Interspecific Complementation between Mouse and Chinese Hamster Cell Mutants Hypersensitive to Ionizing Radiation

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Interspecific and intraspecific hybrids were formed between mouse and Chinese hamster cell mutants hypersensitive to ionizing radiation and their radiosensitivities were examined. Chinese hamster cell mutants irs1, irs2 and irs3 and mouse mammary carcinoma cell mutants SX9 and SX10 have been found to belong to five different complementation groups. A radiosensitive mouse lymphoma cell line L5178Y-S has been demonstrated to be different from the X-ray sensitive mouse cell mutants M10 and LX830, both of which are derived from L5178Y cells, in their complementation groups. L5178Y-S is also distinct from SX9 and SX10.

INTRODUCTION

Nine complementation groups have been reported for Chinese hamster cell mutants hypersensitive to the lethal effect of ionizing radiation¹. We have isolated several ionizing radiation-sensitive mutants from mouse cells²⁴. Complementation tests between mouse cell mutant M10 and Chinese hamster cell mutants irs1, irs2 and irs3 have been performed and it has been found that M10 is different from irs1, irs2 and possibly irs3 in their complementation groups⁵⁶, but the comparison between the mouse mammary carcinoma cell mutants SX9 and SX10, and Chinese hamster cell mutants has not been attempted. A radiation-sensitive mouse lymphoma cell line L5178Y-S has been obtained as a fast-growing variant⁷ and used together with the radiation-resistant strain L5178Y-R in a variety of experiments⁸⁹. We have intentionally selected X-ray-sensitive mutants M10 and LX830 from mutagenized L5178Y cells but
have so far no opportunity to compare these mutants with L517SY-S cells. In this communication we report the results of interspecific complementation tests between radiation-sensitive mouse cell mutants and Chinese hamster cell mutants as well as intraspecific complementation tests between radiation-sensitive mouse cell mutants.

MATERIALS AND METHODS

MEM alpha medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) was used for cell culture. Polyethylene glycol 1500 (PEG1500) and agar Noble were obtained from Boehringer Mannheim, Germany and Difco, Detroit, MI, respectively. Trypsin, ouabain, 6-thioguanine, hypoxanthine, azaserine and thymidine were purchased from Sigma, St Louis, MO.

Cells

Chinese hamster X-ray-sensitive mutants irs1, irs2 and irs3 were generously provided by Dr. J. Thacker. Mouse lymphoma radiosensitive L5178Y-S cells were kindly supplied by Dr. H.H. Evans. Ionizing radiation-sensitive mutants M102 and LX830 were derived from mouse L5178Y cells. SX9 and SX10 were selected from mouse mammary carcinoma FM3A cells for X-ray sensitivity. SR-1 was a derivative of FM3A and used as the wild-type. V79 and L5178Y cells were also used as reference cell lines.

Isolation of drug-resistant cell lines

Spontaneous mutants were obtained by plating cells in 2 mM ouabain and then in 5 μg/ml 6-thioguanine. These drug-resistant mutants had radiosensitivity similar to their drug-sensitive parent lines and were used for hybrid selection.

Cell fusion and hybrid selection

Cells grown in monolayer were detached and dispersed by treatment with trypsin and 10^6 cells of each of two mutants were mixed and centrifuged at 1,000 rpm for 5 min. The pellet was once washed with serum-free medium, spun down and the supernatant was removed by aspiration. The pellet was loosened by brief vibration. To this suspension, 0.5 ml of PEG1500 (50% in 75 mM Hepes buffer) was added. After 1 min at room temperature, 10 ml of serum-free medium was slowly administered. The mixture was left for 10 min and spun down with added serum. The pellet was dispersed, plated in 10-cm dishes and placed in a CO₂ incubator. Selection started on the following day in medium containing HAT/ouabain (50 μM hypoxanthine, 20 μM azaserine, 7.5 μM thymidine, 2 mM ouabain). For hybrid cells which grew in suspension, cells were plated in 0.3% soft agar medium containing 10% fetal bovine serum and HAT/ouabain. We have isolated and examined several hybrid clones and only the typical dose-response curves were presented.
Gamma-ray survival

Radiation-sensitive mutants, wild-type cells and hybrid cells between two mutants were exposed to $^{60}$Co gamma-rays at room temperature at a dose rate of 81 Gy/min and surviving fractions were determined.

