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Low-Dose Radiation Response of Primary Keratinocytes and Fibroblasts from Patients with Cervix Cancer

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The aim of the present study was to examine, using the micronucleus (MN) assay, the low-dose radiation response of normal skin cells from cancer patients and to determine whether the hyper-radiosensitivity (HRS)-like phenomenon occurs in cells of these patients. Primary skin fibroblasts and keratinocytes derived from 40 patients with cervix cancer were studied. After *in vitro* γ irradiation with single doses ranging from 0.05 to 4 Gy, MN induction was assessed. For each patient, the linear-quadratic (LQ) model and the induced repair (IR) model were fitted over the whole data set. In fits of the IR model, an HRS-like response after low doses (seen as the deviation over the LQ curve) was demonstrated for the fibroblasts of two patients and for the keratinocytes of four other patients. The α_s/α_r ratio for the six patients ranged from 2.7 to 15.4, whereas the values of the parameter d_c ranged from 0.13 to 0.36 Gy. No relationship was observed between chromosomal radiosensitivity of fibroblasts and keratinocytes derived from the same donor in the low-dose (0.1–0.25 Gy) region. In conclusion, the fact that low-dose chromosomal hypersensitivity was observed for cells of only six of the patients studied suggests that it is not a common finding in human normal cells and can represent an individual characteristic.

INTRODUCTION

The phenomenon of low-dose hyper-radiosensitivity (HRS), an effect in which cells die from excessive sensitivity to low doses (<0.5 Gy) of ionizing radiation but become more resistant (IRR) to larger doses, has been detected in about 80% of the human tumor cell lines assessed so far (*1–4*). The suggested mechanism for HRS is related to the absence at low doses of an inducible DNA repair mechanism observed at higher doses, above a putative damage threshold. Therefore, cells may show hypersensitivity to X-ray doses that produce damage that is insufficient to activate this process (*5, 6*).

Since HRS was reported after fractionated X irradiation in tumor cell lines, indicating recoverability of HRS between fractions (*5, 7*), there has been a considerable interest in exploiting the HRS phenomenon in radiotherapy of cancer patients. It was suggested that radioresistant (to conventional doses) tumors, such as glioma, could be cured more effectively with multiple very small doses per fraction—''ultrafractionation''—than with conventional radiotherapy (*4, 7*). In a clinical study on the HRS effect in human tumors, Harney *et al.* (*8*) found that an ''ultrafractionated'' radiotherapy (0.5 Gy three times a day) was more effective in growth delay of metastatic melanoma and sarcoma tumor nodules than conventionally fractionated radiotherapy (1.5 Gy/day). If the HRS phenomenon also exists in normal tissues, one of the consequences of greater effectiveness of ultrafractionation could be more severe side effects. This can be important, especially when intensity-modulated radiotherapy is used, because a larger volume of normal tissue receives very small doses per fraction. Therefore, to obtain therapeutic gain with ultrafractionation, critical normal tissues must be less sensitive to low doses than the tumors.

Hyper-radiosensitivity in normal human cells was first discovered *in vitro* in experiments measuring the survival of lung epithelial cells after single doses of X rays (*9*). Three clinical studies of HRS in normal human tissues were focused on the effects of low doses per fraction on skin (*4, 10, 11*). In two of them, the basal cell density in human epidermis was used as the end point. Turesson *et al.* (*4*) found a significant reverse fractionation effect (HRS effect), with greater loss of basal cells after 0.45 Gy than after 1.1 Gy per fraction. However, the effect was lost when dose intensity was taken into account (*4, 11*). To avoid the impact of the time factor, Harney *et al.* (*11*) compared the effects of two regimens of equal dose intensity (0.5 Gy three times a day × 12 days compared to 1.5 Gy/day × 12 days) and did not observe HRS in seven of eight patients after doses of ~0.5 Gy. In one patient, however, an HRS effect, seen as a significant reduction in basal cell density after low doses, was found, suggesting that HRS may be an individual characteristic. The concept of interindividual

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variation in normal tissue radiosensitivity to conventional (>1 Gy) doses is commonly known and was shown in many studies searching for methods that predict normal tissue responses to radiotherapy in cancer patients.

The data on HRS *in vitro* in normal cells are scarce (*4, 9*). No studies have tested the HRS effect *in vitro* in primary normal cells from a group of cancer patients. Therefore, the aim of the present study was to examine, using the micronucleus (MN) assay, the low-dose radiation response of normal skin cells (fibroblasts and keratinocytes) derived from cancer patients and to determine whether an HRS-like phenomenon occurs in cells of these patients. If low-dose chromosomal hypersensitivity is present *in vitro* in the cells of these patients, the future observation of their normal tissue reactions after radiotherapy could address the clinical relevance of this effect.

MATERIALS AND METHODS

Patients

Primary human fibroblast (HFIB) and keratinocyte (HEK) cultures were obtained from normal skin biopsies of 40 cervix cancer patients. The patients were not treated by chemo- and/or radiotherapy before surgery. The mean age of the patients was 46 years (range 36–57). Six patients had FIGO stage IA and 34 patients had stage IB. Informed consent was obtained from all patients. The study was reviewed and approved by the Ethical Committee of the Centre of Oncology.

