

## Evaluation of Adaptive Responses to Cisplatin in Normal and Mutant Cell Lines with Mutations in Recombination Repair Pathways

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**Abstract.** *Cell lines mutant in specific DNA repair pathways were used to determine if these pathways are involved in adaptive responses. For these studies, the effect of deficiencies in homologous recombination repair (HR) were studied in the parental AA8 and mutant  $\text{irs}$ ISF cell line pair and for deficiencies in the nonhomologous endjoining (NHEJ) pathway in the mouse MEF parental and Ku80 mutant cell line pair. The results showed that the XRCC3 mutation in the HR-deficient mutant inhibited adaptive responses to low doses of cisplatin and radiation. The parental lines showed transient adaptive responses to both low-dose cisplatin and radiation treatment. For the mouse MEF and the Ku80 cells, no adaptive responses were observed in either cell line. However, there was an initial transient sensitization response followed by partial recovery. Thus, it appears that the HR repair system may be involved in the adaptive response to cisplatin and radiation. For the NHEJ repair system the question could not be answered since no adaptive responses were evident in the parental line.*

The combination treatment of cisplatin and radiation has been extensively used in cancer treatment (1-8). Studies *in vitro* have shown that cisplatin can be an effective radiation sensitizer (9-12). However, it has also been shown that cells can become resistant to cisplatin treatment and such resistance can affect the degree of radiosensitization. One of the resistance mechanisms is enhanced repair (13-17). Two studies have shown that both the homologous recombination (HR) and the nonhomologous endjoining (NHEJ) systems may be involved in this resistance (18, 19). In addition, it has also been shown that cells can form

transient adaptive responses to various drug treatments, including cisplatin. Activated repair systems have also been implicated (20, 21).

A number of studies have shown that low doses of radiation or low-dose-rate irradiation can cause an adaptive response in mammalian cells (21-34). Such adaptive responses were observed for micronucleus formation and chromosomal aberrations (22, 36), cell survival (32, 34), cell mutation and transformation (29, 36) and in embryogenesis. Our previous results also showed that low doses of radiation result in a decrease in the distance separating homologous chromosomes and may implicate a recombinational repair mechanism (37). This concept is also supported by the observation that adapting doses of radiation can result in reduced micronucleus frequency after irradiation, possibly due to increased repair of chromosomal damage (35, 36).

In order to test whether adaptive responses are affected by recombination repair pathways, we set out to test adaptive responses in cell lines known to have mutations and deficiencies in the recombinational repair pathways. To test the HR repair involvement we used the CHO parental AA8 line and its derivative line  $\text{irs}$ ISF, which is mutant in the XRCC3 gene and is deficient in HR. For the NHEJ repair we used a parental mouse embryo fibroblast line, MEF, and its derivative Ku80 mutant deficient in NHEJ repair.

### Materials and Methods

The cell lines used in this study are described as follows: the mouse embryo fibroblast line MEF and its derivative Ku80<sup>-/-</sup> knockout line deficient in the NHEJ repair were kindly donated by Dr. P. Burgman and Dr. G.C. Li and their culture has been described in detail (38). The CHO cell line AA8 and its derivative  $\text{irs}$ ISF, a knockout mutant of the XRCC3 gene (XRCC3<sup>-/-</sup>) and deficient in HR DNA repair, were kindly donated by Dr. L. Thompson and have been previously described in detail (39).

The mouse and CHO cell lines were grown in a mixture of 1:1 DMEM and F12 medium supplemented with 10% fetal calf serum and 0.1 mM MEM nonessential amino acids. The cells were grown to plateau phase, then re-fed and the experiments were performed

