COMPARISON OF X-RAY DOSE-RESPONSE CURVES OBTAINED BY CHROMOSOME PAINTING, USING CONVENTIONAL AND *PAINT* NOMENCLATURES.

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Abstract

Purpose: To compare the suitability of *PAINT* and Conventional nomenclature systems for the elaboration of chromosome aberration dose-effect curves for X-rays using FISH techniques, and to compare these curves with those bases on solid stained dicentrics analysed in first division metaphases by the FPG technique.

Material and methods: Blood samples were irradiated at 0.1, 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4 and 5 Gy 180 kV X-rays. FISH-painting was performed using probes for chromosomes 1, 4, and 11 in combination with a pan-centromeric probe.

Results: Translocations showed a higher background frequency than dicentrics. This influences the ratio of translocations:dicentrics at the lower doses, and the uncertainties of dose-effect curves for translocations. The dose-effect curves for dicentrics obtained by FISH and solid stain were in close agreement.

Conclusion: For short-term biological dosimetry purposes by FISH, the use of *dic* (BA) (*PAINT* nomenclature) or total dicentrics (conventional nomenclature) should give similar dose estimations. For dose-reconstruction, the use of total or complete translocations result in similar uncertainties.

1. Introduction

The analysis of dicentric chromosome using fluorescence plus Giemsa (FPG) to limit scoring to solid-stained first-division cells is considered the best method for biological dosimetry purposes (IAEA, 1986). This type of analysis is very reliable when used for recent and acute irradiations, but not for chronic or past exposures because the yield of dicentric chromosomes decreases with time after exposure (Awa *et al*. 1978, Buckton 1978, Bauchinger 1989). The introduction of fluorescence *in situ* hybridisation (FISH) techniques (Pinkel *et al*. 1986), particularly of chromosome *painting*, simultaneous with centromere detection, allows one to study both, translocations and dicentrics, and has opened new possibilities for biological dosimetry, such as the retrospective dose estimation through the analysis of symmetrical translocations. Although some studies have shown that the frequency of translocations seems to remain relatively constant with time (Straume *et al*. 1992, Lucas *et al*. 1992a, Salassidis *et al*. 1995, Snigiryova *et al*. 1997, Bauchinger *et al*. 1998, Lloyd *et al*. 1998), others have described a decrease in the frequency of translocations after high acute doses (Natarajan *et al*. 1996, Spruill *et al*. 1996, Matsumoko *et al*. 1998).

So far, relatively few dose-effect curves using FISH techniques have been established (Lucas *et al*. 1992b, Bauchinger *et al*. 1993, Finnon *et al*. 1995, Stephan and Pressl, 1997, Lindholm *et al*. 1998), and in general the chromosome aberrations have been described using the conventional nomenclature (e.g. ISCN, 1985). The use of *painting* techniques has led to the development of two new nomenclatures,

known as *PAINT* (protocol for aberration identification and nomenclature terminology) (Tucker *et al.* 1995a) and S&S (Savage and Simpson 1994 a,b).

The aim of the present study was to elaborate a dose-effect curve for X-rays using FISH painting with whole chromosome specific probes for chromosomes 1, 4 and 11, and a pan-centromeric probe. The dose-effect curves obtained using the conventional and the *PAINT* nomenclatures have been compared. These curves have also been compared with a dicentric curve obtained through the analysis of first division metaphases by the FPG technique (Barquinero *et al.* 1997).

2. Materials and methods

2.1. *Irradiation Conditions*

Peripheral blood samples from a 32 year-old healthy male with no history of exposure to clastogenic agents, including radiation, were obtained by venipuncture and collected into heparinized tubes. The samples were irradiated using an X-ray source, with a beam quality corresponding to a half-value layer of 1.43 mm Cu (180 kV, 9 mA and 0.5 mm Cu filtration). The dose-rate was 0,27 Gy/min. IAEA recommendations (Beninson *et al.* 1986) were followed for the irradiation. Blood samples were irradiated at 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 and 5 Gy.

For the 0 Gy dose, data from eight control individuals, four females and four males, with ages ranging from 23 to 39 (mean 28.9 $\pm$ 5.0) were used.

2.2. *Culture conditions*

Peripheral blood was cultured for 48h in RPMI 1640 medium supplemented with 20% foetal calf serum, antibiotics, phytohaemagglutinin and 12μg/ml of bromodeoxyuridine. Colcemid was added 2h before harvesting. The frequency of first division metaphases, determined by the FPG technique in parallel slides, was higher than 95%.