Primary Cultures

Skin strips (5 × 20 mm) were taken from the pelvic area of patients undergoing hysterectomy, held at 4°C, and processed within 1 h. There was no known previous trauma to this area of skin. The tissue was rinsed three times in calcium- and magnesium-free PBS with antibiotics (200 U/ml penicillin, 200 μg/ml streptomycin and 2.5 μg/ml amphotericin B; Sigma), and then any subcutaneous fat was removed. The remaining tissue was cut into two pieces of different sizes. The smaller pieces (5 × 5 mm) were used for fibroblast preparation as described previously (*12*). Briefly, the pieces of skin were placed in tubes with 0.2% collagenase type I (Sigma) for 24 h at 37°C. The next day the tissue was minced with a sterile scalpel and placed in 25-cm^2 culture flasks (Nunc) containing DMEM supplemented with 10% FCS, 1% Hepes, 1% sodium pyruvate (Biochrom) and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B). The bigger pieces (5 × 15 mm) for keratinocyte preparation were placed in tubes with dispase (12 U/ml; Gibco) for 16 h at 4°C. On the next day, after separation from the dermis, the epidermis was digested with a solution of 0.05% trypsin and 0.02% EDTA (Biochrom) for 15 min at 37°C and shaken for 5 min at room temperature to obtain a single cell suspension. The cells were placed in 25-cm^2 culture flasks containing serum-free keratinocyte basal medium (KBM-2) enriched with supplements and growth factors (KGM-2 SingleQuots). The final concentrations of the supplements in medium were 0.1 ng/ml human EGF, 5 μg/ml insulin, 0.5 μg/ml hydrocortisone, 50 μg/ml gentamicin, 50 ng/ml amphotericin B, 0.15 m*M* calcium and 30 μg/ml bovine pituitary extract (BPE). The keratinocyte culture medium and supplements were purchased from Clonetics® (Cambrex Bio Science Walkersville, MD). All cultures were incubated at 37°C in a humidified atmosphere of 95% air/5% CO_2. The medium was changed every other day, and the cells were subcultured before they reached 70% confluence. Fibroblasts were detached with trypsin/EDTA (0.025%/0.01%) for 3 min and keratinocytes for 10 min.

Micronucleus Assay

The MN assay has been described in detail (*12, 13*). In brief, early-passage cells (third-passage fibroblasts and second-passage keratinocytes) in exponential growth were seeded (at a density of 5×10^4/dish) onto 35-mm petri dishes (Nunc) with 2 ml medium as above, with two dishes per dose. After a 24-h incubation period, the cells were irradiated with single doses ranging from 0.05 to 4 Gy ^{60}Co γ rays at a mean dose rate of 26.2 ± 0.3 cGy/min (range 29.3–23.0). Immediately after irradiation (in less than 30 min), fresh medium with 2 μg/ml cytochalasin B (Sigma) was added to block cytokinesis, but not karyokinesis. This protocol allows us to distinguish between nonproliferating and proliferating cells and to score exclusively micronuclei in binucleated cells, i.e. in cells after their first mitosis. After 48 h (HEK) or 72 h (HFIB) incubation, the cultures were washed with 0.9% NaCl, fixed in 90% methanol, and stained with buffered Giemsa dye (pH 6.8). The duration of incubation was chosen to allow all proliferating cells to accomplish the first karyokinesis, resulting in the formation of binucleated cells for maximum MN yields. The MN assay was performed on fibroblasts of 40 patients and keratinocytes of 35 patients. In five cases, insufficient numbers of keratinocytes were obtained to do the assay. For fibroblasts of 11 patients and for keratinocytes of four patients, the experiment was repeated two to four times. For the remaining patients, the experiment was performed once.

Micronucleus Scoring

All petri dishes were coded and randomized. The percentage of binucleated cells was scored in a total of 200 cells per dose (in two petri dishes). Micronucleus induction was determined in a total of 1000 binucleated cells per dose. The parameters defined were the fraction of binucleated cells with at least one micronucleus (fraction of binucleated cells with micronuclei) and the number of micronuclei per individual binucleated cell. The spontaneous (0 Gy) MN induction was subtracted. Micronuclei were identified as bodies well separated from the two main nuclei, morphologically identical to but smaller than these.

Statistical Analysis

The MN data for each patient were fitted to the linear-quadratic (LQ) model (Eq. 1). To study the possible low-dose hypersensitivity (deviation from the LQ curve), for each patient, the induced-repair (IR) model (Eq. 2) was also fitted over the whole data set (0.05–4 Gy).

$$y = \alpha d + \beta d^2. \quad (1)$$

$$y = \alpha_r[1 + (\alpha_s/\alpha_r - 1)e^{-d/d_c}]d - \beta d^2. \quad (2)$$

The IR model, originally suggested by Joiner and Johns (*14*) to better describe the low-dose response, is based on the LQ model with a modification of the α component, where d is dose, α_r is α extrapolated from the high (conventional)-dose response, and α_s is α derived from the response at very low doses. $\alpha_s > \alpha_r$ represents increased sensitivity at very low doses. d_c is a parameter describing the range of doses over which the transition from hypersensitivity to induced resistance occurs (when α_s to α_r is 63% complete). The β term from the LQ equation remains unmodified. The data (MN induction) were fitted with the IR model and LQ model using nonlinear least-squares regression using the iterative method of Gauss-Newton (Statistica 6.0) to produce the best-fit parameters for each model. The presence of the HRS/IRR dose response is supported by values of α_s higher than α_r, the confidence limits of which do not overlap, and values of d_c significantly greater than 0 (*3*).

