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Review

Combined classical and molecular cytogenetic analysis of cancer☆

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Abstract

While chromosome-banding analysis has set the standard for karyotyping from 1970 onwards, fluorescent *in situ* hybridisation (FISH) has more recently been used to complement the study of chromosomal rearrangements. Especially useful has been the appearance of FISH methodologies with screening abilities, namely comparative genome hybridisation (CGH), multicolour-FISH (m-FISH), and cross-species colour banding (R×FISH). These FISH-based screening techniques are reviewed here together with methodologies using chromosome- or locus-specific probes. Emphasis is put on the strengths and limitations of these FISH techniques to complement standard chromosome banding analysis. Judicious choice from the molecular cytogenetic techniques now available has significantly improved our ability to characterise the genomic rearrangements of cancer cells. © 2002 Published by Elsevier Science Ltd.

Keywords: Chromosome banding; FISH; m-FISH; R×FISH; CGH; Chromosome- and locus-specific probes

1. Introduction

While standard chromosome banding analysis, because of its simplicity and robustness combined with high investigative resolution, remains the technique of choice for the initial screening for karyotypic abnormalities, technical developments based on fluorescence *in situ* hybridisation (FISH) have widened our arsenal to detect and describe the sometimes overwhelmingly complex chromosome changes that are present in neoplastic cells [1]. This relatively new field of molecular cytogenetics, which makes use of a variety of nucleic acid sequences as probes to cellular DNA targets, has helped bridge the gap between molecular genetic and classical cytogenetic analyses.

2. FISH-based screening techniques

Since their introduction more than 30 years ago and until recently, standard chromosome banding techniques stood alone in their ability to examine in a single experiment the entire genome of individual cells for chromosome-level changes. Although FISH with specific probes is nowadays a well-established technique that can help cytogenetic analysis, the selection of probes required a presumption of what the aberration might be, an approach that is prone to mistakes and may necessitate multiple, time-consuming experiments. Recent years, however, have witnessed the appearance of various FISH-based techniques able to generate an unbiased picture of the entire genome of neoplastic cells (Fig. 1).

2.1. Multicolour-FISH

The long searched for goal of multicolour karyotyping was made possible by the availability of several spectrally discrete fluorochromes and the use of combinatorial probe labelling schemes. To identify every human chromosome with an individual colour using painting probes, 24 different probe sets are necessary (22 pairs of autosomes plus the two sex chromosomes). Multicolour-FISH (m-FISH) is very useful to detect and describe cryptic rearrangements and marker chromosomes often found in complex karyotypes, although it is somewhat limited in the identification of intrachromosomal changes and in breakpoint determination. Nevertheless, the combination of chromosome banding

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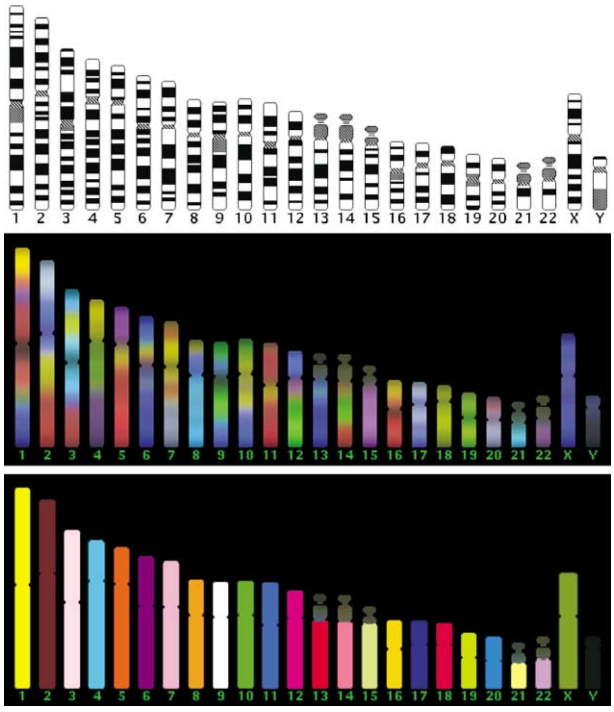

Fig. 1. Ideograms of fluorescence *in situ* hybridisation (FISH)-based screening techniques that are able to analyse all chromosomes at the same time, providing an overview of the karyotypic changes of cancer cells. Top: standard chromosome banding (G-banding); middle: cross-species colour banding (R×FISH); bottom: multicolour-FISH (m-FISH).

analysis with m-FISH has the potential to identify and describe most karyotypic changes of cancer cells. Several partially different methods for m-FISH have now been described.

2.1.1. Spectral karyotyping

Spectral karyotyping (SKY) is based on the simultaneous hybridisation of 24 chromosome-specific painting probes, each one labelled with a different combination of five fluorochromes [2]. The entire emission spectrum is measured through a single custom designed optical filter by means of spectral imaging, which is based on a combination of fluorescence microscopy, Fourier spectroscopy, and charge-coupled device imaging. An interferogram is generated for each pixel in the image that is specific for a given fluorochrome or fluorochrome combination. The spectral classification then assigns a discrete colour to all pixels with identical spectra, thus serving as the basis for chromosome identification. Analysis with SKY has been shown to solve many problems left unsettled by traditional chromosome banding techniques, contributing for a better understanding of the genomic changes that occur in and presumably drive carcinogenesis [3–5].

