

High Sensitivity of Fibroblast Cell Lines Derived from LEC Rats to Heat Treatment

Masanobu HAYASHI¹⁾, Taku HAMASU¹⁾, Daiji ENDOH¹⁾, Ai KAWANA¹⁾, Minako MATSUMOTO¹⁾ and Toyo OKUI²⁾

¹⁾Department of Veterinary Radiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501 and ²⁾Hokkaido Institute of Public Health, Sapporo 060-0819, Japan

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ABSTRACT. A fibroblast cell line derived from LEC rat was approximately twofold more sensitive to heat treatment at 45°C than were that from WKAH rat in terms of heating time required to attain 50% loss of survival in a colony forming assay. The present study was carried out for understanding the mechanism underlying the higher sensitivity of LEC rat cells to heat treatment. Although apoptosis was not found in WKAH rat cells, the percentages of apoptotic cells in LEC rat cells significantly increased after heat treatment. LEC rat cells showed significantly lower sensitivity in induction of cell death and apoptosis to ceramide, a lipid signaling molecule that is associated with heat-induced apoptosis, than did WKAH rat cells. SP600125, an inhibitor of JNK suppressed the induction of cell death in both heated LEC and WKAH rat cells, but SB203580, an inhibitor of p38 mapk, did not. The relative surviving fractions of heated LEC and WKAH rat cells in the presence of both SB203580 and SP600125 were higher than those of cells in the presence of SP600125 alone. The amounts of hsp70 protein in WKAH rat cells increased from 4 to 12 hr after heat treatment, but did not in LEC rat cells. These results suggest that higher thermosensitivity in the fibroblast cell line from LEC rat is due to low inducibility of hsp70 protein after heat treatment.

KEY WORDS: apoptosis, cell death, heat treatment, JNK, LEC rat cell.

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Cellular stresses, including stress caused by heat treatment, impair numerous physiological functions, damage cellular structures, and can lead to cell death. Generally tumor tissue cells have greater sensitivity to heat treatment than do normal tissue cells [14]. Therefore, hyperthermia that achieves temperature in a range from 40 to 45°C are used in clinical practice to treat many types of human and animal malignancies [1]. There is growing evidence of a beneficial effect of hyperthermia when combined with ionizing radiation or anticancer drugs [7, 20, 29]. Hyperthermia sensitizes mammalian cells to ionizing radiation [2, 4]. The potential benefits of combining heat and ionizing radiation in the clinic might be maximized if the molecular mechanisms underlying radiosensitization by hyperthermia are elucidated. Ionizing radiation induces the formation of DNA double strand breaks (DSBs), and DSBs cause major lethal damage to cells unless repaired [23]. Radiosensitization is believed to be caused by an inhibition of repair of radiation-induced DSBs by hyperthermia [6, 22]. However, the mechanism by which heat actually inhibits DSBs repair remains to be elucidated. It has been reported that hyperthermia causes denaturation and aggregation of proteins and that denatured and aggregated proteins mask sites of radiation-induced DNA damage, resulting in interference of the function of repair proteins by limiting their accessibility to DSBs [24, 32]. Alternatively, it has been suggested that inactivation of DNA repair enzymes by hyperthermia is directly responsible for inhibition of repair and radiosensitization [13, 26, 35]. However, Wachsberger and Iliakis [30] reported that hyperthermia does not affect rejoining of DNA DSBs in a cell-free assay. Therefore, the relation between radiosensitization induced by heat treatment and ability of DSBs repair is still controversial. Furthermore, it has been

reported that hyperthermia induces apoptosis in a variety of cells [5, 18]. Ostapenko *et al.* [19] showed that M10 cells, which are deficient in the repair of DNA DSBs and are radiosensitive, are about twofold more thermoresistant than their parental L5178Y cells. However, the relationship between sensitivity of cells to heat-induced apoptosis and cellular ability of DSBs repair remains unclear.

