TECHNICAL REPORT
RERF TR 2-86
業 績 報 告 書

SENSITIVITY TO RADIATION OF HUMAN NORMAL, HYPERTHYROID, AND NEOPLASTIC THYROID EPITHELIAL CELLS IN PRIMARY CULTURE

機能亢進症，腫瘍及び正常のヒト甲状腺に由来する
上皮細胞の初代培養系における放射線感受性

RICHARD C. MILLER, Ph.D.
TOSHIO HIRAOKA, M.D. 平岡敬生
KENNETH J. KOPECKY, Ph.D.
NORI NAKAMURA, Ph.D. 中村 典
MICHAEL P. JONES, Ph.D.
TOSHIO ITO, M.D. 伊藤利夫
KELLY H. CLIFTON, Ph.D.

放影研
RERF

RADIATION EFFECTS RESEARCH FOUNDATION
財団法人 放射線影響研究所

A Cooperative Japan - United States Research Organization
日 米 共 同 研 究 機 関

RERF TECHNICAL REPORT SERIES
放影研業績報告書集

The RERF Technical Reports provide the official bilingual statements required to meet the needs of Japanese and American staff members, consultants, and advisory groups. The Technical Report Series is not intended to supplant regular journal publication.

放影研業績報告書は，日米研究職員，顧問，諮問機関の要求に応えるための日英両語による公式報告記録である．業績報告書は通例の誌上発表論文に代わるものではない．

The Radiation Effects Research Foundation (formerly ABCC) was established in April 1975 as a private nonprofit Japanese Foundation, supported equally by the Government of Japan through the Ministry of Health and Welfare, and the Government of the United States through the National Academy of Sciences under contract with the Department of Energy.

放射線影響研究所（元 ABCC）は，昭和50年4月1日に公益法人として発足したもので，その経費は日米両政府の平等分担により，日本は厚生省の補助金，米国はエネルギー省との契約に基づく米国学士院の補助金とをもって運営されている．

放影研 RERF RADIATION EFFECTS RESEARCH FOUNDATION
財団法人 放射線影響研究所

Research Project 研究課題 18-81

SENSITIVITY TO RADIATION OF HUMAN NORMAL, HYPERTHYROID, AND NEOPLASTIC THYROID EPITHELIAL CELLS IN PRIMARY CULTURE

機能亢進症，腫瘍及び正常のヒト甲状腺に由来する上皮細胞の初代培養系における放射線感受性

RICHARD C. MILLER, Ph.D[1a]; TOSHIO HIRAOKA, M.D. (平岡敬生)[1b]; KENNETH J. KOPECKY, Ph.D.[2]; NORI NAKAMURA, Ph.D. (中村 典)[1]; MICHAEL P. JONES, Ph.D.[c]; TOSHIO ITO, M.D. (伊藤利夫)[d]; KELLY H. CLIFTON, Ph.D.[b]

Departments of Radiobiology[1] and Statistics[2]

放射線生物学部[1]及び統計部[2]

SUMMARY

Samples of thyroid tissue removed surgically from 63 patients were cultured in vitro and X-irradiated to investigate the radiosensitivities of various types of thyroid epithelial cells. A total of 76 samples were obtained, including neoplastic cells from patients with papillary carcinoma (PC) or follicular adenoma (FA), cells from hyperthyroidism (HY) patients, and normal cells from the surgical margins of PC and FA patients. Culturing of the cells was performed in a manner which has been shown to yield a predominance of epithelial cells. Results of colony formation assays indicated that cells from HY and FA patients were the least radiosensitive: when adjusted to the overall geometric mean plating efficiency of 5.5%, the average mean lethal dose D_0 was 97.6 cGy for HY cells, and 96.7 cGy and 94.3 cGy, respectively, for neoplastic and normal cells from FA patients. Cells from PC patients were more radiosensitive, normal cells having an adjusted average D_0 of 85.0 cGy and PC cells a significantly ($p=.001$) lower average D_0 of 74.4 cGy. After allowing for this variation by cell type, in vitro radiosensitivity was not significantly related to age at surgery ($p=.82$) or sex ($p=.10$). These results suggest that malignant thyroid cells may be especially radiosensitive.

要 約

63人の患者から得た甲状腺の手術摘出組織を，試験管内培養した後X線照射して，種々の疾病状態にある甲状腺上皮細胞の放射線感受性を調べた．合計76例の試料が得られ，それらには乳頭癌(PC)患者，あるいは濾胞腺腫(FA)患者由来の腫瘍細胞，甲状腺機能亢進症(HY)患者由来の細胞，及びPC，FA患者の摘出組織周辺部より得た正常細胞が含まれる．細胞の培養は，主に上皮細胞を生じることの明らかにされている手法に従った．コロニー形成法による実験の結果，HY及びFA患者由来の細胞は最も放射線に対する感受性が低いことが分かった．コロニー形成率を相乗平均の5.5%に補正したとき，平均致死線量 D_0 はHY患者由来細胞では97.6cGy，FA患者由来腫瘍細胞では96.7cGy，正常細胞では94.3cGyであった．PC患者の細胞は放射線感受性が高く，正常細胞は補正平均 D_0 値が85.0cGy，またPC細胞は統計的に有意に低い($p=.001$)平均 D_0 値74.4cGyを示した．細胞の種類によってこれらの変動があることを考慮すると，試験管内の放射線感受性は手術時年齢($p=.82$)あるいは性($p=.10$)により影響を受けないことが示された．これらの結果は，甲状腺悪性腫瘍細胞は放射線に特に感受性が高いかもしれないことを示唆している．

a. Radiation Research Laboratory, Columbia University; b. Department of Human Oncology and Radiology, University of Wisconsin; c. Department of Biostatistics, University of Washington; d. Second Department of Surgery, Hiroshima University School of Medicine

a. Columbia 大学放射線研究所 b. Wisconsin 大学人類腫瘍学及び放射線学部 c. Washington 大学生物統計学部 d. 広島大学医学部第二外科学教室

Approved 承認 26 March 1986

Printed 印刷 September 1986

INTRODUCTION

That exposure of the thyroid gland to ionizing radiation increases the risk of thyroid cancer has been confirmed in studies of atomic bomb survivors in Hiroshima and Nagasaki,[1,2] of patients therapeutically exposed to X rays for various medical conditions,[3-13] and of persons exposed to radioactive fallout in the Marshall Islands.[14,15] Recent technical advances in the in vitro culturing of human epithelial cells[16] have made it possible to study the effects of radiation at the cellular level. It has recently been reported[17] that the colony-forming ability of normal human thyroid cells in vitro may be somewhat more sensitive to impairment by X-irradiation than those of human mammary epithelial cells, liver parenchymal cells, or fibroblast cells from various organs.[18-21] However, little has been reported about the radiosensitivities of neoplastic thyroid cells or hyperthyroid cells.[16] Tissue scarcity and limited success with primary culture methods have in the past restricted such comparative studies of human tissue. This report describes the cell survival dose responses of normal, cancer, adenoma, and hyperthyroid cells from the surgically resected thyroids of 63 individuals.

MATERIALS AND METHODS

Cell Origin and Culture Method

Thyroid tissue was obtained from patients undergoing partial or total resection of the thyroid for PC, FA, or HY at the Second Department of Surgery, Hiroshima University School of Medicine. Normal cells were obtained from the margins of the excised tissue of patients with tumors. None of the PC or FA patients received therapy prior to surgery. For approximately one year prior to surgery, HY patients received methylmercaptoimidazole so that serum concentrations of thyroid hormones were within normal limits.