RESULTS

Interspecific complementation between mouse and Chinese hamster cell mutants

Chinese hamster cell V79, its X-ray-sensitive mutant irs1, mouse mammary carcinoma cell SR-1, its X-ray-sensitive mutant SX9, and interspecific hybrids irs1/SX9 and irs1/SR-1 were examined for their gamma-ray survivals. As shown in Fig. 1, irs1/SX9 is far more radioreistant than irs1 or SX9 or even SR-1 and closer to irs1/SR-1 and V79. This result that irs1 and SX9 complement each other, indicates that irs1 and SX9 belong to different complementation groups.

Fig. 1. Gamma-ray-survival curves for SX9, SR-1, irs1, V79, and interspecific hybrids irs1/SX9 and irs1/SR-1. Each point represents the average of at least two experiments and the standard error is omitted, since it is not large. This situation holds also true for the experiments shown in the following figures. The survival curves of V79 and SR-1 in Figs. 2–6 are the same as those in Fig. 1.

Fig. 2. Gamma-ray-survival curves for SX10, SR-1, irs1, V79, and interspecific hybrids irs1/SX10 and irs1/SR-1.
Almost full complementation is observed between irs1 and SX10 (Fig. 2), indicating that irs1 and SX10 belong to different complementation groups.

The hybrid between irs2 and SX9 (irs2/SX9) was much more radioresistant than their respective parent mutants and showed a survival curve similar to those of irs2/SR-1, SR-1 and V79 (Fig. 3). This indicates that irs2 and SX9 are distinct in their complementation groups.

In the combination of irs2 and SX10, irs2 complemented SX10 efficiently (Fig. 4), indicating different complementation groups for irs2 and SX10.

In the case of irs3, this mutant is less radiosensitive than irs1 or irs2 and has a survival curve close to SR-1. But the hybrid irs3/SX9 is more radioresistant than irs3 or SX9 and has a gamma-ray survival curve almost identical to that of irs3/SR-1 and close to that of V79 (Fig. 5). This result will be an indication of positive complementation and hence irs3 and SX9 may belong to different complementation groups. Similar situations hold in the combination between irs3 and SX10 (Fig. 6) and based on the considerations described above, irs3 and SX10 may be distinct in their complementation groups.

Taking the findings into account that irs1, irs2 and irs3 belong to different complementation groups\(^1\) and that SX9 and SX10 complement each other\(^4\), the results of the present interspecific complementation tests indicate that ionizing radiation-sensitive mutants irs1, irs2, irs3, SX9 and SX10 represent five different complementation groups.

Fig. 3. Gamma-ray-survival curves for SX9, SR-1, irs2, V79, and interspecific hybrids irs2/SX9 and irs2/SR-1.

Fig. 4. Gamma-ray-survival curves for SX10, SR-1, irs2, V79, and interspecific hybrids irs2/SX10 and irs2/SR-1.
Intraspecific complementation between mouse cell mutants

L5178Y-S (LY-S) cells are hypersensitive to the killing effect of ionizing radiation and used in many respects since its isolation. We have also selected ionizing radiation sensitive mutants M10 and LX830 from mutagenized L5178Y cells. It is interesting to examine whether or not our mutants complement LY-S. The results are shown in Fig. 7, which indicates that LY-S is different from either M10 or LX830 in their complementation groups, since LY-S complement M10 and LX830 sufficiently and the survival curve of L5178Y is similar to that of L5178Y/LY-S.

