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### Publication Date

1990-10-01



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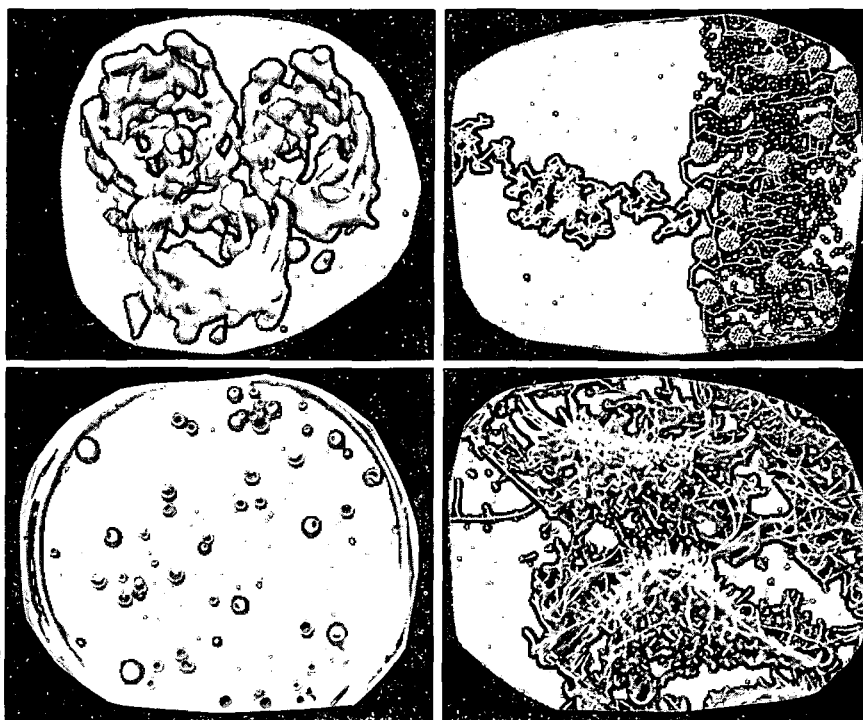
## CELL & MOLECULAR BIOLOGY DIVISION

Presented at the Committee on Space Research  
 XXVIII Plenary Meeting, The Hague, the Netherlands,  
 June 25–July 6, 1990, and to be published in the Proceedings

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November 1990



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This work was supported by the Director, Office of Energy Research, Office of Health and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

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# GENETIC CHANGES IN MAMMALIAN CELLS TRANSFORMED BY HELIUM IONS

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## ABSTRACT

Midterm Syrian Hamster embryo (SHE) cells were employed to study high LET-radiation induced tumorigenesis. Normal SHE cells (secondary passage) were irradiated with accelerated helium ions at an incident energy of 22 MeV/u (9-10 keV/ $\mu$ m). Transformed clones were isolated after growth in soft agar of cells obtained from the foci of the initial monolayer plated postirradiation. To study the progression process of malignant transformation, the transformed clones were followed by monolayer subculturing for prolonged periods of time. Subsequently, neoplasia tests in nude mice were done. In this work, however, we have focused on karyotypic changes in the banding patterns of the chromosomes during the early part of the progressive process of cell transformation for helium ion-induced transformed cells.

## INTRODUCTION

Specific chromosome abnormalities are often associated with specific types of cancers /1,2/. In many cases suppressor genes and/or protooncogenes have been implicated in chromosomal (or DNA) rearrangements or deletions /3/. Although various characteristic genetic changes often appear linked to certain cancers in human and experimental animals /4/, it is not known whether different genes may be involved at different stages of the multistep process towards neoplasia or whether tumorigenicity depends on altered gene function of one or more genes /5/. The present work was initiated to determine if chromosomal (or DNA) changes are non-random in the development of malignant radiogenic transformation *in vitro*. Few studies of this kind exist, but radiation-induced mutation in the *Ki-ras* protooncogene in mouse lymphomas /6/ and activation of *c-myc* and *c-ras* in rat skin carcinomas have been reported /7/. Data from Borek *et al.* /8/ indicate that the same (or distinctive) transforming genes operate in five independently transformed clones derived from X-irradiated SHE cells. This information, however, pertains to the fully tumorigenic transformed cell. Before *in vitro* morphologically transformed cells become tumorigenic, several, if not many, population doublings must have occurred. Our investigation is particularly concerned with genetic changes occurring during this progression stage.

