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Establishment and Characterization of a Human Cell Strain, KT, with High Sensitivity to UV-killing and to Cell Proliferation Inhibition by Interferon

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We have established a human cell line, designated KT, with high susceptibility to both cell proliferation inhibition by interferon and UV-killing, from a metastatic breast carcinoma. A tumor marker, a pregnancy-specific glycoprotein (Schwangerschaftsprotein 1; SP1), and carcinoma characteristics compatible with ductal carcinoma of the breast were seen in KT cells by electron microscopic observation. KT cells were slightly more resistant to X-ray-induced toxicity than fibroblastic cells, termed KS, from the scalp of the patient. But, KT cells had lower cloning efficiency after UV irradiation than did KS cells: D_0 values of 1.5 J/m^2 and 7.2 J/m^2, respectively. KT cells also appeared more susceptible to human interferon (HuIFN) preparations (α, β, γ and natural or recombinant) than did KS cells, as measured by cell colony formation ability, proliferation rates, and [^{3}H]deoxythymidine incorporation levels into acid-insoluble cell materials. The sensitivity of KT cells to UV and HuIFN was greater than that of human RSa cells, a cell line with high sensitivity to both agents. KT cells had more capacity for UV-induced DNA-repair replication synthesis than did RSa cells, the capacity being much the same as that of KS cells. There was no significant difference in levels of antiviral activity induced by HuIFN and binding capacity for ^{125}I-labeled IFN-αA between KT and KS cells. KT cells appeared refractory to cell proliferation inhibition by tumor necrosis factor (TNF) preparations.

Key words: UV — Interferon — Human cell

Mechanisms of the inhibition of cell proliferation by human interferon (HuIFN) are one of the most actively pursued aspects of cytokine research, but they have not been definitively elucidated.[1,2] Recently, we and others have reported that susceptibility of human cells to the anticellular effects of HuIFN correlated with radiosensitivity and/or DNA-repair capacity.[3,4] Up to the present, our experimental studies have focused on an increased resistance and unusually high sensitivity to the anticellular effect of HuIFN, found in 254 nm ultraviolet light (UV)-resistant transformed cell lines and sensitive fibroblast cells, respectively.[4–6] An increased UV-resistance was also found in transformed cell lines already reported to have HuIFN-resistance.[5]

Furthermore, we have found an intriguing action of HuIFN to enhance UV-induced activities such as DNA repair and plasminogen activator.[7] UV is one of the DNA-damaging agents which has been intensively studied to elucidate the cellular response mechanism.[8–11] Thus, comparative studies of UV and IFN actions seem of merit in investigating the relationship of cellular responses to the two agents. The establishment of cell lines suitable for such an investigation is important, but there has so far been no report of human cells originally established from tumor tissues which have distinctively high sensitivity to both agents. Nor has there been a comparative study on the susceptibility of human tumor-derived cell strains to other cytokine preparations and UV. Thus, establishment of a cell strain which has higher sensitivity to UV and HuIFN than cells already reported to be highly sensitive to both agents was attempted from malignant tumor tissues. Here, we present a cell strain, termed KT, which was established from a metastatic brain tumor and

which showed unusually high sensitivity to both HuIFN and UV, but not to tumor necrosis factor (TNF).

Materials and Methods

Cell Lines and Culture Conditions Culture medium was Eagle's minimal essential medium (EMEM) containing 20% heat-inactivated (56°, 20 min) fetal bovine serum and antibiotics (100 μg streptomycin/ml and 100 units penicillin G/ml). The explant method was used for culturing these specimens of the tumor and scalp, which were minced with scissors and cultured at 37° in a humidified atmosphere containing 5% CO_2. After two weeks of culture, one colony containing more than 50 cells was obtained and termed KT. Fibroblastic cells were obtained from a skin specimen of the scalp and termed KS. RSa and HEC-1 cells were described elsewhere.[5]

All the cell strains used here were free of mycoplasma infection, as determined by MYCOTRIM-TC (Hana Biologics Inc., USA).

Histology The patient had been operated for a left mammary tumor 17 months earlier. Tissue samples from the breast and brain tumor were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin (H-E). For electron microscopic study, the KT and KS cell colonies were embedded in Epon by a routine method and examined with a Hitachi D-12 electron microscope at 75 kV.