2.3. *Fluorescence* in situ *hybridisation*

Hybridisation with Cy3 labelled probes for chromosomes 1, 4 and 11 (Cambio, UK) and a fluorescein isothiocyanate (FITC)-labelled pan-centromeric probe (Cambio, UK) was carried out according to manufacturers' protocol. In brief, a mono-coloured cocktail with chromosomes 1, 4 and 11 probes, was denatured for 5 min at 72°C and incubated at 37°C for 60 min. Two-to-three-day old slides were dehydrated in an ethanol series (70, 85 and 100%, 2 min each), air dried and denatured for 2 min at 72°C in 70% formamide / 2xSSC (pH 7.4). After that, the slides were quenched in ice-cold ethanol (70%), dehydrated in an ethanol series as described above, air dried and placed onto a hot plate at 37°C. Meanwhile, the pan-centromeric probe was denatured for 5 min at 72°C and placed in a cold bath (5-10 min maximum). Pan-centromeric and chromosome cocktail probes, were mixed and pipetted onto slides. The slides were overlaid with coverslips, sealed with rubber and incubated overnight at 42°C. After that, slides were washed in 2xSSC at room temperature to remove the coverslip, and in 50% formamide/1xSSC at 42°C (twice for 5min) and in 0.1xSSC (for 5min). Then the slides were washed for 2 min in 1xST buffer (4xSSC plus 0.05% Tween 20) at room temperature. Counterstaining was performed with 4',6-diamidino-

2-phenylindole (DAPI) with a concentration of 1 µg per ml antifade solution (Cambio, UK).

2.4. *Scoring criteria*

Metaphases were examined using a triple-band pass filter, and the painted chromosomes were analysed using the triple, Cy3, FITC, and DAPI filters.

During analysis, each abnormal metaphase was analysed as a unit using the modified *PAINT* nomenclature proposed by Knehr *et al.* (1998), in order to consider the underlying mechanisms of aberration formation. After metaphase description, the chromosome aberrations were converted to conventional and *PAINT* nomenclatures.

In the conventional nomenclature, translocations (*t*), dicentric chromosomes (*dic*), centric and acentric rings (r_c and r_{ace}), acentric fragments (*ace*) and insertions (*ins*) are distinguished. A chromosome aberration is considered as *complete* when all painted portions are rejoined, and as *incomplete* when one or more portions seem to be unrejoined, probably due to the resolution inherent to the *PAINT* technique. As an example, a dicentric was considered incomplete when a metaphase showed a *dic* (BA) plus *ace*(b), a *dic* (BA), or an *ace* (ab). A translocation was considered incomplete when a metaphase showed a *t*(Ba) plus *ace*(b), a *t*(Ba), or a *t*(Ab). Complex exchanges, i.e, those resulting from at least three breaks in two or more chromosomes, were reduced to simple aberration base types. For example a metaphase showing *dic*(BA) plus *t*(Ab) was recorded as a complete dicentric plus a complete translocation, and a metaphase showing *t*(Ba) plus *ace*(ab) was considered as a complete translocation and an acentric fragment.

The *PAINT* nomenclature distinguishes dicentric chromosomes [*dic* (BA)], two types of translocations [*t*(Ab) and *t*(Ba)], acentric fragments [i.e., *ace*(b), *ace*(ab), *ace*(aba)], insertions [*ins*(Aba) and *ins* (Bab)] and centric and acentric rings [*r*(B) and *r*(b).]

2.5 *Statistical methods*

Parameters of dose effect curves were obtained by iteratively reweighted least squares regression, using inverse Poisson variances as weights. Goodness-of-fit was tested with the Pearson χ^2 value (Papworth 1975).

Mean aberration frequencies and curve parameters were calculated for the whole genome, dividing original values and their standard errors (SE) by a factor $2.05 f_p (1-f_p)$ (= 0.318) according to Lucas et al. (1992b), where f_p = 0.1922 is the fraction of painted DNA covered by chromosomes 1, 4 and 11 (Morton 1991). The formula described by Lucas *et al* (1992b) is only applicable for exchanges between painted and unpainted chromosomes involving only two breaks; for this reason complex aberrations should be excluded. However, the score of dicentrics by conventional solid stain includes a heterogeneous ensemble of aberrations (simple and complex). Since no formulae that consider complex aberrations are available, a way to compare the results after solid stain and painting is the reduction of complex aberrations to simple ones.

Comparison of dose-effect curves was performed with a weighted F-test for identity of parameters, accepting a p-value of $p<0.05$ as significant.

3. Results

For the construction of the calibration curves by FISH, a total of 27,341 metaphases was analysed. The 11,789 metaphases at 0 Gy dose correspond to the eight individuals analysed in our laboratory, where a mean of about 1470 metaphases per individual were analysed. In this group, the genomic frequencies of complete and total translocations per 100 cells were 0.64 ± 0.13 and 0.88 ± 0.15 respectively, and 0.03 ± 0.03 and 0.05 ± 0.04 for complete and total dicentrics. Using the Pearson's chi-square test, a homogeneity was observed for all the aberrations considered. The number of cells analysed in the individual used for the elaboration of the dose-effect curve was 1618. For this individual, five complete and one incomplete translocations and one complete dicentric were observed.

The use of the modified *PAINT* nomenclature to analyse all abnormal metaphases, allowed the conversion of aberrations to the conventional and *PAINT* nomenclatures.