The LEC rat strain was established at the Center for Experimental Plants and Animals, Hokkaido University [26]. Rats of this strain suffer from spontaneous fulminant hepatitis associated with severe jaundice at about 4 months of age. Other characteristics of LEC rats are a high incidence of spontaneous liver cancer in long-surviving individuals [33] and an increased sensitivity *in vivo* and *in vitro* to ionizing radiation [9, 16]. In our previous studies, we found that fibroblast cell lines from LEC rats were more sensitive to X irradiation than were cells from a control strain of WKAH rats and that cells from LEC rats had a reduced level of repair of DSBs induced by X irradiation [9, 17]. In the course of our study on the mechanisms of radiosensitizing effects elicited by hyperthermia using DSBs repair-deficient LEC rat cells, we found that a fibroblast cell line from LEC rat is more sensitive to heat treatment than are that from WKAH rat. A number of cellular stress conditions, including hyperthermia, activate the mitogen-activated protein kinase (mapk) cascade, and this signaling pathway has been implicated in mediating the apoptosis process [15, 28, 31, 34]. It has been reported that apoptotic cell death and mapk activation induce by heat treatment are associated with increased levels of a lipid signaling molecule, ceramide [3, 15, 28, 31]. On the contrary, cells respond to stress by adaptive changes that prevent cell death. A level of the thermotolerance is quantitatively related to the absolute levels of a

group of heat-induced proteins, particularly hsp70, first suggested that the heat shock proteins plays a protective role in cell survival [11, 12, 27]. It has been reported that increased level of hsp70 inhibited mapk activation [15]. In the present study, for understanding the mechanism underlying the higher sensitivity of LEC rat cells to heat treatment, we examined the effects of ceramide and mapk inhibitors on the cell death and level of hsp70 in LEC rat cells after heat treatment.

MATERIALS AND METHODS

Culture of cells and drug treatments: Rat fibroblast cell lines were established from lungs of LEC and WKAH rats by SV 40 immortalization as described previously [8]. The cells were grown in a monolayer culture in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum. The cell cultures were kept at ambient humidity and 37°C in an atmosphere containing 5% CO₂.

SB203580 (Wako Chemicals Co.), an inhibitor of p38 mapk, and SP600125 (Calbiochem Co.), JNK inhibitor II, were dissolved in dimethyl sulfoxide (DMSO). Ceramide (N-acetyl-D-sphingosine, Sigma Aldrich Chemical Co.) was dissolved in ethanol.

After the medium had been removed from the plates, the cells were washed with phosphate-buffered saline (PBS), pH 7.2, treated with SB203580 at 40 μ M and/or SP600125 at 50 μ M for 30 min, and then treated at 45°C for 1 hr. After heat treatment, the cells were incubated at 37°C for 4 hr in the presence of inhibitors. After the medium containing the drug had been removed, the cells were washed with PBS. Fresh MEM was added to the plates and the plates were returned to the incubator. The relative surviving fraction was calculated as the surviving fraction of cells with an inhibitor/the surviving fraction of cells without an inhibitor.

The cells were washed with PBS and treated with ceramide at concentrations from 0 to 80 μ M for 1 hr. After the medium containing the drug had been removed, the cells were washed with PBS. Fresh MEM was added to the plates and the plates were returned to the incubator.

Colony-forming assay: Cell survival was determined using the conventional colony-forming assay. Propagated cells were collected by trypsinization, and 2–50 $\times 10^2$ viable cells were plated into 6-cm dishes. After the cells had been treated with heat and/or drugs, the cells were incubated for 2 weeks. The plates were methanol-fixed and stained with May-Grunwald and Giemsa, and then colonies containing more than 50 cells were counted as survivors under a dissecting microscope.

Flow cytometry: Apoptotic cells were labeled with fluorescein-dUTP by using a Mebstain apoptosis kit direct (Medical & Biological Laboratories Co.) according to the manufacturer's instructions. Briefly, after treatment with heat or ceramide, logarithmically growing cells (1×10^6) were incubated in growth medium at 37°C for 0–72 hr and collected. The cells were washed several times with PBS containing 0.2% BSA. The cells were fixed with 0.1 M

NaH₂PO₄ containing 4% paraformaldehyde at 4°C for 30 min, washed 2 times with PBS containing 0.2% BSA, and then pelleted by centrifugation at 500 \times g. The cells were fixed in 5 ml of cold 70% ethanol for 30 min at room temperature and stored at –20°C. Just prior to flow cytometric analysis, individual samples were labeled with fluorescein-dUTP. Fluorescence was measured with a Coulter EPICS EL flow cytometer using a 530-nm filter. The percentage of apoptotic cells was determined using multicycle software.

