Is 24-Color FISH Detection of In-Vitro Radiation-Induced Chromosomal Aberrations Suited to Determine Individual Intrinsic Radiosensitivity?

Alma Kuechler[1], Susann Neubauer[2], Gerhard G. Grabenbauer[2], Uwe Claussen[3], Thomas Liehr[3], Rolf Sauer[2], Thomas G. Wendt[1]

Background: Reliable determination of intrinsic radiosensitivity in individual patients is a serious need in radiation oncology. Chromosomal aberrations are sensitive indicators of a previous exposure to ionizing irradiation. Former molecular cytogenetic studies showed that such aberrations as an equivalent of intrinsic radiosensitivity can be detected by fluorescence in-situ hybridization (FISH) techniques using whole chromosome painting (wcp) probes. However, only one up to three randomly chosen wcp probes have been applied for such approaches until now. As a random distribution of chromosomal rearrangements along the chromosomes is up to now still controversial, the power of the 24-color FISH approach should be elucidated in the present study.
Methods and Material: Lymphocytes derived from lymphoblastoid cell lines of one patient with Nijmegen breakage syndrome (NBS homozygote) and of two NBS heterozygotes and peripheral blood lymphocytes of two controls were analyzed. Samples of each patient/control were irradiated in vitro with 0.0 Gy, 0.7 Gy or 2.0 Gy prior to cultivation. Chromosomal aberrations were analyzed in detail and quantified by means of 24-color FISH as an expression of the individual intrinsic radiosensitivity.
Results: 24-color FISH analyses were done in a total of 1,674 metaphases. After in-vitro irradiation, 21% (0.7 Gy) or 57% (2.0 Gy) of the controls' cells, 15% (0.7 Gy) or 53% (2.0 Gy) of the heterozygotes' cells and 54% (0.7 Gy) or 79% (2.0 Gy) of the homozygote's cells contained aberrations. The highest average rates of breaks per mitosis [B/M] (0.7 Gy: 1.80 B/M, 2.0 Gy: 4.03 B/M) and complex chromosomal rearrangements [CCR] (0.7 Gy: 0.20 CCR/M, 2.0 Gy: 0.47 CCR/M) were observed in the NBS patient. Moreover, the proportion of different aberration types after irradiation showed a distinct increase in the rate of CCR combined with a decrease in dicentrics in the NBS homozygote.
Conclusion: To come to a more complete picture of radiation-induced aberrations and to detect and quantify genetically determined intrinsic radiosensitivity, a 24-color FISH approach using all human chromosome painting probes has been successfully applied on cytogenetic preparation lymphocytes. The controls and NBS heterozygotes were clearly distinguished from the NBS homozygote subject.

Key Words: Radiation-induced chromosomal aberrations · 24-color FISH · Nijmegen breakage syndrome · NBS · Individual radiosensitivity

Strahlenther Onkol 2002;178:209–15
DOI 10.1007/s00066-002-0904-0

Kann die 24-Farben-FISH-Methode zur Bestimmung individueller Strahlenempfindlichkeit verwendet werden?

Hintergrund: Die Verfügbarkeit eines verlässlichen und schnellen prädiktiven Testsystems zur Bestimmung individueller Radiosensitivität von Tumorpatienten ist in der onkologischen Strahlentherapie von enormer Bedeutung. Molekularzytogenetische Studien haben gezeigt, dass intrinsische Radiosensitivität in Form von chromosomalen Aberrationen mittels Fluoreszenz-in-situ-Hybridisierungs-(FISH-)Techniken unter Verwendung von sog. „whole chromosome painting"-(wcp-)Sonden nachgewiesen werden kann. Bisher wurden für solche Ansätze allerdings lediglich maximal drei zufällig ausgewählte wcp-Sonden gleichzeitig eingesetzt. Da eine zufällige Verteilung der induzierten Schäden über das Genom bisher immer noch ungeklärt ist, sollten die Grenzen und Möglichkeiten der 24-Farben-FISH-Methode für die Bestimmung individueller Strahlenempfindlichkeit mit der vorliegenden Studie ermittelt werden.
Methoden und Material: Lymphozyten aus lymphoblastoiden Zelllinien von einem Patienten mit Nijmegen-Breakage-Syndrom (NBS-homozygoter Genträger) und von zwei NBS-heterozygoten Genträgern sowie Lymphozyten aus peripherem Blut von zwei Kontrollen wurden untersucht. Die Proben wurden in vitro mit 0,0 Gy, 0,7 Gy oder 2,0 Gy bestrahlt und anschließend kultiviert. Die chromosomalen Aberrationen wurden mittels 24-Farben-FISH analysiert und können als Maß für die individuelle intrinsische

[1]Department of Radiotherapy, Clinic of Radiology, Jena, Germany,
[2]Department of Radiotherapy, Erlangen, Germany,
[3]Institute of Human Genetics and Anthropology, Jena, Germany.

Received: June 29, 2001; accepted: January 22, 2002

Strahlenempfindlichkeit interpretiert werden.