Table 1 shows the cytogenetic results obtained using the modified *PAINT* nomenclature. For comparison, data from the dose-effect curve for dicentrics, obtained in first division metaphases by the FPG technique (Barquinero *et al*. 1997) have also been included. Using a cocktail of probes for three chromosomes labelled with the same fluorochrome, in some metaphases with multiple rearrangements the relationship among different chromosome aberrations could be misinterpreted. For these metaphases, the aberrations considered were those implicating the minimum number of breaks. For example, a metaphase with a *dic*(BA), an *ace* (ab), a *t*(Ab), and a *t*(Ba), has been considered as containing a *dic*(BA) plus *ace*(ab) (an

apparently simple dicentric, ASD), and a t(Ba) plus t(Ab) (an apparently simple translocation, AST). In our study, the percentages of metaphases with multiple aberrations were 0.04, 0.07, 0.10, 0.75, 2.00, 9.60 and 10.61 at the 0.5, 1, 1.5, 2, 3, 4 and 5 Gy doses, respectively. The percentage of incomplete AST ranged from 14.55 to 42.55, and from 22.2 to 50 for ASD. Using the z test ((t/dic -1)/S.D.) (S.D. = standard deviation of the ratio, calculated by error propagation law), deviations of the expected ratio 1:1 for AST:ASD were observed, mainly at the four lower doses (values Z>1.96).

The results using conventional nomenclature are shown in table 2. The percentages of incomplete translocations and incomplete dicentrics ranged from 10.81 to 37.74 and from 16.67 to 50 respectively. No relation with dose was found. Deviations of the expected ratio 1:1 for translocations:dicentrics were observed, mainly at the four lower doses, where the number of translocations was significantly higher than the number of dicentrics.

Table 3 shows the results obtained with the *PAINT* nomenclature. This nomenclature does not consider exchange paint patterns as an entity, and for this reason comparisons of the ratio translocations:dicentrics were not performed. The number of t(Ab) (548) was higher than t(Ba) (504), but the deviation from 1:1 was not significant.

For the results using conventional nomenclature, in almost all cases the dispersion u-test (Rao et al. 1956, Savage 1970) indicated that the distribution among cells of translocations and dicentrics (complete or total), followed a Poisson. The same was observed for t(Ba), t(Ab) and dic(BA) (*PAINT* nomenclature).

Table 4 shows the whole genomic dose-effect curve coefficients. The goodness-of-fit test revealed a sufficient result for all endpoints. The F-test did not show differences between the curve for dicentrics established by solid stain and any of the FISH curves for complete dicentrics, total dicentrics (figure 1), or dic(BA) (figure 2). However, curves of complete dicentrics and dic(BA) were significantly different (p<0.002); the difference resulted from the quadratic components (Standard difference test). Curves of total dicentrics and dic(BA) were very close to each other (p>0.95). For translocations, the dose-effect curves obtained by t(Ba), t(Ab) (figure 3) and complete translocations (figure 4) did not show differences, but t(Ba) and t(Ab) curves showed differences with the t_t curve (figure 4) (p<0.001 and p<0.02 respectively). Comparing translocation curves with dicentrics, for both complete and total exchanges, the F-test detected a significant difference (p<0.001) which depended from the C values.

4. Discussion

In the present study, the background genomic frequencies of complete and total translocations were 0.64 and 0.88 per 100 cells respectively. These values are similar to those reported by Lindholm et al. (1998), 0.66 and 0.91 for complete and total translocations. Higher and lower values in the background frequencies of total translocations have been described by some authors, ranging from 1.06 (Finnon et al. 1995) to 0.22 and 0.32 (Fernandez et al. 1996, Stephan and Pressl 1997, respectively). The frequency of translocations has shown an age dependency, indicating a tendency for stable type aberrations to be accumulated (Tucker et al. 1994, Ramsey et al. 1995, Bauchinger et al. 1996, and Johnson et al. 1998). For an

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accurate dose quantification in cases of exposures to low doses and low dose rates, the elevated basal frequency of translocations and its inter-individual variability requires the scoring of a sufficient number of cells at the low dose points of the curve, mainly to reduce the α coefficient uncertainties (Bauchinger 1998).

In the dose range analysed (0.1-5 Gy), the mean percentages of incomplete patterns observed in apparently simple aberrations (32.5±7.7 for AST and 31.1±5.9 for ASD) were similar to those observed after conversion to the conventional nomenclature (27.3±7.1 for translocations and 28.0±5.3 for dicentrics), where complex aberrations were converted to simple complete aberrations. Lindholm *et al.* (1998), using the conventional nomenclature and in the same dose range, described similar percentages of incompleteness (28.1±12.0 for translocations and 28.6±10.1 for dicentrics), although in their study complex aberrations were converted to simple incomplete aberrations. The lowest percentage of incomplete translocations (17.4±9) was described by Stephan and Pressl (1997). Using single chromosome paints and analysing all human chromosomes independently, the small chromosomes (13-22) were found to be more frequently involved in apparently incomplete aberrations than the longer ones (1-12) (Barquinero *et al*. 1998), suggesting that some of the apparently incomplete exchanges could be terminal complete exchanges with a distal signal below the resolution limit of FISH painting techniques. Analysing blood samples of A-bomb survivors, Kodama *et al.* (1997) described that the detectable minimal size for painted segments is 11.1±0.8 Mb, and 14.6±0.6 Mb for unpainted segments. Boei *et al*. (1998), analysed incomplete exchanges using a telomeric PNA probe, and found that a great majority (>85%) of the observed one-way patterns

