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IN VITRO LIFE SPAN AND ULTRAVIOLET SENSITIVITY IN CELLS OBTAINED FROM A MULTIMALFORMED PREMATURE HUMAN FETUS

多奇形を伴った1先天異常児から得られた細胞における試験管内寿命と紫外線感受性

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SUMMARY

Cultured human cells were established from various organs of a congenitally abnormal fetus. The life span of lung cells was equivalent to 62 population doublings, but those of cells from skin, liver, bone marrow, and kidney were very short. Chromosome analysis of cells derived from the lung and skin showed few aberrations, but aneuploid cells and chromosomal aberrations were frequent in the kidney cells. There was no difference in ultraviolet (UV) sensitivity between lung cells and kidney cells; both were about twice as sensitive as normal human fibroblasts. In this study, we did not find a correlation between the UV sensitivity and the length of life span in vitro, the amount of chromosomal aberrations, or tissue origin of the cells.

INTRODUCTION

Hayflick and Moorhead[1,2] first reported that normal diploid cells obtained from the lungs of human fetuses have a limited life span in culture. Martin et al[3] found that the in vitro life span of skin fibroblasts has an inverse correlation to the age of the donor. It is widely believed that the limited growth potential or life span of cultured diploid human cells reflects an aging phenomenon. Cells obtained from patients with certain types of autosomal recessive hereditary diseases are

要 約

ヒト先天異常児の種々の臓器から，培養系で増殖する細胞を樹立した．肺由来細胞の試験管内寿命は62PDN（細胞集団分裂回数）に相当するが，皮膚，肝臓，骨髄あるいは腎臓から樹立した細胞の寿命は非常に短かかった．染色体分析の結果，肺及び皮膚由来細胞には染色体異常は少なかったが，腎臓由来細胞には異数体細胞や染色体異常が多くみられた．肺臓及び腎臓由来細胞の紫外線（UV）感受性は全く同じであり，いずれも，正常ヒト由来線維芽細胞のそれより約2倍の高感受性を示す．本研究では，ヒト由来細胞のUV感受性と，試験管内寿命の長さ，染色体異常の多少，細胞の由来組織の間に関連性を見いだせなかった．

緒 言

ヒト胎児肺より得られた正常二倍体細胞は，培養系において有限の寿命をもつことはHayflickとMoorhead[1,2]によって最初に報告された．Martinら[3]は，皮膚由来線維芽細胞の試験管内寿命がその供与者年齢と逆の相関のあることを見いだしている．すなわち，培養されたヒト二倍体細胞の有限増殖能又は

highly sensitive to radiation or chemical carcinogens. For example, cells obtained from patients with xeroderma pigmentosum[4] and ataxia telangiectasia[5] are sensitive to UV rays and ionizing radiation, respectively. Cells from patients with Fanconi's anemia are highly sensitive to mitomycin-C.[6] These hypersensitivities appear to be related to decreased or defective repair of DNA damage. The defective DNA repair systems may play a role in turn in the high cancer susceptibility of patients with these diseases.

The hypothesis that aging may be attributable to the accumulation of DNA damage is especially interesting,[7] but reports supporting this hypothesis are few.[8,9] Rather, the predominant view is that there is no relationship between aging and repairability of DNA damage.[10-14]

In the current study, fresh tissue samples were taken from the skin, lung, liver, kidney, and bone marrow of a congenitally malformed fetus (MM42) that came to autopsy and proliferating cells were established in culture. Cell survival rates were observed by the colony formation method after exposure to UV. Cells obtained from the fetus (MM42) were approximately twice as sensitive to UV as normal cells. Furthermore, the in vitro life sapn of the cells obtained from this case varied according to the organ of their origin. The life span of cells derived from the lung was approximately eight times as long as that of cells derived from the kidney. However, they were alike in UV sensitivity. Chromosome analysis of cells derived from the lung, skin, and kidney revealed some cells with aberrations in every cell population. Especially in kidney cells, polyploid cells and chromosome aberrations were frequently encountered.

MATERIALS AND METHODS

Culture Medium. Eagle's MEM (Nissui Seiyaku Co., Tokyo) supplemented with 10% fetal calf serum (FCS, Flow Laboratories, Australia) was used as culture medium for subcultivation and maintenance of the cells, and 15% FCS was added to the Eagle's MEM for use in the colony formation-cell survival assays. No antibiotics were added.