Reagents HuIFN and mock HuIFN preparations used here were described elsewhere.[4, 5, 12-15] Natural HuIFN preparations were used, unless otherwise stated. Recombinant TNF, with a specific activity of 2.9×10^6 units/mg by cytotoxicity assay using mouse L-M cells *in vitro*, was provided by Dainippon Pharmaceutical Co. Ltd., Osaka.[16] Other chemical agents and isotopes were purchased from the Nakarai Co. Ltd., Tokyo and the Japan Radioisotope Association, Tokyo, respectively.

Measurements of Cell Survival Conditions of UV-irradiation and colony survival assays were described elsewhere.[17] X-irradiation was carried out with a Hitachi X-ray source (MBR-1505R) operating at 150 kV and 5 mA with 0.1 mm Cu and 0.5 mm Al filters, at a dose rate of 50 rads/min. HuIFN sensitivity of cells was measured as described elsewhere.[6] In cell proliferation studies, culture of fibroblastic cells with low density is convenient as it allows observations for several days before control cell cultures reach saturation.[2, 5] Thus, KS fibroblastic cells were seeded with low density.

Measurements of [^{3}H]Deoxythymidine Incorporation Incorporation of [methyl-^{3}H]deoxythymidine ([^{3}H]dThd) (5 μCi/ml, 60 Ci/mmol, New England Nuclear, Boston) into acid-insoluble cellular materials was measured by pulse labeling, principally according to the method described elsewhere.[6]

Studies on DNA-repair Synthesis Levels of unscheduled DNA synthesis were measured by the direct scintillation counting method as described elsewhere.[17] The cellular DNA content was estimated as described.[17] UV-induced repair replication was estimated principally according to the method described before.[6] Briefly, irradiated or mock-irradiated cells were incubated with EMEM containing [^{3}H]dThd (10 μCi/ml, 20 Ci/mmol, New England Nuclear), 10μM 5-bromodeoxyuridine (BrdUrd), 1μM 5-fluorodeoxyuridine (FrdUrd) and 2.5mM hydroxyurea for 3.5 hr, and then lysed.[17] Cellular DNA was extracted from the cell lysate and analyzed by alkaline CsCl centrifugation.[17]

Assay of the Antiviral Effect of HuIFN The antiviral effect of HuIFN preparations was measured in a yield reduction assay with a strain of the Indiana serotype of vesicular stomatitis virus as described.[6]

Studies on Binding of ^{125}I-labeled HuIFN-αA and TNF to Cells Iodination of cytokines and binding assays were done according to the method described.[18] Scatchard analysis was done of the data obtained from saturation of ^{125}I-IFN-αA (1240 Ci/mmol) or ^{125}I-TNF (1550 Ci/mmol) binding to cells at 37° for 30 min, as described.[18]

Studies on Pregnancy-specific Glycoprotein (Schwangerschafts-protein 1; SP1). Cells, scraped off dishes with a piece of silicone rubber, were prepared for checking of SP1 by an immunoglobulin-enzyme bridge method, as described.[19]

Other Conditions Computerized tomography was performed by a CT-T8600 (Yokogawa Medical Systems, Tokyo). Chromosome analyses of cells were done by a modification of the method of Moorhead *et al.*[20] Results were expressed as the mean of values obtained from more than two independent experiments.

Results

Patient and Establishment of KT Cells Prevalent cell types (KT cells) obtained from a brain tumor are shown in Fig. 1A, in comparison with those (KS cells) from the skull skin of the patient (Fig. 1B). KT cells were smaller and rounder than the skin-derived cells, and exhibited properties characteristic of so-called transformed cell lines including higher saturation density. The tumor tissue, from which KT cells were derived, appeared as a ring-like region of density in the right frontal lobe in a