Cell culture methods are described in detail elsewhere.[16] Briefly, excised thyroid tissue was minced and then incubated at 37°C for two hours in collagenase (300 U/ml). Next, the suspension of cells was passed through a coarse nylon filter (53 μm average pore size) to remove undigested stromal tissue and large cell aggregates. The filtered suspension was then centrifuged at 1,200 rpm for 10 minutes after which the supernatant was discarded and the pellet of

緒　言

広島・長崎の原爆被爆者,[1,2] 種々の病気の治療のためX線照射を受けた患者,[3-13] 及び Marshall 諸島で放射性降下物に被曝した人々[14,15]に関する調査により，甲状腺が電離放射線に被曝した場合，甲状腺癌の危険率が増加することが確認されている．ヒト上皮細胞の試験管内培養に関する最近の技術的進歩[16]により，細胞レベルで放射線の影響を調査することが可能になった．試験管内のヒト正常甲状腺細胞は，ヒト乳房上皮細胞，肝実質細胞，様々な臓器の線維芽細胞[18-21]に比べて，X線照射によるコロニー形成能力の障害を幾分受けやすいかもしれないことが最近報告されている．[17] しかし，甲状腺腫瘍細胞又は甲状腺機能亢進症細胞の放射線感受性についてはほとんど報告されていない．[16] 組織の不足及び初代培養法がうまくゆかないことが原因で，今までこのようなヒト組織の比較研究は十分行われてこなかった．本報では，63人の手術摘出甲状腺から得た正常細胞，癌細胞，腺腫細胞，及び甲状腺機能亢進症細胞の細胞生存能力に関する線量効果反応について述べる．

材料及び方法

細胞の入手源及び培養方法

広島大学医学部第二外科において乳頭癌，濾胞腺腫，甲状腺機能亢進症の治療のため甲状腺の一部，又は全部の摘出を行った患者から甲状腺組織を入手した．正常細胞は腫瘍患者の摘出組織周辺部から入手した．乳頭癌又は濾胞腺腫患者のうち，手術以前に治療を受けていた者はいなかった．手術までの約1年間，甲状腺機能亢進症患者はメチルメルカプトイミダゾールを服用していたので，甲状腺ホルモンの血清濃度は正常な範囲内であった．

細胞の培養方法はほかの報告[16]で詳細に述べられている．簡単に述べると，切除した甲状腺組織を細切した後，コラゲナーゼ（300U/ml）中で2時間，37°Cで処理した．次に，この懸濁液を粗いナイロンのフィルター（孔の大きさは平均53μm）にかけ，未消化の間質組織及び大きな細胞塊を除去した．フィルターを通した懸濁液を10分間，1,200rpmで遠心分離した後，上清液を捨て，底に残った細胞を培地で再懸濁

cells resuspended in medium. Complete medium for primary cultures and experimentation consisted of equal parts of Ham's F-12 and minimal essential medium (α-MEM) supplemented with 2.5% fetal bovine serum and six growth factors (insulin, hydrocortisone, transferrin, glycyl-1-histidyl-L-lysine acetate, somatostatin, and epidermal growth factor). Glucose (1.5g/liter) was added to insure a sufficient nutrient supply, and penicillin and streptomycin were added to prevent bacterial contamination. After allowing for cell attachment, dishes were washed with phosphate buffer solution and filled with fresh complete medium for cells to grow from four to six days. Measurements of thyroid hormone levels in the medium and thyroid stimulating hormone (TSH)-stimulated cyclic adenosine monophosphate (cAMP) of cells have been used to verify the culture of thyroid cells.[16]

Irradiation Procedure

Cells at first passage were used in all experiments reported here. Approximately 18 hours before irradiation, subconfluent primary culture cells were trypsinized for five minutes in 0.1% trypsin with 0.01% ethylenediamine tetraacetic acid (EDTA). Complete medium was added and the suspension of single cells was centrifuged for five minutes at 1,200 rpm to remove the trypsin solution from the cells. Cell density was determined with a hemocytometer, and cells were plated into sets of four replicate 60 mm diameter plastic culture dishes per radiation dose group in sufficient numbers to yield approximately 40 colonies per dish. After the 18-hour incubation at 37°C, the cells were irradiated at room temperature with a Softex X-ray machine operated at 40 kVp and 5 mA with 0.2 mm aluminum external filtration (7.70 Gy/min calculated dose rate). Experiments included nonirradiated controls and at least six exposure doses ranging from 37 to 744 cGy of X rays. The population doubling time of all cell types ranged from 32 to 36 hours and the multiplicity at the time of irradiation was less than 1.01 under the present experimental conditions. No feeder cells are now used because early studies revealed no improvement of plating efficiency (PE) with up to 4×10^4 X-ray-inactivated human skin fibroblast cells per dish. No more than 4×10^4 thyroid cells were seeded into each dish.

した．初代培養及び実験のための完全培地は，最少必須培地（α-MEM）とそれと同量の Ham F-12 に2.5%の胎牛血清及び六つの成長因子（insulin, hydrocortisone, transferrin, glycyl-1-histidyl-L- lysine acetate, somatostatin, 及び上皮成長因子）を加えたものである．更に glucose（1.5g/ℓ）を加えて栄養の供給を十分に行い，細菌感染を防ぐために penicillin 及び streptomycin を添加した．細胞を付着させた後，培養皿をリン酸緩衝液で洗浄し，新鮮な完全培地を加え，細胞を4日ないし6日間増殖させた．甲状腺細胞が培養されていることの確認のため，培地中の甲状腺ホルモン水準及び甲状腺刺激ホルモン（TSH）の刺激による細胞内サイクリック AMP（cAMP）の測定を行った．[16]

照射方法

本報に述べたすべての実験において，第一継代細胞を用いた．照射の約18時間前，初代培養系のコンフルエントに達していない細胞を，0.01%のエチレンジアミン四酢酸（EDTA）を含む0.1%のトリプシンで5分間処理した．完全培地を加え，単細胞の懸濁液を1,200rpm で5分間遠心分離し，細胞からトリプシン溶液を除去した．血球計で細胞濃度を測定し，直径 60mm のプラスチック製培養皿を各線量群当たり4枚ずつ用いて，1皿当たり約40個のコロニーが生じるのに十分な細胞を播種した．37°C で18時間培養した後，40kVp, 5mA の条件で 0.2mm アルミニウム製外部フィルタを装着した Softex X線装置を用いて，室温で細胞を照射した（算定線量率 7.70Gy/min）．照射を受けない対照群と X 線量 37～744cGy の最低6照射群について実験を行った．今回の実験条件では，すべての種類の細胞の倍加時間は32～36時間であり，照射時の multiplicity はもとの1.01未満であった．初期の調査の結果，各皿に X 線により不活性化した最高 4×10^4 個のヒト皮膚線維芽細胞を用いてもコロニー形成率（PE）に変化がみられなかったので，現在 feeder 細胞は用いていない．各皿1枚につき 4×10^4 個以上の甲状腺細胞は播種していない．

Accurate dosimetry of soft X rays was accomplished using Nuclear Associates Type 30-330 PTW, Exrodin Type 2A and Shonka-Wyckoff ionization chambers, and a Keithley Model 602 electrometer. Additionally, thermoluminescent (BeO) chip dosimeters were used to verify dose measurements at the location of cell attachments on the dishes. Beam quality was measured using aluminum absorbers. Dose rate calculations were made based on the identical physical parameters encountered during cell irradiations.

