121. Q-Banding Analysis of Long Acrocentric Chromosomes in HeLa Cells newly Appeared after X-Irradiation

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Considerable information has been available so far on the appearance of marker chromosomes in relation to radioresistance following irradiation (Rhynas and Newcombe 1960; Whitefield and Rixon 1961; Bhaskaran and Dittrich 1963). Muta and Koiwai (1970) have established several radioresistant strains in HeLa-cell populations after repeated X-irradiations. The cells of these strains have been characterized by containing unusually long acrocentric markers (LA) (Koiwai and Muta 1974). In the long term cultivation of one of such strains, its radioresistant activity was deleted without loss of the LA chromosomes (Koiwai *et al.* 1979). The evidence presented seems to indicate that the LA chromosomes have not directly been associated with the radioresistance of this strain. On the other hand, Kubo *et al.* (1978) reported the chromosomes of a morphologically similar nature which appeared spontaneously in the resistant cells without irradiation. Thus, the fact showing that all the radioresistant strain cells so far obtained by us contained without exception such LA elements may be an indication that they serve as an index of radioresistance of cells.

Under the above status, it is highly needed to analyse the structure and origin of those LA chromosomes occurring in the radiation-treated cells with the application of current technical procedures, referring to radioresistance. In this paper are presented the results of the quinacrine-banding (Q) analysis of the LA chromosomes.

Materials and methods. HeLa cells from a population SC established by Koiwai and Muta (1974) were grown as monolayer in Eagle's minimum essential medium, supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Cells were plated after trypsinization in a plastic Petri dish (Falcon 3002) at the concentration of 5×10^4 cells/dish. After 24 hr, the dishes were X-irradiated with 800 rad. X-irradiation was carried out with a Toshiba KXC-18 X-ray source operating at 200 kVp and 25 mA with 0.3 mm Cu and 0.5 mm Al filters, at the mean dose rate of 196 rad/min. On 6 days after irradiation, the medium was replaced with fresh one. Subcultures were made on 10 days and 14 days after irradiation. On

the 16th or 17th day of cultivation, the cells were subjected to colcemid for one hour at a final concentration of $0.05 \mu\text{g/ml}$. Cells were then harvested through trypsinization, treated with the hypotonic solution (0.075 M KCl) for 20 min, and fixed with methanol-acetic acid (3:1). Cells suspended in the fixative were dropped onto slides and air-dried. Slides thus prepared were stained with Giemsa solution and quinacrine mustard-Hoechst solution (Yoshida *et al.* 1975). Control chromosome preparations were made by the same procedure as mentioned above without X-ray treatment. Each experiment was repeated three times.

Results and remarks. The incidence of cells containing unusually long acrocentric chromosomes (LA markers) in the non-treated and X-irradiated populations was presented in Table I. The incidence of cells with LA markers (LA(+) cells) was 2.6% in the control non-treated cells. The previous study indicated that the mean lethal dose

Table I. Frequencies of cells with unusually long acrocentric chromosomes (LA(+) cells) in the untreated and X-irradiated populations

Cells examined	Control	X-irradiated	
		Expected	Observed
Total no. of cells	841	—	353
No. of LA(+) cells	22	—	99
%	2.6	11.1	28

(D_0) value was determined as 160 rad for the control population, while the values for several radioresistant strains distributed around 220 rad (Kubo *et al.* 1980). If the D_0 values of all the LA(+) cells in the control population would distribute around 220 rad, the incidence of the LA markers might be expected as 11.1% in cells irradiated with 800 rad, as shown in Table I. The observed value which was shown as 28%, however, was much higher than the expected one. This result seems to suggest that the radiation-induced LA markers cover a considerable part of all the LA markers occurring in the irradiated population. Thus, it seems important to analyse the nature of the LA markers with the Q-banding method in relation to their origin.