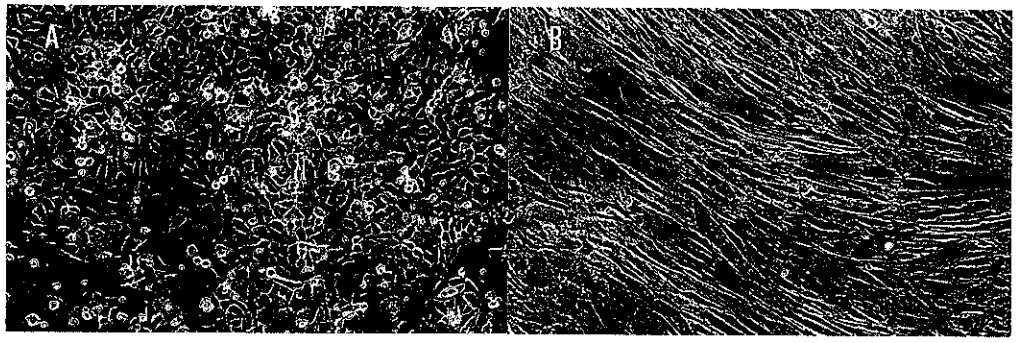

Fig. 1. Morphology of derived cells (phase-contrast photomicrographs). (A) Cells (KT) from brain tumor tissues. (B) Cells (KS) from the skull skin. ×100.

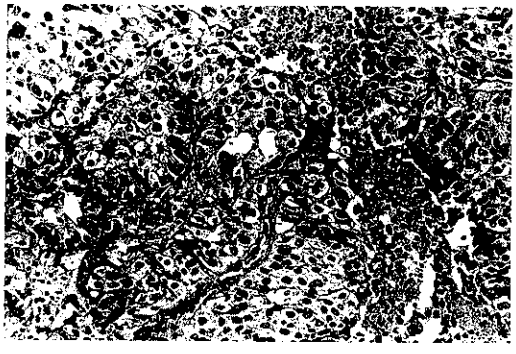

Fig. 2. Photomicrographs of a surgical specimen from tumor tissues. Brain tumor, revealing tumor cell cords with a duct-like structure (arrow). ×400, H-E.

computerized tomographic scan of the patient (data not shown). Light microscopy revealed cells which had round hyperchromatic nuclei and relatively large pale cytoplasm, and were arranged in cords and a gland-like structure (Fig. 2) as in the breast tumor of an invasive ductal carcinoma. Ultrastructurally, KT cells showed tight cell-to-cell attachments, occasional prominent nucleoli, large indented nuclei (Fig. 3A) and intracytoplasmic lumina with microvilli (Fig. 3B). KS cells had convoluted nuclei without any definite cell-to-cell attachments (data not shown). SP1 antibody showed reactivity with the KT cells, whereas it did not react with KS cells (data not shown).

KS cells could be serially subcultured and their morphology remained homogenous. However, KS cells did not replicate after 26 passages.

The modal chromosome number of KT cells was 90–93, greater than the 46 in KS cells.

Radiation Sensitivity Abnormal sensitivity of KT cells to UV-killing was found. As revealed by a cloning efficiency test (Fig. 4A), KT cells showed a lower colony-formation activity throughout the dose range tested as compared with UV-sensitive RSa cells. KS cells appeared much more refractory to UV than did KT and RSa cells, as also shown in Fig. 4 A. The D_0 value of KT was 1.5 J/m^2, smaller than those of RSa and KS cells, 1.9 J/m^2 and 7.2 J/m^2, respectively. On the other hand, KT cells showed a higher colony-formation activity after X-ray irradiation than did RSa cells, and also slight refractoriness, as did KS cells (Fig. 4B). D_0 values were 210 rads for KT, 120 rads for RSa and 190 rads for KS. In the colony formation assays, cloning efficiencies under non-irradiation conditions were 8–10% for KT and RSa cells, and 25–35% for KS cells.

When the repair synthesis activity of KT cells was compared with those of RSa and KS cells by measuring levels of UV-induced DNA-repair replication synthesis, the level of KT cells was about twice that of RSa cells but much the same as that of KS cells (Fig. 5).