Nuclear Associates Type 30-330 PTW, Exrodin Type 2A 及び Shonka-Wyckoff 電離箱，並びに Keithley Model 602 電位差計を用いて軟X線の正確な線量測定を行った．更に，培養皿の細胞付着部分における線量測定の正確性を確認するために，熱ルミネッセンス(BeO)チップ線量計を用いた．アルミニウム吸収板を用いて線質を測定した．細胞照射と同一の物理学的パラメーターに基づいて線量率を計算した．

To facilitate irradiation, dishes contained only 0.5 ml of medium during the irradiation period. An identical procedure, omitting only irradiation, was followed for nonirradiated control cells. After a two-week incubation period in 5% CO_2-95% air at 37°C, the cells were fixed in formalin and stained with Giemsa. Reproductively viable cells were determined as those that had developed into macroscopic colonies containing at least 50 cells. Because the penetrability of 40 kVp X rays is poor and the meniscus at the edge of the dishes certainly resulted in a reduced amount of radiation dosage, colonies at the edge of all dishes were not scored.

照射を容易にするために，照射時に培地を0.5mlだけ培養皿に残した．非照射対照群の細胞については，照射だけを行わないで，その他は同じ手順を踏んだ．5% CO_2-95% 空気中で37°Cで2週間培養した後，細胞をホルマリンで固定しギムザで染色した．少なくとも50個の細胞を含み，肉眼で観察できるコロニーを形成した細胞を増殖能力のある細胞とした．40kVpのX線の浸透性は弱く，培養皿の周縁部が受ける放射線量は確実に低くなるので，すべての培養皿の周縁にあるコロニーは計数対象から除外した．

Statistical Analysis

For each assay the nonirradiated control cell PE was calculated as the number of colonies observed, C_0, divided by the number of cells plated, N_0. The surviving fraction (SF) at each dose D was then calculated as

統計的解析

各解析においては，まず非照射対照細胞について観察されたコロニー数 C_0 を，播種した細胞数 N_0 で割ることによりPEを算出した．次に，各線量Dの生存率(SF)を次の式から求めた．

$$SF_D = C_D/(N_D \times PE) \quad . \qquad [1]$$

Estimates of the mean lethal dose, D_0, and extrapolation number, n, were obtained by fitting the single-hit multitarget model

単一ヒット多重標的モデル〔2〕に適合させることにより，平均致死線量 D_0 及び外挿数 n の推定値を最尤法により求めた．

$$S(D) = 1 - [1 - \exp(-D/D_0)]^n \quad , \qquad [2]$$

using the method of maximum likelihood, assuming the colony counts are realizations of Poisson random distribution. Comparisons of radiosensitivity according to patient's disease, cell type, sex, and/or age were based primarily

コロニー数はポアソン無作為分布が具現したものであるとみなした．患者の疾病，細胞の種類，性，又は年齢別の放射線感受性の比較は，主として分散及び

on analyses of variance and covariance and linear regression analyses of the logarithms of the cell survival parameter estimates. "Average" values of D_0 or n produced by these analyses correspond to geometric rather than arithmetic means. In comparative analyses, each value of $\log(D_0)$ or $\log(n)$ was given a weight inversely proportional to the square of its estimated standard error, to reduce the possibility that comparisons among experiments might be distorted by the least precisely estimated values.

RESULTS

Radiosensitivity assays were performed using 76 samples of cells obtained from 63 patients (54 female, 9 male). Table 1 shows the distribution by sex, patient disease, and cell type, and the ranges and mean values of age at surgery for all experiments. Primary cultures of adenoma cells frequently failed to be established under the present experimental conditions by some unknown reasons. As expected, the HY patients were generally younger than those with neoplastic diseases. The 76 experiments in Table 1 include 26 which used autologous normal and neoplastic cells from eight PC patients and five FA patients; detailed analysis of these 13 cases is given elsewhere.[22]

共分散の分析，並びに細胞生存パラメーター推定値の対数の線形回帰解析に基づいて行った．これらの解析によって得られた D_0 又は n の〝平均〟値は，相加平均ではなく，むしろ相乗平均である．比較解析においては，適合させた曲線から大きく外れた推定値によって実験間の比較に歪みが出る可能性を少なくするために，$\log(D_0)$ 又は $\log(n)$ の各値にその推定標準誤差の2乗に反比例する加重値を与えた．

結　果

63人の患者（女性54人，男性9人）から得た76例の細胞を用いて放射線感受性の測定を行った．表1は性，疾病及び細胞の種類別の分布，並びにすべての実験における手術時年齢の範囲及び平均値を示す．本実験条件では，その理由は不明であるが，腺腫細胞の初代培養系の樹立はしばしば不成功に終わっている．予想されたとおり，甲状腺機能亢進症患者は一般的に腫瘍患者より年齢が低かった．表1に示した76回の実験の中の26回は，乳頭癌患者8例及び濾胞腺腫患者5例から得た同じ個体由来の正常細胞及び腫瘍細胞の実験である．これら13例の詳細な解析は別報に述べられている．[22]

TABLE 1　DISTRIBUTION OF EXPERIMENTS AND AGES AMONG 54 FEMALE AND 9 MALE PATIENTS WITH THYROID DISEASE

表1　女性54人及び男性9人の甲状腺疾病患者の実験及び年齢分布

Patient disease	Cell type	Number of experiments			Patient age at surgery		
		Female	Male	Total	Minimum	Mean	Maximum
PC	Normal	10	5	15	31	51.0	75
PC	Neoplastic	13	2	15	31	51.0	75
FA	Normal	15	0	15	15	42.5	68
FA	Neoplastic	7	0	7	34	50.4	68
HY	Hyperthyroid	21	3	24	15	28.9	53
All	All	66	10	76	15	41.2	75

PC = Papillary carcinoma, FA = Follicular adenoma, HY = Hyperthyroidism

PC＝乳頭癌，FA＝濾胞腺腫，HY＝甲状腺機能亢進症

Fourteen samples of cells were assayed in duplicate, one in triplicate, and one sample was used in four replications; the remaining 60 samples were each assayed only once. It has been shown[16] that replicate experiments using cells in first passage produce consistent results if performed within a period of a few days. Since 11 of the 16 sets of replicated assays were performed within periods of two days or less, and since the aim of the present report is to examine differences between cell types rather than between replications, common estimates of D_0 and n were calculated for each set of replications.

Figures 1a-e display the fitted cell survival curves for each of the 76 samples. This display of multiple survival curves in the panels of Figure 1 portrays two kinds of variability that exist in the data: the variability among experiments using different cell strains of a common type (i.e., within single panels) which arise from experimental "noise" and from the natural variation of radiosensitivity among cells from different persons; and any heterogeneity among the five types of cells (i.e., between panels). The relative magnitudes of these two kinds of heterogeneity would be quite difficult to discern if each survival curve were plotted in a separate panel, as is possible and commonly done in reports involving much smaller numbers of experiments. However, the display of individual data points in Figure 1 is impractical, because of the large number of assays. Therefore, the goodness of fit of the 76 survival curves in Figure 1 is illustrated in Figure 2, which is a histogram of the ratios of the observed SFs (SF_D from equation [1]) to the corresponding fitted values based on the estimates of D_0 and n calculated for each experiment (S(D) from [2]). For 438 (74%) of the 592 SFs that were calculated, the observed value is between 75%-125% of the fitted value, and for only 30 (5%) do the two estimates differ by more than a factor of two. With the exception of five instances for which the observed SF was zero, i.e., for which no colonies were observed, the observed and fitted SFs always differed by less than an order of magnitude. Results for all cell types are combined in Figure 2, since a similar pattern occurred for each cell type. It is apparent from Figure 2 that the single-hit multitarget model [2] provides an adequate fit to the data.

