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MORPHOLOGICAL TRANSFORMATION INDUCED BY X-RAYS IN SYRIAN／GOLDEN HAMSTER EMBRYO CELLS

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Summary

We investigated the induction of morphological transformation in Syrian／golden hamster embryo cells irradiated with X-rays. While the frequency of morphological transformation increased steeply at lower dosey (0-2 Gy), its increment became smaller with doses above 2 Gy. Compared with morphological transformation, the expression of mutation required an expression time more than 5 days and the induction curves for both phenotypes were different.

A large fraction of morphologically transformed colonies (about 80 %) could be cloned with the use of feeder layer cells. Only the progeny of these clones expressed malignant phenotypes, such as an anchorage-independence and tumorigenicity under the skin of nude mice.

These results suggested that different mechanisms might be responsible for the induction of morphological transformation and mutation and that morphologically transformed cells suspected to have the predisposition to malignant transformation.

Introduction

In vitro transformation system has been used to identify the potential carcinogens and to clarify the mechanisms of neoplastic transformation[1-3]. Especially, Syrian／golden hamster embryo (SHE) cells have widely used because they are primary cells and have diploid karyotype[1, 4-9]. Originally, the usage of SHE cells was reported by Berwald and Sachs[1]. Later, Dipaolo *et al*[4] and Pienta *et al*[5] qualified and standarized this as *in vitro* transformation system. Furthermore, recent study revealed that non-mutagenic drugs, such as diethylstilbestrol[10], asbestos[11] and bisulfite[12], caused morphological transformation of SHE cells. These results proved that SHE cells could efficiently detect the carcinogenic potentials of a variety of drugs including DNA damaging agent and non-mutagenic agent[13, 14].

Although the morphologicak transformation of SHE cells is suitable for an identification of environmental carcinogen, the biological significance has not be fully understood yet. Therefore, our present study designed to compare the expression dynamics of morphological transformation and gene mutation, and also to determine the position of morphological transformation during the multistep process of neoplastic transformation *in vitro*.

Materials and Methods

Cell cultures

Primary SHE cells were obtained by trypsinization of 12-14-day-old embryos as reported previously[8, 15]. Cells were cultured in Dulbecco's modified Eagle's minimum essential medium (Nissui Seiyaku Co., Tokyo) supplemented with 10 % fetal bovine serum (M. A. Bioproducts, MD), and subcultured every 3 days as described previously[8, 15].

Assay for morphological transformation

The procedure for morphological transformation was reported elsewhere[15]. Briefly, 48 hr after subculture from either passage 1 or 2, feeder layer cells were irradiated with 50 Gy and seeded into 60-mm plastic dishes at 500 cells per cm^2. After 24 hr, 200 target cells were irradiated with various doses of X-rays and seeded into the dishes. Cultures were incubated for 7-10 days in

a CO_2 incubator at 37 ℃. Two kinds of colonies, designated as type A and type B colony, were identified as morphlogically transformed colonies.

Assay for Mutation

As described elsewhere[8], sufficient numbers of target cells were irradiated to insure at least 10^6 survivors. The cells were grown for various periods up to 14 days after X-irradiation, resuspended into a medium containing 40 μM of 6-thioguanine (6 TG) or 8-azaguanine (8 AG) and plated onto plastic dishes at 2.5×10^3 cells/cm^2. The cells were incubated in a CO_2 incubator for 16 - 20 days.

Assay for anchorage-independence

The procedure for anchorage-independent assay was reported previously[16]. Briefly, the cells suspended in 1 ml of DMEM containing 0.33 % Noble agar (Difco, Detroit, MI) and supplemented with 20 % fetal bovine serum, were plated into dishes that contained 5 ml of freshly-solidified bottom agar (made with the same culture medium, except containing 1 % Noble agar.) The cultures were incubated in a CO_2 incubator for 2 weeks and colonies with a diameter equal to or greater than 100 μm.

Results and Discussion

The dose response curves for cell survival, mutation and morphological transformation are shown in Figure 1. While the mutation frequency increased exponentially with doses above 2 Gy, transformation frequency exhibited a steep increase at doses from 0 to 2 Gy. The expression dynamics of mutation and morphological transformation during the expression time after X-irradiation are presented in Table 1 and Table 2. For all doses, maximum induction of mutant cells was obtained after an expression time of 8 days. In contrast, morphological transformation occurred at highest frequency immediatedly after irradiation and its frequency adruptly decreased during further expression time.