Susceptibility to HuIFN Action Susceptibility of KT cells to HuIFN-α was compared

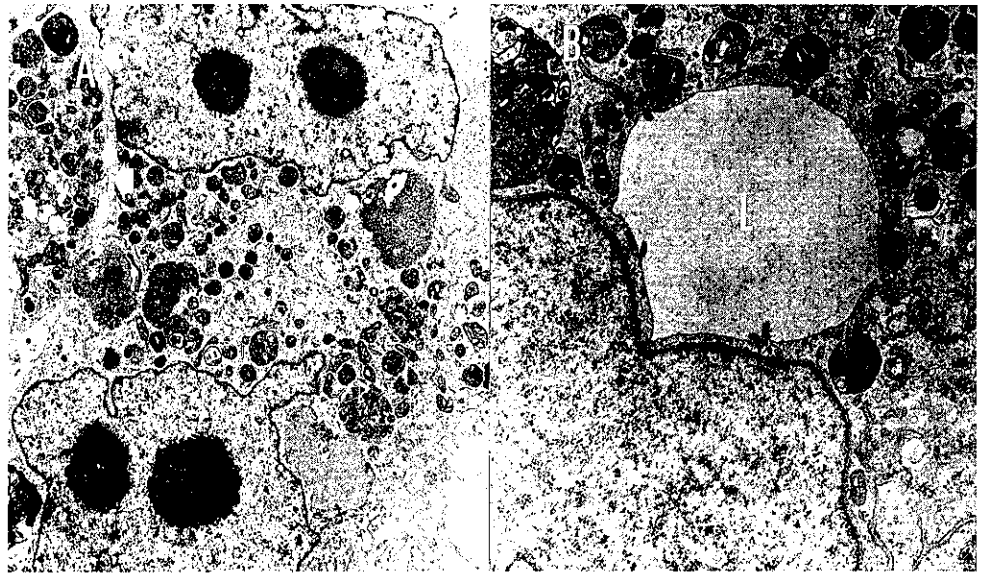

Fig. 3. Electron-micrographs of KT cells. (A) Cells which show a binucleated large cell with prominent nucleoli and tight cell-to-cell attachment (arrow). ×5000. (B) Note presence of intracytoplasmic lumen (L) with scant microvilli. ×8000.

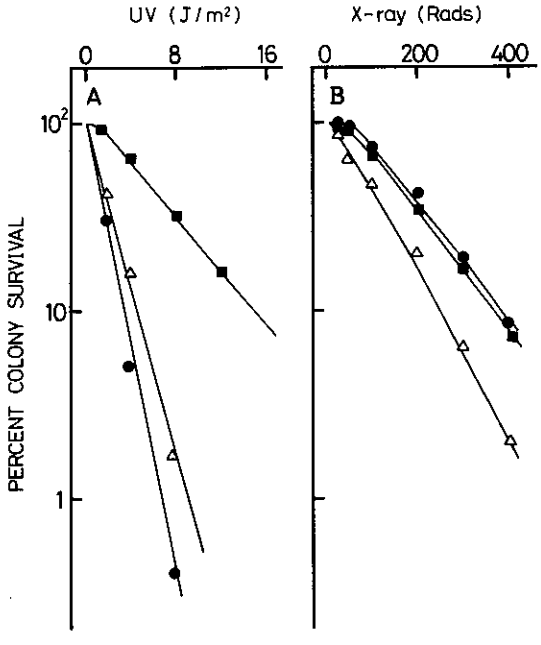

Fig. 4. Comparison of sensitivity to UV (A) and X-rays (B) in KT, KS and RSa cells. ●, KT cells; ■, KS cells; △, RSa cells.

with that of RSa cells, already reported to have unusually high sensitivity. Marked inhibition of KT cell proliferation was evident on continuous exposure to 500 units/ml HuIFN-α for 6 days, and was greater than the inhibition of RSa cells (Fig. 6A). Cloning efficiency of KT cells was impaired more than that of RSa cells at all concentrations examined (100–500 units/ml) (Fig. 6B). Tests of DNA synthesis inhibition by measuring [^{3}H]dThd incorporation into acid-insoluble materials further revealed that KT cells exposed to HuIFN-α for 2 days had less incorporation than did RSa cells (Fig. 6C), while cell growth and DNA synthesis of KS fibroblast cells were less impaired by the HuIFN-α exposure (Fig. 6).

Even when KS cells were seeded in combination with KT or HuIFN-resistant HEC-1 cells in the same dish, neither fibroblastic KS cells nor HEC-1 cells were impaired and cells of the two lines proliferated actively; but KT cells were impaired and their number decreased after continuous HuIFN-exposure (data not shown).