Ergebnisse: Mittels der 24-Farben-FISH Methode wurden insgesamt 1674 Metaphaseplatten analysiert. Nach In-vitro-Bestrahlung mit 0,7 bzw. 2,0 Gy zeigten 21% bzw. 57% der Zellen der beiden Kontrollen, 15% bzw. 53% der Zellen der beiden NBS-Heterozygoten und 54% bzw. 79% der Zellen des NBS-Homozygoten Aberrationen. Die höchsten durchschnittlichen Bruchraten (Brüche pro Mitose = B/M) (0,7 Gy: 1,80 B/M, 2,0 Gy: 4,03 B/M) und Raten an komplexen chromosomalen Rearrangements (= CCR) (0,7 Gy: 0,20 CCR/M, 2,0 Gy: 0,47 CCR/M) wurden in den Zellen des NBS-homozygoten Patienten nachgewiesen. Nach Bestrahlung zeigte sich ein deutlicher Anstieg bezüglich der Anzahl an CCR bei einem gleichzeitigen Abfall der Anzahl an dizentrischen Chromosomen beim NBS-Homozygoten.

Schlussfolgerung: Die Methode der 24-Farben-FISH unter Verwendung aller menschlichen wcp-Sonden wurde erfolgreich zu Nachweis, Charakterisierung und Quantifizierung strahleninduzierter chromosomaler Aberrationen in Lymphozyten eingesetzt. Darüber hinaus ist diese Methode geeignet, um ein vollständigeres Bild der entstandenen chromosomalen Rearrangements zu erhalten. Kontrollen und NBS-Heterozygote konnten klar von einem NBS-homozygoten Genträger unterschieden werden.

Schlüsselwörter: Strahleninduzierte Chromosomenaberrationen · 24-Farben-FISH · Nijmegen-Breakage-Syndrom · NBS · Individuelle Strahlenempfindlichkeit

Introduction

In clinical radio-oncological therapy tumor cell killing and tumor control probability mainly depend on the total radiation dose at the target volume, which is limited by the tolerance of surrounding normal tissue. It is well-known that there is a wide variation between individuals in normal-tissue tolerance and in intrinsic radiosensitivity to the same applied dose [23, 27]. The radiotherapy of choice would be to use a fractionated radiation dose, which on one hand keeps the rate of severe normal-tissue complications acceptably low and on the other hand increases the probability of local control [5]. Even though different approaches for determination of radiosensitivity have been tested (e.g. clonogenic cell survival [7], G2-Assay, [19]), there is still no fast and reliable routine predictive assay available to identify hypersensitive or less sensitive patients prior to treatment and to modulate the therapeutic dose. However, the most promising techniques in that context are molecular cytogenetic approaches analyzing chromosomal aberrations as they are sensitive indicators of an exposure to ionizing irradiation (e.g. [26]). Fluorescence in-situ hybridization (FISH) using one up to three whole chromosome painting probes simultaneously have been successfully applied to detect spontaneous and radiation-induced chromosome instability in peripheral blood lymphocytes (e.g. [8, 9, 15, 16]). However, in former analyses the used chromosomes have been selected by chance – even though a random distribution of chromosomal rearrangements along the chromosomes is up to now still controversial (e.g. [3, 6], reviewed in [29]). To overcome this problem by analyzing the whole karyotype, 24-color FISH was performed, using a probe mix of the 24 different human whole chromosome painting (wcp) probes. This approach, first described by Speicher et al in 1996 [25] and Schröck et al in 1996 [22], allows the simultaneous visualization of all chromosomes within each metaphase in different specific colors. Nearly all chromosomal aberrations (as reciprocal and non-reciprocal translocations, complex rearrangements, ring chromosomes, acentric fragments, dicentric fragments or insertions) can be detected, characterized and defined in more detail.

The present study was focussed on five persons: one person with an increased radiosensitivity due to a Nijmegen breakage syndrome (NBS homozygous), two NBS heterozygous persons and two controls – one tumor patient with normal clinical reaction after radiotherapy and one healthy proband. NBS is a rare autosomal recessive chromosomal instability syndrome with known hypersensitivity to ionizing radiation [16, 28]. Thus, NBS can serve as kind of positive control for a predictive assay. The in vitro irradiation-induced chromosomal aberrations in NBS homozygote, NBS heterozygotes and controls were analyzed and compared with each other. The value of 24-color FISH for the detection of individual intrinsic radiosensitivity was investigated.

Material and Methods

Probands and Patients

One patient suffering from NBS (NBS homozygous male, 7 years), two NBS heterozygotes (NBS-ht1, female, 36 years; NBS-ht2, male, 40 years) and two controls were included into this study. The NBS homozygote was the son of the two heterozygotes. The two controls were one healthy proband (control-1; female, 28 years) and one patient who subsequently underwent radiotherapy for an ENT tumor and showed normal clinical reaction (control-2; male, 62 years). Heparinized peripheral blood samples or lymphocytes from a lymphoblastoid cell line of the NBS homozygote were irradiated in vitro with 0.7 Gy and 2.0 Gy using a 6 MV linear accelerator (Mevatron, Siemens, Germany) with a dose rate of 2.2 Gy/min. One sample, each, remained as unirradiated control.