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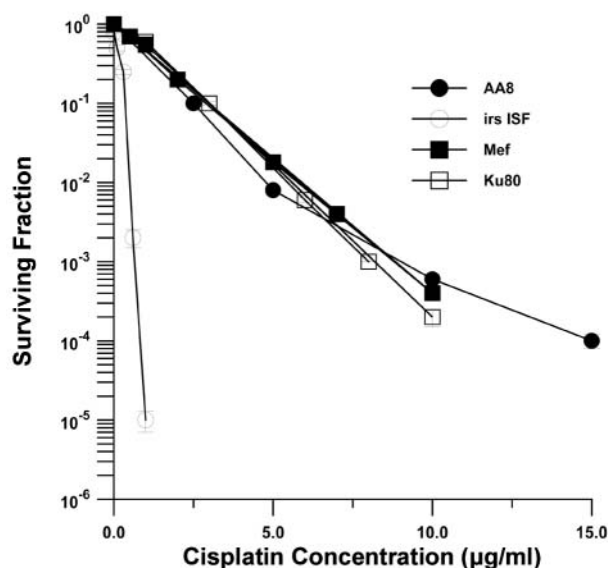


Figure 1. The response to cisplatin is shown for 2 cell line pairs. The AA8 and *irs*ISF are the CHO parental and XRCC3 HR-deficient mutant cell lines, respectively, and the MEF and Ku80 are the mouse MEF parental and the NHEJ mutant cell lines, respectively. Cisplatin exposure lasted 1 hour.

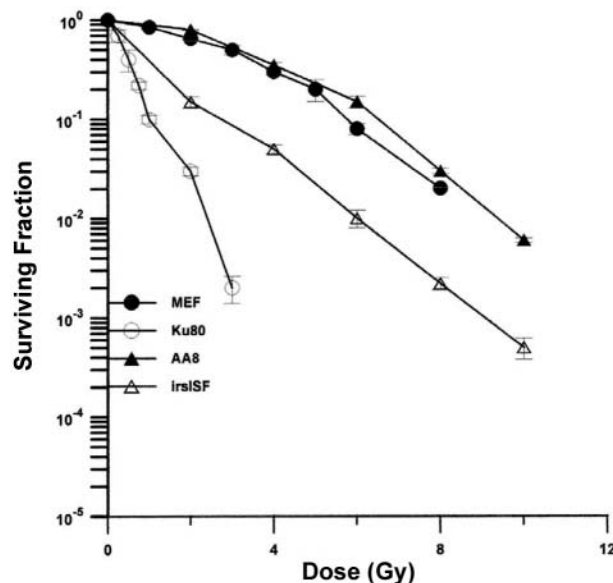


Figure 2. The response to radiation is shown for 2 cell line pairs. The AA8 and *irs*ISF are the CHO parental and XRCC3 HR-deficient mutant cell lines, respectively, and the MEF and Ku80 are the mouse MEF parental and the NHEJ mutant cell lines, respectively.

after 48 h. The plating efficiencies were 80-90% and 50-70% for the AA8 and *irs*ISF cell lines and 80-90% and 70-80% for the MEF and Ku80 cell lines, respectively.

The cells were irradiated in 25-cm<sup>2</sup> tissue culture flasks at room temperature, using a Pantak Bipolar Series model HF320 X-ray unit operating at 250 kVp with 1.87 mm base aluminium filtration giving a dose rate of 168 cGy/min.

For cisplatin treatment, cisplatin obtained from David Bull Canada Inc. in isotonic saline (1 mg/ml) was diluted into the culture medium at the required concentration. For the concentration of 1 µg/ml, the dilution factor is 1000 and has no significant effect on the medium. These solutions were pH buffered at 7.2 and were added directly to the cells. At the end of treatment, the solutions were removed, cultures rinsed with warm isotonic buffer and the medium was then added. Fresh solutions were used for each experiment. After treatments had been completed, the mouse and CHO cells were trypsinized, counted and plated at numbers to give approximately 50 to 100 colonies per 6-cm tissue culture plate. Survival was assayed using the colony forming assay. All dishes were placed in a 37°C incubator until colonies of 50 cells or more were visible, at which point all colonies were stained and counted. Each experiment was repeated 3 times and the error bars represent the standard error of the mean for the replicates. The results for hyperthermia plus radiation were normalized for the results from hyperthermia alone.