(Simpson and Savage 1996) were the result of terminal exchanges. In the present study a non-significant increase of t(Ab) versus t(Ba) has been observed (1.09:1). Using a cocktail of three chromosome probes labelled with a single fluorochrome, Finnon *et al*. (1995) described a non-significant increase of t(Ab) versus t(Ba) (1.14:1), and Tucker *et al.* (1995b) described a significant 1.32:1 ratio. However in two studies where single chromosome paints were used, the number of t(Ab) was lower than t(Ba), 0.94:1 (Knehr *et al*. 1996) and 0.92:1 (Barquinero *et al.* 1998). The differences in the t(Ab):t(Ba) ratio in studies in which three or even only one chromosome were painted, could be ascribed to the different percentage of the DNA that was painted. Moreover, an inherent excess of t(Ab) patterns can arise when one considers all the possible patterns of complex exchanges described in the S&S nomenclature (Savage and Simpson 1994a,b).

The use of the modified *PAINT* nomenclature allows an easy description of all painting patterns. However, at high doses the inter-relationship among all signals in metaphases with multiple rearrangements could be misinterpreted (Savage and Tucker 1996), although in some cases the morphology of the resulting abnormal chromosomes allows their characterisation. In our study, these metaphases were mainly those carrying an apparently simple translocation and a apparently simple dicentric. The percentage of complex aberrations ranged from 0 to 18% and seemed to be dose-dependent. Finnon *et al.* (1995) and Lindholm *et al*. (1998) after X and γ-irradiation respectively, observed a dose-related increase of cells containing complex aberrations. Knehr et *al*. (1999) described a LET dependence of the relative proportion of complex aberrations, that was larger for fission neutrons than for X-

rays. In our study the percentage of metaphases with multiple rearrangements indicates that the misinterpretation level could be negligible for doses below 3 Gy.

The unequal frequencies of translocations and dicentrics described in tables 1 and 2, are related to the higher background yield of translocations. When the yield of translocations observed at the dose-range analysed (0.1-5Gy) was corrected by the subtraction of the background frequencies, only at 0.5 Gy the deviation from the 1:1 ratio remained significant for total translocations and dicentrics. A similar influence of the background translocation yield has been described by Finnon *et al.* (1995) and by Lindholm *et al.* (1998).

Several FISH studies have produced ambiguous results on the ratio of radiation-induced translocations and dicentrics (see Lucas *et al.* 1996). The differences in the scoring criteria make comparison of these studies difficult, and, in part, they could explain the differences observed. However, when comparing studies in which the same scoring criteria have been used, the discrepancies between the ratios of translocations and dicentrics induced by radiation are also evident. While in the present study no deviations from 1:1 ratio were observed, in the study by Barquinero *et al.* (1998), where all human chromosomes were independently analysed, significant deviations from the theoretical 1:1 ratio became apparent for some chromosomes.

In the present study, the dispersion u-test did not show any deviation from a Poisson distribution. In contrast Finnon *et al.* (1995) and Tucker *et al.* (1995b) using the *PAINT* nomenclature described an overdispersion for translocations, but in these

studies t(Ba) and t(Ab) were scored as two independent figures, and pooled for the calculation of the dispersion parameter.

Comparing the dose-effect coefficients for dicentrics obtained by solid stain and by FISH, several authors have described higher linear and quadratic coefficients in the solid stain curves (Bauchinger *et al*. 1993, Tucker *et al.* 1993, Schmid *et al*. 1995, Lindholm *et al.* 1998). In the present study the dose-effect curve obtained by solid stain falls within the curves for complete and total dicentrics obtained by FISH (Figure 1).

The dose-effect curves of *dic*(BA) and total dicentrics are nearly identical; the small differences noted can be explained by the consideration of bicoloured acentrics *ace*(ab) as incomplete dicentrics in the conventional nomenclature scores. It is interesting to note that the frequency of incomplete dicentrics scored from *ace*(ab) was very low. Using the different nomenclature systems, Knehr *et al.* (1998) described a great similarity in the frequencies of *dic*(BA) (PAINT nomenclature) and total dicentrics (conventional nomenclature). These results suggest that for short-term biodosimetry purposes, the use of *dic*(BA) or complete dicentrics should give similar dose estimations. For dose reconstruction purposes, the curves of complete translocations, t(Ba) and t(Ab) should also give similar results.

When the dose-effect curves obtained for translocations and dicentrics were compared, the differences observed results from the higher background frequencies of translocations. However, at higher doses the frequency of radiation-induced translocations and dicentrics were similar.