14例の細胞は2回，1例は3回，1例は4回反復実験し，残り60例の細胞は各々1回ずつ測定した．第1継代の細胞を用いて反復実験を2～3日以内に行えば，一貫した結果が得られることが示されている．[16] 16組の反復測定のうち11組は2日以内に実施した．本報の目的は反復実験間の差異ではなく，細胞の種類による差異を調査することなので，反復実験の各々について D_0 及び n の共通推定値を算出した．

図1a-eは，76例の細胞についての適合細胞生存曲線を示す．図1の中の多数の生存曲線からデータには2種類の変動要因が含まれることが分かる．その一つは，同一種類（すなわち，同一パネル中）の異なる細胞株を用いて実験を行う際に，実験ノイズ及び異なる対象者から得た細胞間の放射線感受性の自然な差異によって起こる実験間の変動性であり，他の一つは，5種類の細胞間（すなわち，パネル間）の異質性である．もしも，個々の生存曲線を別々の図に示そうとすると，これら2種類の変動性の相対的な大きさを判別することは極めて困難となる．しかし，数の少ない実験の場合にはそのような表示も可能であるが，本報告の場合には測定の回数が多いため，個々のデータ値を図1に表示することは不可能である．したがって，図1の生存曲線76本の適合度を図2に示す．これは，観察された生存率（方程式〔1〕から得られる SF_D）と各実験について算出した D_0 及び n の推定値に基づく対応する適合値（〔2〕から得られる S(D)）の割合を表す柱状グラフである．算出した生存率592個のうち438個（74%）については，観察値は適合値の75%～125%であり，30個（5%）についてのみ，この二つの推定値のうちの一つが他の2倍を超えていた．観察された生存率がゼロ，すなわちコロニーが観察されなかった5例を除き，生存率の観察値と適合値の差は常に一桁未満であった．各種類の細胞について類似するパターンが認められたので，全種類の細胞に関する結果を図2にまとめた．図2から，多重標的単一ヒットモデル〔2〕がデータによく適合することは明らかである．

Figure 1. X-ray dose-response cell survival curves for human thyroid epithelial cells in primary culture. Each curve extends to the maximum dose for its experiment(s). a. Normal cells from patients with papillary carcinoma (15 experiments). b. Papillary carcinoma cells (15 experiments). c. Normal cells from patients with follicular adenoma (15 experiments). d. Follicular adenoma cells (7 experiments; arrow indicates one experiment which included maximum X-ray dose of 10.49 Gy). e. Hyperthyroid cells (24 experiments).

図1　初代培養系のヒト甲状腺上皮細胞のX線線量と細胞生存率の反応．各曲線はその実験の最高線量まで描かれている．a．乳頭癌患者由来の正常細胞（実験15回）．b．乳頭癌細胞（実験15回）．c．濾胞腺腫患者由来の正常細胞（実験15回）．d．濾胞腺腫細胞（実験7回．矢印は，その実験で最高X線量10.49Gyを照射したことをす）．e．甲状腺機能亢進症患者由来の細胞（実験24回）．