High LET radiation induces mutations and transformation in mammalian cells more efficiently per unit dose than low LET radiation does /9/. In the case of cell transformation *in vitro*, one explanation may be that the initial lesions such as complicated DNA double-strand breaks (DSBs), possibly arising from clustering of strand scissions, produce severely damaged regions in DNA effecting genetic alterations, whereas for low LET radiation the same endpoint may perhaps only be reached after an accumulation of lesser genetic modifications, gradually accumulated as a consequence of the radiation. High LET radiation, such as alpha particles, induces DSBs efficiently with an RBE (relative biological effectiveness) of 4 or more at optimum LET of 100-200 keV/ $\mu$ m /10,11/. The induction of certain chromosome aberrations also follows this relationship /9/. It is therefore rational to expect that induction of genetic lesions in transformed cells capable of becoming malignant may also show maximum RBE at 100-200 keV/ $\mu$ m as indicated by Yang *et al.* /12/.

## MATERIALS AND METHODS

### Primary Syrian Hamster Embryo (SHE) Cells

A frozen stock of primary cells from a 14 day old Syrian hamster fetus was preserved in liquid nitrogen. Thawed cells were used in secondary passage for the irradiations and in secondary or tertiary passages for

karyotyping of control, unirradiated cells. A normal female karyotype of  $2N = 44$  was characterized. Cells were grown in Falcon plastic flasks or dishes in McCoy's medium (Gibco-BRL) supplemented with 10% fetal calf serum (FCS) from HyClone Laboratories. Incubations were done at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  humidified atmosphere. Normal cells began to show senescence after a number of passages and were unable to form colonies in soft agar as also reported previously by Borek and Sachs /13/ for SHE cells. Whereas rodent fibroblasts may be transformed relatively easily by an acute dose of ionizing radiation, this has not been possible in the case of many other mammalian cell types or normal human fibroblasts, although cell immortality has been achieved by repeated treatment with X-rays /14/.

### Irradiation Conditions

Secondary passage cells were plated into  $25\text{ cm}^2$  culture flasks and irradiated well before confluence. Immediately prior to irradiation, the flask was filled with complete culture medium and irradiated in vertical position with a 22 MeV/u incident energy helium ion beam at 0.5 cm residual range from the Bragg peak. The charged particle beam entered the biological sample through the bottom of the flask into the cells. The LET at this position was  $10\text{ keV}/\mu\text{m}$ . Total doses of either 2.5 or 3.5 Gy were used. Low LET irradiations were done for comparison where samples were irradiated with 6 Gy of 230 kVp X-rays. For both cases 10 to 20 percent surviving fractions were achieved. After irradiation, cells were incubated at  $37^{\circ}\text{C}$  in fresh culture medium for *circa* 48 hours after which time, the samples were trypsinized and plated into 100 mm diameter dishes for focus formation at densities varying from 0.15 to  $1.25 \times 10^3$  cells/cm<sup>2</sup>.

### Isolation of Morphologically Transformed Cells

Opaque foci were clearly visible after 2 to 4 weeks growth. Selected foci were picked from the 100 mm diameter dish by localized trypsinization and were expanded into mass cultures following a protocol developed by us /15/. The morphology was not the same in these different foci and much variation in phenotype stability and growth rate was observed. Determination of population doublings (PDN) is complex and somewhat elusive during the early period of the step-wise progression process of transformation as discussed /16/. We have recorded cell growth in passage numbers (passage at confluency) during expansion into mass culture of a recovered focus. The actual transformed genotype comes, however, from recovery of a single colony after growth in agar of single cells from the mass culture. Noble agar (Gibco Laboratories) of 0.3% in complete McCoy's medium was used. Population doubling numbers are difficult to measure during foci formation (time span B in Fig. 1) and also just after isolation of the colony from agar for growth in monolayer. During the early progression phase which is of special interest to us in this communication, cell growth as passage numbers (passage at confluency) was recorded. The actual PDN may be obtained from counts of the number of cells plated at each passage and the number of cells recovered at confluency, but accurate counts of viable cells are difficult to obtain from a colony removed from soft agar and from populations consisting of a low number of cells.