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Using the dose-effect curves obtained, the presence of three dicentrics per 1000 cells produce significant differences from background frequency, whereas five translocations are needed to indicate an overexposure. Similar results have been obtained by Edwards (1997).

5. Conclusions

The background frequency of translocations is higher than that for dicentrics. For this reason, to obtain a reliable dose-effect curve for translocations, it is necessary to analyse a higher number of cells at the lowest dose points. Moreover, and considering the inter-individual variability in the background frequency of translocations described by several authors, to quantify low-dose exposures it would be necessary for each laboratory to carry out its own dose-effect curve, analysing a control population for the 0 Gy dose.

For short-term dosimetry, our results suggest that the different dose-effect curves obtained could be used, due to the similarities in their uncertainties. A study simulating whole body exposures at different doses would be necessary to validate the applicability of the different dose-effect curves. For dose reconstruction, the curves of complete translocations, t(Ba) and t(Ab) should give similar results. However, more studies on the persistency of translocations, mainly after high doses, are needed.

Acknowledgements

6. References

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Table 1: Cytogenetic results using the modified PAINT nomenclature

Dose (Gy)	0	0.1	0.25	0.50	0.75	1	1.5	2	3	4	5
SOLID STAIN*											
Cells scored	8811	5048	2005	2012	1607	1292	682	403	205	204	200
dic	8	13	35	67	100	103	101	108	123	200	304
FISH											
Cells scored	11789	4679	2954	2315	1757	1513	1021	530	401	250	132
Apparently simple (AS) aberrations											
dic (BA) ace (ab)	1	6	9	17	28	31	38	30	39	63	35
dic (BA) ace(b)	0	0	0	3	5	3	6	9	7	17	2
ace (ab)	0	0	2	1	3	3	1	2	1	4	1
dic (AB)	1	2	2	4	4	6	9	13	15	9	7
total ASD (ASD_t)	2	8	13	25	40	43	54	54	62	93	45
% incomplete	50.00	25.00	30.77	32.00	30.00	27.91	29.63	44.44	37.10	32.26	22.22
t(Ba) t(Ab)	24	14	18	27	31	37	35	27	55	53	47
t(Ba) ace (b)	0	2	2	3	2	2	4	4	5	3	2
t(Ab)	7	2	2	11	8	17	13	7	12	18	2
t(Ba)	2	3	2	6	5	2	4	6	4	10	4
total AST (AST_t)	33	21	24	47	46	58	56	44	76	84	55
% incomplete	27.27	33.33	25.00	42.55	32.61	36.21	37.50	38.64	27.63	36.90	14.55
t(Ba) t(Ab):dic (AB)ace (ab)	24.00	2.33	2.00	1.59	1.11	1.19	0.92	0.90	1.41	0.84	1.34
±SD	4.89	0.65	0.5	0.34	0.34	0.25	0.23	0.26	0.22	0.18	0.22
z-test	4.70	2.06	2.01	1.73	1.73	0.78	-0.34	-0.38	1.84	-0.89	1.58
$AST_t:ASD_t$	16.5	2.63	1.85	1.88	1.88	1.35	1.04	0.81	1.23	0.90	1.22
±SD	2.87	0.59	0.40	0.29	0.29	0.21	0.19	0.20	0.17	0.14	0.19
z-test	5.40	2.77	2.09	3.02	3.02	1.68	0.20	-0.91	1.32	-0.69	1.17
r(B) ace (b)	0	0	0	1	2	2	6	6	6	2	4
r(b)	0	0			1	0	1	1	1	0	
other AS aberrations	0	0	2	2	3	0	2	1	1	0	1
total simple aberrations	35	29	39	75	92	103	119	106	146	179	105
Complex aberrations											
t(Ba) ace(ab)	0	0	0	3	4	4	10	4	4	8	3
dic (BA) t(Ab)	0	0	0	3	1	2	5	5	15	15	6
other complex aberrations	0	1	1	1	2	3	5	6	14	13	12
total complex aberrations	0	1	1	7	7	9	20	15	33	36	21
Terminal deletions	2	1	4	6	5	16	10	9	8	18	4

* = Barquinero *et.al.* 1997, ASD = Apparently simple dicentrics, AST = Apparently simple translocations, SD = standard deviation