Figure 1a

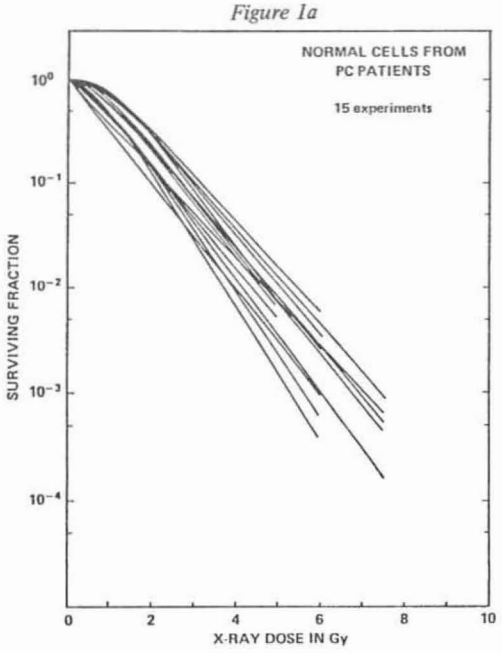


Figure 1b

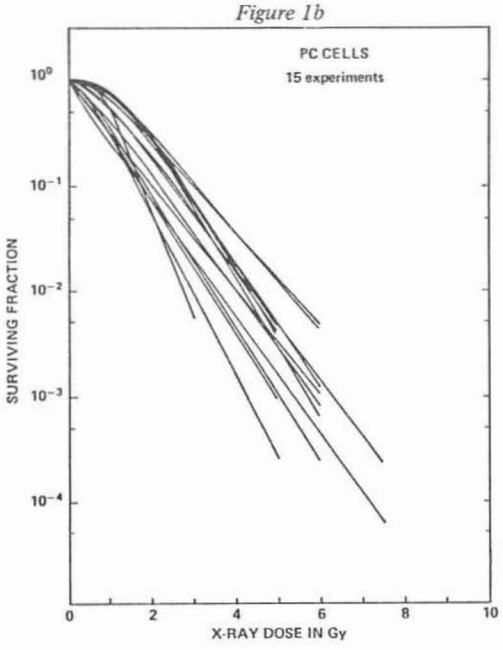


Figure 1c

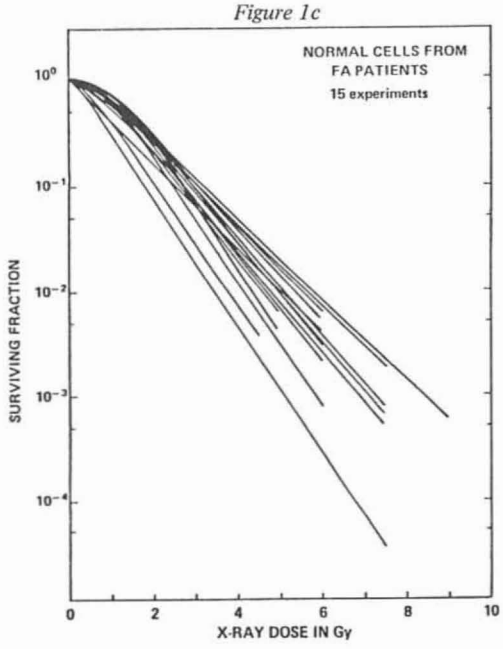


Figure 1d

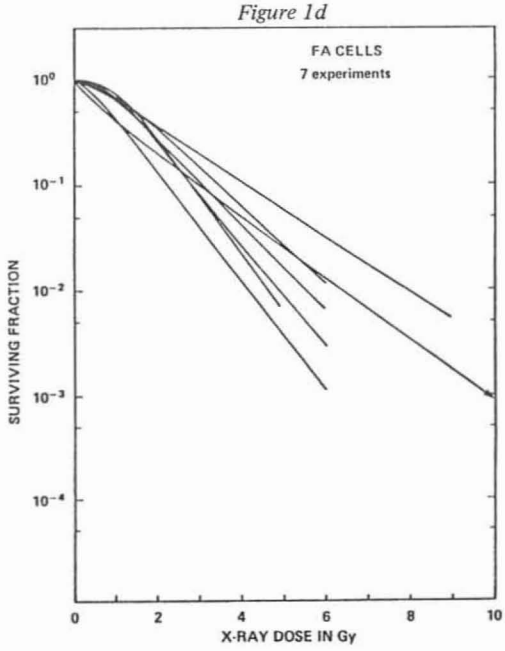


Figure 1e

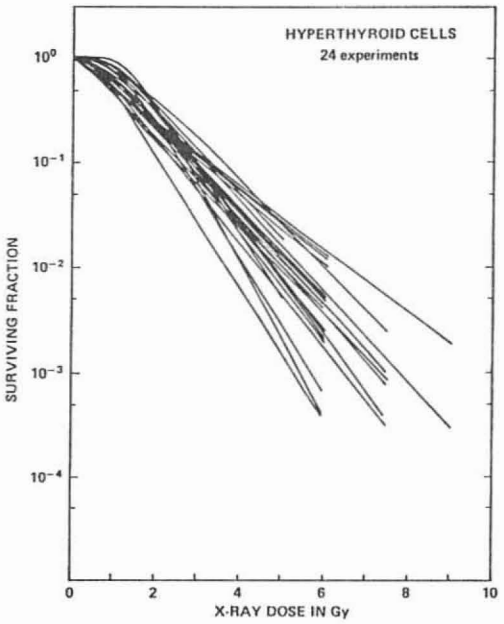


Figure 2. Histogram of ratio of observed [1] to fitted [2] values of surviving fraction, based on all experiments. Actual frequencies are given in the figure for all 592 ratios.

図2　すべての実験に基づいた生存率の適合値〔2〕に対する観察値〔1〕の比率を表すヒストグラム．592個の比率すべてについて実際の頻度を数字で示す．

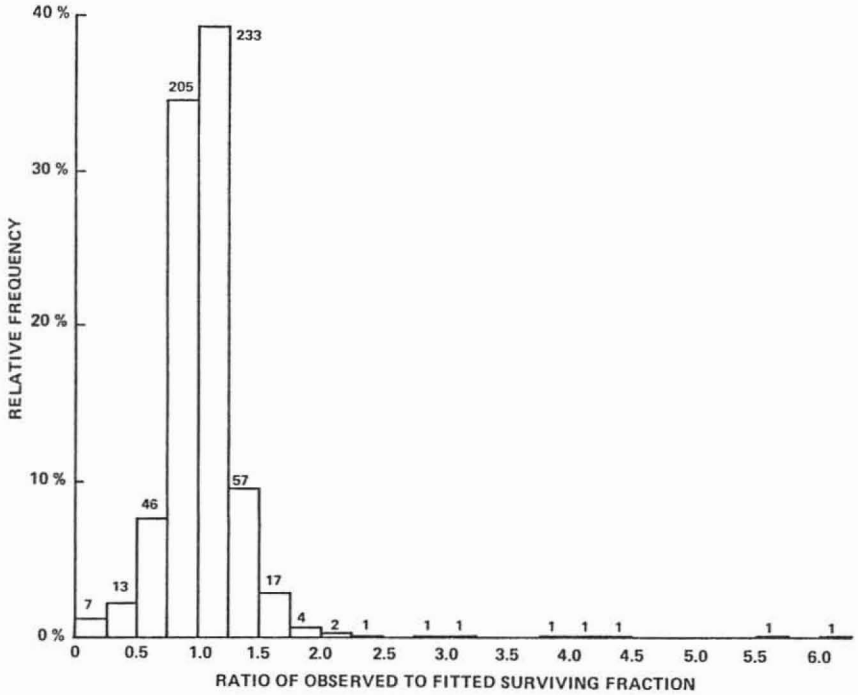
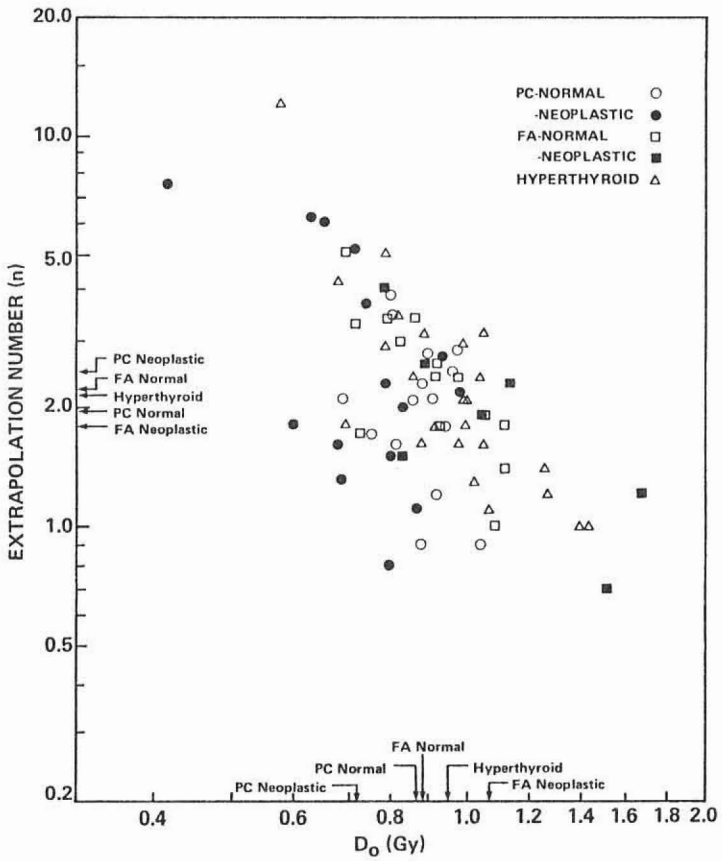


Figure 3 displays the estimates of the cell survival parameters D_0 and n for each of the 76 samples. There is substantial overlapping in Figure 3 among the five types of cells considered, but the heterogeneity of D_0 among the five cell types is nevertheless highly significant ($p<.001$). All experiments with PC cells yielded values of D_0 less than 100 cGy. For ease of comparison, the unweighted, unadjusted geometric mean values of D_0 and n for each cell type are indicated at the margins of Figure 3. From Figures 1b and 3 it is apparent that one experiment with PC cells yielded an exceptionally low D_0 value of 41.1 cGy. It is important to note that this individual point does not unduly influence the comparison among the five cell types: with that point eliminated the heterogeneity of D_0 among cell types is still significant ($p=.004$).

図3は76例の細胞の各々に関する細胞生存パラメーター D_0 及びnの推定値を示す．図3では，対象にした5種類の細胞間にかなりの重複が見られるが，それにもかかわらずこの5種類の細胞の D_0 の異質性は極めて有意である（$p<.001$）．乳頭癌細胞を用いたすべての実験においては，D_0 値が100cGy未満であった．比較を容易にするために，各種類の細胞について D_0 及びnの加重・補正されていない相乗平均値を図3の端に示した．図1b及び3によると，乳頭癌細胞を用いた1回の実験では，例外的に低い D_0 値 41.1cGy が得られた．この値が5種類の細胞間の比較に過度の影響を及ぼさないように留意することは重要である．この値を除いた場合も，細胞の種類間の D_0 の異質性は依然有意である（$p=.004$）．

Figure 3. Cell survival parameter estimates D_0 and n for 76 experiments. Unweighted geometric mean values (not adjusted for PE) are indicated at the margins.