Immunoblotting: After treatment of the cells (1×10^6 to 5×10^6) at 45°C for 1 hr, the cells were incubated at 37°C for 0–12 hr, harvested by trypsinization, washed with PBS, and then pelleted by centrifugation at 500 \times g. The cells were counted and solubilized in Laemmli sample buffer as described by Kastan *et al.* [10]. A modified Lowry assay was used to quantitate relative protein levels in the samples, and extracts were loaded so that equivalent cell numbers and/or equivalent protein amounts were loaded in each comparable set of lanes. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis and wet electrotransfer (Mini TransBlot Cell, BioRad) to polyvinylidene fluoride (PVDF) paper (Immobilon-P, Millipore), equivalent protein loading was confirmed by staining the PVDF paper with amido black. A polyclonal goat IgG antibody to hsp70 and a peroxidase-labeled anti-goat IgG antibody were obtained from Santa Cruz Biotechnology Inc. The proteins were detected with Super signal West Pico (Pierce Co.) according to the protocols provided by the manufacturers.

Statistics analysis: Statistical analysis was performed using Student's *t* test and $p < 0.05$ was considered significant. Data are expressed as the mean \pm standard deviation.

RESULTS

When the cytotoxic effect of heat treatment at 45°C on cell survival was examined using a colony-forming assay, it was found that the surviving fractions decreased in both fibroblast cell lines from LEC and WKAH rats in a heating time-dependent manner and that LEC rat cells showed significantly higher sensitivity to heat treatment than did WKAH rat cells (Fig. 1). The periods of treatment required to reduce cell survival to 50% (ET₅₀) were approximately 1 hr for LEC rat cells and 2 hr for WKAH rat cells. To determine whether the high sensitivity of LEC rat cells to heat treatment is due to induction of apoptosis by heat treatment, the apoptotic cells were analyzed using a flow cytometer after heat treatment for 1 and 2 hr at 45°C. A significant percentage of apoptotic cells was not found in WKAH rat cell populations from 0 to 72 hr after heat treatment (Fig. 2). In contrast, the percentages of apoptotic cells had significantly increased at 72 hr after heat treatment for 1 hr and at 48 and 72 hr after heat treatment for 2 hr in LEC rat cells. The proportion of apoptotic cells in the total LEC rat cell population was approximately 70% at 72 hr after heat treatment for 2 hr (Fig. 2).

We examined the effects of treatment of LEC rat cells with ceramide on cell death and induction of apoptosis to

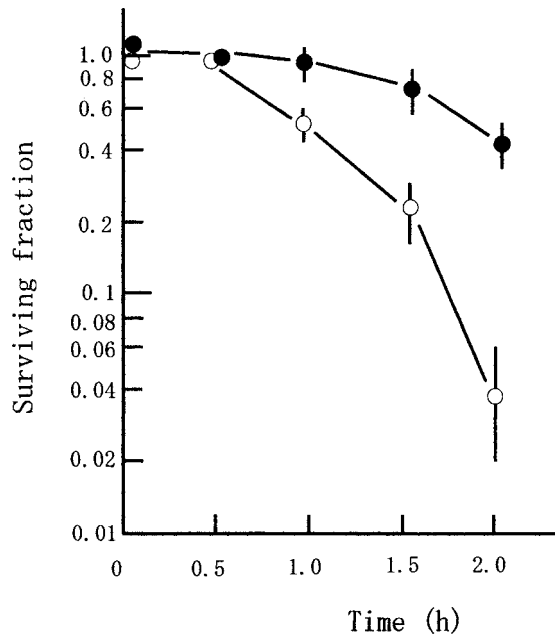


Fig. 1. Cytotoxic effects of heat treatment on the survival of fibroblast cell lines from LEC and WKAH rats. Fibroblast cell lines from LEC (○) and WKAH (●) rats were heated at 45°C. Each value represents the average from four separate experiments. Error bars represent the standard deviations of mean values.