Exposure to recombinant HuIFN-α also inhibited KT cell replication to a greater extent than with RSa cells, but not KS cells (data not shown). Further, HuIFN-β inhibited cell replication of KT to a much greater degree than that of RSa cells, and slightly inhibited replication of KS cells (Fig. 7). HuIFN-γ also inhibited cell replication of KT cells more

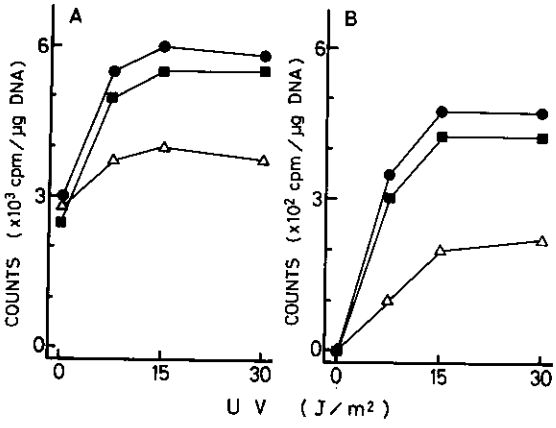

Fig. 5. Comparison of levels of unscheduled DNA synthesis (A) and DNA-repair replication synthesis (B) in UV-irradiated KT, KS and RSa cells. ●, KT cells; ■, KS cells; △, RSa cells.

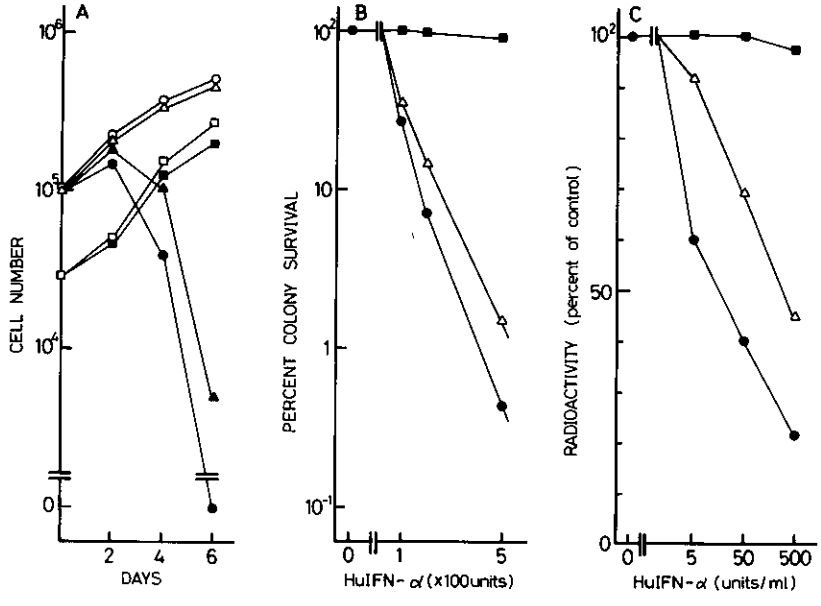

Fig. 6. Effects of HuIFN-α on cell replication during continuous exposure (A), colony formation after 2 days of exposure (B) and [^{3}H]dThd incorporation into acid-insoluble materials after 2 days of exposure (C). (A) ○, KT cells treated with mock HuIFN-α corresponding to 500 units/ml HuIFN-α; ●, 500 units/ml HuIFN-α-treated KT cells; □, KS cells treated with mock HuIFN-α corresponding to 500 units/ml HuIFN-α; ■, 500 units/ml HuIFN-α-treated KS cells; △, RSa cells treated with mock HuIFN-α corresponding to 500 units/ml HuIFN-α; ▲, 500 units/ml HuIFN-α-treated RSa cells. (B) and (C) ●, KT cells; ■, KS cells; △, RSa cells.

