RESPONSE OF THE AMES TEST TO DIFFERENT TYPES OF IONIZING RADIATION

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NEOPLASTIC TRANSFORMATION OF MOUSE C3H 10T1/2 AND SYRIAN HAMSTER EMBRYO CELLS BY DIFFERENT TYPES OF IONIZING RADIATION

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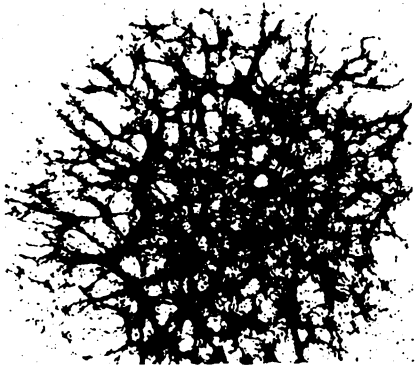

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Carcinogenesis is seen as the dominant risk of low doses of ionizing radiations. Risk estimates are based on epidemiological studies and animal experiments. After the revision of the dosimetry in Hiroshima one has very little epidemiological data for densely ionizing radiation. This and the impending revision of the quality factors has given added importance to transformation studies with densely ionizing radiations.

We have performed transformation studies with α-particles and various heavy ions to investigate the effectiveness of radiation of high ionization density and the possible role of the dose rate. The work is also directed towards the elucidation of the cellular and molecular mechanisms of radiation carcinogenesis.

In Vitro Transformation Assays

The transformation studies were performed with C3H 10T1/2 mouse embryo-fibroblasts in order to make our data comparable to those from other laboratories. The convential focus assay was utilized which is illustrated in Figure 1. The Figure includes a transformed cell clone, a focus of type III.

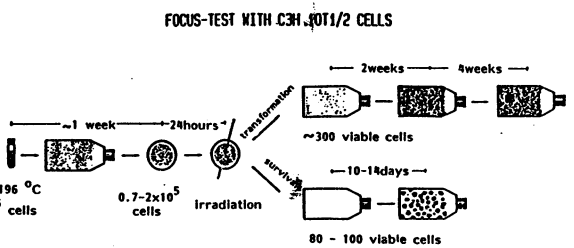

Figure 1:
Scheme of the transformation assay with C3H 10T1/2 cells

For the investigation of growth parameters, chromosomal status, tumorigenicity and oncogene expression Syrian Hamster embryo cells (SHE) were transformed by different types of ionizing radiation. The primary SHE cells age during a few passages and only the immortalized or transformed cells overgrow the dying, aged cells.

Inactivation and Transformation of C3H 10T1/2 Cells

The inactivation curves of C3H 10T1/2 cells exposed to γ-rays and α-particles are shown in Figure 2. The survival relation after γ-irradiation has a pronounced shoulder, in contrast to the survival curve after α-irradiation. The D_{37} for α-particles was 0.6 Gy.

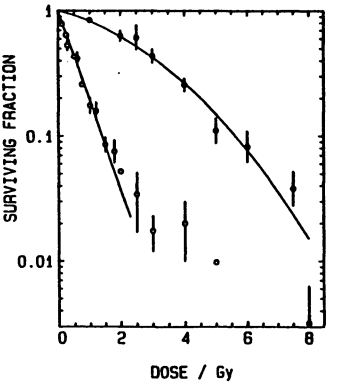

Figure 2:
Inactivation of C3H 10T1/2 cells by γ-rays (●) and α-particles (○).

Elkind and coworkers have reported a greatly enhanced transformation efficiency in C3H 10T1/2 cells exposed to low doses of fission-spectrum neutrons protracted over several hours (Hill et al., Int.J.Radiat.Biol., 46, 11-15, 1984). These results were highly unexpected in view of accepted biophysical considerations, and they are of sufficient pragmatic importance to make analogous investigations with other densely ionizing radiations mandatory.

In our studies with α-particles, we have followed the experimental protocol of Hill et al. with only minor changes, but we do not see any reversed dose rate effect on transformation efficiency. The results are compared with those of Hill et al. in Figure 3. The transformation efficiency of α-particles at high dose rate is somewhat lower than that of neutrons at high dose rate. The dose dependencies for α-exposures at high and low dose rates are the same.

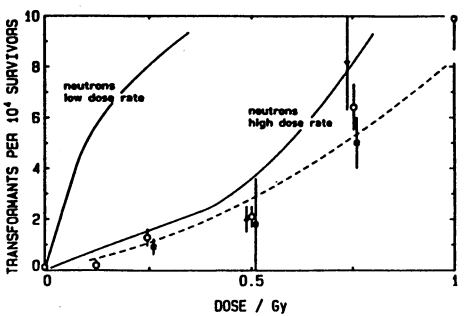

Figure 3:

Transformation rates after exposure to α-particles at different dose rates: (■) 0.83 mGy/min, (▲) 1.7 mGy/min, (▼) 2.5 mGy/min, and (○) 0.2 Gy/min. For comparison the results of Hill et al. (1984) with neutrons at high and low dose rates are shown as solid lines.