RESULTS

The raw data obtained in the dose–response study for fibroblasts and keratinocytes of each patient are summarized in Tables 1 and 2, respectively. Data are shown for

TABLE 1
Number of Binucleated Cells with Micronuclei Scored in 1000 Binucleated Fibroblasts

Patient no.	Age (years)	0 Gy	0.05 Gy	0.1 Gy	0.15 Gy	0.2 Gy	0.25 Gy	0.5 Gy	0.75 Gy	1 Gy	1.5 Gy	2 Gy	4 Gy
1.*	48	2 ± 0	8 ± 2	11 ± 2	16 ± 2	20 ± 2	31 ± 3	43 ± 4	77 ± 9	95 ± 4	179 ± 17	257 ± 19	500 ± 20
2.*	38	6 ± 0	10 ± 9	13 ± 9	14 ± 8	18 ± 7	24 ± 12	32 ± 13	50 ± 9	94 ± 11	187 ± 26	235 ± 40	573 ± 17
3.**	37	40 ± 14	13 ± 1	23 ± 2	43 ± 7	45 ± 8	65 ± 8	67 ± 7	82 ± 11	119 ± 10	204 ± 12	273 ± 20	562 ± 19
4.*	36	23 ± 0	21 ± 2	26 ± 2	33 ± 1	41 ± 3	50 ± 2	96 ± 15	125 ± 1	178 ± 18	311 ± 4	360 ± 17	690 ± 6
5.*	52	10 ± 0	12 ± 3	14 ± 4	15 ± 5	19 ± 4	31 ± 1	45 ± 1	88 ± 12	126 ± 24	176 ± 14	239 ± 13	569 ± 69
6.*	48	20 ± 0	13 ± 7	27 ± 1	28 ± 19	18 ± 12	42 ± 14	67 ± 12	95 ± 24	113 ± 13	171 ± 16	261 ± 54	503 ± 16
7.**	52	21 ± 3	13 ± 2	23 ± 2	32 ± 3	45 ± 5	53 ± 7	77 ± 6	94 ± 10	127 ± 7	199 ± 17	242 ± 17	554 ± 27
8.**	49	28 ± 3	16 ± 3	21 ± 6	33 ± 3	48 ± 4	61 ± 8	96 ± 17	138 ± 10	191 ± 20	239 ± 12	299 ± 13	546 ± 31
9.**	46	40 ± 4	10 ± 4	18 ± 6	22 ± 6	27 ± 9	32 ± 7	63 ± 11	86 ± 15	122 ± 29	160 ± 33	224 ± 50	458 ± 59
10.**	49	10 ± 0	23 ± 11	38 ± 13	39 ± 5	44 ± 2	44 ± 5	46 ± 5	75 ± 1	90 ± 8	136 ± 8	188 ± 6	399 ± 31
11.**	55	26 ± 3	13 ± 4	19 ± 6	23 ± 5	30 ± 5	43 ± 3	86 ± 7	83 ± 11	105 ± 8	175 ± 25	228 ± 17	415 ± 39
12.	45	19	8	22	18	32	44	59	110	142	202	266	496
13.	44	13	3	24	22	43	44	67	107	151	202	245	447
14.	41	11	5	10	22	32	35	81	101	119	173	242	449
15.	53	22	2	10	13	25	37	59	85	110	169	214	401
16.	37	45	12	13	37	43	42	87	129	141	205	324	602
17.	41	23	12	14	22	18	31	81	91	129	184	245	485
18.	45	56	0	25	20	27	27	76	91	139	230	284	557
19.	41	52	6	21	28	36	38	74	101	163	213	298	503
20.	45	23	13	14	18	17	34	91	107	125	201	255	477
21.	50	9	6	16	19	24	28	71	121	135	151	226	481
22.	54	55	11	17	31	41	37	61	104	109	240	289	510
23.	56	15	12	16	21	27	33	65	88	132	198	265	475
24.	51	13	10	3	17	15	20	40	73	111	145	192	382
25.	43	15	9	12	12	23	26	50	104	125	198	303	503
26.	57	26	9	12	24	25	40	52	80	108	202	254	489
27.	56	20	10	17	22	29	39	68	92	127	188	253	503
28.	53	10	7	28	22	43	43	77	103	137	210	290	560
29.	35	42	10	20	27	28	44	89	108	148	209	270	538
30.	37	23	1	17	17	19	27	70	66	132	184	255	442
31.	37	31	13	19	25	25	50	76	141	159	227	278	582
32.	52	16	4	12	10	17	11	16	39	48	70	115	254
33.	43	20	16	20	22	26	25	71	91	118	157	256	497
34.	48	10	3	9	10	7	20	47	50	73	139	173	413
35.	47	18	0	10	12	13	16	48	55	109	123	186	352
36.	57	10	11	19	25	31	41	69	95	128	195	257	509
37.	50	19	4	13	21	36	48	96	103	174	243	349	618
38.	45	4	5	15	20	14	25	67	98	112	166	214	363
39.	42	10	8	10	13	15	31	43	76	140	165	216	500
40.	44	15	12	20	25	20	37	56	91	129	220	254	556

Note. The spontaneous (0 Gy) micronucleus induction was subtracted from that observed in irradiated cells.
* Mean ± SEM of two independent experiments.
** Mean ± SEM of four independent experiments.

the number of binucleated cells with micronuclei only. However, mean values (±SEM) of percentages of binucleated cells, fractions of binucleated cells with micronuclei, and the numbers of micronuclei per single binucleated cell for the patients' cells are listed in Table 3. Since, in the present study, exactly the same results for two MN induction parameters (fraction of binucleated cells with micronuclei and micronuclei/binucleated cells) were observed, for clarity, the results for only one (fraction of binucleated cells with micronuclei) are presented.