図3　76回の実験についての細胞生存パラメーター推定値 D_0 及びn．非加重相乗平均値（PE について補正しない）を図の端に示す．



Among all 76 experiments, there is a moderately strong positive association between PE and the cell survival parameters: for log(PE) and log(D_0), Pearson's coefficient of correlation is r=0.22 (one-tailed p=.03); for log(PE) and log(n), r=0.13 (p=.14). Therefore, to compare the survival parameters among the groups, the weighted geometric mean values of D_0 and n, adjusted for regression on log(PE) are shown in Table 2; the corresponding survival curves are illustrated in Figure 4. (The results in Table 2 and Figure 4 are all calculated for the overall mean PE of 5.5%) The effect of weighting is most apparent for neoplastic cells from FA patients (Table 2). This group has the largest unweighted geometric mean D_0, 1.07 Gy (Figure 3); however, its adjusted and weighted mean, 0.97 Gy, is slightly lower than that for HY cells. This occurs because the two largest values of D_0 from the seven experiments with FA cells were estimated with quite large standard errors. Nevertheless, the average survival curve for neoplastic cells from FA patients lies above that for HY cells over the entire dose range 0-6 Gy even when the adjustment for correlation with PE is included (Figure 3). After allowing for the marginally significant (p=.06) variation of D_0 among HY, PC (normal and neoplastic combined), and FA (combined), there is a highly significant difference between the average D_0 values of neoplastic and normal cells from PC patients (74.4 and 85.0 cGy, respectively; one-tailed p=.001), but not between those of FA patients (p=.36).

76回の実験すべてについて，PEと細胞生存パラメーターの間に比較的強い明確な関連が認められる．log(PE)とlog(D_0)についてPearsonの相関係数はr=0.22（片側検定p=.03）であり，log(PE)とlog(n)についてはr=0.13(p=.14)であった．したがって，各群間の生存パラメーターを比較するために，log(PE)の回帰について補正したD_0及びnの加重相乗平均値を表2に示し，その対応する生存曲線を図4に示す（表2及び図4の結果はすべて，PEの全体平均値5.5%について算出したものである）．加重の影響は，濾胞腺腫患者由来の腫瘍細胞について最も顕著である（表2）．この群のD_0の非加重相乗平均は最も大きく1.07Gyであった（図3）．しかし，それを補正・加重した平均値0.97Gyは甲状腺機能亢進症患者由来の細胞のものよりやや低い．これは，濾胞腺腫患者由来の細胞を用いた7回の実験で得られたD_0値の中の，最も高い2個に極めて大きい標準誤差があるからである．しかし，濾胞腺腫患者由来の腫瘍細胞の平均生存曲線は，PEとの相関について補正を行った場合でも，0～6Gyの線量域全体において甲状腺機能亢進症患者由来の細胞の平均生存曲線の上方にある（図3）．甲状腺機能亢進症，乳頭癌（正常及び腫瘍細胞の合計），及び濾胞腺腫（合計）には，わずかに有意な（p=.06）D_0変動性があり，それを考慮すると，乳頭癌患者由来の腫瘍及び正常細胞との平均D_0値には極めて有意な差異が認められるが（各々74.4cGy及び85.0cGy；片側検定p=.001），濾胞腺腫患者の場合は有意ではない（p=.36）．

TABLE 2 PLATING EFFICIENCY (PE) AND AVERAGE CELL SURVIVAL PARAMETERS BY PATIENT DISEASE AND CELL TYPE

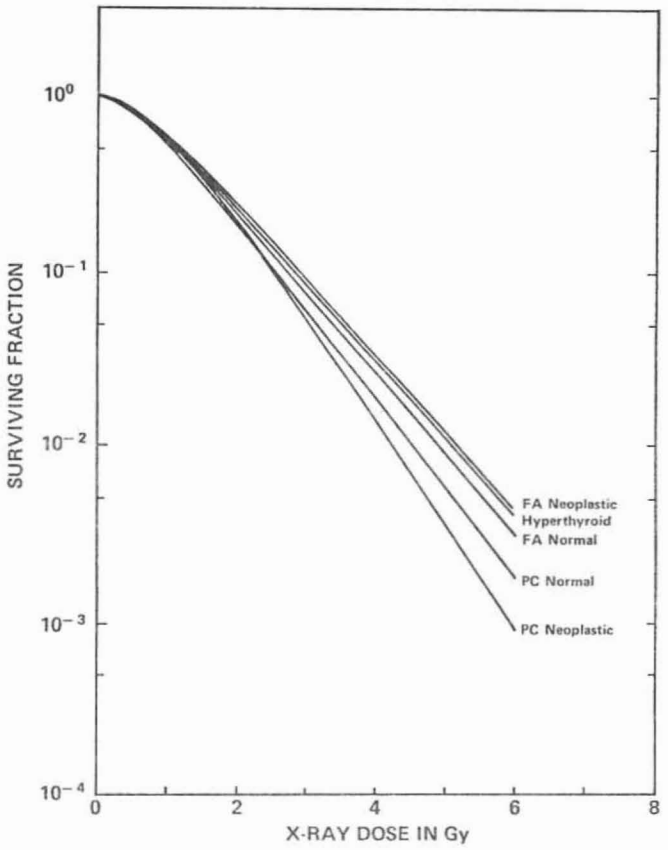
表2 患者の疾患及び細胞種別のコロニー形成率(PE)及び平均細胞生存パラメーター

Patient disease	Cell type	No. of experiments	PE(%)		Average cell survival parameters*					
					D_0			n		
			GM	Range	GM	95%	CI	GM	95%	CI
PC	Neoplastic	15	2.8	0.8-14.7	74.4	(65.3,	84.8)	3.0	(2.5,	3.6)
PC	Normal	15	6.2	1.2-15.5	85.0	(77.4,	93.4)	2.1	(1.6,	2.7)
FA	Normal	15	9.4	2.9-20.6	94.3	(87.1,	102.2)	1.8	(1.6,	2.0)
FA	Neoplastic	7	4.6	0.2-16.3	96.7	(85.5,	109.4)	2.1	(1.5,	2.9)
HY	Hyperthyroid	24	6.0	0.4-30.9	97.6	(91.3,	104.4)	1.8	(1.5,	2.1)

*Estimated by weighted least squares, with weights inversely proportional to estimated sampling variances, and adjusted for regression on $\log_{10}$PE; estimates are given for overall geometric mean (GM) of PE (5.5%) with 95% confidence interval (CI).

推定標本分散に反比例する加重値を用いて加重最小二乗により推定し，$\log_{10}$PEの回帰について補正．PE(5.5%)の全体相乗平均(GM)について推定値を算出．信頼区間(CI)は95%．

Figure 4. X-ray dose-response cell survival curves corresponding to weighted geometric mean values of cell survival parameters, adjusted for PE.
図4　PEについて補正し，細胞生存パラメーターの加重相乗平均値に対応するX線線量と細胞生存率の曲線．



There was no evidence that the extrapolation number n varied significantly between the five cell types (p>.20). This was true whether or not the analysis involved adjustment for the association between log(n) and log(PE) and/or log(D_0).

Based on the weighted analysis with adjustment for the association between D_0 and PE and for differences between the five cell types in Table 2, there was no significant association between D_0 and either patient age at surgery (p=.82) or sex (p=.10).

