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EDITORIAL

Karyotyping in the era of genome sequencing

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Since 1959, the emergence of cytogenetics as a diagnostic tool was made possible due to the microscopic observation of chromosome preparations during the metaphase stage. In this year, Lejeune proved by using this technique that down syndrome patients' karyotype contains a third copy of chromosome 21. After that, other aneuploidies and several deletion and duplication syndromes were described during the 1960s and 1970s using karyotype analysis, particularly after the use of the G-banding technique in 1971.

For several decades, this technique designated today as "conventional cytogenetics" remained the gold standard genetic analysis technique offering a pangenomic view of chromosomal aberrations even with the limited resolution of approximately 6 Mb and the requirement of subjective visual morphologic interpretation of chromosomes abnormalities that is dependent on staff expertise and experience.

Since the 1990s, fluorescence in situ hybridization (FISH) methods based on nucleic acid hybridization and fluorescent microscope observation emerged as a powerful molecular cytogenetic method for the analysis of target chromosomal rearrangements with greatly expanded sensitivity to detect submicroscopic abnormalities such as microdeletions, microduplications, and cryptic translocations. FISH also has the added advantage to analyze uncultured interphase cells, single-cells, and paraffin sections tissue and has a superiority in identifying low-level mosaicism compared with karyotype. However, FISH remains a tool for targeted analysis and does not allow the global genomic view as karyotyping. Muli-FISH and spectral karyotyping analysis developed at the end of the 1990s by a combination of the karyotype and FISH advantages failed to become a routine cytogenetics test and to replace the traditional banded karyotype.

Based on the principle of FISH, a new methodology was developed in 1998 by the hybridization of patient and control DNA on a microarray containing genomic clones providing extensive coverage of the genome. This technique named comparative genomic hybridization (CGH) array entered for the first time the arena of clinical diagnostics in 2004 and redefined cytogenetic testing, including the new terminology of copy-number variant adopted to describe small deletions and duplications that is >1 kb in size. For the first time the term "molecular karyotyping" was used.

For more than 10 years, the CGH array was routinely used with increasing resolution and became the first-tier test for chromosomal analysis in cases of developmental disorders and/or multiple congenital anomalies with around 20% improved diagnostic rate compared to the 3% observed with conventional karyotyping. CGH array allowed us to identify numerous new recurrent microdeletions and microduplication syndromes. Nonetheless, molecular karyotyping by CGH array is not able to detect low-level mosaicism and balanced rearrangements, and above all, it cannot determine the mechanism of the detected imbalance, which is essential to elucidate a correct interpretation and achieve correct genetic counseling. For that reason, we still continue to perform our "old-fashioned" and famous banded karyotype to verify molecular karyotyping results at the chromosomal level to distinguish between: (1) a derivative chromosome result of unbalanced translocation, (2) an intrachromosomal duplication, (3) an insertion, and (4) a small supernumerary marker chromosome, from a duplication detected by CGH array, and to see if one of the parents is a carrier or not of the balanced form of this rearrangement, which is essential for genetic counseling.

Recently, whole genome sequencing (WGS) joined the panel of routine diagnostic tools thanks to the improvement of next-generation sequencing and data analysis. In its original form, WGS was used to look for sequence mutations using short-read sequencing techniques. Recently, WGS using long-read sequencing coupled with chromosomal reconstitution by alignment and quantitative analysis software started being used to detect balanced structural chromosome rearrangements and copy-number variations. This NGS-based pangenomic technique is recently called "next-generation karyotyping" with a high potential to replace CGH array "molecular karyotyping" during the next few years in routine clinical testing.

It is exciting to imagine WGS as an "all-in-one" routine testing allowing complete analysis starting from

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structural chromosomal rearrangements, going through copy-number changes, and ending with sequence-level variants.

But is it finally the end of our famous G-banded karyotype? Not sure at all!

Metaphasis karyotype is still the only way to allow us to have a whole genomic view at a single cell level compared to the cell pooling DNA analysis carried out during WGS. For that, low-level mosaicism, clonal evolution in cancer, and rearrangements lying within large repetitive, unmappable regions such as Robertsonian translocations and centromeric translocations remain the private territory of conventional karyotyping. Microscopic observation of stained chromosomes still resists new molecular technologies to preserve a place in cytogenetics laboratories making the happiness of old-school cytogeneticists.

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