Consultations in Molecular Diagnostics

Application of Multicolor Banding for Identification of Complex Chromosome 18 Rearrangements

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Multicolor chromosome banding (mBAND) is a recently developed technique that allows the delineation of chromosomal regions with a resolution of a few megabase pairs. The resolution of mBAND is slightly below that of conventional chromosome banding; however, the color bands have a great value in identifying chromosomal abnormalities, particularly complex chromosome rearrangements, and intrachromosome exchanges (ie, inversions, deletions, duplications, and insertions). These abnormalities cannot be defined easily by conventional cytogenetic analysis or chromosome paint. In this report, we present the application of the mBAND analysis for identification of complex intrachromosome rearrangements of chromosome 18 in a child with dysmorphic features. (J Mol Diagn 2006, 8:521–525; DOI: 10.2353/jmoldx.2006.060001)

Cytogenetic techniques have been widely used for the identification of chromosomal abnormalities in patients with mental retardation, congenital malformations, dysmorphic features, amenorrhea, reproductive wastage, infertility, and neoplastic diseases. However, complex chromosome rearrangements cannot be identified easily using regular banding techniques alone. These rearrangements involve more than two chromosomes or have more than two breaks in one chromosome. Molecular cytogenetic techniques including fluorescence *in situ* hybridization (FISH) and spectral karyotyping (SKY; multicolor FISH) have been used as complementary tools for

analysis of such cases. FISH with chromosome-specific paint probes has been routinely used as a confirmatory test for chromosome abnormalities detected by conventional cytogenetic analysis.¹ SKY, which utilizes 24 chromosome-specific paint probes, has been used to identify complex chromosome rearrangements between nonhomologous chromosome pairs, such as complex translocations, and for identification of additional chromosome material with unknown origin.² More recently, microarray comparative genomic hybridization (array CGH) has been used to define the critical regions for deletion or duplication.³ Isolated paint probes, or SKY, cannot be used to identify intrachromosomal rearrangements, such as deletions, duplications, inversions, and insertions. Array CGH cannot be used to detect balanced rearrangements, such as reciprocal translocations, inversions, and insertions. The multicolor banding (mBAND) technique can be used to overcome some of these difficulties.^{4,5} The mBAND DNA probe contains a mix of region-specific partial chromosome paint probes (PCPs) that are generated by microdissection of a particular chromosome and labeled with three to five different fluorophores. The neighboring PCPs partially overlap each other. Consequently, the overlapping of the neighboring PCPs decreases the fluorescent intensity toward the margins of the signals leading to a consistent variation of fluorescent intensity ratios along the longitudinal axis of the chromosomes. These unique color combinations can be identified with the mFISH/mBAND module of the FISH imaging software Isis (MetaSystems, Altlußheim, Germany). The final banding resolution is about 500 bands per genome.⁴ Although this technique was developed in 1999, it has not been widely used for analysis. In the current report, we demonstrate the application of mBAND as a complementary method to standard cytogenetics and routine FISH analysis for identification of complex chromosome 18 rearrangements in a child with developmental delay and dysmorphic features.

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Materials and Methods

Conventional Cytogenetics

Cytogenetic analysis was performed using phytohemagglutinin-stimulated peripheral blood lymphocyte cultures, and metaphase chromosomes were banded by GTG banding technique. Lymphocyte culture and GTG-banding were performed using standard protocols described in the AGT Cytogenetics Laboratory Manual.⁶ Karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN 2005).

FISH Analysis

The most distal region on a chromosome arm that is comprised of unique DNA sequences is referred to as the "subtelomere." Chromosome 18 subtelomeric probes for FISH analysis included probes for the subtelomeric region of the short arm (18ptel) and the long arm (18qtel) of chromosome 18 purchased from Vysis (Downers Grove, IL). The hybridization was performed according to the manufacturer's protocol.

mBAND Analysis

The mBAND analysis was performed by MetaSystems (Altussheim, Germany). The dissected mBAND chromosome 18 probes were directly labeled with three different fluorochromes, fluorescein isothiocyanate, SpectrumOrange, and diethylamino-coumarin, and the mBAND slides were imaged with the efficient FISH imaging system Isis.