Chromosomal radiosensitivities of fibroblasts as well as keratinocytes, expressed as the induction of micronuclei per unit dose (fraction of binucleated cells with micronuclei/Gy), varied significantly between cancer patients ($P < 0.0005$). The high interindividual variation in MN induction was observed after low (<0.5 Gy) and higher doses (Tables 1, 2). For example, there was a sixfold difference at 0.25 Gy between the least responsive and the most responsive patients. As shown in Table 1, there was no relationship between the age of the patients and MN induction at low and high doses.

A linear-quadratic dose response for MN induction was observed for most of the patients, and the dose response approximated linearity for only a few patients. To study the possible low-dose hypersensitivity (HRS), for each patient, the IR model was fitted to the complete data set covering the whole dose range, from 0.05 to 4 Gy. In the fits of the IR model, the deviation over the LQ curve for MN induc-

TABLE 2
Number of Binucleated Cells with Micronuclei Scored in 1000 Binucleated Keratinocytes

Patient no.	0 Gy	0.1 Gy	0.15 Gy	0.2 Gy	0.25 Gy	0.5 Gy	0.75 Gy	1 Gy	2 Gy	4 Gy
1.	25	5	11	—	16	26	33	45	85	170
2.	18	6	7	—	9	23	35	40	68	152
3.	23	4	8	—	12	24	36	40	80	131
4.	28	11	11	—	27	42	50	68	118	228
5.	13	11	22	—	25	57	83	93	115	130
6.	35	14	12	—	24	28	67	111	136	241
7.	22	19	19	—	22	43	73	100	134	260
8.	17	5	15	—	23	35	41	99	145	211
9.	13	8	11	—	19	29	29	62	86	194
10.	25	11	11	—	14	31	46	97	116	203
11.	20	7	9	—	12	55	65	89	145	194
12.	19	3	7	—	13	17	28	48	75	165
13.	—	—	—	—	—	—	—	—	—	—
14.	—	—	—	—	—	—	—	—	—	—
15.	20	8	4	—	13	36	43	61	69	76
16.	24	10	3	—	17	18	27	45	67	119
17.	27	15	14	—	16	31	59	80	113	193
18.	19	9	17	—	17	32	51	62	106	206
19.	21	5	5	—	11	23	37	74	135	219
20.	—	—	—	—	—	—	—	—	—	—
21.	—	—	—	—	—	—	—	—	—	—
22.	20	10	15	—	15	30	53	25	65	105
23.*	20 ± 0	20 ± 0	16 ± 1	—	25 ± 2	29 ± 0	48 ± 3	50 ± 0	88 ± 3	193 ± 8
24.	—	—	—	—	—	—	—	—	—	—
25.	9	15	15	—	20	31	43	45	112	214
26.	22	11	11	—	16	33	49	59	93	155
27.	16	9	10	—	11	16	39	48	120	250
28.	18	15	15	—	24	33	44	54	86	154
29.*	10 ± 0	20 ± 8	20 ± 9	—	32 ± 9	38 ± 5	—	40 ± 6	85 ± 9	200 ± 16
30.	20	16	17	—	25	36	48	58	92	162
31.	12	1	1	11	11	8	13	18	26	75
32.*	23 ± 0	20 ± 8	27 ± 5	40 ± 8	50 ± 8	45 ± 8	47 ± 10	77 ± 10	102 ± 5	187 ± 10
33.	20	14	15	—	23	33	44	54	85	154
34.	20	16	18	—	34	50	67	86	160	280
35.*	23 ± 3	43 ± 1	43 ± 10	51 ± 1	57 ± 9	43 ± 5	37 ± 2	53 ± 6	76 ± 11	153 ± 23
36.	10	15	15	16	14	26	44	51	69	76
37.	10	15	11	—	24	33	43	54	86	154
38.	12	7	10	8	16	23	30	48	68	169
39.	10	16	15	16	33	40	62	84	108	209
40.	10	17	18	—	24	26	40	43	80	153

Note. The spontaneous (0 Gy) micronucleus induction was subtracted from that observed in irradiated cells.
* Mean ± SEM of two independent experiments.

tion after low doses, which is an indicative of an HRS-like response, was demonstrated for the fibroblasts of two patients (Fig. 1) and for the keratinocytes of four other patients (Fig. 2). Values of parameters of the two models for the six patients are shown in Table 4. Values of α_s (derived from the response at very low doses) higher than α_r (derived from the response at high doses) whose confidence limits do not overlap were found for them. The interindividual variability of parameter α_s was higher than of parameter α_r, and it gave a difference between the six patients in the α_s/α_r ratio that ranged from 2.7 to 15.4. The values of d_c were significantly greater than 0 and ranged from 0.13 to 0.36 Gy. For the patients without a low-dose hypersensitive response, the α_s/α_r ratio was ~1, and the fits of two models were equivalent in the low- and high-dose regions (data not shown).

When the mean MN induction per unit dose (for the whole group of patients) was compared for low and high γ-ray doses, it appeared that doses in the range 0.05–0.25 Gy were significantly ($P < 0.0001$) more effective per gray than higher doses in both fibroblasts and keratinocytes (Fig. 3). For fibroblasts, the mean effect per gray increased by a factor of ~1.5 from 0.13 at a dose of 1 Gy to 0.19 at a dose of 0.05 Gy, whereas in keratinocytes the mean effect per gray increased by a factor of ~3 from 0.05 at a dose of 1 Gy to 0.15 at a dose of 0.1 Gy. If the data were reanalyzed excluding the six patients presenting an HRS-like response, the mean MN induction per unit dose was still significantly higher (~1.5 times) at doses <0.5 Gy than at higher doses in fibroblasts and keratinocytes ($P < 0.0001$). The doses in the range 0.75–4 Gy were equally effective per unit dose in MN induction.