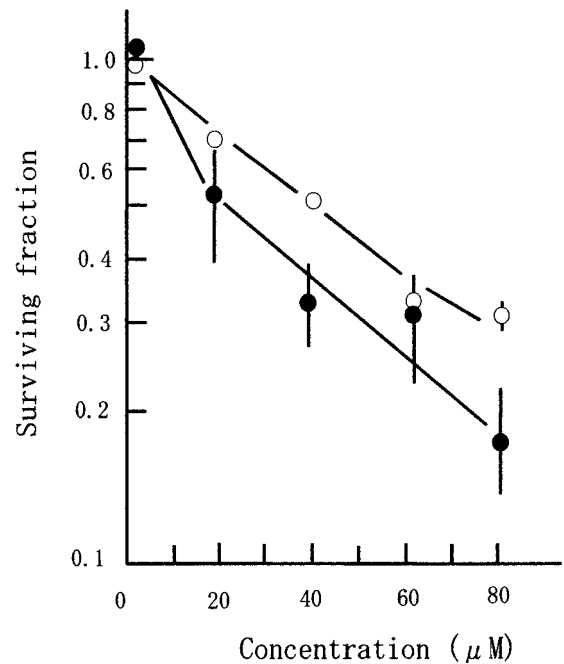


Fig. 3. Cytotoxic effects of ceramide on the survival of LEC and WKAH rat cells. LEC (○) and WKAH (●) rat cells were treated with ceramide at 37°C for 1 hr. Error bars represent the standard deviations of mean values (n=4). The standard deviations were within symbols at some points.

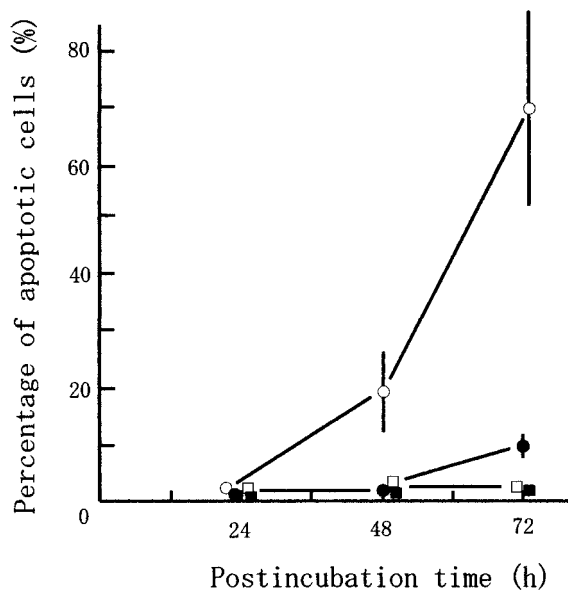


Fig. 2. Induction of apoptosis in LEC and WKAH rat cells after heat treatment. After heat treatment of LEC (○, ●) and WKAH (□, ■) rat cells at 45°C for 1 hr (●, ■) and for 2 hr (○, □), the cells were incubated at 37°C for 0–72 hr, and the number of apoptotic cells was counted using a flow cytometer at each incubation time. Error bars represent the standard deviations of mean values (n=4). The standard deviations were within symbols at some points.

determine whether the higher sensitivity of LEC rat cells to heat treatment is due to the higher sensitivity to ceramide. When the cytotoxic effects of ceramide on cell survival were examined using a colony-forming assay, it was found that the surviving fractions decreased in both LEC and WKAH rat cells in a dose-dependent manner by treatment with ceramide and that LEC rat cells showed significantly lower sensitivity to ceramide than did WKAH rat cells (Fig. 3). When the apoptotic cells were analyzed after treatment with ceramide at 80 μM, the percentages of apoptotic cells significantly increased in a post-incubation time-dependent manner after treatment with ceramide. The proportion of apoptotic cells in the total WKAH rat cell population was significantly higher than the proportion of apoptotic cells in the total LEC rat cell population at 48 and 72 hr after treatment with ceramide (Fig. 4).

To investigate whether mapk pathway is associated with high sensitivity of LEC rat cells to heat treatment, we examined the inhibitory effects of SB203580, an inhibitor of p38 mapk, and SP600125, a c-Jun N-terminal kinase (JNK) inhibitor on induction of cell death of LEC rat cells by heat treatment. The relative surviving fraction of cells was normalized in such a way that each surviving fraction of LEC and WKAH rat cells treated at 45°C for 1 hr without an inhibitor was 1.0. SB203580 showed no inhibitory effect on cell death in either LEC or WKAH rat cells (Table 1). The surviving fractions of both heat-treated LEC and WKAH rat