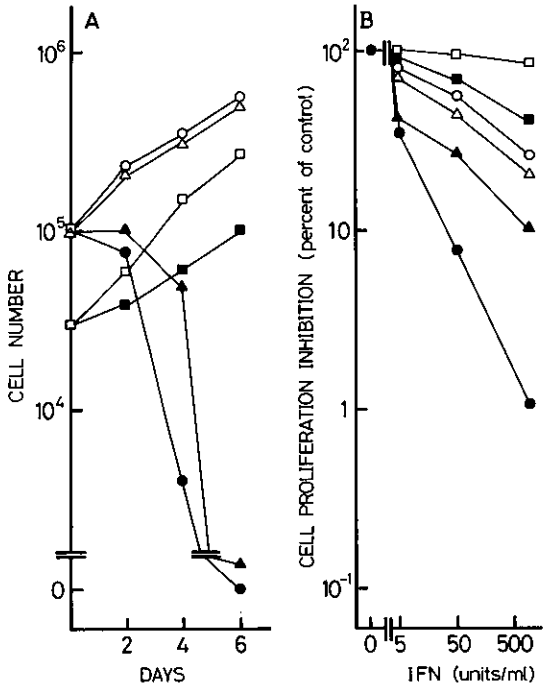

Fig. 7. Effects of continuous exposure to 500 units/ml HuIFN-β on cell replication (A), and the survival percentage ratio 4 days after exposure to HuIFN-β and to HuIFN-γ (B). (A) ○, KT cells treated with mock HuIFN-β corresponding to 500 units/ml HuIFN-β; ●, KT cells with 500 units/ml HuIFN-β treatment; □, KS cells treated with mock HuIFN-β corresponding to 500 units/ml HuIFN-β; ■, KS cells treated with 500 units/ml HuIFN-β; △, RSa cells treated with mock HuIFN-β corresponding to 500 units/ml HuIFN-β; ▲, RSa cells treated with 500 units/ml HuIFN-β. (B) ● and ○, KT cells treated with HuIFN-β and -γ, respectively; ■ and □, KS cells treated with HuIFN-β and -γ, respectively; ▲ and △, RSa cells HuIFN-β and -γ, respectively.

than it did that of RSa cells, although to a lesser extent than did HuIFN-β preparations when the corresponding antiviral activities were compared (Fig. 7B).

In contrast to the apparent discrepancy between KT and other cells in their susceptibility to cell growth inhibition by HuIFN, the level of antiviral activity of HuIFN-α in KT cells was not significantly different from those of RSa and KS cells (Table I). Analysis of the binding of ^{125}I-labeled HuIFN-αA showed the total number of binding sites per cell and the dissociation constant for KT cells to be almost the same as those for KS cells but smaller than those for RSa cells (Table II).

Susceptibility to TNF Cell proliferation of HuIFN-sensitive RSa was not affected by TNF preparations under the exposure conditions used (1–10^3 units/ml TNF for 10 days), whereas that of HuIFN-resistant HEC-1 was greatly affected (Fig. 8). KT cells appeared refractory to TNF, as did RSa cells (Fig. 8).

Analysis of the binding of ^{125}I-labeled TNF showed that the total number of binding sites per cell and the dissociation constant did not differ notably among the cells examined (Table III).

Table II. Estimated Number of HuIFN-αA-binding Sites

	Cell strain		
	KT	KS	RSa
Binding sites/cell	510	480	1100
Dissociation constant ($\times 10^{-10}M$)	7.3	7.0	8.7

Table I. Sensitivity of KT Cells to the Antiviral Action of HuIFN-α

	Titer of vesicular stomatitis virus (log TCID50/0.2 ml)			
Cells	Cont.	10 units/ml	100 units/ml	1000 units/ml
KT	7.5	3.8	2.5	1.7
KS	7.5	3.5	2.2	1.5
RSa	7.0	3.5	2.7	1.5

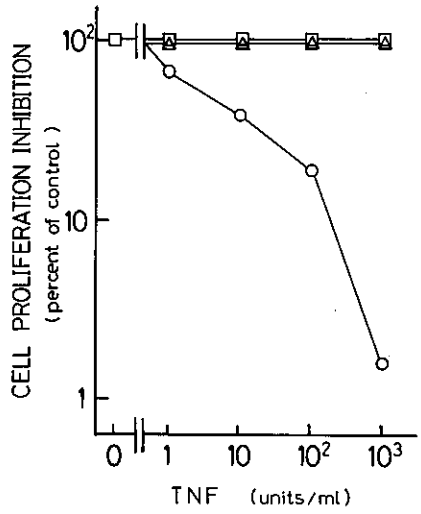

Fig. 8. Effects of continuous exposure to recombinant TNF for 10 days. □, KT cells; △, RSa cells; ○, HEC-1 cells.