Origin of Cells. All cells for this study were established in our laboratory. The samples were

寿命は，細胞レベルにおける老化現象を反映するものであると広く受け入れられている．ある種の常染色体性劣性遺伝病患者より得られた細胞は，放射線あるいは化学発癌剤に対して感受性が高いことが知られている．例えば，色素性乾皮症患者[4]やataxia telangiectasia 患者[5]から得られた細胞は，各々UVや電離放射線に対して感受性が高い．Fanconi's anemia 患者の細胞はマイトマイシン－C に対して感受性が高い．[6] これらの高感受性は，DNA 損傷に対する修復能の減少あるいは欠損によると思われている．すなわち，DNA 障害修復能の低下がこれら疾患の高発癌性の一因となると考えられている．

老化機構を考える上でも，老化が DNA 損傷の蓄積に起因するかもしれないと考える仮説は特に興味深い．[7] しかし，この仮説を支持する報告は少ない．[8,9] むしろ，老化と DNA 損傷修復能との関連性はないとみるのが大勢である．[10-14]

本研究では，病理解剖に付された先天異常胎児（MM42）の皮膚，肺，肝，腎，及び骨髄から新鮮な組織を分離し，培養系で増殖する細胞を樹立した．これらの細胞に種々の線量の UV を照射後，コロニー形成法によって生残率を調べた．1 先天異常児（MM42）から得られた細胞は，正常細胞より約 2 倍も UV 感受性が高かった．更に，この個体より得られた細胞の試験管内寿命は由来臓器によって異なる．肺由来細胞の寿命は，腎由来細胞のそれの約 8 倍である．しかし，両者の UV 感受性は同じである．肺，皮膚及び腎由来の細胞についての染色体分析では，いずれの細胞集団にも染色体異常をもつ細胞が含まれている．特に腎細胞には，多倍性細胞の出現頻度が高く，染色体異常も多かった．

材料及び方法

培養液． 継代培養及び細胞維持に用いる培養液は，10％胎牛血清（Flow Laboratories, Australia）を添加した Eagle's MEM（日水製薬，東京）を用いた．コロニー形成－細胞生残率検査を行うときには，Eagle's MEM に15％の胎牛血清を加えた．抗生物質は特に添加していない．

細胞の起源． この研究でのすべての細胞は我々の研究室で樹立されたものである．材料は，広島通信

obtained from the fetal body (MM42) which was referred from the Hiroshima Communications Hospital to our laboratory for autopsy. Small sections of tissues (0.5-2 cm^3) were separated from the lung, liver, kidney, breast skin, and femoral bone marrow within 15 hours of death.

All of the tissue sections were washed well with a buffer solution to which 500 μg/ml of streptomycin and 500 U/ml of penicillin were added. They were cut into small pieces less than 1 mm^3 in size in a plastic tissue culture plate containing a small volume of growth medium. These were put in Eagle's MEM culture medium containing 20%-30% FCS and cultured at 37°C in a 5% CO_2-95% air atmosphere. After 3-10 days, the culture solution was replaced with Eagle's MEM culture medium containing 10% FCS.

In the UV sensitivity studies, cells established from other lung specimens were used as controls. These specimens were obtained from bodies of four children 0-4 years of age and one male 66 years old, autopsied at the Department of Pathology, Hiroshima Atomic Bomb Hospital and our laboratory, and which showed no abnormalities of the lungs.

Estimation of Life Span in Vitro. Fibroblast-like cells which propagated from the autopsy fragments were cultured in 6 cm petri dishes (Falcon Plastics, Oxnard, California) according to a method described previously.[15,16] Cells were subcultured as soon as they became confluent. All of the cells were subcultured or fed with fresh medium regularly every 5-7 days. The cells were harvested with a solution of 0.125% trypsin plus 0.01% EDTA, resuspended in a fresh medium, counted, and plated at $2X10^5$ cells per plate. As the age of the cells entered the latter half of the life span, the monolayer cell density gradually decreased. Thus, the number of cells obtained at subculture became less than twice the inoculum size (2.0×10^5 cells/plate). From this point of time, the cells were subcultured at 1:2 split ratio. Frequency of cell population doubling during each subculture was calculated based on the number of cells obtained at each subcultivation. When the cells lose their monolayer forming activity, the cumulative number of population doublings were summed and used as the in vitro life span of the strain (i.e., population doubling number, PDN).[15,16]

病院から当研究室に移され，病理解剖に当てられた胎児個体（MM42）から採られたものである．死後15時間以内に，肺臓，肝臓，腎臓，胸部皮膚及び大腿骨骨髄の組織小片（0.5－2 cm^3）を分離した．

各組織片は500μg/ml streptomycin と 500 U/ml penicillin を添加した緩衝液で十分に洗滌した．少量の培養液を入れたプラスチック組織培養皿中の各組織片を1 mm^3 以下になるよう細かく切り刻み，20%－30%の胎牛血清を含む Eagle's MEM 培養液中で，37°C，5 % CO_2 空気圧95%インキュベーター内で培養した．3－10日後に，10%胎牛血清を含む Eagle's MEM 培養液に交換した．

UV感受性を調べる実験においては，0－4歳の4体の児童及び66歳の男性1体の剖検時に得られた肺由来細胞をコントロールとして用いた．これらの材料は，広島原爆病院病理部及び当研究室で病理解剖に当てられた個体から得られたもので，すべての肺組織に異常は認められなかった．