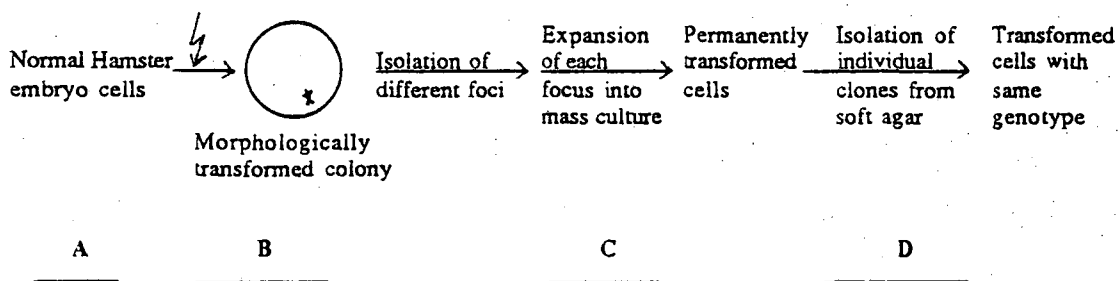


Fig. 1. Procedure for the isolation of a culture of transformed cells of same genotype.

## Metaphase Chromosome Spreads and G-Banding

Exponentially growing cell cultures were treated with Colcemid (0.1 or 0.4  $\mu\text{g/ml}$ ) for 2 hours. Mitotic cells were collected preferentially by centrifugation of cells released from the monolayer by partial trypsinization. Following hypotonic treatment (0.075 M KCl at room temperature for 20 to 30 min.) and fixation in methanol:acetic acid (3:1) /17/, spreads were made on cold, wet slides (Clay Adams, Gold Seal) and air dried. The number of bands varies greatly according to the stages of the cell division /18/. For G-banding, late prophase chromosomes give the highest resolution with 2-3 times more bands than are visible in mid-metaphase /18,19/. Our spreads contained a mixture of cell division stages including late prophase. Several modifications of the basic technique for preparations of chromosome spreads exist /19/ which will favor different characteristics including an increase in late prophase cells and preparations suitable for high quality banding.

G-banded chromosomes were made after the slides were kept at 40°C for 4-6 days by the following method: pancreatin treatment (0.1%) for 10-40 seconds, two 1 min washes in 2% methanol and 3-5 min staining in 5% Giemsa (Gurr). All solutions were made in 0.06 M phosphate buffer at pH 6.8. Stained chromosomes were photographed using either Kodak Technical Pan or Kodak TMX-100 films.

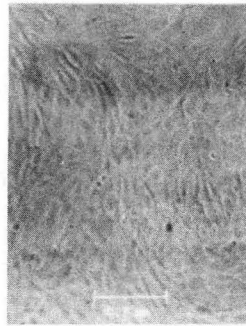
## RESULTS AND DISCUSSION

### Changes in Growth and Morphology of Transformed Cells

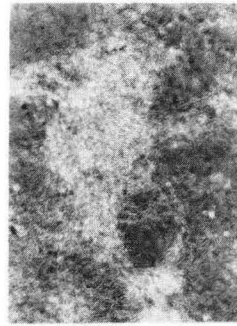
Our procedural steps are shown schematically in Fig. 1. Irradiated cells were plated in 100 mm diameter dishes for foci isolation. Initially nontransformed, surviving cells formed a monolayer which deteriorated due to senescence after a few weeks. Transformed cells proliferated and piled up in loci. Different growth rates and morphologies were evident. Loci were removed from the dish after two to four weeks and plated for further growth in 35 mm diameter or smaller dishes. Tests were made of these cells for ability to grow in 0.3% agar. Generally, cells from the early passages during this time space (marked C in Fig. 1) did not grow in agar. Subsequently it was possible to isolate clones from the agar dishes. At this point (time span D in Fig. 1), cells proliferated easily. Both morphological and karyotypic changes continued to go on during further subculturing. Not all foci which were picked from the 100 mm dish after growth span B in Fig. 1 consisted of cells capable of continued proliferation. Work is in progress to maximize recovery of the "transformed" cells from these foci /15/. It is general practice to score piled up fusiform cells as transformed, but cells from different foci show very different growth characteristics. Our aim was to examine chromosomal changes as early as possible after isolation of a transformed phenotype and also to follow this karyotype during progression toward malignancy. Quite possibly genotypic alterations occur during timespan B shown in Fig. 1, but we were unable to examine these heterogeneously mixed cell cultures for characteristic chromosome abnormalities. The earliest time when karyotypic studies may be done with some degree of confidence is during time span C shown in Fig. 1. Unfortunately, subpopulations may exist primarily due to cell diversification existing in a single focus. Spontaneous transformation of SHE cells is very rare and would mainly occur after many PDNs at very low frequency/16,20/.