Chromosome Preparation

After irradiation, lymphocyte cultures were set up according to cytogenetic standard protocols (1 ml whole blood, 9 ml RPMI 1640 medium including 15% fetal calf serum, 2.5% phytohemagglutinine and 1% penicillin-streptomycin) and cultured for 48 hours (37 °C). 3 hours before harvesting, colcemid (0.1 µg/ml) was added to each blood culture for mitotic block. Chromosome preparation was done according to standard protocols.

24-Color FISH

Chromosome suspension was dropped onto slides and pretreated for 24-color FISH hybridization according to Liehr et al [12]. 24-color FISH was performed according to Senger et al [24]. After 72 hours incubation (37 °C) with the probe mix, posthybridization-washes and detection, metaphase images were captured on a fluorescence microscope (Axioplan 2, Zeiss, Germany) with a PCO VC45 CCD camera (PCO, Kehl, Germany) and suitable filter combinations (DAPI/FITC/SpectrumOrange/TexasRed/Cyanine 5/Cyanine 5.5) using isis3-software (MetaSystems, Altlussheim, Germany). Using this method, each of the 24 different chromosomes (22 autosomes, X, Y) was labeled with a specific combination of fluorochromes that allows to identify unambiguously the origin of the chromosomal material.

Aberration Analysis

At least 100 metaphases (100–148 metaphases) per irradiation dose and patient/proband were analyzed (at least 300 metaphases per person) for chromosomal aberrations. Aberration types and involved chromosomes were characterized in detail. The rearrangements were classified as reciprocal and non-reciprocal translocations, complex rearrangements, ring chromosomes, acentric fragments, dicentric fragments, inversions and insertions. Translocations, insertions and complex rearrangements were visible because of a color change along a rearranged chromosome. Each color change was registered as one break event per each involved chromosome. Dicentrics and ring chromosomes could easily be identified using the inverted DAPI picture. The frequency of break events constituting the observed aberrations was estimated as the minimal number of breaks considered to be necessary for producing the aberrations in each metaphase. Complex chromosomal rearrangements (CCR) consisted per definition of at least two chromosomes with in summary three or more breaks [20, 21].

The total number of break events in each patient/control and irradiation dose was summed up and divided by the number of metaphases analyzed to get the average rate of breaks per mitosis (B/M). The radiosensitivity of lymphocytes was expressed as number of radiation-induced B/M after 0.7 Gy or 2.0 Gy, respectively, subtracted by the 0.0-Gy control value to correct the influence of spontaneous basic aberration frequencies.

The frequency distribution of different aberration types (see above) in the homozygote was compared to those in heterozygotes and controls. Moreover, the proportion of breaks involved in the formation of CCR was evaluated in comparison to the total number of breaks, as CCR are suspected to occur more often in increased radiosensitivity [16].

Results

24-color FISH analyses were performed in a total of 1,674 metaphases; 386 metaphases were analyzed of the NBS homozygote, 670 metaphases of the two NBS heterozygotes and 618 metaphases of the two controls. Representative 24-color FISH results are shown in Figure 1.

In 0.0 Gy, no spontaneous aberrations or complex rearrangements could be detected for control-1, three breaks, and one reciprocal translocation (two breaks) for control-2, one break event in NBS heterozygote-1 and a non-reciprocal translocation (two breaks) in one metaphase of NBS heterozygote-2 (i.e. 100% normal cells without aberrations in control-1, 96% in control-2, 99% in heterozygotes). In the unirradiated probe of the NBS homozygote, 37 spontaneous breaks (0.25 B/M) and two complex chromosomal rearrangements (0.01 CCR/M) could be observed in 148 studied metaphases (i.e. 13.5% cells with aberrations).

As the results of two NBS heterozygotes were relatively uniform as well as those of the tumor patient compared to the proband (Tables 1 and 2), the data of the corresponding two individuals are presented and discussed together. After in-vitro irradiation using 0.7 Gy, 21% of the controls' cells, 15% of the heterozygotes' cells and 54% of the homozygote's cells contained aberrations. With an irradiation dose of 2.0 Gy, the percentage was as follows: 57% (controls), 53% (heterozygotes) and 79% (homozygote) of the cells presented with chromosomal rearrangements. Furthermore, all cases and doses were analyzed with regard to the absolute number of breaks present in the individual metaphase. There was no cell in heterozygotes and control containing more than five or nine breaks after 0.7 Gy or 2.0 Gy irradiation, respectively, whereas the NBS homozygote's cells showed up to eleven (0.7 Gy) and 17 breaks (2.0 Gy). The relative frequency distribution of breaks per cell is summarized in Table 1.

The NBS patient showed in both irradiation doses the highest average rate of breaks per mitosis (0.7 Gy: 1.80 B/M, 2.0 Gy: 4.03 B/M) and complex chromosomal rearrangements (0.7 Gy: 0.20 CCR/M, 2.0 Gy: 0.48 CCR/M), whereas no differences could be observed between NBS heterozygotes and controls (all results are listed in detail in Table 2).