Case Report

A female infant was delivered by cesarean section due to breech position at 36 weeks to a 15-year-old G1P0 mother. The birth weight was 1729 g, and the length was 40.5 cm. Both of these measurements are below the 10th percentile for adjusted gestational age. At birth, the physical examination revealed a prominent forehead, side hair whirl, epicanthal folds, down-slanting palpebral fissures, hypertelorism, and widely spaced nipples. The anterior fontanel was 2 × 2 cm in size. Growth restriction was first noticed at 31 weeks gestation by ultrasound. The pregnancy was uneventful with the exception of significant morning sickness. The mother denied recreational drug exposure; however, she did take the prescribed medications Zofran, Paxil, and Neurontin. At adjusted age of 6 weeks, the newborn's weight was 2.92 kg, which is less than fifth percentile for adjusted age of 5 weeks. She appeared dysmorphic due to a prominent forehead and a very flat nasal bridge. Her inner canthal distance of 2.9 cm was greater than 2 standard deviations above the mean. She had strabismus and a right-side preauricular pit. Her ears were not low set. She had small feet, and her thumbs and toes appeared a little short. She was noted to have an intracranial cyst by MRI and was diagnosed with Chiari malformation. She was found to have an anteriorly

Figure 1. Partial High-resolution G-banded karyotypes of the cell lines showing ring chromosome 18 (left) and derivative chromosome 18 (right).

displaced anus, without stricture or fistula. She had poor eating and weight gain. At the age of 2, her height was 74 cm and her weight was 8.8 kg, which are less than the fifth percentile. She also had obstructive sleep apnea and subsequently underwent tonsillectomy and adenoidectomy. Developmental and speech delays were noted.

Results

Cytogenetic Analysis

Cytogenetic analysis at the 625-GTG-band level showed a mosaic karyotype. Eleven of the 20 cells examined by traditional cytogenetics were missing a #18 chromosome but contained a ring chromosome that appeared to be composed of chromosome 18 material (Figure 1). Breakage and reunion was estimated to have occurred at bands 18p11.3 and 18q21.3. The segments 18p11.3 to 18pter and 18q21.3 to 18qter appeared to have been lost. The remaining nine cells examined contained an apparent terminal deletion of chromosome 18q (Figure 1), with breakage at band 18q22.1. The original karyotype based on the traditional cytogenetic analysis was 46,XX, r(18)(p11.3q21.3)[11]/46XX,del(18)(q22.1)[9].

FISH Analysis

Metaphase FISH analysis revealed the absence of both the short arm and the long arm subtelomere probes on the ring chromosome 18, as expected. Therefore, these cells contained a partial monosomy for the short arm of chromosome 18 (18p) and for the long arm of chromosome 18 (18q). However, the cells of the second cell line containing the apparent terminal deletion of 18q showed a signal corresponding to the 18p telomere on the terminal ends of both the p arm and the deleted q arm (Figure 2a). No signal for the 18q telomere probe was seen on this chromosome (Figure 2b). These results indicate that the cells with this apparently deleted chromosome 18 had a partial trisomy for 18p and a partial monosomy for 18q. It appeared that only the subtelomeric region of the 18p was duplicated by FISH analysis.

mBAND Analysis

mBAND analysis was performed to further characterize the deletion and duplication of the abnormal chromosome 18s. Figures 3 and 4 show the images of the

Figure 2. A: FISH with the 18ptel probe showing two signals on each arm of the derivative chromosome. **B:** The derivative chromosome 18 negative for the 18qtel probe.

derivative chromosome 18 and the ring chromosome 18, respectively, along with images of the normal chromosome 18. The results of the mBAND indicated that the derivative chromosome 18 had a deletion from band q21.3 to the terminal region that stained dark blue, pink, and orange on the normal 18 and a duplication of the region from 18p11.3 to 18pter that stained light blue and yellow in color. The deleted region was larger than the region defined by conventional cytogenetics. The last band on the q arm of the derivative chromosome 18 was in fact the duplicated region 18p11.3 to 18pter and not a terminal deletion at 18q22.1 as previously defined by conventional cyto-genetic analysis. Therefore, the karyotype of the cell line with the derivative 18 was reinterpreted as 46,XX,der(18)(pter->q21.3::p11.3->pter). For the ring chromosome 18, the mBAND results were consistent with the cytogenetic findings. Although the breakpoints shown by mBAND analysis and the cytogenetic analysis were slightly different, we believe that the correct chromosome breakpoints are p11.3 on both the derivative 18 and the ring 18 chromosomes as shown by the high resolution G-banding (Figure 1). The slight differences in breakpoints may be due to the limitation of the mBAND technique, such as the nature of the probe and flaring of the fluorophores. However, as a complementary technique mBAND analysis was helpful in detecting the larger duplication that was not visible by traditional cytogenetic analysis. Furthermore, FISH analysis indicated

only a subtelomeric duplication of 18p on the derivative 18, whereas mBAND analysis showed a larger duplicated fragment from 18p11.3 to 18pter.