Fig. 2 shows a typical focus of piled-up transformed cells induced by helium ion irradiation. The left panel represents normal nonirradiated SHE cells at secondary passage. The photomicrograph to the right represents a focus at the boundary between the transformed cell population and the monolayer of the surviving irradiated cells. After transfer of the focus to a culture dish for monolayer growth and expansion into mass cultures (time span C in Fig. 1), records of population doublings were kept from this time. In our hands colony forming ability in soft agar of these cells did not occur during the early PDN periods. Independently isolated transformed cells were derived from isolated clones growing in soft agar; however in this communication we have concentrated on comparisons of cell populations in the very early progression period (time span C) in the transformation process; i.e., before successful isolation of a single clone from a culture growing in agar.

TRANSFORMATION OF SHE CELLS BY 22 MEV/U HELIUM IONS



UNIRRADIATED CULTURE



TRANSFORMED CELLS  
AT PERIPHERY OF FOCUS

Fig. 2. Normal and transformed SHE cells growing in plastic dishes. The left panel shows a secondary culture of normal cells. A focus of helium ion transformed cells growing in a piled-up manner is shown to the right.

XBB 906-4644A

Detection of Karyotypic Abnormalities in Cells Transformed by Helium Ions

In Fig. 3, the photomicrograph depicts an abnormal karyotype of metaphase chromosomes from helium ion induced transformed SHE cells. The banding pattern is for Giemsa-stained pancretin treated spreads. Criteria used in identifications of the chromosomes were as described /21/ as well as from ideograms supplied by Dr. Suzuki, Yokohama City University, Japan. The arrow points to monosomic X. Normal karyotype is XX.

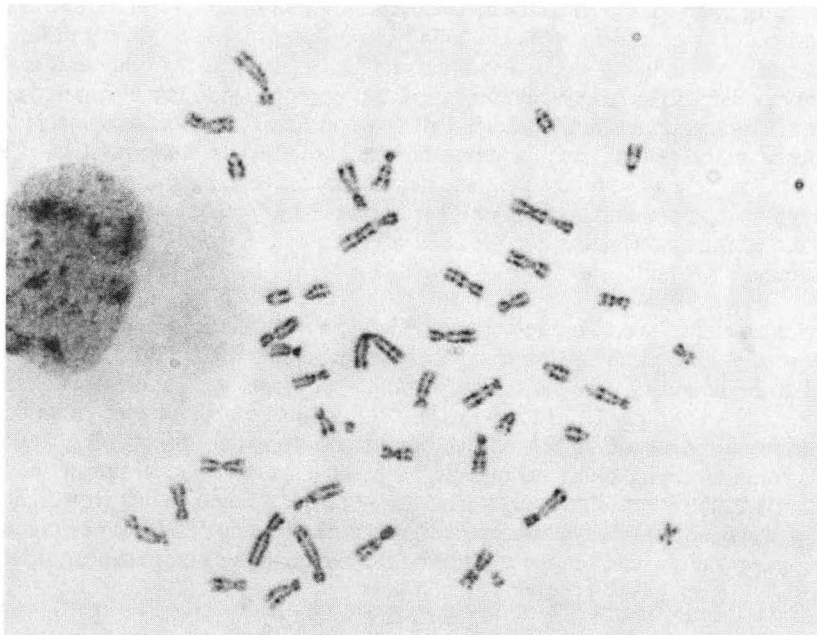


Fig. 3. Giemsa-banded metaphase chromosome spread obtained from helium ion transformed cells at very low passage numbers (PDN of 5 to 12).