2.1.2. Multiplex-FISH

Multiplex-FISH (M-FISH) uses a combinatorial labelling scheme with five spectrally distinguishable fluorochromes similar to the one described for spectral karyotyping, but the method to detect and discriminate the different combinations of fluorescence signals differs. This is done by capturing separate images for each of the five fluorochromes using narrow bandpass microscope filters, which are then combined by dedicated software [6]. Uniquely distinctive pseudocolours are assigned to each chromosome based on their specific fluorochrome signature. The filter-based approach has the advantage of permitting the visualisation of the signal from each fluorochrome individually, thereby allowing control of the results. This technique has already proven useful to characterise complex chromosome aberrations found in cancer cells [7,8].

2.1.3. Combined binary ratio FISH

Combined binary ratio FISH (COBRA-FISH) uses ratio labelling in addition to the combinatorial labelling described above [9]. With this approach, human chromosome painting in 24 colours is achieved using only four fluorochromes detected using custom designed filters. Three fluorochromes are used pair-wise for ratio labelling of a set of 12 chromosome painting probes and the second set of 12 probes is given a binary label in addition (fourth fluorochrome). Despite the fact that ratio labelling is far more challenging than pure binary approaches, the use of this methodology with a fifth fluorochrome opens up the possibility of arm-specific painting [10] or the combination of m-FISH with locus-specific probes [11].

2.2. Cross-species colour banding

Cross-species colour banding (also known as R× FISH standing for rainbow cross-species FISH) has the particularity of using flow-sorted, differentially labelled gibbon chromosomes as DNA probes [12]. Because of the extensive sequence homology between gibbon and human DNA (98%) and the many chromosomal rearrangements that have occurred during evolution, the hybridisation of these probes onto human metaphase plates gives rise to a specific colour banding pattern for each chromosome. This feature makes it useful to characterise not only inter-, but also intrachromosomal rearrangements like deletions and inversions [13,14].

Despite the advantage of its banding capability, current R×FISH technology uses combinations of only three fluorochromes to label the probes, which of necessity implies that several segments of different chromosomes share the same colour. Furthermore, the present-day banding resolution of R×FISH is less than 100 bands compared with the nearly 400 bands attainable by standard G-banding. This notwithstanding, it has been shown that the combined use of standard chromosome banding analysis with R×FISH allows the resolution of most chromosomal rearrangements

(Fig. 2), both in haematological malignancies [13,15] and in solid tumours [14,16].

2.3. Comparative genomic hybridisation

All FISH-based screening methods mentioned above have a common feature, which is shared with standard chromosome banding analysis: their dependence on the availability of metaphases from the neoplastic cells. Whenever the mitotic rate *in vitro* is poor, the chromosome analysis by these techniques is hindered. This is not the case for Comparative genomic hybridisation (CGH), since this technique is based on the *in situ* hybridisation of differentially labelled total genomic tumour DNA and normal reference DNA to normal human metaphases [17]. The ratio of the fluorescence intensities of tumour and normal DNAs is measured along every chromosome, allowing one to obtain an overview of DNA sequence copy number changes (losses and gains) in the neoplastic cells mapped on normal chromosomes.

Besides analysing cancer cells that grow poorly *in vitro*, CGH is also useful in cases with complex karyotypes containing markers, double minutes, homogeneously staining regions and additions of unknown material. The limitations are that CGH only detects the imbalances that are present in a substantial proportion of the tumour sample and that it is unable to detect balanced chromosomal rearrangements. Since most pathogenetically important karyotypic changes in haematological cancer are balanced, CGH has mostly been used to detect copy number changes in solid tumours. A non-random pattern of genomic imbalances has emerged from these studies [18], and these data are now guiding more detailed analysis with microarray-based CGH [19]. Especially informative are those studies that combine traditional G-banding with CGH analysis of the same tumours [20]. Furthermore, the possibility of making use of genomic DNA extracted from archival material after tissue microdissection and DNA amplification by universal primer polymerase chain reaction (PCR), allows the analysis of minute subregions of tumours or even individual cells, making possible the comparison of different stages of tumour progression.

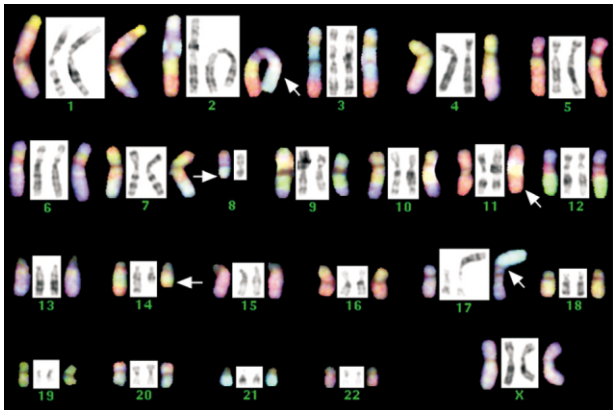

Fig. 2. Composite karyogram after sequential G-banding and R×FISH colour banding of the same metaphase cell from an uterine sarcoma. Arrows point to chromosomes with structural abnormalities. The combined analysis showed the karyotype to be 45,XX,t(2;8)(p25;q13),-8, inv(11)(p15q23),del(14)(q22q32),der(17)t(8;17)(q11;p13).