## Results

The results of the exposure to cisplatin of the two parental cell lines and the two derivative mutants are shown in Figure 1. For the AA8 and *irs*ISF cell lines, the parental

cell line was much more resistant to cisplatin than the mutant. The difference in sensitivity at the 10% survival level was about 10-fold. Regarding the MEF and the Ku80 cell lines, the data revealed no significant difference in response to cisplatin.

The radiation responses for the parental and mutant cells are depicted in Figure 2. In both cell line pairs, the mutants were more sensitive than the parental cell lines. This difference was much larger for the MEF, Ku80 pair of cell lines compared to the difference observed between the AA8 and *irs*ISF cell lines.

The results obtained for the parental CHO AA8 and its mutant *irs*ISF cell line when an inducing dose of cisplatin was followed by incubation and then a challenge dose of cisplatin are illustrated in Figure 3. The adaptive dose for the mutant was 10-fold less compared to the parental line because of increased sensitivity, as shown in Figure 1. The 1.0 µg/ml inducing treatment of 1 h resulted in resistance to a subsequent treatment of 4 µg/ml for 1 h. This resistance was maximum between 24 and 48 h and then declined at 72 h. In the mutant cell line, the inducing dose of 0.1 µg/ml for 1 h cisplatin did not cause resistance to the subsequent 0.4 µg/ml for 1-h treatment. Instead, there was an initial decline in survival, which was greatest after 8 h and then the cell survival increased, but never exceeded the initial survival. The zero-hour incubation treatments were the inducing treatment followed immediately by the challenge treatment.

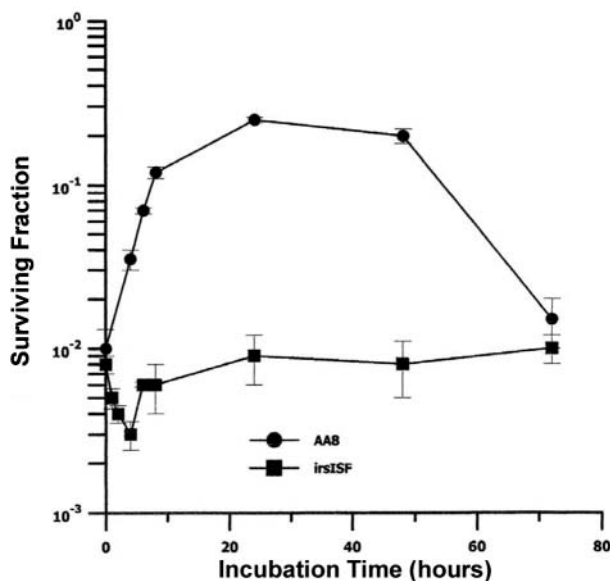


Figure 3. Responses to inducing and challenge doses of cisplatin are shown for the parental AA8 and mutant *irsISF* cell lines. Treatments for AA8 were 1  $\mu$ g/ml followed by 4  $\mu$ g/ml and 0.1  $\mu$ g/ml, followed by 0.4  $\mu$ g/ml for *irsISF*. All treatments lasted 1 h.