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A comparison of karyotypes from the helium-ion transformed cells during early progression phase gave the following results: (1) Minor changes in chromosome morphology or G-banding were found generally. (2) Karyotypes with missing homologs were found occasionally. (3) Hardly ever were complex rearrangements or unidentified chromosomes or extra chromosomes such as trisomy detected. Briefly, minor structural changes rather than numerical irregularities were observed. At this point in the progression process, growth in soft agar was not possible. We intend to follow the transformation process during later phases where one may expect more severe chromosome abnormalities to show up. It will be of interest to establish the karyotypic abnormalities at the points where growth in soft agar occurs and where tumorigenesis becomes possible. It will be important to extend these initial studies (LET = 10 keV/ $\mu\text{m}$ ) to higher LETs.

#### Comparison of Chromosomal Abnormalities in Syrian Hamster Cells Transformed by Various Agents

We have been able to examine karyotypes from various later stages in the multistep progression process for X-ray-induced SHE transformed cells. In these cases it was found that: (1) the karyotypes became progressively more abnormal as the PDNs increased; (2) translocations, minute ring chromosomes (chromosome markers) and extra chromosomes, often of abnormal morphology were detected; but (3) a non-random pattern was not detected. Invariably, extra chromosomal material appeared as the cells became tumorigenic.

In Fig. 4 we have illustrated a few of these abnormalities. In particular we have detected abnormality in chromosome number 11 (t[11;X]) and number 9 trisomy. Other abnormalities were additional unidentified chromosomes and the presence of minute ring chromosomes. Monosomy was detected of various chromosome numbers. The labeled arrows in the figure point to the Xq-translocation, to one abnormal chromosome 11 and to one normal chromosome 11. The three unmarked arrows point to 9 trisomy. The two marker chromosomes are labeled M1 and M2. Abnormalities in chromosomes 9 and 11 have been reported by other researchers /24,25/ for photon-induced cell transformation in SHE cells. The X-ray-induced transformed cells formed tumors in nude mice 10-12 weeks post inoculation of  $10^6$  cells.

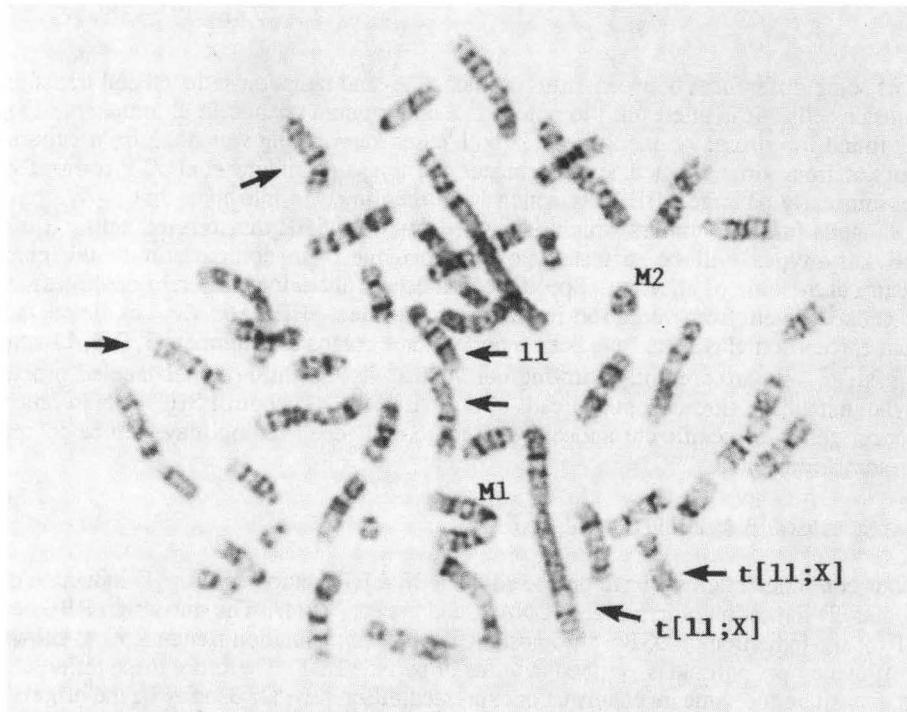


Fig. 4. Illustration of chromosomal rearrangements detected in X-ray induced SHE transformed cells before tumorigenicity. Arrows point to various abnormalities as discussed in the text.