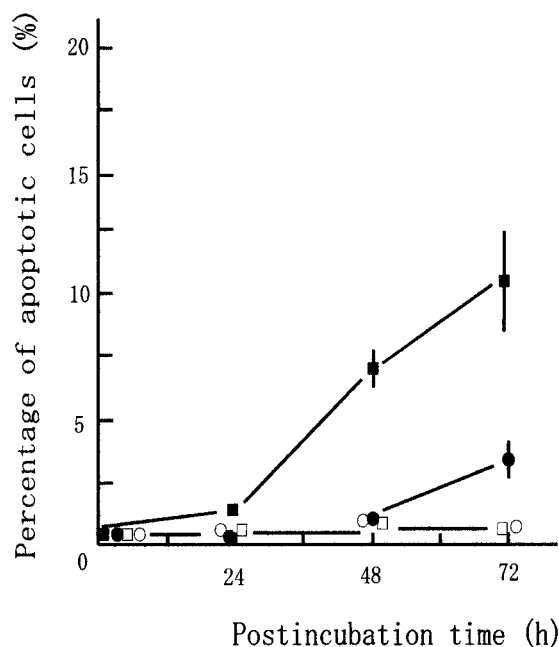


Fig. 4. Induction of apoptosis in LEC and WKAH rat cells after treatment with ceramide. LEC (○, ●) and WKAH (□, ■) rat cells were treated with 80 μ M of ceramide at 37°C for 1 hr (●, ■) or were not treated with ceramide (○, □), and then the cells were incubated at 37°C for 0–72 hr and the number of apoptotic cells was counted using a flow cytometer at each incubation time. Error bars represent the standard deviations of mean values ($n=4$). The standard deviations were within symbols at some points.

Table 1. Effects of mapk inhibitor on the cell death of LEC and WKAH rat cells

Cell	Relative surviving fraction			
	No drug	SB203580	SP600125	SB203580 + SP600125
WKAH	1.0	1.0 \pm 0.1	2.1 \pm 0.3	3.1 \pm 0.3
LEC	1.0	1.0 \pm 0.1	1.4 \pm 0.2	2.6 \pm 0.4

LEC and WKAH rat cells were heated at 45°C for 1 hr in the presence or absence of SB203580 and/or SP600125, as described in the text. The relative surviving fraction of the cells was normalized in such a way that each surviving fraction of the WKAH and LEC rat cells treated at 45°C for 1 hr without an inhibitor was 1.0. The relative surviving fraction was calculated as the surviving fraction of cells with an inhibitor/the surviving fraction of cells without an inhibitor. Each value represents the average from four separate experiments (\pm SD).

cells increased in the presence of SP600125 compared with those of heat-treated cells without SP600125. The relative surviving fraction of WKAH rat cells was significantly higher than that of LEC rat cells in the presence of SP600125. Furthermore, the relative surviving fractions of LEC and WKAH rat cells in the presence of both SB203580 and SP600125 were higher than those of cells in the presence of SP600125 alone.

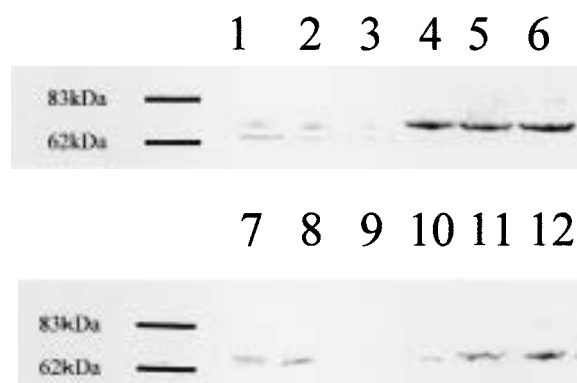


Fig. 5. Expression of hsp 70 protein in LEC and WKAH rat cells after heat treatment. After WKAH rat cells (lanes 1–6) and LEC rat cells (lanes 7–12) had been heated at 45°C for 1 hr, the cells were incubated at 37°C for 0 (lanes 2 and 8), 2 (lanes 3 and 9), 4 (lanes 4 and 10), 6 (lanes 5 and 11) and 12 (lanes 6 and 12) hr. Lanes 1 and 7 represent unheated WKAH and LEC rat cells, respectively.

The amounts of hsp70 protein in LEC and WKAH rat cells were analyzed by immunoblotting after heat treatment at 45°C for 1 hr and typical results were shown in Fig. 5. In WKAH rat cells, intensified bands of hsp70 protein were observed from 4 to 12 hr after heat treatment compared with the bands of hsp70 protein from untreated cells. In LEC rat cells, the band intensity had not increased at 4 hr after heat treatment compared with the bands of hsp70 protein from untreated cells. Intensity of the bands in LEC rat cells was weaker than that in WKAH rat cells from 6 to 12 hr after heat treatment.

