editorial
Haematologica 1995; 80:95-97

FISH (FLUORESCENT IN SITU HYBRIDIZATION): THE SECOND YOUTH OF CYTOGENETICS

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The introduction of high resolution chromosome banding at the beginning of the eighties significantly improved the techniques for cancer cytogenetics. New criteria for classification and monitoring of leukemia and lymphoma have been established, and new insights into the understanding of the genetic bases of malignancies have been gained.[1] The limits of classical cytogenetics, however, are determined by the low number of metaphases available for analysis in some types of malignant hemopathies as well as by possible misinterpretations of banding in complex exchanges.

Thus, much excitement was generated when the new techniques of molecular biology were introduced to investigate malignant hemopathies at the gene level. FlSH combines the resolution of molecular techniques with cytogenetics and allows the precise localization of nucleic acid sequences of interest on chromosomes or in interphase nuclei.[2] A single-stranded DNA probe hybridizes with a single-stranded sequence on a slide. Probes labeled with molecules such as biotin or digoxigenin may be detected after hybridization by using fluorochrome-conjugated molecules with green (fluorescein) and red (Texas red) coloration, respectively. DNA probes commonly available include centromeric α satellite probes, used in interphase analyses,[3] whole-chromosome specific probes, obtained from libraries of single chromosomes, which allow precise assignment of chromosome material (so-called chromosome painting),[4] locus-specific probes which detect restricted chromosome regions.[5] This last group of probes includes lambda phages, yeast artificial chromosome (YAC) and cosmids, which are vectors of DNA sequences of different sizes. In addition to DNA probes, RNA probes can localize transcription products.

Furthermore morphology and immunophenotyping may be combined with molecular cytogenetics in both metaphase and interphase. This technology may be applied on cryostat sections as well as on cytospin preparations, where metaphases are spread within an intact cytoplasmic membrane.[6,7]

Two recent papers in this journal show the usefulness of FISH in clinical hematology.[8,9] Mancini et al.[8] studied a patient with a clinical picture suggestive of chronic myelogenous leukemia (CML), cytogenetically characterized by a t(9;21;22) with no clear involvement of chromosome 9. The dual color FISH technique performed by using specific painting probes for chromosomes 9, 21 and 22, and a bcr-abl translocation probe enabled them to confirm the diagnosis of CML by detecting bcr-abl rearrangement on chromosome 22q and involvement of chromosome 9 in a variant translocation t(9;21;22).

The paper by Fugazza et al.[9] is an example of the association of interphase FISH with simultaneous evaluation of cell morphology. Results show that in a case of sideroblastic anemia monosomy 7 was present in all leukocytes but absent in lymphocytes. These data are in accord with previously published studies on myelodysplastic syndromes (MDS) by Gerritsen et al.[10] who performed FISH analysis in myeloid and lymphoid subpopulations of MDS with monosomy 7 after separation with a cell sorter. Similar results have also been documented in MDS associated with trisomy 8, where simultaneous evaluation of *in situ* hybridization with a centromeric probe and cell morphology showed only two chromosomes 8 in lymphocytes.[11,12] Moreover, FISH demonstrated that both monosomy 7 and trisomy 8 may be restricted to distinct subpopulations in the myeloid system.[12] Thus, in some cases only monocytes and granulocytes were affected,[7] while in others erythrocytes were also

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involved.[12] Whether this cell lineage variability reflects differences in clinical-hematological features is still undetermined. The involvement of lymphocytic cells in some MDS must also be better defined since clonal lymphocytic subpopulations have been detected either by polymorphism studies or by standard cytogenetics.[13,14]

In acute myeloid leukemia (AML), FISH associated with cell identification by monoclonal antibodies showed that the translocation t(15;17) in M3 type is only found in myeloid cells, while in acute leukemia of the M2 type with t(8;21) translocation the involvement of some erythroid cells is still controversial.[10] Interphase cytogenetics for numerical anomalies such as monosomy 7, trisomy 8, and trisomy 12 added information to classical cytogenetics by increasing the incidence of positive clonal cases.[15-18] However, since false negative results also exist, large prospective studies comparing classical cytogenetics and FISH should be performed to establish whether FISH is suitable for detecting minimal residual disease in cases with numerical changes.