3. Chromosome- and locus-specific FISH

Although whole-genome information of cancer cells should ideally be obtained by one or more of the methods described above, it must be recognised that this is not always possible or warranted. An alternative is to perform FISH analysis with chromosome- and/or locus-specific probes. It must, however, be emphasised that this approach enables one to find only what one is looking for, genomic rearrangements involving areas not analysed with our *a priori* selection of probes remain undetected. This type of analysis is especially relevant when trying to solve eventual doubts remaining after the initial genome screening and when one is only interested in detecting already known, pathogenetically or prognostically relevant, rearrangements in a particular neoplasia.

3.1. Chromosome painting

A whole-chromosome painting probe is made of sequences from the entire length of a given chromosome labelled with a fluorochrome. These chromosome-specific probes can be generated by chromosome flow sorting or microdissection of normal metaphase chromosomes, followed by sequence-independent DNA amplification by DOP-PCR and fluorochrome labelling [21,22]. The probes can be hybridised to metaphases obtained from neoplastic cells to detect cytogenetic changes involving the specific chromosomes they target, being especially useful to delineate the identity of rearranged chromosomes for which one already has some clue [23]. Small intrachromosomal changes, like deletions, duplications or inversions, remain undetectable with this approach.

Microdissection has the major advantage over flow sorting of being able to generate region-specific probes (from entire arms to a single band) in addition to whole chromosome paints. Differentially labelled, overlapping microdissection libraries can be used as probes for multicolour banding of individual chromosomes [24], thereby allowing the identification of intrachromosomal rearrangements on those chromosomes that are probed.

3.2. Reverse painting

The same approach described above to produce probes for direct chromosome painting can also be used

for reverse chromosome painting [21]. The trick is to isolate the abnormal chromosomes one is interested in by flow sorting or chromosome microdissection and, after amplification and labelling, use them as painting probes on normal metaphases (CGH is also considered a reverse FISH technique since it, too, uses tumour DNA and normal metaphases). If microdissection is used, the unknown part of an abnormal chromosome can be isolated and have its identity revealed by reverse painting [25]. Since normal chromosomes are easily identified by inverted DAPI counterstaining, it becomes possible to determine the identity of the abnormal chromosomes initially isolated by determining where the probes hybridise. In addition to G-banded chromosomes, microdissection can also be performed on m-FISH metaphases to determine the breakpoints involved in already identified interchromosomal changes [26].

3.3. Repetitive-sequence probes

There are presently two types of probes that are based on chromosome-specific repetitive sequences. The first are centromeric probes targeting chromosome-specific satellite sequences of pericentromeric heterochromatin, which are used to enumerate chromosomes. These probes can be useful when numerical chromosomal changes carry diagnostically or prognostically relevant information, as is the case in a subgroup of childhood acute lymphoblastic leukaemia. This is especially important when no or only poor-quality metaphases are available, since these probes are also informative in interphase cells [27]. The second type of probes based on repetitive sequences are chromosome-specific subtelomeric probes, which are able to identify the ends of all chromosomes individually [28]. These probes can be useful to detect cryptic translocations that may escape the resolution level of G-banding or FISH painting probes.

3.4. Locus-specific probes

Single locus probes are aimed at detecting sequences normally present in only one copy in the haploid genome. Locus-specific probes can be useful to confirm specific rearrangements that can be masked in complex or cryptic chromosomal changes. However, their main utility in cancer genetics is the detection of deletions, amplifications, inversions and translocations that are often disease-specific [29–31]. The possibility of using these probes in interphase cells makes them useful not only for the diagnosis of neoplasias with a low mitotic activity, but also for the detection of minimal residual disease and monitoring in cases that are well characterised genetically. Furthermore, the possibility of using locus-specific (as well as chromosome enumeration) probes in interphase cells from archival material allows genetic analysis in the context of cellular phenotype and tissue architecture.

4. Conclusion

It is important to emphasise that the FISH methodologies reviewed here complement, but do not replace, standard chromosome banding analysis. Every technique has its strengths and limitations. The initial screening for cancer-associated cytogenetic changes should, whenever possible, be performed by standard chromosome banding analysis, followed by the appropriate FISH method depending on what questions remain to be answered. This combined approach should ideally be performed on the same cells (Fig. 2) to avoid interpretation errors caused by cell-to-cell genetic heterogeneity [14]. The combination of G-banding with the newer FISH technologies has already allowed the detection of novel recurrent chromosome changes in human neoplasia, as exemplified by the t(12;21) in acute lymphoblastic leukaemia and the t(5;11) in acute myeloid leukaemia [32,33]. A cost–benefit analysis should guide the choice of which method or combination of methods should be used in a particular clinical or research setting.

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