Table 2: Cytogenetic results using the conventional nomenclature

Aberration type	Dose (Gy)										
	0	0.1	0.25	0.5	0.75	1	1.5	2	3	4	5
t_c	24	14	18	33	36	46	55	42	84	82	66
t_i	9	7	6	20	15	21	21	17	21	34	8
t_t	33	21	24	53	51	67	76	59	105	116	74
% t_i	27.27	33.33	25.00	37.74	29.41	31.34	27.63	28.81	20.00	29.31	10.81
dic_c	1	6	9	20	29	34	45	38	62	82	50
dic_i	1	2	4	9	12	12	16	24	24	32	10
dic_t	2	8	13	29	41	46	61	62	86	114	60
% dic_i	50.00	25.00	30.77	31.03	29.27	26.09	26.23	38.71	27.91	28.07	16.67
t_c / dic_c	24	2.33	2.00	1.65	1.24	1.35	1.22	1.11	1.36	1.00	1.32
±SD	4.89	0.65	0.50	0.32	0.25	0.24	0.20	0.22	0.19	0.15	0.20
z-test	4.70	2.06	2.01	2.06	0.95	1.48	1.10	0.47	1.92	0.00	1.64
t_t / dic_t	16.5	2.63	1.85	1.83	1.24	1.46	1.25	0.95	1.22	1.02	1.23
±SD	2.87	0.59	0.40	0.27	0.21	0.21	0.17	1.18	0.15	0.13	0.17
z-test	5.40	2.77	2.09	3.09	1.15	2.19	1.42	-0.27	1.46	0.14	1.34
ace	2	1	4	9	9	21	23	15	21	31	14
r_{ace}	0	0	0	0	3	0	1	1	0	1	0
r	0	0	1	2	1	3	8	7	9	3	5
ins	0	1	0	2	2	3	1	2	4	4	2
inv	0	0	1	1	2	0	2	1	0	0	1

t_c, t_i and t_t = complete, incomplete and total translocations respectively; dic_c, dic_i and dic_t= complete, incomplete and total dicentrics, respectively; ace = acentric fragment; r_{ace} =acentric ring; r= ring; ins = insertion; inv = inversion.

Table 3: Cytogenetic results using the *PAINT* nomenclature

	Dose (Gy)										
	0	0.1	0.25	0.5	0.75	1	1.5	2	3	4	5
Aberration type											
t(Ba)	26	19	22	39	42	46	56	43	72	79	60
t(Ab)	31	16	20	41	40	59	58	43	92	85	63
dic(BA)	2	8	11	28	38	43	60	60	85	110	59
ace(ab)	1	6	11	21	35	38	52	37	48	71	45
ace(b)	2	3	6	13	14	24	26	30	30	49	13
r(B)	0	0	1	2	3	3	8	7	9	3	5
r(b)	0	0	0	0	1	0	1	1	1	1	0
ins(Bab)	0	1	0	0	1	0	0	1	0	3	1
ins(Aba)	0	0	0	2	1	3	1	1	4	1	1
inv(B)	0	0	1	1	2	0	2	1	1	0	1

Table 4: Coefficients of the dose-effect curves.

	$C \pm SE$ (x10^{-2})	$\alpha \pm SE$ (x10^{-2}Gy^{-1})	$\beta \pm SE$ (x10^{-2}Gy^{-2})	χ^2 goodness-of-fit[a]
		$Y = C + \alpha D + \beta D^2$		
SOLID STAIN [b]				
dic	0.08 ±0.04	3.31 ±0.6	5.30 ±0.4	13.87
FISH				
conventional nomenclature				
t_c	0.63 ±0.12	3.64 ±1.0	5.42 ±0.5	3.32
t_t	0.85 ±0.14	6.55 ±1.1	6.46 ±0.6	7.25
dic$_c$	0.03 ±0.03	2.78 ±0.7	4.71 ±0.5	4.12
dic$_t$	0.05 ±0.04	4.12 ±0.9	6.37 ±0.6	9.33
PAINT nomenclature				
t(Ba)	0.71 ±0.12	5.32 ±1.0	4.42 ±0.5	3.09
dic (BA)	0.05 ±0.04	3.68 ±0.9	6.32 ±0.6	8.62
t(Ab)	0.78 ±0.13	4.94 ±1.0	5.28 ±0.6	8.06