DISCUSSION

In the present study, we found that LEC rat fibroblast cell lines showed significantly higher sensitivity to heat treatment at 45°C than did WKAH rat cells in colony forming assay (Fig. 1). LEC rat cells were approximately twofold more sensitive to heat treatment than were WKAH rat cells in terms of ET_{50} . LEC rat cells also showed a higher sensitivity to induction of apoptosis by heat treatment. Therefore, the higher sensitivity of LEC rat cells in cell death to heat treatment may be at least partly responsible for the higher sensitivity to induction of apoptosis. Ostapenko *et al.* [19] showed that M10 cells, which are deficient in the repair of DNA DSBs and are radiosensitive, are about twofold more thermoresistant than their parental L5178Y cells. However, since LEC rat cells are highly radiosensitive and are deficient in DSBs repair [9, 17], present results suggest that sensitivity of cells to heat-induced cell death is not directly associated with the radiosensitivity and the ability of repair of DSBs.

It has been reported that apoptosis induced by heat treatment is associated with increased levels of ceramide [3, 28, 31]. LEC rat cells did not show high sensitivity to ceramide

in cell death and induction of apoptosis. Therefore, sensitivity to ceramide can not account for the high sensitivity of LEC rat cells to heat-induced apoptosis. The reason why LEC rat cells showed less sensitivity than WKAH rat cells to ceramide in induction of cell death and apoptosis is not known. It has been shown that various treatments, including hyperthermia, treatment with inflammatory cytokines, and UV and ionizing radiation, activate mapk, resulting in cell death [31, 34]. Although SB203580, an inhibitor of p38 mapk, showed no inhibitory effect on cell death in either LEC or WKAH rat cells, the surviving fractions of both heat-treated LEC and WKAH rat cells increased in the presence of SP600125, a JNK inhibitor, compared with those of heat-treated cells without SP600125 (Table 1). These results suggest that JNK plays an important role in induction of cell death of both LEC and WKAH rat cells by heat treatment but that p38 mapk does not. However, since the relative surviving fractions of LEC and WKAH rat cells in the presence of both SB203580 and SP600125 were higher than those of cells in the presence of SP600125 alone, a pathway *via* p38 mapk, which induced cell death, may be activated by heat treatment when JNK does not work. The relative surviving fractions of LEC rat cells after heat treatment in the presence of SB203580 and SP600125 were the same as those of heat-treated WKAH rat cells in the presence of SB203580 and SP600125. Therefore, a pathway *via* p38 mapk, which induced cell death, may be more strongly activated in LEC rat cells by heat treatment when JNK does not work. A study on the activation pathway of p38 mapk by heat treatment is now in progress.

Increase of hsp70 protein was observed in WKAH rat cells from 4 to 12 hr after heat treatment at 45°C for 1 hr compared with unheated cells (Fig. 5). In contrast, the band intensity did not increase in LEC rat cells at 4 hr after heat treatment. The band intensity in LEC rat cells was weaker than that in WKAH rat cells from 6 to 12 hr after heat treatment. It is well known that heat-induced proteins, particularly hsp70, play a protective role in cell survival against heat treatment [11, 12, 27]. Since it has been suggested that hsp70 protein is able to block apoptosis by inhibiting signaling events upstream of JNK activation [15], the increase in hsp70 protein from 4 to 12 hr after heat treatment may inhibit the induction of cell death in WKAH rat cells. On the contrary, the very slightly or no increase in hsp70 protein in LEC rat cells may not inhibit the activation of JNK pathway resulting in the high sensitivity in cell death after heat treatment. Furthermore, it has been reported that transfection of inducible *Hsp70* to NIH 3T3 mouse embryo cells conferred radioresistance to the cells as assayed by clonogenic survival [21]. Weak inducibility of hsp70 protein after heat treatment may be one factor of the higher radiosensitivity of LEC rat cells. Whether expression of hsp70 may affect the radiosensitivity in LEC rat cells is also now in progress.

The present study suggests that higher thermosensitivity in the fibroblast cell line from LEC rat is due to low inducibility of hsp70 after heat treatment. However, whether

other cells, including primary fibroblast, derived from LEC rat show the higher thermosensitivity and low inducibility of hsp70 remains unelucidated yet. A comparison of thermosensitivity of LEC rat cells with that of cells derived from other rat strains than WKAH rat also may be needed. Since a LEC rat cell line show higher sensitivity to heat treatment and ionizing radiation, and deficiency in DSBs repair, the cell line provides a useful model for understanding the mechanisms underlying radiosensitization by hyperthermia.