Transformation experiments were, as shown in Table 1, also performed with a variety of heavy ions with LET from 170 to 15 700 keV/µm. The transformation yields decrease with increasing ionization density. This is the case not only with regard to the frequencies per unit absorbed dose but even to the frequencies per particle, i.e. per unit fluence. For the heaviest ions, i.e. uranium with different energies, no transformations were obtained.

Ion	Energy	LET	Transformants per 10^4 survivors at fluences ϕ/cm^2							
	MeV/u	keV/µm	2.5×10^5	5.0×10^5	1.0×10^6	1.5×10^6	2.0×10^6	3.0×10^6	4.0×10^6	6.0×10^6
C	8.0	170		<0.4	<0.5	1.3±1.3	<0.6	<0.8	1.3±1.3	
C	5.5	220		<0.4	<0.5	<0.4	1.5±0.9	0.5±0.5	2.3±1.2	
O	9.0	275				2.2±1.1		2.0±0.9		1.6±0.8
Ar	18.6	780				5.0±3.5		2.6±1.2		1.7±1.0
Ar	9.6	1800	1.1±0.8	0.7±0.7	1.5±0.9		2.7±1.2		1.1±0.8	
Fe	17.3	1500	<0.6	0.7±0.7	1.3±0.9		1.4±1.0		2.4±1.1	
Kr	16.7	3200				1.7±1.2		2.3±1.4		<0.4
Kr	8.5	4300				0.8±0.8		0.6±0.6		<0.5
Pb	7.8	13900	0.6±0.6	0.6±0.6	0.7±0.7		<0.6		<0.6	
U	16.3	12800				<0.5		<0.4		<0.4
U	9.0	15300				<0.9		<0.4		<0.4
U	5.0	15700	<0.5	<0.4	<0.5	<0.6	<0.5	<0.5		

Table 1:
Transformation frequencies per surviving C3H 10T1/2 cell for various heavy ions

Tumorigenicity and Growth Parameters of Transformed SHE Cells

All radiation transformed cell lines induced tumors in athymic nude mice, as shown in Table 2. Syrian Hamster embryo cells which immortalized spontaneously were only tumorigenic after more than 30 subcultures. The latency period of tumor induction was between 3 and 6 weeks.

Cell line	Type of radiation	Dose (Gy)	Passage number	Latency period (w)	Tumor cell line
82-9			5		—
A0			30		—
A0			43	17	T 2339
A0			70	5	T 2337
A38Ia1	^{60}Co-γ	2.5	21	3	T 2744
A38Ib1	^{60}Co-γ	5.0	25	4	T 2741
A38Ic1	^{60}Co-γ	7.5	22	4	T 2748
A38IIa1	^{241}Am-α	0.25	30	3	T 2760
A38IIb1	^{241}Am-α	0.75	22	3	T 2739
A38IIc2	^{241}Am-α	1.5	21	4	T 2764
A40I-2	Carbon	0.14	5	6	T 2792
A40II-2	Carbon	0.54	5	6	T 2794
A40III-2	Carbon	1.08	5	5	T 2793

Table 2:

Tumorigenicity of transformed SHE cells exposed to γ-rays, α-particles and carbon ions.

Table 3 lists the plating efficiencies of transformed and tumor cell lines under normal culture conditions and in semisolid medium (soft agar). The cloning efficiency in soft agar of tumor cells was always higher than that of the corresponding transformed cell line from which they were derived. This indicates that additional events may take place during tumor development. The doubling times of transformed and tumor cells are shorter than the doubling times of primary SHE cells.

