press (2006).

Hoshi M, Otagiri N, Shiwaku HO, Asakawa S, Shimizu N: Detailed deletion mapping of chromosome band 14q32 in human neuroblastoma defines a 1.1-Mb region of common allelic loss. Br J Cancer 82:1801–1807 (2000a).

Hoshi M, Shiwaku HO, Hayashi Y, Kaneko Y, Horii A: Deletion mapping of 14q32 in human neuroblastoma defines an 1,100-kb region of common allelic loss. Med Pediatr Oncol 35:522–525 (2000b).

Iehara T, Hamazaki M, Sawada T: Cytogenetic analysis of infantile neuroblastomas by comparative genomic hybridization. Cancer Lett 178: 83–89 (2002).

Ishkanian AS, Malloff CA, Watson SK, DeLeeuw RJ, Chi B, et al: A tiling resolution DNA microarray with complete coverage of the human genome. Nat Genet 36:299-303 (2004).

Jones TA, Flomen RH, Senger G, Nizetic D, Sheer D: The homeobox gene *MEIS1* is amplified in IMR-32 and highly expressed in other neuroblastoma cell lines. Eur J Cancer 36:2368–2374 (2000).

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, et al: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258:818–821 (1992).

Kaneko Y, Kanda N, Maseki N, Sakurai M, Tsuchida Y, et al: Different karyotypic patterns in early and advanced stage neuroblastomas. Cancer Res 47:311–318 (1987).

Kuroda H, White PS, Sulman EP, Manohar CF, Reiter JL, et al: Physical mapping of the *DDX1* gene to 340 kb 5′ of *MYCN*. Oncogene 13:1561–1565 (1996).

Lastowska M, Nacheva E, McGuckin A, Curtis A, Grace C, et al: Comparative genomic hybridization study of primary neuroblastoma tumors. United Kingdom Children's Cancer Study Group. Genes Chromosomes Cancer 18:162–169 (1997).

Look AT, Hayes FA, Nitschke R, McWilliams NB, Green AA: Cellular DNA content as a predictor of response to chemotherapy in infants with unresectable neuroblastoma. N Engl J Med 311: 231–235 (1984).

Lucito R, Healy J, Alexander J, Reiner A, Esposito D, et al: Representational oligonucleotide microarray analysis: a high-resolution method to detect genome copy number variation. Genome Res 13:2291–2305 (2003).

Manohar CF, Salwen HR, Brodeur GM, Cohn SL: Co-amplification and concomitant high levels of expression of a DEAD box gene with *MYCN* in human neuroblastoma. Genes Chromosomes Cancer 14:196–203 (1995).

Maris JM, Matthay KK: Molecular biology of neuroblastoma. J Clin Oncol 17:2264–2279 (1999).

Maris JM, Weiss MJ, Guo C, Gerbing RB, Stram DO, et al: Loss of heterozygosity at 1p36 independently predicts for disease progression but not decreased overall survival probability in neuroblastoma patients: a Children's Cancer Group study. J Clin Oncol 18:1888–1899 (2000).

Molenaar JJ, van Sluis P, Boon K, Versteeg R, Caron HN: Rearrangements and increased expression of cyclin D1 (*CCND1*) in neuroblastoma. Genes Chromosomes Cancer 36:242–249 (2003).

Mosse Y, Greshock J, King A, Khazi D, Weber BL, Maris JM: Identification and high-resolution mapping of a constitutional 11q deletion in an infant with multifocal neuroblastoma. Lancet Oncol 4:769–771 (2003).

Mosse YP, Greshock J, Margolin A, Naylor T, Cole K, et al: High-resolution detection and mapping of genomic DNA alterations in neuroblastoma. Genes Chromosomes Cancer 43:390–403 (2005).

Plantaz D, Mohapatra G, Matthay KK, Pellarin M, Seeger RC, Feuerstein BG: Gain of chromosome 17 is the most frequent abnormality detected in neuroblastoma by comparative genomic hybridization. Am J Pathol 150:81–89 (1997).