TABLE 3
Mean Values (±SEM) of the Percentage of Binucleated Cells, Fraction of Binucleated Cells with Micronuclei and Micronuclei/Binucleated Cells in Micronucleus Assay for Fibroblasts of 40 and Keratinocytes of 35 Cervix Cancer Patients

	Fibroblasts (n = 40)			Keratinocytes (n = 35)		
Dose (Gy)	Percentage binucleated cells	Fraction binucleated cells + micronuclei	Micronuclei/ binucleated cells	Percentage binucleated cells	Fraction binucleated cells + micronuclei	Micronuclei/ binucleated cells
0	67.6 ± 1.4	0.022 ± 0.002	0.024 ± 0.002	61.4 ± 5.4	0.020 ± 0.002	0.020 ± 0.002
0.05	65.6 ± 1.4	0.009 ± 0.001	0.011 ± 0.001	—	—	—
0.1	63.7 ± 1.5	0.017 ± 0.001	0.020 ± 0.001	60.5 ± 5.2	0.015 ± 0.003	0.017 ± 0.004
0.15	62.3 ± 1.6	0.022 ± 0.001	0.025 ± 0.001	58.8 ± 5.1	0.015 ± 0.004	0.018 ± 0.004
0.2	60.7 ± 1.5	0.028 ± 0.002	0.032 ± 0.002	36.0 ± 5.1	0.034 ± 0.012	0.041 ± 0.014
0.25	58.9 ± 1.5	0.036 ± 0.002	0.041 ± 0.002	56.5 ± 5.3	0.024 ± 0.005	0.027 ± 0.005
0.5	55.0 ± 1.5	0.066 ± 0.003	0.075 ± 0.004	54.1 ± 5.1	0.033 ± 0.004	0.037 ± 0.004
0.75	51.7 ± 1.4	0.092 ± 0.004	0.106 ± 0.004	51.7 ± 5.9	0.044 ± 0.005	0.048 ± 0.006
1	48.1 ± 1.5	0.126 ± 0.004	0.148 ± 0.006	50.6 ± 4.7	0.055 ± 0.007	0.060 ± 0.008
1.5	43.5 ± 1.6	0.189 ± 0.006	0.227 ± 0.009	—	—	—
2	37.3 ± 1.3	0.252 ± 0.007	0.316 ± 0.011	46.7 ± 4.8	0.086 ± 0.009	0.093 ± 0.011
4	21.6 ± 1.0	0.493 ± 0.013	0.688 ± 0.025	40.0 ± 4.7	0.155 ± 0.016	0.168 ± 0.016

Comparison of the means of MN induction for fibroblasts and keratinocytes obtained after irradiation showed statistically significant differences between them ($P < 0.0001$). In general, keratinocytes were at least twice as resistant to MN induction as fibroblasts (Table 3).

The analysis of 35 patients showed no significant relationship between the chromosomal radiosensitivity of fibroblasts and keratinocytes from the same donor in the low-dose (0.1–0.25 Gy) region of the radiation response (r = 0.02, P = 0.84). The correlation was observed in the high-dose (0.5–4 Gy) region only (r = 0.82, P = 0.0001, Fig. 4). The same result was encountered when the relationship was analyzed for six patients presenting a low-dose HRS-like response. For fibroblasts of two such patients, the calculated correlation coefficients were r = 0.98, P = 0.0001 and r = 0.05, P = 0.93 for high and low doses, respectively, whereas for keratinocytes of the remaining four patients, the values were r = 0.88, P = 0.0001 and r = −0.33, P = 0.25 for high and low doses, respectively.

DISCUSSION

The present study showed high interindividual variation in low-dose (<0.5 Gy) chromosomal radiosensitivity, expressed as MN induction, in a group of patients with cervix cancer. This gave us a reason to investigate whether an HRS-like phenomenon occurs in normal cells of these patients. The data presented here suggest that low-dose chromosomal hypersensitivity is not a common finding in human normal cells and can represent an individual characteristic. An HRS-like response after low doses (seen as the deviation over the LQ curve) was demonstrated only for the fibroblasts of two patients (Fig. 1) and for the keratinocytes of four other patients (Fig. 2). Although an in-

