Guest Editorial

Advances in the detection of ploidy differences in cancer by in situ hybridization

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Abstract

Three main techniques allow the detection of changes in the cellular genomic content. The karyotyping procedure on metaphase spreads can give specific information on chromosome number and structural chromosome changes, but analyses are restricted to a limited number of chromosome spreads. Furthermore, cell culturing of (in particular solid) cancer specimens can result in selection of a minor tumor cell population with a high proliferative capacity. On the other hand, flow cytometry allows the analyses of large numbers of cells, but does not detect small variations in the DNA content or structural changes. The fluorescent in situ hybridization (FISH) procedure combines the advantages of the two former procedures, in that relatively large numbers of cells can be analysed easily and specific chromosomal changes can be detected.

Key words: Flow cytometry; Karyotyping; In situ hybridization; DNA content; Chromosome aberrations

Introduction

Changes in the cellular DNA content and the proliferative fraction of certain malignancies are regarded as clinical prognosticators. As a result, the flow cytometric (FCM) analysis of such lesions has become a widely used and objective screening method for the quantitation of the DNA content and for the estimation of the individual cell cycle fractions \cite{10}. This technique, however, is limited in that it does not normally detect small variations in DNA content and provides no information about specific chromosome aberrations. Karyotyping of solid tumours is a more precise approach in this respect and detects numerical and/or structural chromosomal defects \cite{15}. However, when tumour cells are cultured to obtain more and better metaphases as compared to the direct analysis, a potential danger of loss of genetic material and selection of certain fast growing subpopulations exists. A recently developed technique for the detection of specific genetic aberrations is the fluorescent in situ hybridization (FISH) procedure using specific DNA probes. This technique allows the detection of numerical and structural chromosome aberrations in non-mitotic cells \cite{12}. The method, also termed interphase cytogenetics, has already been
applied to several types of malignancies [1,2,4–6,11,16–19]. It has been demonstrated that this approach enables routine screening of large tumour cell populations in, for example, transitional cell carcinomas of the urinary bladder [6–9,11–14,19]. Furthermore, FISH enables the detection of minor cell populations or heterogeneity in chromosome copy number within one tumour.

**DNA probes**

Several types of DNA probe can be used to detect numerical and structural chromosome aberrations in cancer cells. DNA probes recognizing highly repetitive sequences, mostly in the centromeric and telomeric regions, are now routinely applicable in daily practice [16,20]. In most instances, probes of the satellite or alphoid family have been used. The targets of these probes occur in several hundreds up to several thousands of higher-order repeats. This results in DNA targets of several thousand kilobase pairs (kb) localized in the compact centromere or telomere regions of the individual chromosomes. Since these chromosome regions also appear as distinct DNA clusters within the interphase nucleus, and since moreover their number is constant during S-phase [3], these probes can be used reproducibly to detect chromosome copy number (Fig. 1a,b). For the detection of whole chromosomes, or chromosome arms, so-called chromosome library probes are being used, which comprise a large range of DNA probes for one specific chromosome. Such probes mark complete chromosome regions, rather than only a fraction of a chromosome. Double- and triple-target FISH procedures, combining such library probes with centromere and telomere probes, already allow the detection of structural chromosome aberrations.

Several methods for FISH detection of DNA targets have been described [14]. They are based on the enzymatic or chemical introduction of a reporter molecule into the DNA probe that can then be detected immunocytochemically after hybridization. Recently, directly labeled fluorochrome probes have become (commercially) available. FISH procedures have been applied to single cell suspensions prepared from solid tumours, tumour cell lines, imprints, cytologic preparations, as well as paraffin sections and frozen sections of solid tumours [14].