t_c and t_t = complete and total translocations, respectively; dic_c and dic_t= complete and total dicentrics, respectively. [a] = 8 *df* for FISH and solid stain, [b] = Barquinero *et.al.* 1997
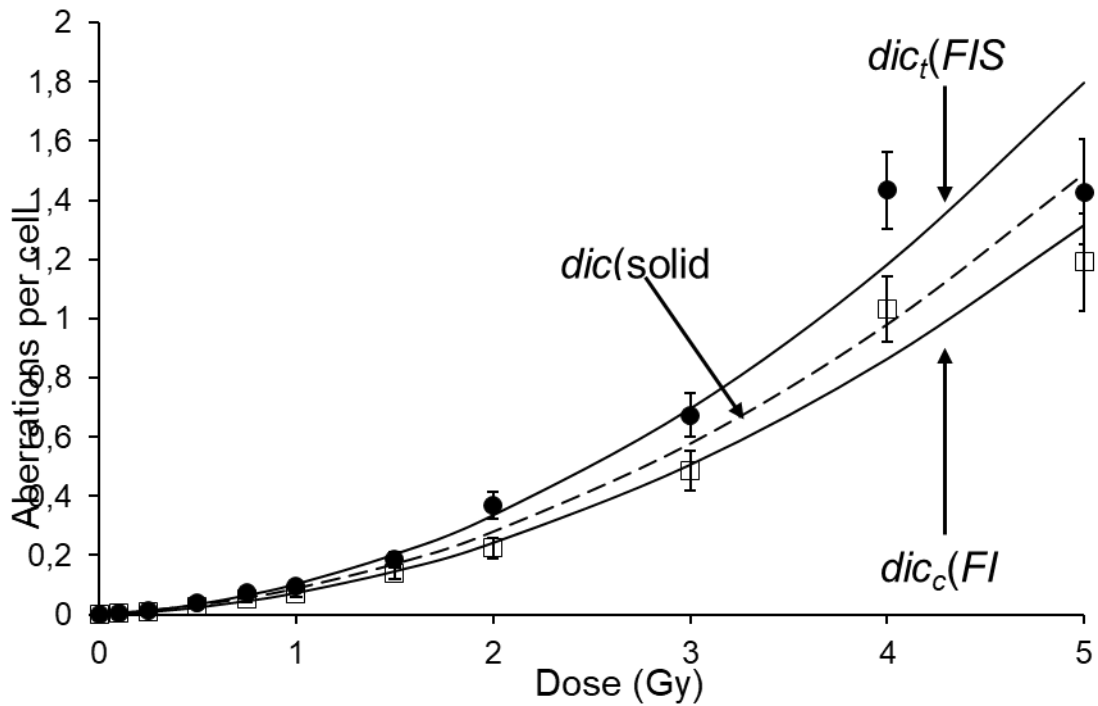
Figure 1: FISH dose-effect curves for dicentrics (conventional nomenclature). Solid lines show the curves by FISH for complete and total dicentrics (dic_c and dic_t, respectively). Squares and black circles indicate the genomic frequencies of dic_c and dic_t, respectively, and error barrs show the standard errors. For comparison, the dotted line shows the curve by dicentric solid stain analysis.
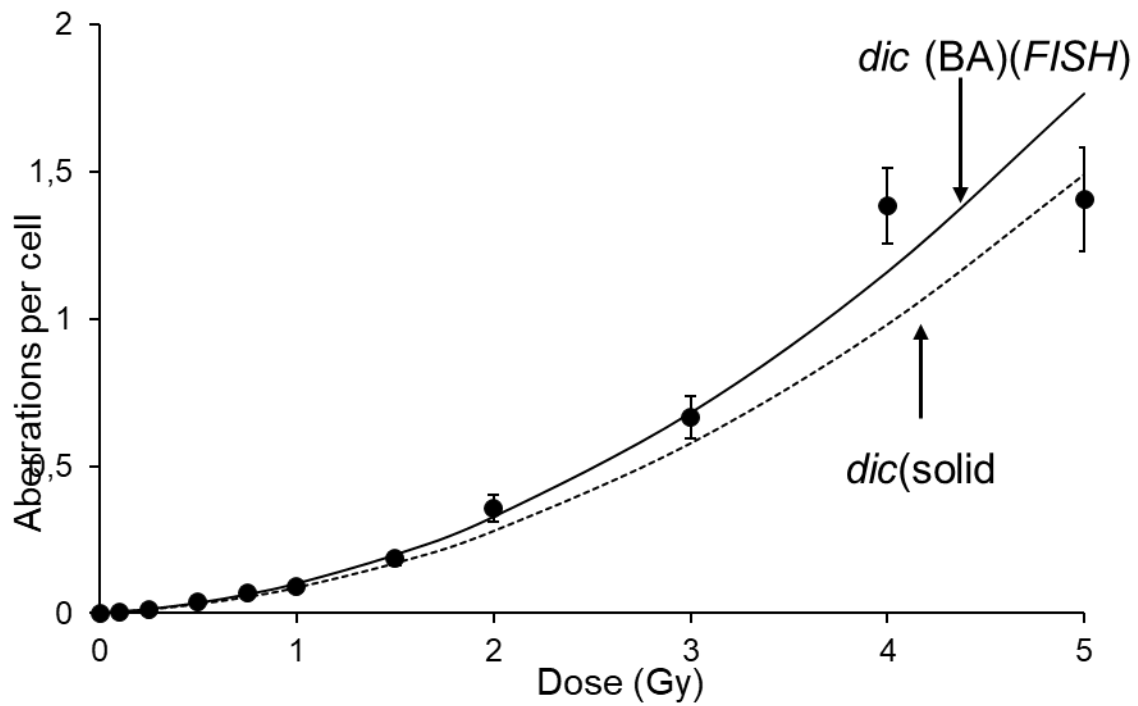