Table III. Estimated Number of TNF-binding Sites

	Cell strain		
	KT	RSa	HEC-1
Binding sites/cell	290	370	260
Dissociation constant ($\times 10^{-11} M$)	4.0	3.7	3.1

Discussion

Since we preliminarily reported their establishment more than 3 years ago,[21] KT cells have continued to replicate, in contrast to the finite lifespan of KS fibroblastic cells. Furthermore, the KT cells have exhibited multilayering and criss-crossing in a continuous culture, distinct from contact-inhibited KS cells, and have formed colonies over monolayers of the KS cells. Such morphological characteristics, shown by electron microscopic observations (Fig. 3), and the presence of SP1 in KT cells, suggest that the KT cells are derived from cells which existed in tissues of the brain tumor, although the tumor tissues were not examined for tumor markers and KT cells have not formed tumors in mice to date. The brain tumor may have been a metastatic carcinoma because of its histological resemblance to breast cancer (Fig. 2). Reactivity with the antibody of SP1 was frequently detected in breast cancer[22] and intracytoplasmic lumina in KT cells seems to be consistent with one of the characteristics of breast carcinoma.[23] Therefore, KT cells might be derived from cells which were present in the metastatic carcinoma.

KT cells appeared more refractory to X-ray killing than did KS cells, whereas the former were more highly sensitive to UV killing and the anticellular effects of various kinds of HuIFN preparations (Figs. 4, 6 and 7). Brouty-Boye *et al.*[24] reported a decrease of IFN-sensitivity in association with X-ray-induced transformation in mouse cells. The present patient had received radiation therapy after removal of the mammary lesion, but no chemotherapy. Thus, the phenotype of the susceptibility of KT cells to UV and HuIFN might be due to modification in association with tumorigenic changes of normal tissue cells *in vivo* or that caused by therapeutic radiation.

Interferon - susceptibility varies among different cell types,[1-3] but in some cell strains there may be rules which govern this susceptibility.[5] The pair of KT and KS cells resembles the pair of RSa and UV^r-1 cells in the following points: difference in susceptibility to UV-killing and similarity in capacity of UV-induced DNA-repair replication synthesis (Fig. 5, Refs. 4 and 5). However, KT is an intriguing cell line because it has higher susceptibility to both UV (Fig. 4) and HuIFN (Figs. 6 and 7) than has RSa cells.

Induction of antiviral action by HuIFN and similar capacity of HuIFN-binding in KT cells to that in KS cells (Tables I and II) imply that the intracellular effects of HuIFN may differ in the two cell types, as described for RS and its UV-resistant variants which have different susceptibility to cell proliferation inhibition by HuIFN.[4, 6]

We earlier suggested that repair or related functions, except for excision-repair detected by DNA-repair replication assay, are responsible for the susceptibility to HuIFN.[4] As KT cells have normal capacity for DNA-repair replication, defects of the DNA-repair mechanism other than that detected by the DNA-repair assays used here might be one cause of

the abnormal sensitivity of these cells to the inhibitory effect of HuIFN on cell proliferation and DNA synthesis. We are now exploring human genes which can control HuIFN-susceptibility and we have recently transformed RSa cells into cells with increased resistance to HuIFN-α in association with UV^r-1 cells.[25]

The lack of correlation between TNF-susceptibility and TNF-binding capacity among KT, RSa and HEC-1 cells (Fig. 8 and Table III) may imply that the TNF-resistance of KT cells is due to mechanisms other than TNF-binding steps. KT cells also appeared refractory to X-rays (Fig. 4B). It has been suggested that UV- and X-ray response mechanisms differ.[11] Therefore, in addition to the relationship between UV- and HuIFN-susceptibility, comparative studies between X-ray- and HuIFN- or TNF-susceptibility are necessary. Further characterization of KT cells in comparison with RSa and HEC-1 cells may provide a model for investigating the rules underlying the difference in susceptibility of human cells to cytokines and radiation.

Acknowledgments

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