Complementation tests between LY-S and SX9 or SX10 were also carried out. As shown in Fig. 8, the hybrids are much more radioresistant than their respective parents and close to wild-type cells. Hence LY-S, SX9 and SX10 belong to three different complementation groups.

DISCUSSION

Ionizing radiation-sensitive mutant cell lines of rodent origin have been classified into nine complementation groups, which are represented respectively by group 1(EM9), 2(irs1), 3(irs1SF), 4(XR-1), 5(xrs), 6(irs2), 7(irs3), 8(BLM2), and 9(V-3).
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Fig. 7. Gamma-ray-survival curves for LX830, M10, L5178Y-S(LY-S), and hybrids LX830/LY-S, M10/LY-S and L5178Y/LY-S. The survival curve of L5178Y is similar to that of L5178Y-LY-S (data not shown).

Fig. 8. Gamma-ray-survival curves for SX9, SX10, L5178Y-S(LY-S), and hybrids SX9/LY-S, SX10/LY-S and SR-1/LY-S.

We have shown in this communication that the ionizing radiation-sensitive mouse cell mutants SX9 and SX10 which we have isolated are classified into different complementation groups from those of Chinese hamster cell mutants irs1(group 2), irs2 (group 6) or irs3(group 7), and that the ionizing radiation-sensitive mouse lymphoma cell mutant L5178Y-S (LY-S) belongs to a complementation group different from those of radiosensitive mouse cell mutants M10, LX830, SX9 or SX10. Complementation tests with the mutants of other groups are under way.

It has been reported that M10 is different from EM9(group 1), xrs(group 5), V3(group 9),irs1(group 2) or irs2(group 6) in their complementation groups and that the hybrids of irs3/M10 and XR-1/M10 are more resistant than the component mutant lines but do not achieve wild-type resistance. These findings are reasonably understood since M10 is deficient in DNA double-strand break (dsb) repair, whereas irs1 and irs2 are normal in dsb repair, although irs1 misrejoins double-strand scissions. Since the mutation in M10 cells is complemented by human chromosome 5 and XR-1 corresponds to XRCC4 which is located on human chromosome 5, it is possible that M10 and XR-1 belong to the same complementation group. There are three groups defective in DNA dsb repair, group4 (XR-1), group 5 (xrs6) and group 9 (V-3, scid). The relationship of the mutants with reduced rejoining capability, M10, SX9 and SX10, with the above groups is being studied.
L5178Y-S (LY-S) cell line has been obtained after prolonged cultivation of L5178Y cells in vitro. In contrast, M10 and LX380 mutants have been intentionally selected for their radiosensitivity\(^2,3\). The similarity between LY-S and M10 is that both show longer mitotic lag and higher chromatid aberrations after irradiation compared with their wild-type counterparts\(^8,17\). But they have many contrasting features. LY-S cells have a shorter doubling time in suspension culture and a higher plating efficiency in soft agar than L5178Y-R (LY-R) cells\(^9\), whereas M10 and LX380 cells have longer doubling times and lower plating efficiencies than L5178Y cells. These features tend to be maintained in the hybrid cells but influenced by the partners. LY-S is less mutable at the hypoxanthine/guanine phosphoribosyl transferase (hprt) locus by radiation than LY-R\(^10\), while M10 and LX380 are hypermutable at the hprt locus by radiation in comparison with L5178Y\(^18,19\). However, it should be noted that LY-R is not necessarily the same as L5178Y which we have used as the wild-type. LY-R is X-ray-resistant but hypersensitive to the killing effect of ultraviolet light (UV)\(^9\) but L5178Y is resistant to both X-rays and UV\(^2,20\). LY-S\(^21,22\) as well as M10 and LX380\(^12\) have been demonstrated to be defective in DNA dsb repair. These results indicate that M10 and LY-S represent two steps involved in the repair of DNA double-strand breaks in the mouse L5178Y cell line.

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REFERENCES