**Comparison of FISH and karyotyping**

Chromosome banding techniques play an important role in the detection of genetic aberrations of hematological malignancies for example. They are used in determining

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**Fig. 1.** (a) FISH with a chromosome 1 centromere probe on a chromosome spread and an interphase nucleus of normal human lymphocytes. (b) Double-target FISH of a lymphocyte with centromere probes for chromosome 1 (red) and chromosome 18 (green). (c) Nuclei from a DNA diploid bladder carcinoma, targeted with a probe for chromosome 1, showing three spots per nucleus. Occasionally, split spots are detected in these preparations (upper left nucleus). (d) Double-target FISH on leukemic cells with centromere probes for chromosome 7 (green) and chromosome 17 (red). Co-localization of chromosome centromeres (arrow) indicates a translocation between these two chromosomes. (e) Double-target FISH on nuclei from a near-diploid bladder carcinoma. Red spots represent chromosome 1 centromere regions, while the green spots result from the chromosome 18 centromere target. Note three spots for chromosome 1 and two spots for chromosome 18. (f) The same case as in (e) showing a polyplloid cell with six spots for chromosome 1 (red) and four spots for chromosome 18 (green).
diagnostic and prognostic parameters, since well established knowledge of chromosome aberrations in several malignancies exists. Recently, the results of the interphase cytogenetic FISH procedure have been compared to conventional cytogenetic analyses of cell lines derived from solid tumours, in neoplastic cells from bone marrow and peripheral blood [1,11,16]. Although the detection of structural aberrations is limited when centromeric DNA probes are used, double-target FISH experiments on interphase nuclei can, for example, detect translocations between chromosomes (Fig. 1d) [11]. In some cases this could be done only retrospectively, although recently marker chromosomes could be recognized by colocalization of FISH signals in double-target FISH procedures. In these latter cases the FISH data were used to explain cytogenetically non-characterized marker chromosomes. With the FISH method it is also feasible to detect low numbers of host cells in sex-mismatched bone marrow transplant recipients [17], where male cells could be detected in a female population on the order of 0.01% with a Y-chromosome-specific probe.

By means of conventional karyotyping, non-random chromosome aberrations involving chromosomes 1, 5, 7, 9 and 11 have frequently been detected in bladder cancer (for a review, see ref. 14). Monosomy for chromosome 9 was observed in many of these cases, where in about 40% of the bladder carcinomas this aberration was the only karyotypic change observed. Loss of chromosome 9 has been recognized as an early event in bladder carcinogenesis. With progression of the disease, numerical and structural abnormalities of chromosomes 1, 7 and 11 have been proposed to occur in a high percentage of tumours. In more detail, comparison of karyotyping data with recent results of FISH in bladder cancer reveals the following correlations [6–9, 13, 14, 19].

For bladder cancer, aberrations involving a monosomy for chromosome 9, and trisomy for chromosomes 1 and 7, have been described by FISH. Such analyses of flow cytometrically analysed transitional cell carcinomas have shown that about 60% of low grade/low stage tumours show a numerical aberration for at least one of chromosomes 1, 7 and 9 (Fig. 1c) [6–9, 13, 19]. Most striking was the finding of a monosomy for chromosome 9 in about 30% of these low grade, low stage transitional cell carcinomas. Trisomy of chromosomes 1 and 7 was detected in about 30 and 20% of the cases studied, respectively.

Aberrations such as +1p- and duplication of 1q are frequently encountered chromosomal anomalies in bladder cancer. Similarly, extra chromosome 1 copy numbers were detected by FISH in diploid cases. We examined in more detail 22 cases with a centromeric (1q12) and a telomeric-associated (1p36) DNA probe, as well as a library DNA probe from sorted human chromosome 1 in single- and double-target FISH procedures [13]. All flow cytometrically determined DNA diploid bladder carcinomas that showed three spots for 1q12 had two spots for 1p36. Since the library DNA probe showed three separate domains in the nuclei of these cases, the additional copy for 1q12 could be explained as an extra chromosome 1p-, containing the 1q12 target. This means that, in cases with a trisomy for chromosome 1 (Fig. 1c), which occurred at a frequency of about 20% in low grade, non-invasive bladder carcinomas, 1p36 was specifically deleted. In the flow cytometric DNA tetraploid/aneuploid bladder tumours the results were more complex. In a considerable number of cases we observed an overrepresentation of 1q12 as compared to 1p36, also suggesting the presence of extra copies of 1p- chromosomes.

