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ASSOCIATION BETWEEN THE CYTOTOXICITY OF THYMIDINE AND TUMORIGENICITY OF CLONES DERIVED FROM C3H/10T1/2 MOUSE EMBRYO FIBROBLASTS

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SUMMARY

We have measured the cytotoxicity of thymidine to C3H/10T1/2 mouse embryo fibroblasts derived from morphologically transformed foci of cells from cultures exposed to chemical carcinogens. Four of these cell lines have previously been shown to be tumorigenic in irradiated syngeneic hosts and were all more sensitive to the lethal effects of thymidine than were the non-transformed cells. Strikingly, the most tumorigenic of the cell lines were most sensitive to thymidine. Differences in plating efficiencies or growth rates of the various cell lines were not associated with differences in thymidine sensitivity.

INTRODUCTION

Cells from various human cancers, mouse cells that are oncogenically transformed, and malignant human T cells are abnormally sensitive to the lethal effects of thymidine (1,2,3). In the present study the cytotoxicity of thymidine was tested in the C3H/10T1/2 Cl 8 line of transformable mouse embryo fibroblasts, in three lines of morphologically transformed C3H/10T1/2 Cl 8 cells that were not originally found to be tumorigenic, and in four lines of morphologically transformed C3H/-10T1/2 Cl 8 cells that were originally found to be tumorigenic (4).

METHODS

The transformed C3H/10T1/2 Cl 8 cell lines were cloned from morphologically transformed foci (4) produced by treating the parent cells with 1.0 μg/ml DMBA (DMBA clones) or 1.0 μg/ml MCA (MCA clones). The foci were characterized by three different morphologies: Type I, foci of cells piled up in 2-3 layers without loss of parallel alignment; Type II, foci of cells extensively piled up without loss of parallel alignment; Type III, foci of cells extensively piled up with loss of parallel alignment as judged from the appearance of the edges of the foci (4).

The abbreviations are: DMBA, 7,12-dimethylbenz[a]anthracene; MCA, 3-methylcholanthrene

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For one measurement of toxicity, 200-1,000 cells were seeded in four 60 mm plastic petri dishes (Corning) in 5 ml of Eagles' basal medium supplemented with 10% fetal calf serum (Gibco). One day later, thymidine or deoxycytidine dissolved in 0.1 ml of phosphate buffered saline was added to the medium. After 7-10 days incubation at 37° C, the cells were fixed with methanol, stained with Giemsa, and colonies containing 50 or more cells were counted. Each data point in the figure shows the mean and standard error of 2-4 measurements. Population doubling times were determined by linear regression analysis of cell numbers 24, 48, 72 and 96 hours after plating 1.5×10^4 cells/60 mm dish.

For the original measurements of tumorigenicity, 3-6 week old syngeneic C3H mice, were irradiated with 550 rads of γ radiation and, 24 hours later, were inoculated subcutaneously with $2\text{-}5 \times 10^6$ cells (4). For the present study, syngeneic female mice, 4 weeks old, were whole-body x-irradiated with 550 rads and were inoculated 24 hours later with 10^6 cloned cells under the skin of the breast.

RESULTS

In the original assays of tumorigenicity, with $2\text{-}5 \times 10^6$ cells inoculated per C3H mouse, the latent period for tumor production was 1-20 weeks (4). In the present study, with 1×10^6 cells inoculated per mouse, the shortest latent period was 2 weeks (MCA 16, Table 1), clone MCA 15 gave a latent period of 23 days, and the other clones, including two that were tumorigenic in 1973, gave no tumors in 9 months. These findings suggest that differences between the original and the recent assays may be due to the 2-5 fold differences in the numbers of cells inoculated, or to changes that occurred during cryopreservation.

The shapes of the dose response curves for survival in medium containing thymidine were different for different clones (Fig. 1). For example, the curves for DMBA 2 and C3H/10T1/2 Cl 8 cells were straight lines passing through the origin, the curve for DMBA 3 cells had a shoulder, while that for MCA 16 cells showed an initial steep slope at low concentrations of thymidine and a more shallow but linear decrease in survival at concentrations of thymidine greater than 10^{-5} M. However, the tumorigenic clones were 2.5-8 fold more sensitive to the cytotoxic effects of thymidine than were the nontumorigenic clones (Fig. 1). Fig. 1 also shows that deoxycytidine at concentrations from 100-400 μM completely reversed the highly cytotoxic effects of 200 μM of thymidine in MCA 16 cells.

The concentrations of thymidine (D_o doses) required to reduce the surviving fraction by 1/e in the linear portions of survival curves such as those in Fig. 1 are given in Table 1, together with the original classification of the morphology of the

TABLE 1

TUMORIGENICITY AND SENSITIVITY TO THE CYTOTOXICITY OF THYMIDINE OF C3H/10T1/2 Cl 8 CELLS AND OF MORPHOLOGICALLY TRANSFORMED CLONES DERIVED FROM THEM

Clone	D_o* $Mx10^{-5}$	Plating Efficiency(%)	Focus Morphology Type	Tumorigenicity Assays Original (ref 4)	Present
C3H/10T1/2 Cl 8	32±6.9	27 ± 3.2	-	0/5	0/10
DMBA 2	38±6.8	50 ± 2.5	I	0/2	0/10
DMBA 5	36±3.0	29 ± 6.0	II	0/2	0/10
DMBA 8	30±2.8	21 ± 1.5	III	0/2	0/10
DMBA 4	12±2.8	28 ± 3.0	II	1/2	0/10
DMBA 3	8.9±0.59	54 ± 2.5	III	2/2	0/10
MCA 15	4.7±0.68	15 ± 0	III	2/2	10/10
MCA 16	3.6±0.18	44 ± 4.2	III	2/2	10/10

*Thymidine concentration, see text.