The percentage of breaks involved in the formation of CCR among the total number of breaks differed between the three entities. CCR were not present in the NBS heterozygotes and the controls after in-vitro irradiation with 0.0 Gy and 0.7 Gy apart from one single CCR with three breaks and two involved chromosomes in control-2. After 2.0 Gy irradiation, 24.0% (controls), 23.4% (heterozygotes) and 47.0% (homozygote) of all breaks were involved in CCR.

When comparing the proportion of different aberration types after irradiation (0.7 and 2.0 Gy) in controls, NBS heterozygotes and NBS homozygote (Figure 2), a distinct increase in the rate of CCR combined with a decrease in dicentrics could be observed in the NBS homozygote.

Discussion

The present study showed that genetically determined intrinsic radiosensitivity can be detected and quantified by 24-color FISH on cytogenetic preparations of lymphocytes. Probands

Table 1. Percentage of cells with n breaks per metaphase (NBS-ht1: NBS heterozygote-1; NBS-ht2: NBS heterozygote-2; NBS-hm: NBS homozygote).

Tabelle 1. Prozentualer Anteil an Zellen mit n Brüchen pro Metaphase (NBS-ht1: NBS-Heterozygoter 1; NBS-ht2: NBS-Heterozygoter 2; NBS-hm: NBS-Homozygoter).

	Percentage of cells with n breaks per metaphase (%)																	
Pat.-ID + irrad. dose	**n = 0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Control-1 – 0.0 Gy	100																	
Control-1 – 0.7 Gy	73.8	10.7	14.6	1.0														
Control-1 – 2.0 Gy	43.0	9.3	21.5	10.3	10.3	1.9	1.9	1.9										
Control-2 – 0.0 Gy	96	3.0	1.0															
Control-2 – 0.7 Gy	85.0	5.0	9.0			1.0												
Control-2 – 2.0 Gy	43.0	5.0	27.0	8.0	8.0	3.0	3.0	1.0	1.0	1.0								
NBS-ht1 – 0.0 Gy	99.0	1.0																
NBS-ht1 – 0.7 Gy	84.1	8.4	6.5	0.9														
NBS-ht1 – 2.0 Gy	46.8	9.9	18.9	11.7	6.3	2.7	2.7	0.9										
NBS-ht2 – 0.0 Gy	99.0		1.0															
NBS-ht2 – 0.7 Gy	86.0	6.0	7.0	1.0														
NBS-ht2 – 2.0 Gy	47.2	4.2	23.9	9.2	7.0	2.8	3.5		0.7	1.4								
NBS-hm – 0.0 Gy	86.5	5.4	5.4	2.0	0.7													
NBS-hm – 0.7 Gy	45.5	10.4	11.2	6.7	9.0	4.5	4.5	3.0	3.0	0.7	0.7	0.7						
NBS-hm – 2.0 Gy	21.2	4.8	18.3	6.7	8.7	9.6	3.8	6.7	6.7	2.9	1.0	1.9	1.9	3.8	1.0			1.0

can clearly be distinguished from a genetically determined radiosensitivity syndrome, in our case NBS. These data are in concordance with data obtained by three-color FISH and conventional cytogenetic data (e.g. [9]). Even though 1,674 metaphases had to be studied in one homozygote, two heterozygotes and two controls (range 300–386 metaphases per analyzed person), this is a reduced number of metaphase spreads compared to other FISH approaches (e.g. in three-color FISH with a total of 1,600 evaluated metaphases per case).

The frequency of spontaneous aberrations (without irradiation, 0.0 Gy) in the NBS homozygous patient differed from that of the other investigated unirradiated probes. The analyzed lymphocytes of this case are derived from the B cell line, as a lymphoblastoid cell line had to be established due to insufficient cell growth of T lymphocytes in phytohemagglutinine-stimulated blood cultures. Comparative studies of PHA-stimulated vs EBV-transformed lymphocytes of ataxia teleangiectasia (AT) patients came to contradictory results concerning their susceptibility to radiosensitivity [13, 30].

The presence of stable aberrations in radiation-exposed individuals was proven by FISH analysis [1]. Nevertheless, no clonal aberrations were detected in 386 analyzed metaphases of our studied NBS patient. It cannot be excluded that clonal ones could be detected when analyzing a higher number of cells.

Table 2. Summary of all metaphases (MP) analyzed of each person and irradiation dose: total number of analyzed MP, percentage of normal vs aberrant MP, average number of breaks per mitosis and average number of complex chromosomal rearrangements (CCR) per mitosis (NBS-ht1: NBS heterozygote-1; NBS-ht2: NBS heterozygote-2; NBS-hm: NBS homozygote). All 0.7 and 2.0 Gy-values of "breaks per mitosis" and "CCR per mitosis" are corrected by the 0.0 Gy-value (if this is not 0.00).

Tabelle 2. Übersicht über alle pro Person und Bestrahlungsdosis untersuchten Metaphasen (MP): Gesamtanzahl untersuchter MP, prozentualer Anteil normaler vs. aberranter MP, durchschnittliche Anzahl an Brüchen pro Mitose und komplexen chromosomalen Rearrangements (CCR) pro Mitose (NBS-ht1: NBS-Heterozygoter 1; NBS-ht2: NBS-Heterozygoter 2; NBS-hm: NBS-Homozygoter). Alle 0,7- und 2,0-Gy-Werte von „Brüchen pro Mitose" und „CCR pro Mitose" wurden um den Nullwert korrigiert, wo dieser nicht 0,00 war.