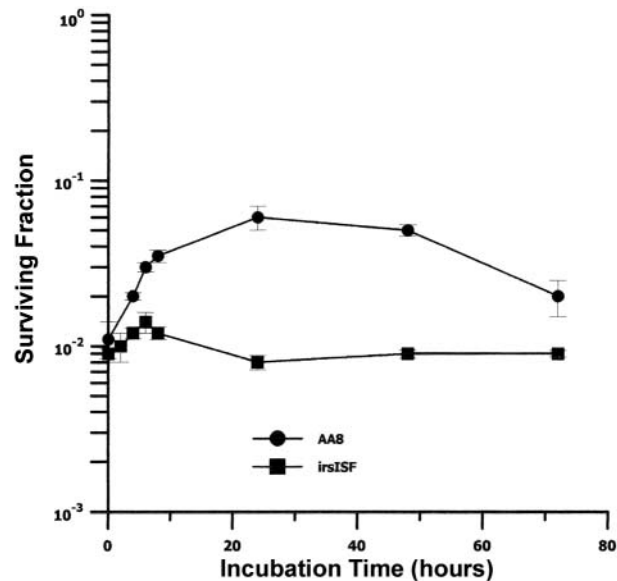


Figure 4. Responses of parental AA8 and mutant *irsISF* lines are shown for inducing doses of 1-h cisplatin treatments of 1  $\mu$ g/ml followed by 8 Gy for the parental and 0.1  $\mu$ g/ml followed by 6 Gy for the mutant cell line, respectively.

The adaptive responses for the AA8 and *irsISF* lines given treatments of 1 and 0.1  $\mu$ g/ml for 1 h, respectively, followed by incubation and then irradiation with 8 and 6 Gy, respectively, are illustrated in Figure 4. The radiation doses were chosen to match survival (see Figure 2). The parental cell line showed an adaptive response which was maximum at 24 h and then declined at 72 h. The mutant line showed an initial very small adaptive response, which reached a maximum at 6 h and then declined back to the initial non-adapted level.

The data depicted in Figure 5 correspond to the radiation-inducing treatments followed 24 h later by a challenge radiation dose. Initially, small doses of 5 and 10 cGy were given followed 24 h later by challenge doses of 8 and 6 Gy for the AA8 and *irsISF* cell lines, respectively.

The data showed an adaptive response for the parental cell line and a sensitizing response for the mutant cell line.

The data for the MEF and its mutant Ku80 for cisplatin treatments of 1.0  $\mu$ g/ml over 1 h, followed by incubation and then a challenge treatment of 6  $\mu$ g/ml for 1 h are shown in Figure 6. Note from Figure 1 that the cisplatin response was the same for both cell lines and, thus, the same doses were chosen. The data reveal that initially there was a substantial degree of sensitization, which was followed by a recovery which was greater for the parental line. However, neither recovered to the zero incubation level. The inset in the figure shows the nature of the initial decline in more detail over the first hour of incubation. The experiments were repeated 6

times in order to clearly confirm this result. Experiments were also carried out for small doses of radiation followed by larger challenge doses and no adaptive response was observed in either cell line. In addition, experiments on chicken DT40 cells using the parental line and the DT40Rad54 line deficient in HR and the DT40Ku70 line deficient in NHEJ repair revealed that neither the parental line nor the mutants showed any adaptive response to cisplatin and radiation. These data are not presented.

## Discussion

A number of studies have shown that adaptive responses to low levels of cisplatin and radiation can occur in mammalian cells (20-38). Our earlier studies indicated that an adaptive response to cisplatin occurred at the DNA repair level (21). The studies of Caney *et al.* (40, 41) indicated that low doses of radiation could induce adaptive responses to cisplatin but that cisplatin could not induce adaptive responses to radiation. In addition, our studies and those of others showed that the DNA HR repair system was involved in cisplatin and radiation responses (18-19).

The data in this study confirmed that inhibition of HR in the XRCC3 mutant cell line *irsISF* increased its sensitivity to radiation compared to the AA8 parental cell line. Our data also showed that low-dose cisplatin treatment could induce a transient adaptive response to challenge treatment of cisplatin or radiation. However, the mutant *irsISF* cell line