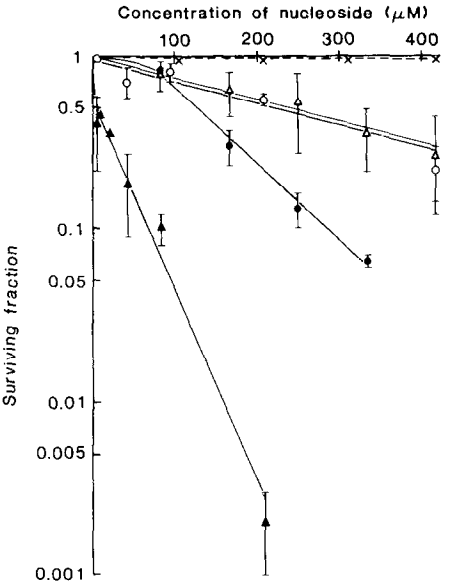

FIGURE 1

The cytotoxicity of thymidine and of thymidine plus deoxycytidine to various clones of C3H/10T1/2 mouse embryo fibroblasts. ▲, clone MCA 16 - a highly tumorigenic cell line; ● , clone DMBA 3 - moderately tumorigenic; △ , clone DMBA 2 - nontumorigenic but morphologically transformed; ○ , C3H/10T1/2 Cl 8 cells - nontumorigenic, non transformed; × clone MCA 16 cells treated with 200 μM thymidine plus 90-440 μM deoxycytidine.

foci from which clones were derived, plating efficiencies in media free of added nucleosides, and the original (4) and recent analyses of the tumorigenicities of 7 cloned lines of chemically transformed C3H/10T1/2 Cl 8 cells. The plating efficiencies did not correlate with thymidine sensitivity or tumorigenicity, and doubling times of the highly tumorigenic and non-tumorigenic cells were not significantly different (16.7 $\pm$ 0.24 hr, MCA 16; 18.3 $\pm$ 2.0 hr, C3H/10T1/2 Cl 8), which is in agreement with previous data (4). Moreover, the morphologies of the transformed foci were not always diagnostic of the tumorigenicities of the clones derived from them. However, all the clones that had been shown to be tumorigenic were at least 2.5 fold more sensitive to thymidine than non-tumorigenic clones, and the clones that were most tumorigenic in the original or the current assays were most sensitive (7-8 fold more sensitive than C3H/10T1/2 Cl 8) to thymidine cytotoxicity (Table 1).

DISCUSSION

The data in Table 1 are consistent with other reports of the selective lethal effect of thymidine on neoplastic cells (1-3). In addition, our experiments indicate a quantitative association between tumorigenicity and sensitivity to thymidine in C3H/10T1/2 cells. Moreover, previous studies of the selective lethal effect of thymidine employed concentrations of thymidine 1-2 orders of magnitude higher than we have used.

Differences in thymidine sensitivity between malignant and non-malignant cells have been attributed to differences in the activities of enzymes of thymidine metabolism (3). On the other hand, we have previously shown that increasing the propensity of Chinese hamster cells to undergo mutation, by treating them with mutagenic alkylating agents, also increases the sensitivity of the cells to thymidine (5). Furthermore, it is considered that highly tumorigenic cells are hypermutable, or have an increased propensity to undergo mutation (6,7). Therefore, to further explore our observation, we are considering the possibility that hypermutable tumorigenic cells could be selectively killed by thymidine nucleotide pool imbalances that further increase the mutability of the cells to a lethal level. We are currently

testing this hypothesis by measuring the mutability of discrete genetic loci in the transformed and non-transformed C3H/10T1/2 Cl 8 cells

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REFERENCES

1. Lee, S.S., Giovanella, B.C., and Stehlin, J.S. Jr. (1977) J. Cell Physiol. 92, 401-405.

2. Lee, S.S., Giovanella, B.C., Stehlin, J.J. Jr. and Brunn, J.C. (1979) Cancer Res. 39, 2928-2933.

3. Carson, D.A., Kaye, J., Mutsumoto, S., Seegmiller, J.E., and Thompson, L. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 2430-2433.

4. Reznikoff, C.A., Bertram, J.S., Brankow, D.W. and Heidelberger, C. (1973) Cancer Res. 33, 3329-3249.

5. Peterson, A.R. and Peterson, H. (1979) Mutation Res. 61, 319-331.

6. Loeb, L.A., Springgate, C.F. and Battula, N. (1974) Cancer Res. 34, 2311-2321.

7. Cairns, J. (1975) Nature 255, 197-200.