DISCUSSION

Early cultures of human thyroid epithelial cells from 63 patients (54 female, 9 male; age at surgery 15-75 years) with PC, FA, or HY were exposed to graded doses of soft X rays. Since PE was seen to have a moderate association with the cell survival parameters, comparisons of the

5種類の細胞間で外挿値nが有意に変化することを示す証拠はなかった(p>.20)．それはlog(n)及びlog(PE)又はlog(D_0)の関連についての補正が解析において行われているか否かに関係しなかった．

表2におけるD_0とPEの関連及び5種類の細胞間の差異について補正を行った加重解析によると，D_0と患者の手術時年齢(p=.82)又は性(p=.10)との間に有意な関連は認められなかった．

考　察

乳頭癌，濾胞腺腫，又は甲状腺機能亢進症患者63人(女性54人，男性9人，手術時年齢15～75歳)から得たヒト甲状腺上皮細胞の初期の培養系に種々の線量の軟X線を照射した．PEと細胞生存パラメーターとの間に適度な関連があることが認められたので，異なる種類の細胞間のパラメーター値の比較を

parameter values among cell types were adjusted for PE. This ensures that the differences in cell survival shown in Table 2 and Figure 4 do not arise as an artifact due to variability of PE. Additionally, in these comparisons, the cell survival parameters were weighted in inverse proportion to their estimated sampling variances, thus reducing the possibility that the most poorly estimated values might distort comparisons among the five cell types. The weighted, adjusted analysis showed that neither patient age at surgery nor patient sex had a significant effect on the cell survival parameters.

There was highly significant heterogeneity of cell survival among the five types of cells ($p<.001$), however HY, FA normal, and FA neoplastic cells all gave similar results (Table 2 and Figure 4). The average D_0 values of neoplastic (74.4 cGy) and normal (85.0 cGy) cells from PC patients, on the other hand, were substantially lower. There were no significant differences in the average value of n among the five groups.

The present results may appear to suggest that human thyroid epithelial cells are more radiosensitive than other types of human cells. For example, D_0 values for human normal mammary epithelial cells were reported to range between 109-148 cGy for 225 kVp X-irradiation[18] and between about 110-130 cGy for Cesium gamma rays.[20] Experiments with human normal skin fibroblasts have yielded D_0 values between 140-152 cGy for 220 kVp X rays,[19] and between 101-160 cGy for ^{60}Co γ rays.[21] (The latter results were used by the authors of that work to deduce a "normal range" of 97-180 cGy.[21]) However, recent results for human skin fibroblasts using the same soft X-ray machine as in the present study yielded much lower D_0 values between 61-83 cGy.[23] Because of the discrepancy between results using fibroblasts, the present results concerning thyroid epithelial cells must be viewed with some caution.

A number of reasons may explain the discrepancy between D_0 values for skin fibroblasts. One is the difference of X-ray energy used at the different laboratories. It has been shown that low energy photons are more effective for killing cultured mammalian cells than are higher energy photons. Zeitz et al[24] using HeLa cells estimated the relative biological effectiveness

PE について補正した．これにより，表2及び図4に示す細胞生存の差異が PE の変動性により人為的に発生したものでないことが確認できる．更に，これらの比較では，推定標本分散に反比例して細胞生存パラメーターを加重したので，最も正確さが劣る推定値により5種類の細胞間の比較に歪みができる可能性は減少した．加重及び補正を行った解析では，患者の手術時年齢及び性は，細胞生存パラメーターに有意な影響を与えないことが示された．

5種類の細胞間には極めて有意な細胞生存の異質性が認められたが（$p<.001$），甲状腺機能亢進症患者由来の細胞，濾胞腺腫患者由来の正常細胞及び腫瘍細胞はすべて類似した結果を示した（表2及び図4）．一方，乳頭癌患者由来の腫瘍細胞（74.4cGy）及び正常細胞（85.0cGy）の平均 D_0 値は極めて低かった．5群間には平均 n 値に有意な差はなかった．

本研究の結果は，ヒト甲状腺上皮細胞の放射線感受性が他の種類のヒト細胞に比べ高いことを示唆していると考えられるかもしれない．例えば，ヒト正常乳腺上皮細胞の D_0 値は 225kVp の X 線照射では 109～148cGy,[18] セシウムγ線では約 110～130cGy[20] と報告されている．ヒト正常皮膚線維芽細胞を用いた実験により得られた D_0 値は，220kVp の X 線では 140～152cGy,[19] ^{60}Co γ線では 101～160cGy[21] であった（その報告の著者は後者の結果を用いて〝正常範囲〟97～180cGy を推論した[21]）．しかし，最近，本研究と同じ軟X線装置を用いてヒト皮膚線維芽細胞の実験を行ったところ，極めて低い D_0 値 61～83cGy が得られた．[23] 線維芽細胞を用いた研究結果に食い違いが見られるので，甲状腺上皮細胞に関する本研究結果は慎重に考察する必要がある．

皮膚線維芽細胞の D_0 値の不一致には幾つかの原因が考えられる．一つは異なる研究室で用いられたX線エネルギーの差異である．低エネルギー光子は高エネルギー光子に比べ，培養哺乳動物細胞を殺傷する効果が高いことが示されている．Zeitz ら[24] は HeLa 細胞を用いて，^{60}Co γ線に対する低エネルギー

(RBE) of low energy (≤40 kVp) X rays compared to ^{60}Co γ rays (i.e., the ratio of γ-ray D_0 to X-ray D_0) to be 1.1-1.4, with higher dose rates having lower RBE. Preliminary results indicate that under the experimental conditions reported here, a reasonable estimate of RBE for D_0 is about 1.4 for Chinese hamster V79 cells, compared to ^{60}Co γ rays (manuscript in preparation).

(≤40kVp) X線の生物学的効果比(RBE, すなわち, γ線 D_0 に対するX線 D_0 の割合)は1.1～1.4となり, 線量率が高くなればRBEが低くなることを観察した. 本報告と同じ実験条件では, ^{60}Co γ線と比較し, チャイニーズ・ハムスターV79細胞の D_0 の適切なRBE推定値は1.4となることを予備的結果は示している(原稿作成中).

Another possible reason for the discrepancy in results with fibroblasts is the difference in methods for irradiating cells. In the experiments of other investigators, cells were irradiated, either while in suspension or while attached to dishes, and then trypsinized for plating at the desired densities. This permits low-density cell plating regardless of radiation dose, since the number of dishes for high-dose cells can be increased as needed to obtain adequate numbers of colonies. Because of the low penetrability of soft X rays, the method used in both the present study and the recent study of fibroblasts carried out at RERF[23] required that trypsinized cells be plated into dishes prior to irradiation. Moreover, due to constraints on time available for irradiation, the number of dishes that could be used was limited. Therefore, cells exposed to high radiation doses had to be plated at comparatively high densities in order to obtain adequate colony yields.

線維芽細胞を用いた実験結果に不一致がある原因としてほかに考えられるのは, 細胞への照射方法の違いである. ほかの研究者の実験では, 懸濁状態又は培養皿に付着した状態で細胞を照射し, トリプシン処理を行い適切な密度で播種している. 適切な数のコロニーを生じさせるためには, 必要に応じて高線量照射細胞の培養皿の数を増加させることができるので, 前記の方法では放射線量にかかわらず低密度細胞播種が可能になる. 軟X線の浸透性は低いので, 本研究及び最近放影研で実施された線維芽細胞の研究[23]では, トリプシン処理した細胞を照射の前に培養皿に播種することが必要であった. 更に, 照射に費やすことができる時間には制約があったので, 使用できる培養皿の数は限られていた. したがって, 適切なコロニー形成結果を得るためには, 高線量の放射線を照射した細胞は比較的高い密度で播種しなければならなかった.