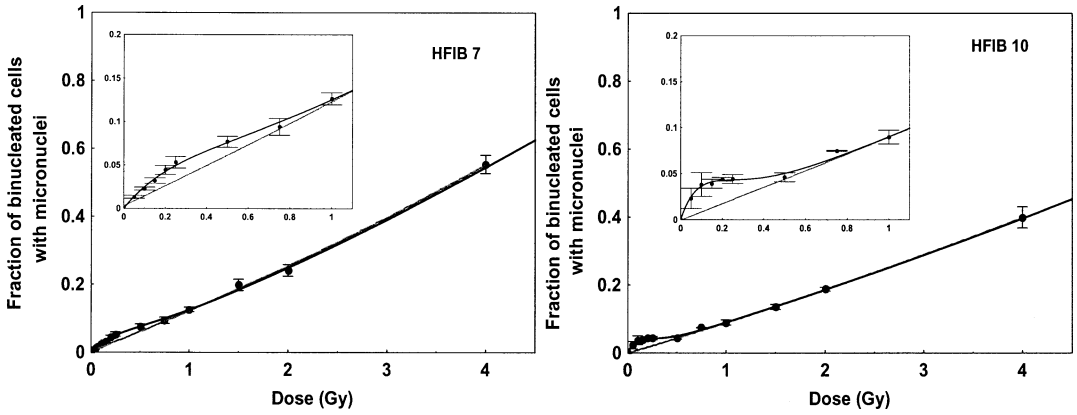

FIG. 1. Induction of micronuclei in fibroblasts (HFIB) of two patients (no. 7 and 10) showing chromosomal hypersensitivity after single low doses of γ radiation. Each point represents the mean ± SEM of four experiments. The solid line and dotted line show the fits of the induced-repair (IR) and linear-quadratic (LQ) models, respectively. The insets show the low-dose region of the MN induction and demonstrate the increased effectiveness of doses below 0.5 Gy compared to the LQ model prediction.

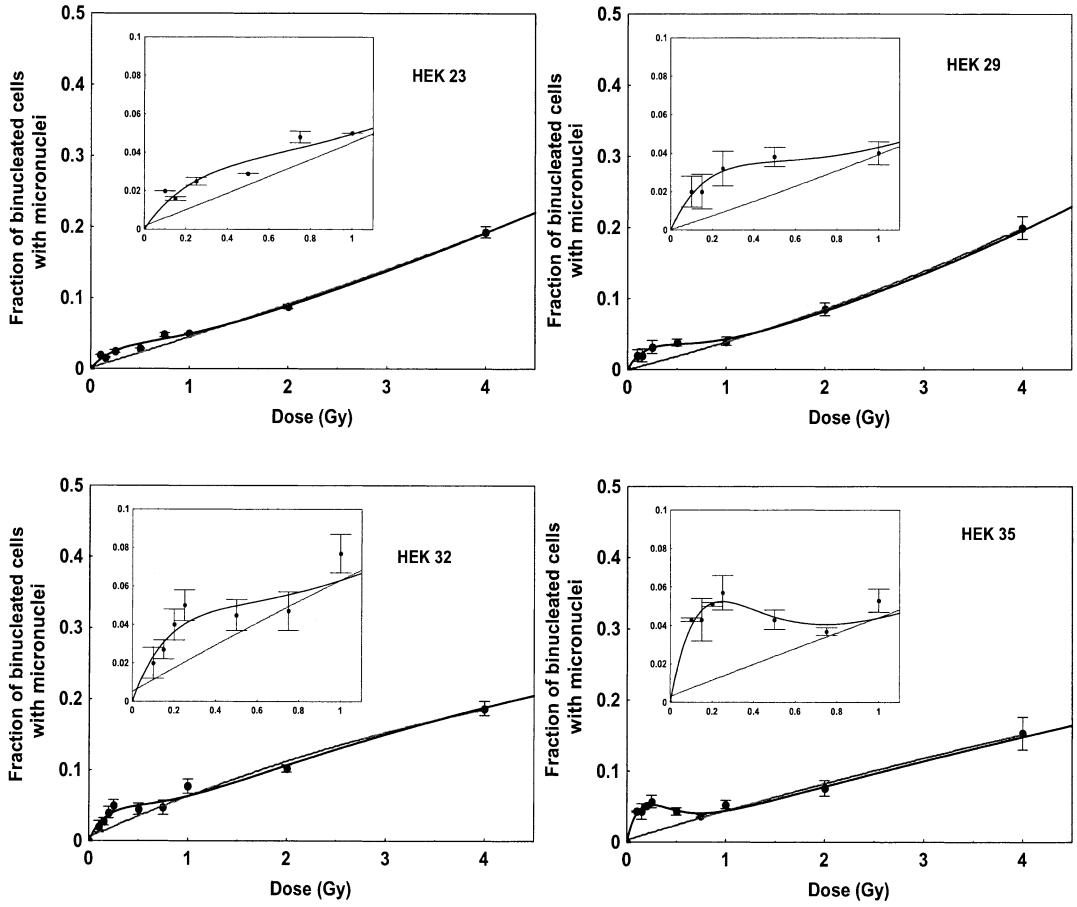

FIG. 2. Induction of micronuclei in keratinocytes (HEK) of four patients (no. 23, 29, 32 and 35) showing chromosomal hypersensitivity after single low doses of γ radiation. Each point represents the mean ± SEM of two experiments. The solid line and dotted line show the fits of the induced-repair (IR) and linear-quadratic (LQ) models, respectively. The insets show the low-dose region of the MN induction and demonstrate the increased effectiveness of doses below 0.5 Gy compared to the LQ model prediction.

creased effectiveness of low doses (<0.5 Gy) in comparison with higher doses in mean MN induction per unit dose was observed for the whole group of patients, the fibroblasts of 38 patients and keratinocytes of 31 patients did not demonstrate any HRS-like effect.