Figure 2: Solid line shows the FISH dose-effect curve for *dic*(BA) (*PAINT* nomenclature). Black circles indicate the genomic frequencies of *dic*(BA). Error barrs show the standard errors. For comparison, the dotted line shows the curve by dicentric solid stain analysis.
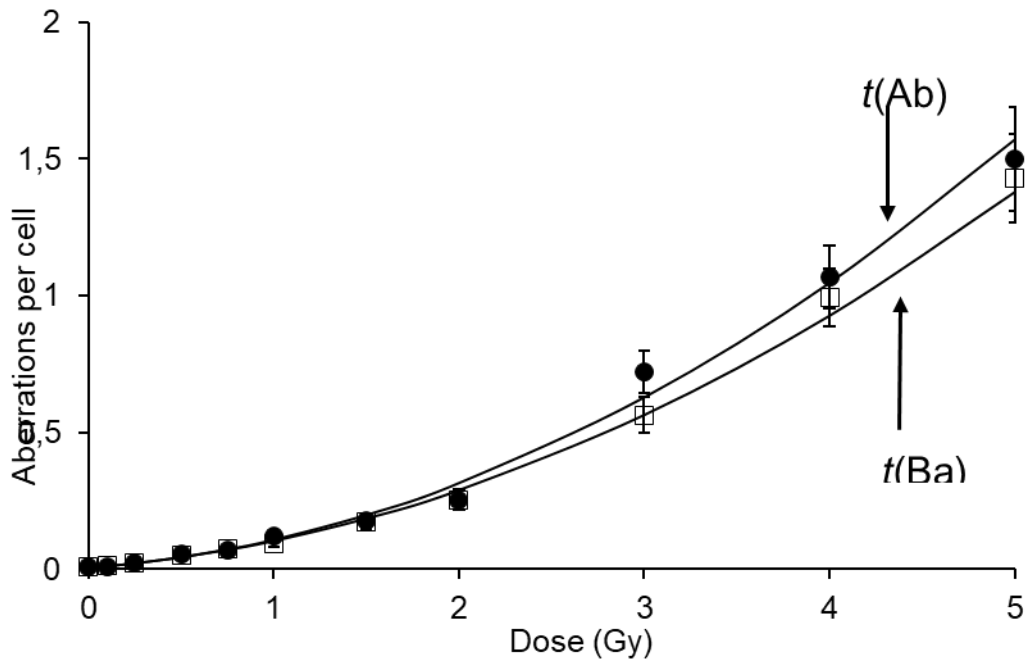
Figure 3: Solid lines show the FISH dose-effect curves for *t*(Ba) and *t*(Ab) (*PAINT* nomenclature). Squares and black circles indicate the genomic frequencies of *t*(Ba) and *t*(Ab), respectively. Error barrs show the standard errors.
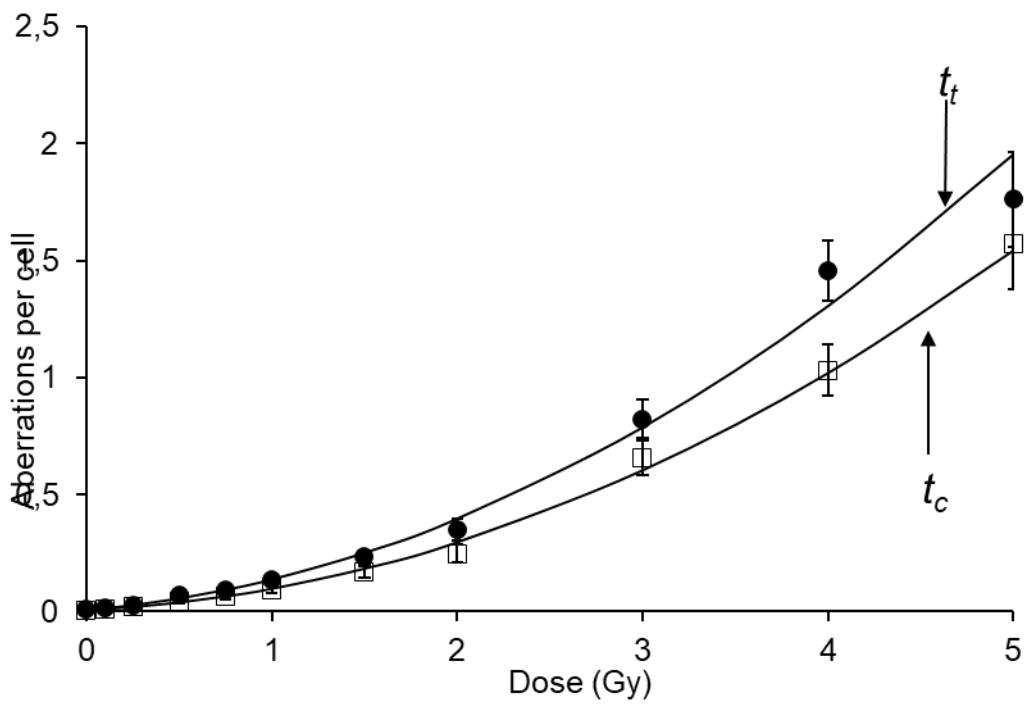
Figure 4: Solid lines show the FISH dose-effect curves for complete (t_c) and total (t_t) translocations (conventional nomenclature). Squares and black circles indicate the genomic frequencies of t_t and t_c, respectively. Error barrs show the standard errors.