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REFERENCES

1. Alexander, H. R. Jr. 2003. Hyperthermia and its modern use in cancer treatment. *Cancer* **15**: 219–221.
2. Ben-Hur, E., Elkind, M. M. and Bronk, B. V. 1975. Thermally enhanced radioresponse of cultured Chinese hamster cells: inhibition of repair of sublethal damage and enhancement of lethal damage. *Radiat. Res.* **58**: 38–51.
3. Cuvillier, O., Pirianov, G., Kleuser, B., Vanek, P. G., Coso, O. A., Gutkind, J. S. and Spiegel, S. 1996. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature (Lond.)* **381**: 800–803.
4. Dewey, W. C., Sapareto, S. A. and Betten, D. A. 1978. Hyperthermic radiosensitization of synchronous Chinese hamster cells: Relationship between lethality and chromosome aberrations. *Radiat. Res.* **76**: 48–59.
5. Dieing, A., Ahlers, O., Kerner, T., Wust, P., Felix, R., Loffel, J., Riess, H. and Hidebrandt, B. 2003. Whole body hyperthermia induces apoptosis in subpopulations of blood lymphocytes. *Immunobiology* **207**: 265–273.
6. Dikomey, E. and Franzke, J. 1992. Effect of heat on induction and repair of DNA strand breaks in X-irradiated CHO cells. *Int. J. Radiat. Biol.* **61**: 221–233.
7. Dyson, J. E. D., Simmons, D. M., Daniel, J., McLaughlin, J. M., Quirke, P. and Bird, C. C. 1986. Kinetics and physical studies of cell death induced by chemotherapeutic agents or hyperthermia. *Cell Tissue Kinet.* **19**: 311–324.
8. Hayashi, M., Ishimori, K., Maeda, A., Watanabe, T., Arai, S. and Okui, T. 1996. Radioresistant DNA synthesis in fibroblast cell lines derived from LEC strain rats. *Mutat. Res.* **352**: 117–121.
9. Hayashi, M., Okui, T., Endoh, D., Sato, F., Kasai, N. and Namioka, S. 1994. Radiation-hypersensitivity of LEC strain rats controlled by a single autosomal recessive gene. *Mutat. Res.* **314**: 135–142.
10. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B. and Craig, R. W. 1991. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* **51**: 6304–6311.
11. Landry, J., Bernier, D., Chretien, P., Micole, L. M., Tanguary, R. M. and Marceai, N. 1982. Synthesis and degradation of heat shock protein during development and decay of thermotolerance. *Cancer Res.* **42**: 2457–2461.
12. Li, G. C. and Werb, Z. 1982. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **79**: 3218–3222.
13. Matsumoto, Y., Suzuki, N., Morimatsu, A. and Murofushi, A.