Discussion

Cytogenetic analysis provides useful information for clinical diagnosis, prognosis, and management of many genetic disorders. Patients with ring 18 often share clinical features with 18q deletion or 18p deletion syndromes or a combination of both.⁷ The most common features of the 18p deletion syndrome are short stature, facial dysmorphism, mental retardation, and skeletal and cardiac anomalies.⁸ The main phenotype of the 18q deletion syndrome includes mental retardation, short stature, mid-face hypoplasia, deeply set eyes, abnormal genitalia, hypotonia, malformed ears, and foot deformities.⁹ However, patients with a deletion of chromosome 18, particularly the long arm of chromosome 18, may have a phenotype that varies considerably depending on the size of the deletion and location of the breakpoint. Without mBAND analysis we would have underestimated the sizes of the 18q deletion and 18p duplication in the cell line with the derived chromosome 18.

Patients with mosaic duplication of 18 and ring 18 have been reported⁷; however, those patients had a cell line with ring 18 and a cell line with both ring 18 and duplicated 18. The present case has unique cytogenetic findings. The duplication of 18p in the cell line with derivative 18 (deletion of 18q and duplication of 18p) may compensate for the deletion of the 18p in the ring 18 cell line to a certain degree, although the compensation may not be complete. Both cell lines contained a deletion of 18q, therefore the patient was expected to have the phenotypic features of the 18q deletion syndrome. Some of the most common features, eg, developmental and speech delays, short stature, hypertelorism, epicanthal folds, and short thumbs and toes were observed in our patient. Because our patient had a mosaic karyotype for the deleted/duplicated 18 and ring chromosome 18, precise phenotype and karyotype correlation was not possible.

Two possible mechanisms for the formation of the two cell lines in our patient are illustrated in Figure 5. The first model (Figure 5A) suggests that the formation of two abnormal cell lines most likely occurred due to two postzygotic events in early embryonic development. Perhaps just after fertilization during the first mitotic cycle at the G2 stage, a chromosome 18 was broken at q21.3 and one of the broken chromatids also had a break at p11.3. This was followed by separation of the two derivative chromatids. In one of the daughter cells, the ends of the chromatid with deletion of p11.3 and q21.3 were fused to form a ring chromosome 18, while the chromatid with only the deletion of q21.3 was fused with the chromatid fragment of p11.3->pter to form the derivative chromosome 18 in the second daughter cell. The fragments of q21.3 to qter were lost. The second hypothesis (Figure 5B) suggests that one of the parents may be a carrier for a pericentric inversion involving 18p11.3 and 18q21.3. As a result of a crossover during meiosis, the zygote received a recombinant chromosome,

Figure 3. mBAND image and profiles showing the normal 18 (top) and the derivative chromosome 18 with deletion and duplication (bottom).

Figure 4. mBAND image and profiles showing the normal 18 (top) and the ring chromosome 18 (bottom).

Figure 5. A and B: Schematic representation of two suggested mechanisms for origin of the two derivative cell lines.

rec(18)dup(18p)inv(p11.3q21.3) (Figure 5B). At the early stage of embryonic development, chromosome fragments from p11.3 to pter at both ends of the recombinant chromosome were cut off, and the ends of the remaining chromosome material were fused to form a ring 18. It has been noted that the maternal family has a history of multiple miscarriages. The parental blood samples were not available for further study, therefore, we could not exclude the possibility that one of the parents carries a balanced rearrangement involving chromosome 18.

In conclusion, we have applied the technique of mBAND to identify complex chromosome 18 rearrangements in a child with dysmorphic features. Using FISH and mBAND techniques we confirmed the size of the deleted fragments in the ring chromosome of one cell line and redefined an apparently terminal deletion on chromosome 18 as a derivative 18 with deletion of q21.3 to qter and duplication of p11.3 to pter in the second cell line. Precise delineation of deletions and duplications can provide useful information for clinical management of the patient. Our study demonstrated that mBAND technique is a useful complementary technique for analysis of complex intrachromosome abnormalities.

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