Trisomy 12 in chronic lymphocytic leukemia (CLL) may be detected by FISH not only in cases without mitoses, but also in those with a normal karyotype.[18-21] Moreover, atypical morphology and/or immunology are usually found in cases with trisomy 12 detected either by FISH or by classical cytogenetics.[20,21] In addition, FISH confirmed that this change only affects a portion of the malignant B cells identified by immunological investigations.[21] The significance of this result is still not clear, although we know that it is not related to the clinical-hematological phase of the disorder.[20]

New insights into the incidence and biological significance of other recurrent trisomies in hematological malignancies are expected from interphase FISH. In a large FISH screening of trisomy 11 in MDS/AML, the number of positive cases was no greater than that found by karyotyping.[16] Moreover, preliminary results on hypoplastic MDS with trisomy 6 in a small clone revealed the presence of the trisomy in all dyserythropoietic cells.[23]

FISH has already made major contribution to the improvement of classical cytogenetics by chromosome painting.[4] The conventional cytogenetic definition of marker to refer to an unidentifiable chromosome on banding will possibly disappear from future cytogenetic nomenclature thanks to chromosome painting, which allows us to specifically *color* single chromosomes or pieces of them. Thus, even very complex structural rearrangements may be identified; mistakes from banding interpretation may be corrected and cryptic translocations may be revealed.[10,24]

As a further refinement to chromosome painting, locus-specific probes are helpful for exploring in detail the chromosomal region involved in a structural rearrangement. From classical cytogenetics we knew that a cytogenetic subgroup in CLL with a 13q– chromosomic change existed. Speculations were made about involvement of the retinoblastoma gene on 13q14 in this deletion. On interphase FISH, Stilgenbauer et al.[25] showed that Rb-1 deletions do indeed occur in B cells of CLL. Interestingly, deletions of Rb-1 have also been detected by in situ hybridization in another B cell neoplasia, multiple myeloma, in which classical cytogenetics has frequently been unsuccessful.[26]

A new typical translocation t(12;21) in a subgroup of acute lymphoblastic leukemia has been characterized at the molecular level by the use of YAC clones, which localize the breakpoint at 21q22.2.[27] Molecular restriction of breakpoints in chromosome translocations via the identification of probes (YAC or cosmids) spanning the translocated region, may be a first step in strategies for the cloning of new genes.[1,28]

A spectacular application of interphase cytogenetics is the so-called dual color-FISH. This technique is based on knowledge of the molecular composition of the hybrid gene underlying specific chromosome translocations. The paradigm is once more represented by the Philadelphia chromosome with a juxtaposition of the abl gene from chromosome 9 and bcr sequences from chromosome 22.[29] The simultaneous use of a biotinylated probe for abl and a digoxigenin-labeled probe for bcr results in three signals in cells bearing the Philadelphia chromosome; one green, one red, and one green/red together. This technique represents an additional tool to PCR for monitoring the Philadelphia chromosome in the evaluation of minimal residual disease.

Another elegant application of this procedure was recently[4] published by Garicochea et al.[30] who excluded the presence of the Philadelphia rearrangement in T lymphocytes from chronic myeloid leukemia. In addition to the Philadelphia rearrangement, interphase FISH has already demonstrated other typical translocations in hematological malignancies, such as the t(15;17) (PML/RARα at the gene level) of M3 acute leukemia and the t(8;14) (myc/IgH at the gene level) of Burkitt lymphoma.

It is easy to forecast a burst of papers on the applications of FISH in hematological malignancies in the near future. Ledbetter,[2] in a recent review oh FISH technology and its impact on the scientific community, underlined that each time a new technology is transferred to diagnostic procedures there is immediate excitement about the opening of new horizons. When the first limitations and pitfalls are discovered, however, disappointment may arise. Only when people have become quite expert will the technique be properly used. Ledbetter concluded that with regard to FISH we still are in the excitement phase!

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