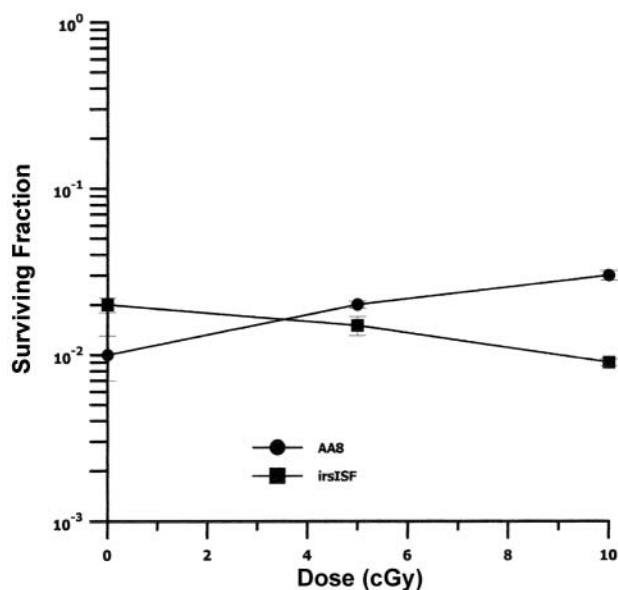


Figure 5. Adaptive responses to radiation are shown for the parental AA8 and mutant *irsISF* cell lines. Cells were given 0, 10 and 20 cGy inducing doses followed 24 h later by 8 and 6 Gy challenge doses for the parental and mutant lines, respectively.

was not able to undergo an adaptive response to low levels of cisplatin treatment followed by either challenge treatment of cisplatin or radiation. These results implicate the involvement of the HR repair system in these adaptive responses. In addition, we also showed that small doses of radiation caused an adaptive response in AA8 but not in the mutant *irsISF* cell line, again implicating HR involvement in this adaptive response. In contrast to our results, those of Caney *et al.* (41) for cisplatin given before X-rays showed no adaptive response, while X-rays before cisplatin did show an adaptive response. The reasons for this difference are not clear, however the adaptive response may be very cell line-dependent (24), as confirmed in our earlier studies (32) and in the current studies in which no adaptive responses were seen in the MEF and Ku80 cell lines. In addition, we also could not evoke any adaptive responses in the DT40 chicken cell lines. Studies in these cell lines were not pursued further.

The results for the MEF and Ku80 cell lines were similar and neither showed an adaptive response, thus the involvement of the NHEJ repair system could not be further investigated. However, the initial response, indicating an initial increased sensitivity with a partial recovery for incubation times exceeding 2 h, is of interest. The initial hypersensitivity may be similar to that observed by Caney *et al.* (42). The later recovery may, in fact, be an adaptive response, but both parental and mutant cell lines showed such a response, thus ruling out potential NHEJ involvement.

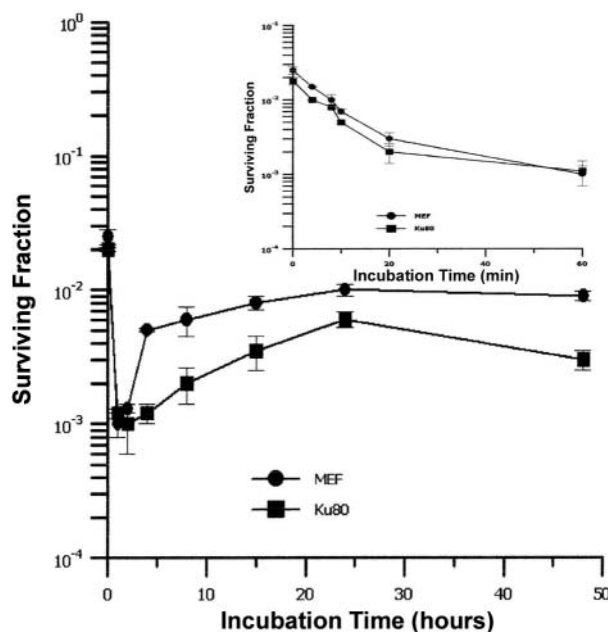


Figure 6. Responses to inducing and challenge treatments of cisplatin are shown for the parental MEF and the mutant Ku80 cell lines. Both cell lines were given a 1  $\mu$ g/ml cisplatin inducing treatment followed by a 6  $\mu$ g/ml cisplatin challenge treatment. All cisplatin treatments lasted 1 h.

In summary, the HR repair system appears to be involved in the adaptive responses to cisplatin and radiation while the involvement of the NHEJ system remains unclear.

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