Probe-ID + irrad. dose	**Total No. of MP**	**Normal MP (%)**	**Aberrant MP (%)**	**Breaks per mitosis**	**CCR per mitosis**
Control-1 – 0.0 Gy	108	100	0	0.00	0.00
Control-1 – 0.7 Gy	103	73.8	26.2	0.43	0.00
Control-1 – 2.0 Gy	107	43.0	57.0	1.58	0.10
Control-2 – 0.0 Gy	100	96.0	4.0	0.05	0.00
Control-2 – 0.7 Gy	100	85.0	15.0	0.23	0.01
Control-2 – 2.0 Gy	100	43.0	57.0	1.67	0.13
NBS-ht1 – 0.0 Gy	105	99.0	1.0	0.01	0.00
NBS-ht1 – 0.7 Gy	107	84.1	15.9	0.23	0.00
NBS-ht1 – 2.0 Gy	111	46.8	53.2	1.43	0.11
NBS-ht2 – 0.0 Gy	105	99.0	1.0	0.02	0.00
NBS-ht2 – 0.7 Gy	100	86.0	14.0	0.21	0.00
NBS-ht2 – 2.0 Gy	142	47.2	52.8	1.59	0.10
NBS-hm – 0.0 Gy	148	86.5	13.5	0.25	0.01
NBS-hm – 0.7 Gy	134	45.5	54.5	1.80	0.20
NBS-hm – 2.0 Gy	104	21.2	78.8	4.03	0.48

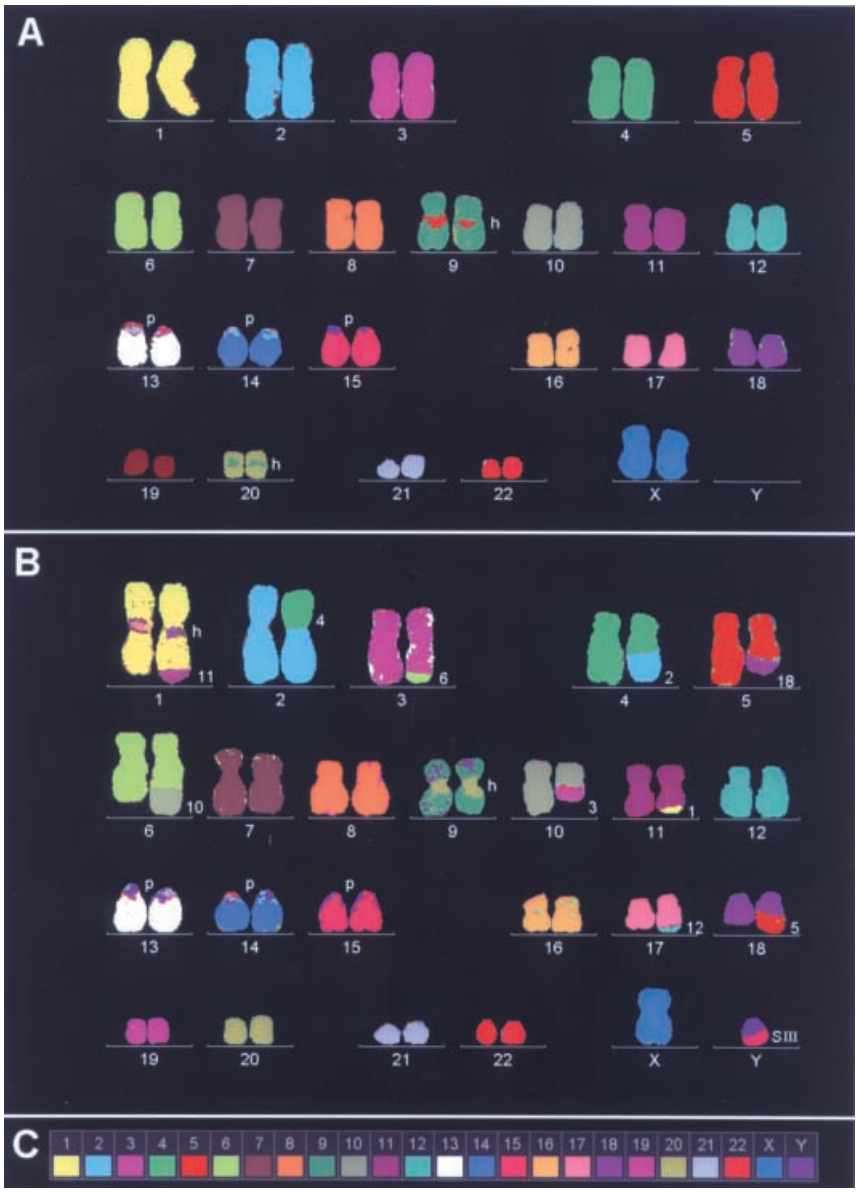

Figure 1. Example of 24-color FISH results (pseudocolor representations). Images were captured with the ISIS3 digital FISH imaging system (MetaSystems, Altlussheim, Germany) using a PCO VC45 CCD camera (PCO, Kehl, Germany) on an Axioplan 2 microscope (Zeiss, Jena, Germany). A. Karyogram of an unirradiated cell (control-1 after 0.0 Gy) showing a normal karyotype. – B. Karyogram of the NBS homozygote after in-vitro irradiation with 0.7 Gy. The karyotype can be described according to the ISCN 1995 [14] as follows:

46,XY,t(1;11),t(2;4),t(5;18),der(3)t(3;6),der(6)t(6;10),der(10)t(3;10),der(17)t(12;17),del(12).