Involvement of chromosome 7 in invasion and tumour progression has been
reported for several malignancies. In 2 out of 24 non-invasive, superficially growing bladder carcinomas with a normal DNA index, an extra chromosome 7 copy was detected. With progression, chromosome 7 was affected in 3 of 9 cases of our series of invasive diploid bladder carcinomas [6].

Our recent results as well as those from Waldman et al. [19] confirmed the cytogenetic observations concerning loss of chromosome 9 in low grade, low stage bladder carcinomas. In a high percentage of non-invasive, low grade bladder carcinomas a monosomy for chromosome 9 was detected. The loss of chromosome 9 is most probably not limited to a small part of 9q, since as a result of the DNA target in the heterochromatin on 9q, close to the centromere, only complete loss of this heterochromatin region would result in a complete disappearance of the chromosome 9 signal. In most cases, only one clear FISH signal was observed, suggesting complete loss of complementary chromosome 9 or at least the entire 9q arm.

In brain tumours cytogenetic studies revealed polysomy for chromosome 7 and loss of chromosomes 10, 22 and sex chromosomes. Screening for these chromosomal aberrations using centromeric DNA probes by interphase cytogenetics, apart from a discrepancy in the frequency of appearance of chromosomal aberrations as described in the literature, resulted in the same cytogenetic aberrations being found [2].

**Comparison of FISH and flow cytometry**

Before going into detail it is important to realize that the number of FISH signals, in the case of the chromosome centromere probes for example, is constant during the different cell cycle stages, including G₀G₁, S and G₂M [3]. This enables the discrimination between a flow cytometrically determined G₂M population of a diploid tumour and a G₀G₁ population of a tetraploid tumour. In these cases, two FISH signals and four FISH signals for each chromosome centromere were detected, respectively.

In order to illustrate the advantages of the FISH technique over FCM in the characterization of malignancies, we will summarize the results obtained with this interphase cytogenetic procedure in several different types of tumours that were analysed in parallel by FCM.

ISH studies on bladder cancers, which had a DNA index of approximately 1.0 as measured by FCM, have shown that, in diploid transitional cell carcinomas, chromosome copy number which deviated from normal could frequently be detected [5,7]. Moreover, tetraploid cells which were present at low frequency (<5%) could easily be detected. In many of these FCM diploid cases, which were abnormal as determined by FISH, no indication of an abnormal genomic content was found by flow cytometry.

As mentioned above, in diploid, non-invasive bladder cancer cases, evident clonal abnormalities for chromosomes 1, 7 and 9 were detected. Generally, with tumour progression the DNA index increases. The complexity of the chromosome copy numbers, at that stage, hampers a simple comparison of DNA index, chromosome copy numbers, and karyotyping data from the literature. No correlation seems to exist between the flow cytometrically determined DNA index and chromosome copy number as detected by FISH.
Invasive bladder carcinomas with a tetraploid/aneuploid DNA index showed a large chromosome heterogeneity both with respect to the range of chromosome copy numbers in tumours with the same DNA index, as well as with respect to different chromosomes within the same tumour. In these aneuploid bladder carcinomas the copy numbers for different chromosomes ranged from 1 to 7, depending on the DNA index. Close examination of the DNA aneuploid/tetraploid cases shows that the chromosome 9 copy number was frequently under represented as compared to the other chromosomes. This means that whenever four or more spots for other chromosomes are detected, a high percentage of these cells shows only two spots for chromosome 9. Recently, screening of extensive series of bladder carcinomas for chromosomes 15 and 17 has shown that chromosome 15 was frequently disomic and trisomic in DNA-aneuploid cases, thus showing an underrepresentation as compared to chromosomes 1, 7 and 17.