1997. A possible mechanism for hyperthermic radiosensitization mediated through hyperthermic lability of Ku subunits in DNA-dependent protein kinase. *Biochem. Biophys. Res. Commun.* **234**: 568–572.
14. Meyer, J. L. 1984. The clinical efficacy of localized hyperthermia. *Cancer Res.* **44**: 4745–4751.
15. Mosser, D. D., Caron, A. W., Bourget, L., Denis-Larose, C. and Massie, B. 1997. Role of human heat shock protein hsp 70 in protection against stress-induced apoptosis. *Mol. Cell. Biol.* **17**: 5317–5327.
16. Okui, T., Endoh, D., Arai, S., Isogai, E. and Hayashi, M. 1996. Cross-sensitivity of X-ray-hypersensitive cells derived from LEC strain rats to DNA-damaging agents. *J. Vet. Med. Sci.* **58**: 1067–1071.
17. Okui, T., Endoh, D., Kon, Y. and Hayashi, M. 2002. Deficiency in nuclear accumulation of G22p1 and Xrcc5 proteins in hyper-radiosensitive Long-Evans Cinnamon (LEC) rat cells after X irradiation. *Radiat. Res.* **157**: 553–561.
18. O'Neill, K. L., Fairbraim, D. W., Smithe, M. J. and Poe, B. S. 1998. Critical parameters influencing hyperthermia-induced apoptosis in human lymphoid cell lines. *Apoptosis* **3**: 369–375.
19. Ostapenko, V. V., Wang, X., Ohnishi, K., Takahashi, A., Yamamoto, I., Tanaka, Y. and Ohnishi, T. 1999. Increased resistance of the radiosensitive M10 mutant cells of the L5178Y mouse lymphoma cell line to heat-induced apoptosis. *Radiat. Res.* **152**: 321–327.
20. Overgaard, J. 1989. The current and potential role of hyperthermia in radiotherapy. *Int. J. Oncol. Biol. Phys.* **16**: 535–549.
21. Park, S.-H., Lee, S.-J., Chung, H.-Y., Kim, T.-H., Cho, C.-K., Yoo, S.-Y. and Lee, Y.-S. 2000. Inducible heat shock protein 70 is involved in the radioadaptive response. *Radiat. Res.* **153**: 318–326.
22. Radford, I. R. 1983. Effects of hyperthermia on the repair of X-ray induced DNA double strand breaks in mouse L cells. *Int. J. Radiat. Biol.* **5**: 551–557.
23. Radford, I. R. 1986. Evidence for a general relationship between the induced level of DNA double-strand breakage and cell-killing after X-irradiation of mammalian cells. *Int. J. Radiat. Biol.* **49**: 611–620.
24. Roti Roti, J. L., Wright, W. D. and VanderWaal, R. 1997. The nuclear matrix: A target for heat shock effects and a determinant for stress response. *Crit. Rev. Eukaryot. Gene Expr.* **7**: 343–360.
25. Sasaki, M., Yoshida, M. C., Kagamori, K., Takeichi, N., Kobayashi, H., Dempo, K. and Mori, M. 1985. Spontaneous hepatitis in an inbred strain of Long-Evans rats. *Rat News Lett.* **14**: 4–6.
26. Spiro, I. J., Denman, D. L. and Dewey, W. C. 1982. Effect of hyperthermia on CHO DNA polymerase α and β . *Radiat. Res.* **89**: 134–149.
27. Subject, J. R., Sciandra, J. J. and Johnson, R. J. 1982. Heat shock proteins and thermotolerance; a comparison of induction kinetics. *Br. J. Radiol.* **55**: 579–584.
28. Verheij, M., Bose, R., Lin, X.H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Szabo, E., Zon, L. I., Kyriakis, J. M., Haimovitzy-Friedman, A., Fuc, A. and Kolesnick, R. N. 1996. Requirement for ceramide-initiated SAPK/JNK signaling in stress-induced apoptosis. *Nature (Lond.)* **380**: 75–79.
29. Vernon, C. C., Hand, J. W., Field, S. B., Machin, D., Whaley, J. B., van der Zee, J., van Putten, W. L., van Rhooen, G. C., van Dijk, J. D. and Sherar, M. 1996. Radiotherapy with or without hyperthermia in the treatment of superficial localized breast cancer: Results from five randomized controlled trials. *Int. J. Radiat. Oncol. Biol. Phys.* **35**: 731–744.
30. Wachsberger, P. R. and Iliakis, G. 2000. Hyperthermia does not affect rejoining of DNA double-strand breaks in a cell-free assay. *Int. J. Radiat. Biol.* **76**: 313–326.
31. Westwick, J. K., Bielawska, A. E., Dbaibo, G., Hnnun, Y. A. and Brenner, D. A. 1995. Ceramide activates the stress-activated protein kinase. *J. Biol. Chem.* **270**: 22689–22692.
32. Wong, R. S. L., Dynlacht, J., Cedervall, B. and Dewey, W. C. 1995. Analysis by pulsed field gel electrophoresis of DNA double-strand breaks induced by heat and/or X-irradiation in bulk and replicating DNA of CHO cells. *Int. J. Radiat. Biol.* **68**: 141–152.
33. Yoshida, M. C., Masuda, R., Sasaki, M., Takeichi, N., Kobayashi, H., Dempo, K. and Mori, M. 1987. New mutation causing hereditary hepatitis in the laboratory rat. *J. Hered.* **78**: 361–365.
34. Zaneke, B.W., Boudreau, K., Rubie, E., Winnett, E., Tibbles, L. A., Zon, L., Kriakis, J., Liu, F. F. and Woodgett, J. R. 1996. The stress activated protein kinase pathway mediated cell death following injury induced by cis-platinum, UV irradiation or heat. *Curr. Biol.* **6**: 606–613.
35. Zhu, W.-G., Seno, J. D., Beck, B. D. and Dynlacht, J. R. 2001. Translocation of Mre11 from the nucleus to the cytoplasm as a mechanism of radiosensitization by heat. *Radiat. Res.* **156**: 95–102.