This result can be summarized as eleven breaks per mitosis. The derivative chromosomes 3, 6 and 10 form a complex chromosomal rearrangement (p: short arms of the acrocentric chromosomes #13, #14 and #15 consisting of repetitive DNA, pseudocolored in a different paint; h: heterochromatic DNA, which is polymorphic and can be present at #1, #9 and #16; SIII: polymorphic heterochromatic DNA [satellite 3 type] in chromosome Yq12). – C. Pseudocolors for each individual chromosome.

Abbildung 1. Beispiel für ein 24-Farben-FISH-Ergebnis in Falschfarbdarstellung. Die Bildanalyse erfolgte mit dem ISIS3 Digital-FISH-Imaging-System (MetaSystems, Altlussheim, Germany) unter Verwendung einer PCO-VC45-CCD-Kamera (PCO, Kehl, Germany) und eines Axioplan-2-Mikroskops (Zeiss, Jena, Germany). A. Metaphase einer unbestrahlten Zelle (Kontrolle 1 nach 0,0 Gy) mit einem normalen Karyotyp. – B. Metaphase des NBS-Homozygoten nach In-vitro-Bestrahlung mit 0,7 Gy. Der Karyotyp kann nach ISCN 1995 [14] beschrieben werden als:

46,XY,t(1;11),t(2;4),t(5;18),der(3)t(3;6),der(6)t(6;10),der(10)t(3;10),der(17)t(12;17),del(12).

Alternativ kann man es auch als elf Brüche pro Mitose zusammenfassen. Die derivativen Chromosomen 3, 6 und 10 bilden ein komplexes chromosomales Rearrangement (p: kurze Arme der akrozentrischen Chromosomen 13, 14 und 15 bestehend aus repetitiver DNA, welche in der Falschfarbendarstellung in einer anderen Farbe dargestellt werden; h: heterochromatische DNA, die polymorph ist und auf den Chromosomen 1, 9 und 16 vorhanden sein kann; SIII: polymorphe heterochromatische DNA [Satellite-3-Typ] in Chromosom Yq12). – C. Verwendete Falschfarben für die 24 verschiedenen menschlichen Chromosomen.

The distribution of different aberration types after in-vitro irradiation, as summarized in Figure 2, is similar in the three studied entities, apart from two exceptions. The number of CCR is approximately twice as high and the number of dicentrics is markedly lower in NBS homozygote compared to NBS heterozygotes and controls. However, the lacking dicentrics are "hidden" in the CCR (data not shown). Dicentrics and rings are the chromosome aberrations, which are preferentially scored in conventional cytogenetic approaches to estimate individual doses of previous exposures to irradiation [2]. In the present study these two aberration types represented only between 24% and 35% of the total aberrations (see Figure 2). The majority (i.e. about 70%) of all other aberration types listed in Figure 2 would be missed using conventional cytogenetic approaches. Additionally, a detailed analysis of the data revealed that dicentrics (including those involved in CCR) tend to be underrepresented among the other aberration types after irradiation with 0.7 Gy and overrepresented after 2.0 Gy (data not shown).

The NBS homozygote differed from the NBS heterozygotes and the controls in five parameters: 1. the percentage of cells presenting aberrations was approximately twice as high