In cases of primary breast cancers Devilee et al. detected numerical aberrations for the target sites of chromosome 1 and/or chromosome 18. Although all cases showed a single peak in DNA flow measurements, with no shift in DNA content, most of the cases appeared to be heterogeneous with respect to the number of chromosome FISH spots per nucleus.

In the prevention of testicular germ cell cancer, diagnosis at an early stage, i.e. carcinoma in situ (CIS), is important. The so-called atypical germ cells that characterize this lesion morphologically resemble neoplastic cells in seminoma. The hyperdiploid DNA content, as measured by flow cytometry, is one of the markers of seminomas, together with numerical aberrations of chromosome 1. In collaboration with Giwercman [5] we developed a non-invasive procedure for the screening of carcinoma of the testis in situ by means of interphase cytogenetics on semen, using a centromere probe for chromosome 1. Samples from patients with isolated CIS as well as patients in whom CIS accompanied by a tumour were identified, based on the percentage of hyperdiploid cells. For detection of aneuploid cells in semen, FISH may prove to be a more sensitive technique than flow cytometry.

Van Dekken et al. [17] applied the FISH procedure to assess the presence of numerical aberrations for several chromosomes in nuclei isolated from moderately or poorly differentiated gastric adenocarcinomas. The chromosome aneuploidies as detected by FISH was shown to parallel the DNA-ploidy as detected by FCM. Moreover, the FISH technique detected the loss of the Y chromosome in a high percentage of cells in a high percentage of tumours from male patients, while in other cases a possible loss of chromosome Y was observed in a small proportion of tumour cells.

Differential diagnosis of complete and partial hydatidiform moles and hydropic abortions can be difficult when based on histology alone. Therefore, van de Kaa et al. [18] used the ISH technique on paraffin sections of such specimens using probes for chromosome 1, X and Y, in order to discriminate between maternal and trophoblast cells. Using the FISH technique, the diploid or triploid character of a hydatidiform mole could be established. Such information can be of great value in the differential diagnosis between complete and partial moles. Furthermore, as a result of the discrimination between maternal and trophoblast cells of complete moles, the cytogenetic heterogeneity as detected in the proliferating cytotrophoblasts confirmed the relatively high hyperdiploid fraction observed by FCM analyses.
Detection of polyploidy and tumour heterogeneity by FISH

The process of tetraploidization or polyploidization, in which random as well as non-random loss of chromosomes can lead to selection and growth of aneuploid cells, is a generally accepted concept in tumour progression. This phenomenon can be studied by cytogenetic and molecular approaches. We recently showed [6] that FISH enables the detection of tetraploidization in cancers in which no aneuploidy could be detected by FCM. This was only possible because the FISH procedure can discriminate between a cell in G2M containing 2C DNA and a tetraploid cell in G0G1 also containing 2C DNA. These cells will contain two or four FISH signals in the interphase nucleus, respectively [3]. Using double-target hybridization we could demonstrate [6] doubling of numerical aberrations, resulting in ‘minor’ cell populations that contain, for example, 4 copies of chromosome 1 and 2 copies of chromosome 9 (see also Figs. 1e and 1f). As a result, interphase cytogenetics is a simple instrument for the study of genetic tumour heterogeneity. Using this approach genetic aberrations can be correlated with heterogeneity and tetraploidization. Therefore, FISH enables the detection of tetraploidization in tumours in which no aneuploidy could be detected by flow cytometry. Also, FISH is able to detect ‘minor’ cell populations with an aberrant chromosome content within tumours with a diploid DNA content, as well as tumours which had undergone tetraploidization. In bladder cancer these minor fractions could be detected by a doubling of the numerical aberration as detected in the major tumour cell population. Finally, double-target FISH can be used to detect the imbalance between chromosomes within these minor tumour cell populations, since the different chromosomes are detected simultaneously.

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References