Plantaz D, Vandesompele J, Van Roy N, Lastowska M, Bown N, et al: Comparative genomic hybridization (CGH) analysis of stage 4 neuroblastoma reveals high frequency of 11q deletion in tumors lacking *MYCN* amplification. Int J Cancer 91:680–686 (2001).

Raghavan M, Lillington DM, Skoulakis S, Debernardi S, Chaplin T, et al: Genome-wide single nucleotide polymorphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. Cancer Res 65:375–378 (2005).

Reiter JL, Brodeur GM: High-resolution mapping of a 130-kb core region of the *MYCN* amplicon in neuroblastomas. Genomics 32:97–103 (1996).

Reiter JL, Brodeur GM: *MYCN* is the only highly expressed gene from the core amplified domain in human neuroblastomas. Genes Chromosomes Cancer 23:134–140 (1998).

Savelyeva L, Corvi R, Schwab M: Translocation involving 1p and 17q is a recurrent genetic alteration of human neuroblastoma cells. Am J Hum Genet 55:334–340 (1994).

Scaruffi P, Parodi S, Mazzocco K, Defferrari R, Fontana V, et al: Detection of *MYCN* amplification and chromosome 1p36 loss in neuroblastoma by cDNA microarray comparative genomic hybridization. Mol Diagn 8:93–100 (2004).

Schleiermacher G, Janoueix-Lerosey I, Combaret V, Derre J, Couturier J, et al: Combined 24-color karyotyping and comparative genomic hybridization analysis indicates predominant rearrangements of early replicating chromosome regions in neuroblastoma. Cancer Genet Cytogenet 141:32–42 (2003).

Schwab M: Oncogene amplification in solid tumors. Semin Cancer Biol 9:319–325 (1999).

Schwab M, Alitalo K, Klempnauer KH, Varmus HE, Bishop JM, et al: Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. Nature 305:245–248 (1983).

Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, et al: Association of multiple copies of the *N-myc* oncogene with rapid progression of neuroblastomas. N Engl J Med 313:1111–1116 (1985).

Selzer RR, Richmond TA, Pofahl NJ, Green RD, Eis PS, et al: Analysis of chromosome breakpoints in neuroblastoma at sub-kilobase resolution using fine-tiling oligonucleotide array CGH. Genes Chromosomes Cancer 44:305–319 (2005).

Shiloh Y, Shipley J, Brodeur GM, Bruns G, Korf B, et al: Differential amplification, assembly, and relocation of multiple DNA sequences in human neuroblastomas and neuroblastoma cell lines. Proc Natl Acad Sci USA 82:3761–3765 (1985).

Snijders AM, Nowak N, Segraves R, Blackwood S, Brown N, et al: Assembly of microarrays for genome-wide measurement of DNA copy number. Nat Genet 29:263–264 (2001).

Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, et al: Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer 20:399–407 (1997).

Speicher MR, Gwyn Ballard S, Ward DC: Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 12:368–375 (1996).

Speleman F, Bown N: 17q gain in neuroblastoma, in Brodeur GM, Sawada T, Tsuchida Y, Voûte PA (eds): Neuroblastoma (Elsevier Science, Amsterdam, 2000).

Spieker N, van Sluis P, Beitsma M, Boon K, van Schaik BD, et al: The *MEIS1* oncogene is highly expressed in neuroblastoma and amplified in cell line IMR32. Genomics 71:214–221 (2001).

Stallings RL, Nair P, Maris JM, Catchpoole D, McDermott M, et al: High-resolution analysis of chromosomal breakpoints and genomic instability identifies *PTPRD* as a canidate tumor suppressor gene in neuroblastoma. Cancer Res 66: 3673–3680 (2006).

Su WT, Alaminos M, Mora J, Cheung NK, La Quaglia MP, Gerald WL: Positional gene expression analysis identifies 12q overexpression and amplification in a subset of neuroblastomas. Cancer Genet Cytogenet 154:131–137 (2004).

Takita J, Hayashi Y, Kohno T, Shiseki M, Yamaguchi N, et al: Allelotype of neuroblastoma. Oncogene 11:1829–1834 (1995).