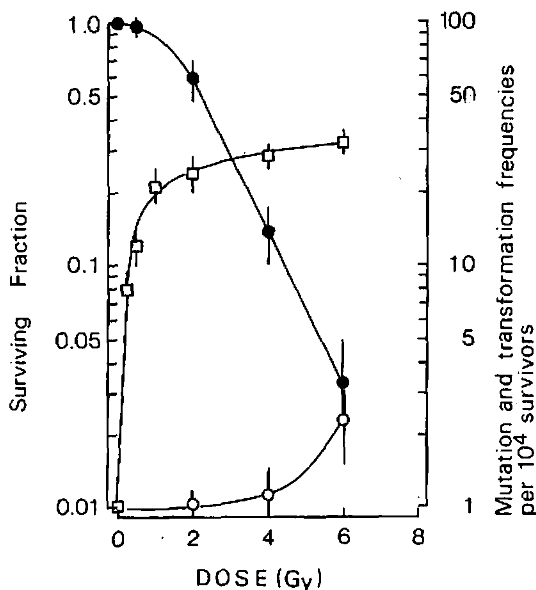

Fig. 1 . Survival and frequencies of morphological transformation and mutation. ● ; survival, □ ; transformation, ○ ;mutation.

Table 1 Comparison of the mutation frequency at each passage after X-irradiation.

Passage	Mutation frequency ($\times 10^{-5}$) X-ray dose 0 Gy	1 Gy	2 Gy	4 Gy
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)[a]
1	0.0 (1.8)	0.0 (2.5)	0.0 (1.5)	0.9 (1.4)
2	0.0 (4.6)	0.3 (4.6)	0.7 (4.0)	2.4 (3.3)
3	0.0 (7.2)	0.3 (6.9)	0.5 (5.2)	0.9 (5.2)
4	0.0 (9.3)	0.3 (9.2)	0.4 (8.5)	0.9 (7.8)

[a]Numbers in parenthesis show the number of cell doublings.

Table 2 Comparison of the transformation frequency at each passage after X-irradiation.

Passage	Transformation frequency (x 10^{-3})			
	X-ray dose			
	0 Gy	1 Gy	2 Gy	4 Gy
0	0.0 (0.0)	2.1 (0.0)	2.7 (0.0)	3.0 (0.0)[a]
1	0.0 (2.3)	1.3 (2.2)	1.8 (2.0)	2.3 (2.0)
2	0.0 (4.5)	0.0 (4.2)	0.9 (4.3)	1.5 (4.0)
4	0.0 (9.3)	0.0 (9.2)	0.0 (8.5)	0.0 (7.8)

[a]Numbers in parenthesis show the number of cell doublings.

These results demonstrated that the expression dynamics of mutaion and morphological transformation were completely different[8]. Since morphological transformation induced efficiently with doses corresponding to the shoulder region than that to the exponential region of the survival curve, repair processes which might be responsible for the shoulder would be associated with the steep induction of morphological transformation. On the other hand, the induction of mutation increased exponentially with doses corresponding to the exponential region of the survival curve. Furthermore, the maximum mutation frequency was obtained by 3 to 4 cell population doublings after X-irradiation, while morphological transformation required no cell population doubling. Therefore, it was predicted that genetic change, other than single gene mutation, might be responsible for the induction of morphological transformation. Recently, we found that a common chromosome change occurred in transformed cells[17-19] and these, so called chromosome mutation, supposed to be one of the cellular changes associated with morphological transformation[19].

In order to determine the significance of morphological tranformation during the malignant progression of X-irradiated cells, we tried to picked up morphologically transformed colonies. More than 80% of morphologically transformed colony could survive with feeder layer cells, while most colony was senescent without feeder layer cells. We isolated ten morphologically transformed clones and found that all clones could grow in soft agar medium and aquired tumorigenicity under the skin of nude mice. Although the latent period for tumor growth varied between clones, normal cells did not give rise to tumors in any circumstances.

Since the morphological alteration was the first noticeable change, the examination of malignant phenotypes in morphologically altered cells was required for evaluation of the usage of morphological transformation as an reliable marker for identification of potential carcinogens. One of our findings was a requirment of feeder layer cells to isolated morphologically transformed cells. It was suggested that the medium is nutritionally inadequate for the growth of these cells as low density. The other finding was that all morphogically transformed clones expressed anchorage-independence and tumorigenicity. Although stepwise changes were required for the malignant progression of morphologically transformed cells[19], only the progeny of these clones found to give rise to these malignant cells. Therefore, morphological transformation was merely first observable phenotype, but it was determined to be closely related to the malignant transformation thereafter.

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