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Figure 5 illustrates the gradual morphological changes taking place in radiation-induced transformed cells. Competence for clonal growth also changes dramatically as the cells become tumorigenic.

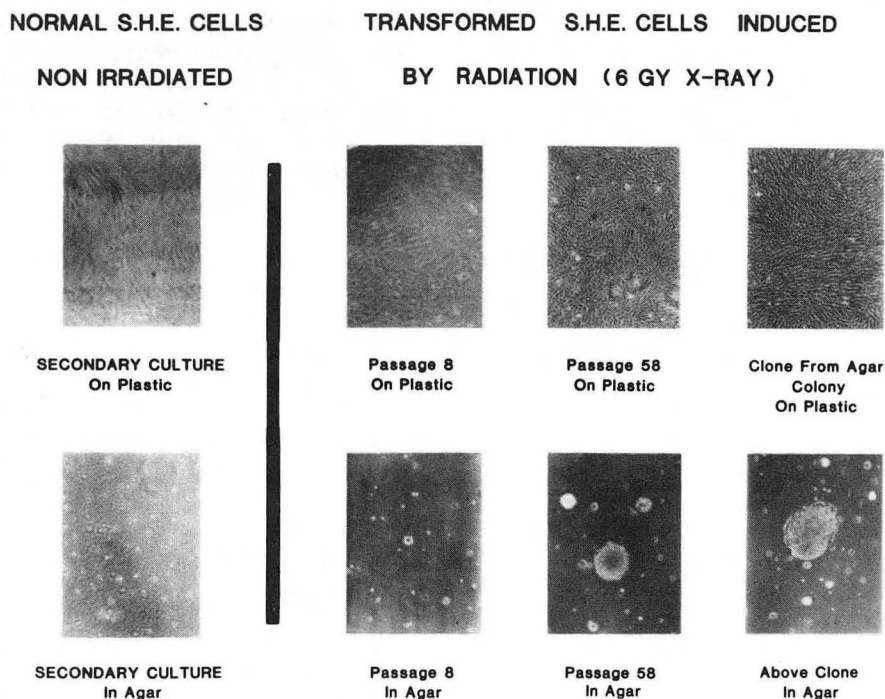


Fig. 5. Photographs showing the gradual changes in the morphology as well as the clonogenic potential of cells isolated from one focus derived from X-irradiated SHE cells.

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Table 1 lists some differences between viral- or oncogene- and radiation-induced cell transformation in Syrian hamster cells. It is interesting to note that a non-random change in chromosome 15 to monosomic was found for viruses or oncogenes. In both cases karyotyping was done from cultures derived from tumors or from virus infected Syrian hamster cell lines. Oshimura et al. /23/ cotransfected *c-ras* and *c-myc* into early passage SHE cells which were then injected into nude mice. We have not yet analyzed the cells from the tumors which developed from our SHE transformed cells. It is likely the tumor cell karyotypes will be different post tumorigenesis in comparison to the chromosome characteristics at the time of injection. Specific chromosome alterations (3, or 3q trisomies) were found in tumor cells derived from radiation-induced transformed SHE cells /24/ as listed in Table 1. Nonrandom chromosomal defects have been reported for chromosome numbers 3, 7, 9, 11 and 20 in the SHE cell system. We are presently carrying out *in situ* hybridization of  $^3\text{H}$ -labeled probes (cancer genes) to the metaphase chromosome spreads of normal ( $2N = 44$ ) control SHE cells in order to locate various cancer genes to specific chromosomes. The normal gene location may then be compared to its location in transformed cells.

#### Critical Lesions in DNA as a Function of LET

In a previous communication we have discussed the RBE-LET relationship for (1) induction of DSBs in cell DNA and (2) formation of irreparable DNA strand breaks /10,11/. The maximum RBE occurs at the same LET for the induction of DSBs, chromosome damage and mutation frequencies as shown by Kraft /9/ when efficiency per particle is graphed in terms of particle LET. Therefore these different biological endpoints may share the same mechanisms or expressed differently, DSBs may be the original cause for the formation of these lesions. The data in Table 2 show this RBE-LET relationship for induction of DSBs by alpha particles in cell DNA /11/. An RBE of 2 to 3 has been reported /26/ for helium ion induced transformation of SHE cells at 36 and 77 keV/ $\mu\text{m}$ , respectively. It would seem logical to expect that the unrepaired lesions and grossly misrepaired lesions may be the damages leading to cell killing;