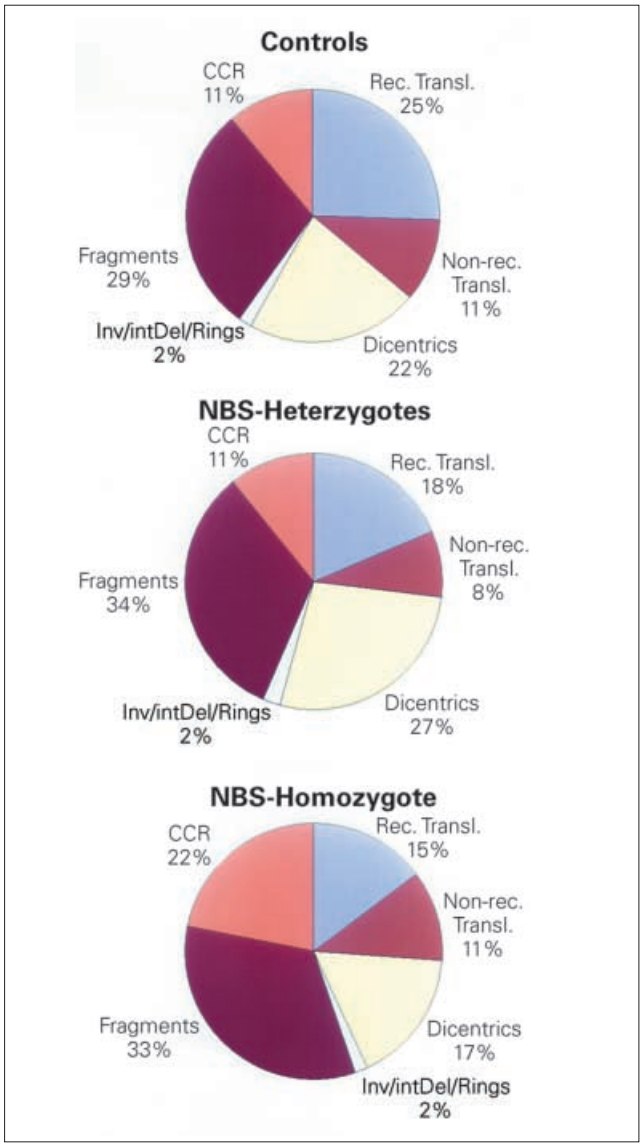

Figure 2. Relative proportion of the different aberration types in NBS homozygote, NBS heterozygotes and controls (Rec. Transl.: reciprocal translocations; Non-rec. Transl.: non-reciprocal translocations; Inv: inversions; intDel: interstitial deletions; CCR: complex chromosomal rearrangements; Rings: ring chromosomes; Dicentrics: dicentric chromosomes).

Abbildung 2. Anteil der unterschiedlichen Aberrationstypen bei NBS-Homozygoten, NBS-Heterozygoten und Kontrollen (Rec. Transl.: reziproke Translokationen; Non-rec. Transl.: nicht reziproke Translokationen; Inv: Inversionen; intDel: interstitielle Deletionen; CCR: komplexe chromosomale Rearrangements; Rings: Ringchromosomen; Dicentrics: dizentrische Chromosomen).

in the NBS homozygote; the same was true for 2. the average rate of B/M, 3. the maximal appearing number of breaks in one cell, 4. the average rate of CCR/M (exception: at 2.0 Gy a four-fold higher rate in the NBS homozygote) and 5. the percentage of break events involved in CCR formation at 2 Gy. To exclude the influence of spontaneous aberrations in the NBS patient, the 0.0-Gy value was subtracted from the 0.7- and 2.0-Gy values.

Comparing the data concerning CCR in NBS homozygote/-heterozygotes and controls, it is pointed towards that this parameter could be suited to distinguish individual radiosensitivity. These observations are in concordance with the results described in Neubauer et al 1997 [16]. Until now, it was not possible to separate NBS heterozygotes from normal controls using predictive assays, while this was done for AT heterozygotes [S. Neubauer, in preparation].

In summary, the 24-color FISH method yields new detailed additional information for the characterization of radiation-induced chromosomal damages. Much more and potentially up to now unrecognized chromosomal aberrations can be characterized and described in detail using the 24-color FISH technique. These advantages have been mentioned by others before [10, 18].

However, this technique has also its limitations, for instance, when exact breakpoint localization of translocations is required, or in case of small intrachromosomal rearrangements such as interstitial deletions or inversions. To overcome these restrictions it has been proposed to apply chromosome-arm specific probes [4] or the recently developed multicolor-banding (MCB) technique for characterization of highly complex aberrations [11]. However, these two methods do not allow to analyze the whole karyotype at once, in contrast to the 24-color FISH technique.

Further investigations are necessary to clarify whether it is possible to detect NBS heterozygotes among healthy probands using suitable 24-color FISH parameters. Additional investigations with more patients are required to find out whether there exist specific distribution patterns of chromosomes involved in radiation-induced aberrations for different diseases/malignancies and whether there exist distinct chromosomes which are more suited to detect radiosensitivity than others. It has to be demonstrated if this approach is suited as predictive cytogenetic test system. Then it could help to receive better tumor control together with reduced side effects, both in normal and radiosensitive cancer patients, by controlled enhancement or reduction of the curative dose, respectively.

Acknowledgments

The technical assistance of I. Hartmann (Jena), E. Müller (Erlangen) and M. Ziegler (Jena) and the financial support of Carl Zeiss Jena GmbH are gratefully acknowledged.

References

1. Bauchinger M, Salassidis K, Braselmann H, et al. FISH-based analysis of stable translocations in a Techa River population. Int J Radiat Biol 1998;73:605–12.
2. Bauchinger M. Quantification of low-level radiation exposure by conventional chromosome aberration analysis. Mutat Res 1995;339:177–89.