In the first place, the Q-banding karyotype was analysed in a dozen of the non-treated cells in the SC population. The results are summarized in Table II. Cells under study contained 44 to 51 intact human chromosomes in addition to 14 to 21 marker chromosomes. There were 28 types of marker chromosomes of different origin in this population. The marker chromosomes, m 1, m 2, m 3 and m 9,

occurred in all the karyotypes here dealt with. Most cells contained markers, m 4-6, m 10, m 11, m 16, m 18 and m 21. Chromosomes similar in appearance to m 3 and m 10 were reported to occur in

Table II. Different types of marker chromosomes and their frequencies in twelve HeLa cells observed

Marker no.	No. of cells studied	Possible origin
1	12	t(1;2)(1pter→1q42::2q31→2qter)
2	12	t(1;3;7)(1q32→1cen::3q12→3q27::7q22→7qter)
3	12	t(1;3)(1qter→1cen::3cen→3qter)
4	10	t(3;6)(3pter→3cen::6cen→6q23)
5	11	t(2;8)(2p23→2cen::8cen→8qter)
6	11	t(4;10)(4pter→4cen::10cen→10q24)
7	4	del(4)(pter→q25)
8	8	t(17;18)(17pter→17cen::18cen→18qter)
9	12	i(8)(pter→cen→pter)
10	11	i(5)(pter→cen→pter)
11	11	i(11)(pter→cen→pter)
12	2	i(16)(pter→cen→pter)
13	5	t(9;2;10;3)(9qter→9q13::2p13→2cen::10cen→10q24::3p13→3pter)
14	9	t(5;8)(8pter→8q24::5q31→5qter)
15	9	t(8;12)(12pter→12q13::8q12→8qter)
16	10	t(7;?)(7pter→7q36::?)
17	1	t(13;16)(16pter→16q13::13q12→13qter)
18	10	t(12;19)(19qter→19cen::12q13→12qter)
19	2	del(9)(pter→q22)
20	8	del(3)(pter→q11)
21	10	t(22;?)(22pter→22q13::?)
22	4	t(3;7)(7pter→7q32::3p13→3pter)
23	1	del(1)(pter→q25)
24	2	t(3;5)(5pter→5q12::3q13→3qter)
25	3	t(2;10;3)(2p13→2cen::10cen→10q24::3q13→3pter)
26	6	del(5)(pter→q31)
27	1	del(4)(pter→q25)
28	4	t(4;12)(12pter→12cen::4cen→4qter)

HeLa cells by Hennen (1976) and Rin and Goldstein (1974). Fig. 1 shows a Q-banding karyotype in one of the non-treated cells from the SC population (Fig. 1A). Some other cells of the same population contained additional markers as shown in Fig. 1B.

In cells received 800 rad, 95 cells with the LA markers were karyotypically analysed. Fig. 2 illustrates five types of the LA chromosomes structurally different which were observed in those LA(+) cells. LA 1 seems to be recognized in origin as the marker, m 13 or m 25, occurring in the non-treated karyotype. The origin of

LA 2 may be the long arm of m 2. The possible origin of the five types of the LA chromosomes is shown in Table III, together with their frequencies obtained in the ninety-five karyotypes. The incidences of LA 1 and LA 2 were 83.1% and 11.6%, respectively, observed in LA (+) cells. LA 3, LA 4 and LA 5 were seen each in a single LA (+) cell. The above evidence suggests that the LA markers are not of single origin, although considered so in the former paper (Koiwai and Muta 1974), but of multiple origin, particularly in relation to at least

Fig. 1. Q-banded chromosomes in HeLa cells. A: Q-banding karyotype of a cell (SC-population) consisting of 46 intact chromosomes with 21 marker chromosomes. B: 7 additional markers occurring in other cells.

5 structurally different markers. The above features allow the following conclusion that the LA chromosomes do not always serve as the index of the radioresistance. Thus, it appeared that all the strains containing the LA chromosomes are not always radioresistant, since there are cells containing LA chromosomes which do not deal with radioresistance. The investigations of the five LA chromosomes in relation to radioresistance have been in progress.

Fig. 2. 5 Q-banded unusually long acrocentric chromosomes (LA 1-5) observed in the X-irradiated cells, and normal no. 1 chromosome (No. 1) for comparison.

Table III. Different types of unusually long acrocentric chromosomes observed in the irradiated HeLa-cell population and their frequencies in ninety-five cells

LA marker	No. of cells observed	Possible origin
LA1	87	t(3;10)(10cen→10q24::3p13→3pter)
LA2	11	t(3;7)(3q12→3q27::7q22→7qter)
LA3	1	t(?;2)(?:2q23→2qter)
LA4	1	t(13;1;2)(13pter→13q14::1q25→1q42::2q31→2qter)
LA5	1	t(?;12)(?:12q13→12qter)

Summary. The nature and origin of unusually long acrocentric marker chromosomes were investigated in relation to radioresistance with the application of the Q-banding method. In cells receiving 800 rad, there were five types of unusually long acrocentric markers, different in structure. Evidence presented seems to suggest that all the strains with the LA markers are not always radioresistant.

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