but it may be expected that much more subtle genetic changes may be observable in viable but transformed cells. We have stressed that many PDNs pass before malignancy becomes detectable. Unfortunately the transformed cells cannot be examined during some of the early PDN-periods as illustrated in Fig. 1. Our data to date indicate that initially structural changes rather than numerical changes occur in the karyotypes of transformed cells. As the transformed cells acquire further chromosomal abnormalities, the chromosome number increases numerically. It becomes important to investigate in a systematic manner whether there is a similar RBE-LET relationship for karyotypic abnormalities after cell transformation as has been shown for the induction of DSBs. In this report we have data for helium ion irradiated SHE cells at an LET of 10 keV/ $\mu\text{m}$ . We hope to continue these studies with helium ion irradiations at comparable LET values as shown in Table 2.

Table 1. Chromosomal Abnormalities in Transformed Syrian Hamster Cells

Agent	Cells	Abnormalities		References
		Nonrandom	Random	
Viruses	Tumor derived cultures or cell lines	15 Monosomy		Pathak <i>et al.</i> , 1981
Oncogenes	Cells from tumors derived from transfected SHE cells	15 Monosomy	Monosomies; trisomies; extra abnormal or unidentified deletions	Oshimura <i>et al.</i> , 1985
Ionizing Radiation	Transformed SHE cells	3, 11 Trisomy		Watanabe <i>et al.</i> , 1990
	Tumors derived from above cells	3, or 3q <sup>-</sup> Trisomy		
	Transformed SHE cells	7, 20 Trisomy		Suzuki <i>et al.</i> , 1989
	Tumors derived from above cells	7, 9 Trisomy		
	Transformed SHE cells	Xq translocation; monosomies; trisomies; extra chromosomes; minute ring chromosome		This report

Table 2. RBEs for the Induction of DSBs in Yeast and Mammalian Cells by Alpha Particles

Cell type	Energy (MeV)	LET (keV/ $\mu\text{m}$ )	Relative Effectiveness (RBE)
Yeast (wild type)	3.5	65	2.6 <sup>a</sup>
Chinese hamster	-	50	1.8 <sup>b</sup>
	-	100	3.3
	-	160	4.4
Ehrlich	3.4	65	1.6 <sup>c</sup>

The initial yields of DNA DSBs were calculated from neutral sucrose gradient sedimentation profiles after alpha particle irradiation.

<sup>a</sup>Relative to 30 MeV electrons [Frankenberg *et al.*, *Radiat. Res.* 88, 524 (1981)].

<sup>b</sup>Relative to <sup>60</sup>Co  $\gamma$ -rays [Kampf and Eichhorn, *Studia Biophysica* 93, 17 (1983)].

<sup>c</sup>Relative to 140 kVp X-rays [Blöcher, *Int. J. Radiat. Biol.* 54, 761 (1988)].

## ACKNOWLEDGMENTS

These studies were supported by the Istituto Nazionale di Fisica Nucleare and by the U.S. Department of Energy (contract DE-AC03-76SF00098). We also wish to express our sincere thanks for excellent help by Ms. L. Craise, Ms. R. Lara, and Ms. L. Lopez.