Cell line	Type of radiation	Dose (Gy)	Passage number	PE	DT (hours)	PE in soft agar ($\% \times 10^{-3}$)
82-9			4	0.25	20	< 0.001
A0			30	0.53	11.2	< 0.17
A0			46	0.97	9.1	< 0.13
T 2339			3	0.71	16.5	40
AC			70	0.49	10.3	< 0.1
T 2337			9	0.65	13.3	> 50
A38Ia1	^{60}Co-γ	2.5	25	0.81	15.8	0.5
T 2744			3	0.71	13.6	12.0
A38Ib1	^{60}Co-γ	5.0	27	0.57	15.4	0.7
T 2741			3	0.72	10.3	10.1
A38Ic1	^{60}Co-γ	7.5	24	0.60	16.5	0.3
T 2748			3	0.62	14.1	27.8
A38IIa1	^{241}Am-α	0.25	29	0.91	13.3	< 0.01
T 2760			4	0.07	19.8	1.4
A38IIb1	^{241}Am-α	0.75	24	0.76	14.1	< 0.01
T 2739			4	0.53	16.1	9.4
A38IIc2	^{241}Am-α	1.5	23	0.85	15.4	< 0.01
T 2764			3	0.63	15.8	> 50
A40I-2	Carbon	0.14	7	0.60	14.1	< 0.01
T 2792			4	0.31	20.4	35.8
A40II-2	Carbon	0.54	7	0.63	13.6	< 0.01
T 2794			4	0.53	14.4	4.6
A40III-2	Carbon	1.08	7	0.60	13.3	0.44
T 2793			4	0.55	12.6	3.9

Table 3:

Growth parameters of transformed SHE cells and tumor cell lines derived from tumors in nude mice after subcutaneous injection of $2x10^6$ cells.

The three histograms of the chromosome numbers of SHE cell lines transformed by γ-rays, α-particles, and carbon ions are examples for the chromosomal status of radiation transformed SHE cells (Figure 4). The transformed cell populations contain various fractions of cells with a diploid, aneuploid and tetraploid set of chromosomes. Primary diploid Syrian Hamster cells have 40 chromosomes. Experiments are underway to characterize various subclones of different transformed cell lines.

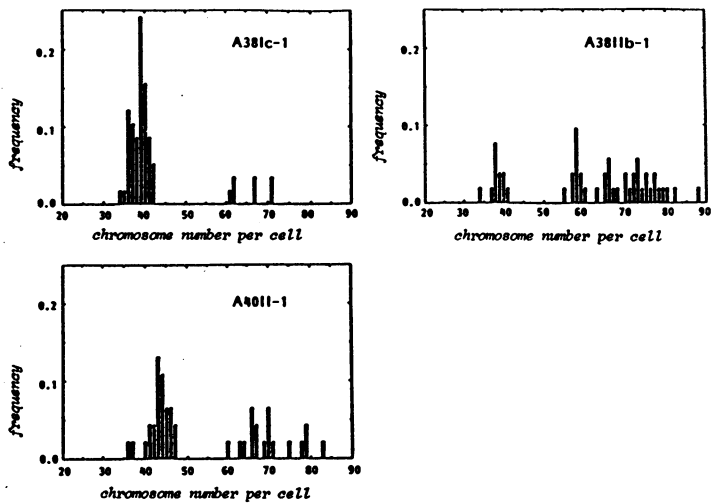

Figure 4:

Histograms of the chromosome number of transformed SHE cell lines exposed to γ-rays (A38Ic-1), α-particles (A38IIb-1), and carbon ions (A40II-1).

It has been demonstrated that transformed cell lines and cells of various human tumors tend to express a mutated ras oncogene or show enhanced expression of ras genes. It is of particular interest to investigate the oncogene expression in cells transformed by different types of ionizing radiation in comparison to chemically induced transformants. Figure 5 is a Northern analysis for the ras oncogene of various transformed and tumor cell lines derived from SHE cells. The cell line A37IIc3 (transformed by 7.5 Gy γ -rays) and, to a higher degree, the corresponding tumor cell line T 2662 show enhanced expression of the Ha-ras oncogene. For comparison the nylon filter was cohybridized with an actin probe. The other cells appear to lack enhanced ras expression. These preliminary results have to be confirmed, and further experiments are needed in order to elucidate the mechanisms for enhanced ras expression in radiation induced transformation and radiation carcinogenesis.

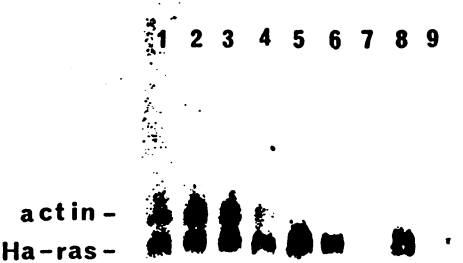

Figure 5:
Northern Blot for the ras gene of various transformed and tumor cell lines

1 T 2760 0.25 Gy α-rays
2 T 2653 1.5 Gy α-rays
3 T 2655 spontaneous
4 T 2718 7.5 Gy x-rays
5 T 2662 7.5 Gy γ-rays
6 T 2643 5µg/ml iso-IQ
7 A37 Ko spontaneous
8 A37IIc3 7.5 Gy γ-rays
9 A37Ic1 7.5 Gy x-rays