In the present study we used the MN assay, a simple chromosomal radiosensitivity test with the end point being the result of DNA damage and repair. The usefulness of the MN assay as a biological indicator of radiation doses as low as 0.1 Gy was confirmed in many studies (*15–17*). These results show that it is possible to observe the deviation over the LQ curve for MN induction after low doses, which is indicative of an HRS-like response. Similarly, in the study of Courdi *et al.* (*16*), the MN assay allowed them

TABLE 4
Values of Parameters and 95% Confidence Limits Obtained with the IR Model and LQ Model for Six Patients whose Cells Demonstrated Low-Dose Chromosomal Hypersensitivity

Patient	IR fit					LQ fit	
no.	α_s	α_r	α_s/α_r	d_c	β	α	β
Fibroblast							
7	0.302 (0.13–0.47)	0.112 (0.09–0.13)	2.7	0.31 (0.04–0.58)	0.006 (0.001–0.011)	0.117 (0.10–0.14)	0.005 (−0.001–0.012)
10	0.688 (0.22–1.15)	0.087 (0.08–0.10)	7.9	0.13 (0.05–0.20)	0.003 (−0.001–0.007)	0.086 (0.07–0.10)	0.003 (−0.002–0.009)
Keratinocytes							
23	0.161 (0.10–0.23)	0.040 (0.03–0.05)	4.0	0.36 (0.18–0.54)	0.002 (−0.001–0.005)	0.044 (0.03–0.06)	0.001 (−0.002–0.004)
29	0.249 (0.15–0.34)	0.033 (0.02–0.04)	7.5	0.28 (0.16–0.40)	0.004 (0.002–0.007)	0.036 (0.03–0.04)	0.004 (0.003–0.004)
32	0.313 (0.16–0.47)	0.060 (0.04–0.08)	5.3	0.28 (0.12–0.45)	−0.003 (−0.008–0.002)	0.067 (0.04–0.09)	−0.005 (−0.013–0.002)
35	0.631 (0.38–0.88)	0.041 (0.03–0.06)	15.4	0.20 (0.12–0.27)	−0.001 (−0.005–0.003)	0.045 (0.01–0.08)	−0.002 (−0.011–0.007)

Note. Values of α_s higher than α_r that do not overlap and values of d_c greater than 0 were found.

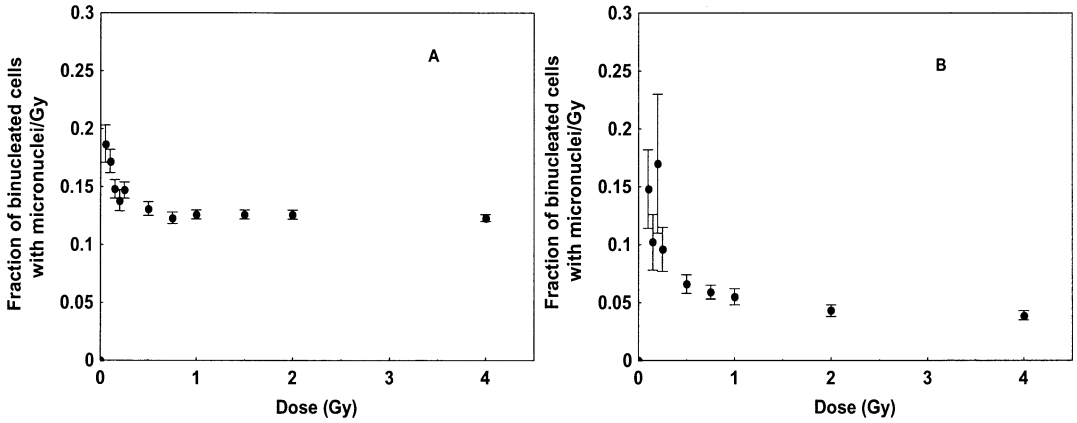

FIG. 3. Induction of micronuclei per unit dose of radiation (Gy) in (panel A) fibroblasts (HFIB) and (panel B) keratinocytes (HEK). Each point represents the mean ± SEM of 40 and 35 patients, respectively. The increased effectiveness of doses below 0.5 Gy in MN induction compared to higher doses is demonstrated.

to observe significant chromosomal hypersensitivity to low doses in some of the human tumor cell lines they examined.

For reasons given below, we cannot exclude the possibility that the HRS response would be seen in more patients if different culture conditions and different radiosensitivity assays were used. It was shown recently that low-dose hyper-radiosensitivity is dependent on the cell cycle and is a consequence of ineffective cell cycle arrest of cells irradiated in G_2 phase with doses less than 0.4 Gy (*18–20*). Short *et al.* (*18*) found that in some cell lines HRS was observed in asynchronous populations but always with the most marked effect in G_2-phase cells. In some cell lines, however, HRS was not found in asynchronous populations, although it was demonstrable in G_2-phase cells (*18, 20*). Therefore, it is possible that at least in some of the patient cells in our study, the HRS response at G_2 phase could be masked in the asynchronous cell cultures we used. Another less likely possibility is that the cells of some patients demonstrate low-dose hypersensitivity, but only at much lower doses than it is possible to study with the MN assay. An increased biological effectiveness per unit dose (1.5 times) of the lowest doses, 0.05 Gy for fibroblasts and 0.1 Gy for keratinocytes in comparison with higher doses, was still observed for the group of patients when the six patients with an HRS-like response were excluded from the group.

In the present study, high variability in the HRS-like response between six patients was observed. The α_s/α_r ratio ranged from 2.7 to 15.4; the difference was due to the high interindividual variation in the values of α_s representing the response at very low doses (Table 4). The parameter d_c in the IR model describes the range of doses over which the transition from HRS to IRR occurs. The d_c values obtained in the present study for six patients with HRS ranged from 0.13 to 0.36 Gy. Therefore, the difference in both parameters between the patients suggests that the HRS phenomenon can be an individual characteristic. The present d_c values are similar to those obtained with the clonogenic assay by other authors (*3*) for cells of human tumor cell lines (0.14–0.47 Gy) but lower than the value of d_c reported by Singh *et al.* (*9*) for normal human lung epithelial cells (0.59 Gy). Only one report on MN induction in tumor cells after low-dose irradiation is known in which the authors found an HRS response in one tumor cell line (*16*), but they did not use the IR model to fit the data, and the d_c value is not known.