## REFERENCES

1. A.G. Knudson, Genetic of human cancer, *Ann. Rev. Genet.* 20, 231 (1986).
2. J.J. Yunis, The chromosomal basis of human neoplasia, *Science* 221, 227 (1983).
3. R. Sager, Tumor suppressor genes: the puzzle and the promise, *Science* 246, 1906 (1989).
4. D. Forman and J. Rowley, Chromosomes and cancer, *Nature* 300, 403 (1982).
5. D.G. Thomassen, T.M. Gilmer, L.A. Annal and J.C. Barrett, Evidence for multiple steps in neoplastic transformation of normal and preneoplastic Syrian hamster embryo cells following transfection with Harvey murine sarcoma virus oncogene (v-Ha-ras), *Cancer Res.* 95, 726 (1985).
6. I. Guerrero, P. Calzada, A. Mayer and A. Pellicer, Activation of c-K-ras oncogene by somatic mutation in mouse lymphomas induced by gamma radiation, *Science* 225, 1159 (1989).
7. M.J. Sawey, A.T. Hood, F.J. Burns and S.J. Garte, Activation of c-myc and c-K-ras oncogenes in primary rat tumors induced by ionizing radiation, *Mol. Cell. Biol.* 7, 932 (1978).
8. C. Borek, A. Ong and H. Mason, Distinctive transforming genes in X-ray-transformed mammalian cells, *Proc. Natl. Acad. Sci. USA* 84, 794 (1987).
9. G. Kraft, Radiobiological effects of very heavy ions: inactivation, induction of chromosome aberrations and strand breaks, *Nucl. Sci. Appl.* 3, 1 (1987).
10. R. Roots, W. Holley, E. Rachal and G. Kraft, The influence of radiation quality on the formation of DNA breaks, *Adv. Space Res.*, 9, #10, 45 (1989).
11. R. Roots, W. Holley, A. Chatterjee, M. Irizarry and G. Kraft, The formation of strand breaks in DNA after high-LET irradiation: a comparison of data from *in vitro* and cellular systems, *Int. J. Radiat. Biol.* in press (1990).
12. T.C. Yang, L.M. Craise, M.T. Mei and C.A. Tobias, Neoplastic cell transformation by heavy charged particles, *Radiat. Res.* 106, S-177 (1985).
13. C. Borek and L. Sachs, *In vitro* cell transformation by X-irradiation, *Nature* 210, 276 (1966).
14. M. Namba, Neoplastic transformation of human diploid fibroblasts (KMST-6) by treatment with Co-60 gamma rays, in: *Carcinogenesis*, vol. 9, eds. J.C. Barrett and R.W. Tennant, Raven Press, New York 1985, p. 217.
15. R. Roots, M. Durante, G. Grossi and A. Chatterjee, Isolation and propagation of mammalian cells from *foci* of radiogenically transformed cells, in preparation.
16. J.C. Barrett and P.O.P. Ts'O, Evidence for the progressive nature of neoplastic transformation *in vitro*, *Proc. Natl. Acad. Sci. USA* 75, 3741 (1978).
17. V.G. Dev and R. Tantravahi, Techniques for chromosome analysis, in: *Techniques in somatic cell genetics*, ed. J.W. Shay, Plenum Press, New York and London 1982, p. 939.

18. J.J. Yunis, J.R. Sawyer and D.W. Ball, The characterization of high- resolution G-banded chromosomes of man, *Chromosoma* 67, 293 (1978).
19. S. Li, S. Pathak and T.C. Hsu, High resolution G-banding patterns of Syrian Hamster chromosomes, *Cytogenet. Cell Genet.* 33, 295 (1982).
20. C. Borek and E.J. Hall, Transformation of mammalian cells *in vitro*, by low doses of X-rays, *Nature* 243, 450 (1973).
21. R.A. Pavia, L.W. Smith and D.M. Goldenberg, An analysis of the G-banded chromosomes of the golden hamster, *Int. J. Cancer* 20, 960 (1977).
22. S. Pathak, T.C. Hsu, J.J. Trentin, J.S. Butel and B. Panigraphy, Nonrandom chromosome abnormalities in transformed Syrian hamster cell lines, in: *Genes, chromosomes, and neoplasia*, ed. F.E. Arrighi, S.V. Rao and E. Stubblefield, Raven Press, New York 1981, p. 405.
23. M. Oshimura, T.M. Gilmer and J.C. Barrett, Nonrandom loss of chromosome 15 in Syrian hamster tumours induced by *v-Ha-ras* plus *v-myc* oncogenes, *Nature* 316, 636 (1985).
24. M. Watanabe, K. Suzuki and S. Kodama, Karyotypic changes with neoplastic conversion in morphologically transformed golden hamster embryo cells induced by X-rays, *Cancer Res.* 50, 760 (1990).
25. K. Suzuki, F. Suzuki, M. Watanabe, and O. Nikaido, Multistep nature of X-ray neoplastic transformation in Golden hamster embryo cells: Expression of transformed phenotypes and stepwise changes in karyotypes, *Cancer Res.* 49, 2134 (1989).
26. M. Suzuki, M. Watanabe, K. Suzuki, K. Nakano, and I. Kaneko, Neoplastic cell transformation by heavy ions, *Radiat. Res.* 120, 468 (1989).

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