3. Barrios L, Miro R, Caballin MR, et al. Cytogenetic effects of radiotherapy. Breakpoint distribution in induced chromosome aberrations. Cancer Genet Cytogenet 1989;41:61–70.
4. Boei JJ, Vermeulen S, Natarajan AT Dose-response curves for X-ray induced interchanges and interarm intrachanges in human lymphocytes using arm-specific probes for chromosome 1. Mutat Res 1998;404:45–53.
5. Burnet NG, Nyman J, Turesson I, et al. Prediction of normal-tissue tolerance to radiotherapy from in-vitro cellular radiation sensitivity. Lancet 1992;339:1570–1.
6. Cigarran S, Barrios L, Barquinero JF, et al. Relationship between the DNA content of human chromosomes and their involvement in radiation-induced structural aberrations, analysed by painting. Int J Radiat Biol 1998; 74:449–55.
7. Cole J, Arlett CF, Green MH, et al. Comparative human cellular radiosensitivity: II. The survival following gamma-irradiation of unstimulated (G0) T-lymphocytes, T-lymphocyte lines, lymphoblastoid cell lines and fibroblasts from normal donors, from ataxia-telangiectasia patients and from ataxia-telangiectasia heterozygotes. Int J Radiat Biol 1988;54:929–43.
8. Dunst J, Gebhart E, Neubauer S. Kann man erhöhte Strahlensensitivität von Patienten durch In-vitro-Testung von Lymphozyten erkennen? Strahlenther Onkol 1995;171:581–6.
9. Gebhart E, Neubauer S, Schmitt G, et al. Use of three-color chromosome in situ suppression technique for the detection of past radiation exposure. Radiat Res 1996;145:47–52.
10. Greulich KM, Kreja L, Heinze B, et al. Rapid detection of radiation-induced chromosomal aberrations in lymphocytes and hematopoietic progenitor cells by mFISH. Mutat Res 2000;452:73–81.
11. Johannes C, Chudoba I, Obe G. Analysis of X-ray-induced aberrations in human chromosome 5 using high-resolution multicolour banding FISH (mBAND). Chromosome Res 1999;7:625–33.
12. Liehr T, Thoma K, Kammler K, et al. Direct preparation of uncultured EDTA-treated or heparinized blood for interphase FISH analysis. Appl Cytogenet 1995;21:185–8.
13. Littlefield LG, Colyer SP, Joiner EE, et al. Chromosomal radiation sensitivity in ataxia telangiectasia long-term lymphoblastoid cell lines. Cytogenet Cell Genet 1981;31:203–13.
14. Mitelman F, ed. ISCN (1995) An international system for human cytogenetic nomenclature. Basel: Karger, 1995.
15. Neubauer S, Gebhart E, Schmitt G, et al. Is chromosome in situ suppression (CISS) hybridization suited as a predictive test for intrinsic radiosensitivity in cancer patients? Int J Oncol 1996;8:707–12.
16. Neubauer S, Dunst J, Gebhart E. The impact of complex chromosomal rearrangements on the detection of radiosensitivity in cancer patients. Radiother Oncol 1997;43:189–95.
17. Nijmegen breakage syndrome. The International Nijmegen Breakage Syndrome Study Group. Arch Dis Child 2000;82:400–6.
18. Sachs RK, Hlatky LR, Trask BJ. Radiation-produced chromosome aberrations: colourful clues. Trends Genet 2000;16:143–6.
19. Sanford KK, Parshad R, Price FM, et al. Enhanced chromatid damage in blood lymphocytes after G2 phase x irradiation, a marker of the ataxia-telangiectasia gene. J Natl Cancer Inst 1990;8:1050–4.
20. Savage JRK, Simpson PJ. On scoring of FISH "painted" chromosome exchange aberrations. Mutat Res 1994;307:345–53.
21. Savage JRK, Simpson PJ. FISH "painting" pattern resulting from complex exchanges. Mutat Res 1994;312:51–60.
22. Schrock E, du Manoir S, Veldman T, et al. Multicolor spectral karyotyping of human chromosomes. Science 1996;273:494–7.
23. Scott D. Chromosomal radiosensitivity, cancer predisposition and response to radiotherapy. Strahlenther Onkol 2000;176:229–34.
24. Senger G, Chudoba I, Plesch A. Multicolor-FISH – the identification of chromosome aberrations by 24 colors. BIOforum 1998;9:499–503.
25. Speicher MR, Gwyn Ballard S, Ward DC. Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 1996;12:368–75.
26. Tucker JD, Ramsey MJ, Lee DA, Minkler JL. Validation of chromosome painting as a biodosimeter in human peripheral lymphocytes following acute exposure to ionizing radiation in vitro. Int J Radiat Biol 1993;64: 27–37.
27. Turesson I, Thames H. Repair capacity and kinetics of human skin during fractionated radiotherapy: erythema, desquamation and teleangiectasia after 3 and 5 years' follow-up. Radiother Oncol 1989;15:169–88.
28. Varon R, Vissinga C, Platzer M, et al. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. Cell 1998;93:467–76.
29. Verdorfer I, Neubauer S, Letzel S, et al. Chromosome painting for cytogenetic monitoring of occupationally exposed and non-exposed groups of human individuals. Mutat Res 2001;491:97–109.
30. Waghray M, Gascon GG, al-Sedairy S, Hannan MA. Cytogenetic investigations in three cell types of a Saudi family with ataxia telangiectasia. Hum Genet 1991;87:285–9.

Correspondence Address
Dr. Thomas Liehr
Institut für Humangentik und Anthropologie
Kollegiengasse 10
07740 Jena
Germany
Phone (+49/3641) 935-533, Fax -502
e-mail: i8lith@mti-n.mti.uni-jena.de