Comparison of the means of MN induction for fibroblasts

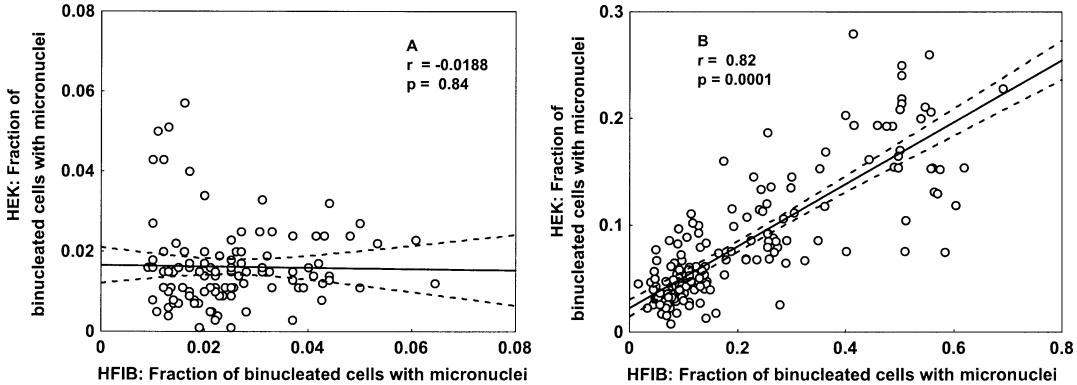

FIG. 4. Comparison between fibroblast (HFIB) and keratinocyte (HEK) MN induction after (panel A) doses below 0.5 Gy and (panel B) higher doses of γ radiation. Statistically significant correlation was observed after higher doses only.

and keratinocytes obtained after irradiation has shown keratinocytes to be at least twice as resistant as fibroblasts (Table 3). In the previous work (*13*), greater MN induction in human fibroblasts than in keratinocytes was found after X irradiation. Similarly, in cell survival studies, Geara *et al.* (*21*) and D'Errico *et al.* (*22*) found keratinocytes to be more radioresistant (higher surviving fraction) than fibroblasts after irradiation.

The difference in radiosensitivity between these two types of cells can be one explanation of the more marked HRS-like effect seen in keratinocytes (higher α_s/α_r ratio ranging from 4–15.4) than in fibroblasts (α_s/α_r ratio ranging from 2.7–7.9). This is consistent with the previous suggestion that the low-dose effect is more pronounced in more radioresistant cells (*3*). In the cell survival studies of Singh *et al.* (*9*) and Short *et al.* (*3*), an α_s/α_r ratio of 7.9 for normal human lung epithelial cells and an α_s/α_r ratio ranging from 13.6 to 21.6 for the most radioresistant tumor cell lines have been reported. In regard to the greatest HRS effect seen in G_2 phase, it was suggested that actively proliferating cell populations may demonstrate a greater increase in radiosensitivity to very low doses compared with quiescent populations (*18*). Therefore, quickly proliferating keratinocytes in comparison with slowly proliferating fibroblasts could have more chance to show the HRS effect.

We observed no correlation between the chromosomal radiosensitivity of fibroblasts and keratinocytes after very low doses (0.1–0.25 Gy), although a positive correlation was seen after higher doses (0.5–4 Gy) (Fig. 4). Therefore, the results seen after low doses suggest that the response to DNA damage can be cell type specific. Flatt *et al.* (*23*) and D'Errico *et al.* (*22*) have reported that there is a difference in the cell cycle response for DNA damage between human keratinocytes and fibroblasts. The G_1 arrest in keratinocytes was attenuated, whereas fibroblasts were arrested in G_1 phase after γ irradiation. Second, our results suggest that cell type specificity for DNA damage can be dependent on the radiation dose. Ding *et al.* (*24*) recently found differences in gene expression of human fibroblasts after low-dose (2 cGy) and high-dose (4 Gy) irradiation. At low dose, genes involved in cell-cell signaling, signal transduction and response to DNA damage were predominantly expressed, whereas at high dose, genes involved in apoptosis and cell proliferation were predominantly expressed. In the study by D'Errico *et al.* (*22*), apoptosis was not observed after high-dose X irradiation in normal human keratinocytes and fibroblasts. Therefore, the difference between the two types of cells after higher doses ($>$0.5 Gy) can be diminished and give a positive correlation between them. Because some genes were found to respond only after low doses (*24*), all this suggests that cell type specificity for DNA damage can be manifested more easily at low doses. This could explain, at least in part, why in our study the low-dose hypersensitive response demonstrated in one type of cells was not seen in another type of cells from the same donors.

In conclusion, our study showed that fibroblasts of two and keratinocytes of four of the 40 patients studied are hypersensitive to radiation doses $<$0.4 Gy and show induced radioresistance in response to higher doses. The data suggest that low-dose chromosomal hypersensitivity is not a common finding in normal human cells and can be an individual characteristic. The existence of HRS in normal cells of some patients can imply, on the one hand, lower cancer risk from small exposures to ionizing radiation, because the elimination of damaged cells protects them from mutation. The first experimental evidence for that was provided by Redpath *et al.* (*20*), who observed the reduction of transformation frequency at low doses as a consequence of low-dose HRS. On the other hand, the HRS effect can be responsible for more severe normal tissue response to radiotherapy. The clinical relevance of the low-dose chromosomal hypersensitivity in the normal cells of the six patients is under investigation after their course of radiotherapy.

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