Even if either or both of these effects influence the outcomes of radiosensitivity assays, it is reasonable to presume that they affect equally the five types of human thyroid epithelial cells used in the present study. Thus, the conclusion that cells from PC patients, and especially the malignant PC cells themselves, are relatively more sensitive to radiation-induced suppression of colony formation than are cells from FA or HY patients appears to be well founded.

これらの原因の一つ又は両方が放射線感受性の測定結果に影響を与えるとしても, それらは本研究で用いた5種類のヒト甲状腺上皮細胞に対しては同等な影響を及ぼすと考えるのが妥当であろう. したがって, 乳頭癌患者由来の細胞, 特に悪性の乳頭癌細胞それ自身は, 濾胞腺腫患者又は甲状腺機能亢進症患者由来の細胞に比べ, 放射線によるコロニー形成能喪失に関して感受性が高いという結論には十分な根拠があると考えられる.

Further studies are planned to examine the possible effect of cell cycle distribution on the interpretation of the present results. That is, it is well established that cellular radiosensitivity changes during cell cycle. An 18-hour incubation of trypsinized cells prior to X-irradiation might result in different cell cycle distribution for PC neoplastic cells compared to the others because their PE was the lowest of all. A new high energy X-ray (220 kVp) apparatus now installed at RERF will enable us to carry out these further studies.

更に本研究結果の解釈に対する細胞周期分布の影響を調査するための研究が計画されている. すなわち, 細胞の放射線感受性は細胞周期中に変化することが確認されている. トリプシン処理した細胞をX線照射前に18時間培養すれば, 乳頭状腺癌患者由来の腫瘍細胞のPEは最も低かったので, その細胞周期分布は他に比べ異なったものになるかもしれない. 現在放影研に設置されている新しい高エネルギーX線(220kVp)装置により, これらの研究を更に進めることができる.

REFERENCES
参考文献

1. SOCOLOW EL, HASHIZUME A, NERIISHI S, NIITANI R: Thyroid carcinoma in man after exposure to ionizing radiation. A summary of the findings in Hiroshima and Nagasaki. New Engl J Med 268:406-10, 1963 (ABCC TR 13-62)

2. PARKER LN, BELSKY JL, YAMAMOTO T, KAWAMOTO S, KEEHN RJ: Thyroid carcinoma after exposure to atomic radiation. A continuing survey of a fixed population, Hiroshima and Nagasaki, 1958-71. Ann Intern Med 80:600-4, 1974 (ABCC TR 5-73)

3. DUFFY BJ Jr, FITZGERALD PJ: Cancer of the thyroid in children: A report of 28 cases. J Clin Endocrinol 10:1296-308, 1950

4. SIMPSON CL, HEMPELMANN LH, FULLER LH: Neoplasia in children treated with X-ray in infancy for thymic enlargement. Radiology 46:840-5, 1955

5. SIMPSON CL, HEMPELMANN LH: The association of tumors and Roentgen-ray treatment of the thorax in infancy. Cancer 10:42-56, 1967

6. CLARK DE: Association of irradiation with cancer of the thyroid in children and adolescents. JAMA 159:1008-9, 1955

7. ROONEY ER, POWELL RW: Carcinoma of the thyroid in children after X-ray therapy in early childhood. JAMA 169:1-4, 1959

8. PIFER JW, TOYOOKA ET, NURRAY RW, AMES WR, HEMPELMANN LH: Neoplasms in children treated with X-rays for thymic enlargement. I. Neoplasms and mortality. J Natl Cancer Inst 31:1333-56, 1963

9. HEMPELMANN LH, HALL WJ, PHILLIPS M, COOPER RA, AMES WR: Neoplasms in persons treated with X-rays in infancy: Fourth survey in 20 years. J Natl Cancer Inst 55:519-30, 1975

10. FAVUS MJ, SCHNEIDER AB, STACHURA ME, ARNOLD JE, YUN RYO U, PINSKY SM, COLMAN M, ARNOLD MJ, FROHMAN LA: Thyroid cancer occurring as a late consequence of head-and-neck irradiation; Evaluation of 1056 patients. New Engl J Med 294:1019-25, 1976

11. MODAN B, MART H, BAIDTZ D: Radiation-induced head and neck tumors. Lancet 1:227-79, 1974

12. EZAKI T, YAGAWA K, HAYASHI Y, NISHIDA T, ISHIMARU T: Thyroid carcinoma after exposure to the atomic radiation of Hiroshima, 1958-79. J Jpn Practic Surg Soc 44:1127-37, 1983

13. MODAN B, RON E, WERNER A: Thyroid cancer following scalp irradiation. Radiology 123:741-4, 1977

14. CONARD RA: Late effects in Marshall Islanders exposed to fallout 28 years ago. In *Radiation Carcinogenesis: Epidemiology and Biological Significance.* Ed by Boice JD Jr and Fraumeni JF Jr. New York, Raven Press, in press

15. CONARD RA: Review of medical findings in a Marshallese population twenty-six years after accidental exposure to radioactive fallout. Medical Department. Brookhaven National Laboratory Associated Universities, Inc. 1980

16. MILLER RC, HIRAOKA T, NAKAMURA N, TENOU H, KOPECKY KJ, JONES MP, GOULD MN: In vitro culture of human thyroid cells: Methods and application to radiation biology. J Radiat Res 26:269-82, 1985 (RERF TR 10-84)

17. HIRAOKA T, MILLER RC, GOULD MN, KOPECKY KJ, EZAKI H, TAKEICHI N, ITO T, JONES MP, CLIFTON KH: Survival of human normal thyroid cells after X-ray irradiation. Int J Radiat Biol 47:299-307, 1985 (RERF TR 13-84)

18. YANG TC, STAMPFER MR, SMITH HS: Response of cultured normal human mammary epithelial cells to X-rays. Radiat Res 96:476-85, 1983

19. WEICHSELBAUM RR, NOVE J, LITTLE JB: X-ray sensitivity of fifty-three human diploid fibroblast cell strains from patients with characterized genetic disorders. Cancer Res 40:920-5, 1980

20. CATHERS LE, GOULD MN: Human mammary cell survival following ionizing radiation. Int J Radiat Biol 44:1-16, 1983

21. ARLETT CF, HARCOURT SA: Survey of radiosensitivity in a variety of human cell strains. Cancer Res 40:926-32, 1980

22. MILLER RC, KOPECKY KJ, HIRAOKA T, EZAKI H, CLIFTON KH: Comparison of radiosensitivities of human autologous normal and neoplastic thyroid epithelial cells. Br J Radiol 59:127-30, 1986

23. MILLER RC, ENNO M, YAMANE M, NISHIKI M: Recovery from X-ray induced damage in primary cultures of human skin fibroblast cells. J Radiat Res 26:339-45, 1985 (RERF TR 1-85)

24. ZEITZ L, KIM SH, KIM JH, DETKO JF: Determination of relative biological effectiveness (RBE) of soft X-rays. Radiat Res 70:552-63, 1977