Theobald M, Christiansen H, Schmidt A, Melekian B, Wolkewitz N, et al: Sublocalization of putative tumor suppressor gene loci on chromosome arm 14q in neuroblastoma. Genes Chromosomes Cancer 26:40–46 (1999).

Thompson PM, Seifried BA, Kyemba SK, Jensen SJ, Guo C, et al: Loss of heterozygosity for chromosome 14q in neuroblastoma. Med Pediatr Oncol 36:28–31 (2001).

van Duin M, van Marion R, Watson JE, Paris PL, Lapuk A, et al: Construction and application of a full-coverage, high-resolution, human chromosome 8q genomic microarray for comparative genomic hybridization. Cytometry A 63: 10–19 (2005).

Van Gele M, Van Roy N, Jauch A, Laureys G, Benoit Y, et al: Sensitive and reliable detection of genomic imbalances in human neuroblastomas using comparative genomic hybridisation analysis. Eur J Cancer 33:1979–1982 (1997).

Van Roy N, Laureys G, Cheng NC, Willem P, Opdenakker G, et al: 1;17 translocations and other chromosome 17 rearrangements in human primary neuroblastoma tumors and cell lines. Genes Chromosomes Cancer 10:103–114 (1994).

Van Roy N, Forus A, Myklebost O, Cheng NC, Versteeg R, Speleman F: Identification of two distinct chromosome 12-derived amplification units in neuroblastoma cell line NGP. Cancer Genet Cytogenet 82:151–154 (1995).

Van Roy N, Jauch A, Van Gele M, Laureys G, Versteeg R, et al: Comparative genomic hybridization analysis of human neuroblastomas: detection of distal 1p deletions and further molecular genetic characterization of neuroblastoma cell lines. Cancer Genet Cytogenet 97:139–142 (1997).

Van Roy N, Van Limbergen H, Vandesompele J, Van Gele M, Poppe B, et al: Combined M-FISH and CGH analysis allows comprehensive description of genetic alterations in neuroblastoma cell lines. Genes Chromosomes Cancer 32:126–135 (2001).

Van Roy N, Vandesompele J, Menten B, Nilsson H, De Smet E, et al: Translocation-excision-deletion-amplification mechanism leading to nonsyntenic coamplification of *MYC* and *ATBF1*. Genes Chromosomes Cancer 45:107–117 (2006).

Vandesompele J, Van Roy N, Van Gele M, Laureys G, Ambros P, et al: Genetic heterogeneity of neuroblastoma studied by comparative genomic hybridization. Genes Chromosomes Cancer 23:141–152 (1998).

Vandesompele J, Speleman F, Van Roy N, Laureys G, Brinskchmidt C, et al: Multicentre analysis of patterns of DNA gains and losses in 204 neuroblastoma tumors: how many genetic subgroups are there? Med Pediatr Oncol 36:5–10 (2001).

Vandesompele J, Baudis M, De Preter K, Van Roy N, Ambros P, et al: Unequivocal delineation of clinicogenetic subgroups and development of a new model for improved outcome prediction in neuroblastoma. J Clin Oncol 23:2280–2299 (2005).

Vettenranta K, Aalto Y, Wikstrom S, Knuutila S, Saarinen-Pihkala U: Comparative genomic hybridization reveals changes in DNA-copy number in poor-risk neuroblastoma. Cancer Genet Cytogenet 125:125–130 (2001).

Wessendorf S, Fritz B, Wrobel G, Nessling M, Lampel S, et al: Automated screening for genomic imbalances using matrix-based comparative genomic hybridization. Lab Invest 82:47–60 (2002).

White PS, Thompson PM, Gotoh T, Okawa ER, Igarashi J, et al: Definition and characterization of a region of 1p36.3 consistently deleted in neuroblastoma. Oncogene 24:2684–2694 (2005).

Zhao X, Li C, Paez JG, Chin K, Janne PA, et al: An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. Cancer Res 64: 3060–3071 (2004).