試験管内寿命の評価法． 剖検組織片から増殖してきた線維芽（様）細胞は，既に報告されている方法[15,16]に従って，6 cmプラスチックシャーレ（Falcon Plastics, Oxnard, California）中で培養した．細胞が単層状に達したときに，細胞を継代培養する．すべての細胞は5－7日ごとに規則正しく継代培養するか又は培養液の交換を行った．細胞は0.125% trypsin＋0.01% EDTA 溶液で剝離し，fresh medium 中に再懸濁し，細胞を数え，プレート当たり 2×10^5 個の細胞を植え込んだ．細胞の加齢が寿命の後半に入ってくると，単層状態での細胞密度は徐々に減少する．したがって，継代培養時に得られる細胞数は植え込み時の細胞数（2.0×10^5 cells/plate）の2倍より少なくなってくる．この時点から，細胞は1：2の分割法で継代培養した．各継代培養時に得られた細胞数を基にして，各継代培養間の細胞集団の分裂回数を計算した．細胞が単層を形成する活性を失ったとき，細胞集団の総分裂回数を計算し，その細胞系統の試験管内寿命，すなわち Population Doubling Number（PDN）として表す．[15,16]

Chromosome Analysis. Chromosome analysis was made 2-4 weeks after the establishment of cells derived from the lung, skin, and kidney. The chromosome slides were prepared by the usual flaming method, with hypotonic treatment of the cells using 0.075 M KCl (2 volumes) + 1% Na citrate (1 volume) buffer solution after one hour colchicine treatment. Chromosome preparations were made according to the conventional method described by Awa et al.[17]

UV Irradiation. Aliquots of proliferating cells (5×10^4) suspended in 0.5 ml phosphate buffer saline (PBS) were placed into 6 cm plastic dishes. The cell suspension was uniformly spread in the dish with a pipet to leave a margin approximately 5 mm from the edge and were irradiated with various doses of UV (15 W UV lamp, Toshiba GL-15). The dose rate was 2 J/m^2/sec as determined with a Topcon UV radiometer (Tokyo Kogaku Co. Ltd., Tokyo).

RESULTS

Materials used in this experiment were obtained from a multimalformed fetus (Table 1). Because of sterility, the mother (26 years old) of this fetus had been administered such ovulation inducing drugs as progesterone, human chorionic gonadotropin, human mesopanse gonadotropin, etc., for one year and 10 months. She had no particular abnormalities except mild diabetes mellitus during her pregnancy.

The fetus was autopsied 15 hours after death. At that time, approximately 0.5-2 cm^3 of tissue sections were separated from the lung, kidney, liver, femoral bone marrow, and breast skin to establish cells which can be propagated in culture. The in vitro life span of these cells is shown in Table 2. The life span of the cells varies very much by the organ of origin. In our culture conditions, the in vitro life span of cells derived from the lung was equivalent to 62 PDN, which is consistent with the results previously reported for cells obtained from normal human lung.[15] The life span of cells derived from the kidney, liver, and bone marrow was very short. The life span of cells derived from the skin was equivalent to 18.9 PDN which was less than half the value reported previously by us for normal diploid cells.[14] In this experiment many of the cells that propagated from skin tissue sections were epithelial-type cells, and subculture was begun from the time few fibroblasts were seen

染色体分析. 肺，皮膚及び腎由来細胞の樹立後2－4週間目に染色体分析を行った．染色体標本はコルヒチン1時間処理後，0.075 M KCl（2容量）＋1％ Na citrate（1容量）緩衝液で低張処理し，阿波ら[17]が述べている通常の火焔法で作成した．

UV照射. 増殖期にある細胞（5×10^4）を0.5ml の燐酸塩緩衝液（PBS）に懸濁し，6 cmプラスチックシャーレに入れる．細胞懸濁液はシャーレの縁から約5 mmを残すようにして，ピペットで均一に広げた後，紫外線ランプ（15W，東芝 GL-15）からの種々の線量の UV を照射した．紫外線線量率はトプコン UV 線量計（東京光学社，東京）で測定し，2 J/m^2/sec の条件で細胞に照射した．

結　果

本実験で用いた材料は，多奇形早産児から得られた（表1）．患者の母親（26歳）は不妊症のため，1年10か月にわたって排卵誘発剤，progesterone，human chorionic gonadotropin, human mesopanse gonadotropin などの投与を受けていた．妊娠中，母親には軽い糖尿病以外，特に異常はなかった．

胎児は，死亡後15時間目に病理解剖に付された．このときに，肺臓，腎臓，肝臓，大腿骨骨髄，胸部皮膚より0.5－2 cm^3 程度の組織片を分離し，培養系で増殖可能な細胞を樹立した．各細胞の試験管内寿命を表2に示す．細胞の試験管内寿命は，由来臓器によって非常に異なる．我々の培養条件では，肺由来細胞の試験管内寿命は62PDN に相当し，この結果は先に報告した正常ヒト肺由来細胞の結果と一致する．[15] 腎臓，肝臓，骨髄由来の細胞の寿命は非常に短かかった．皮膚由来細胞の寿命は18.9PDN に相当し，この値は我々が先に報告した正常二倍体細胞の結果[14] の2分の1以下にすぎなかった．本実験では，皮膚組織片から増殖してきた細胞の多くは上皮様細胞（図1）であり，線維芽細胞がほとんど認められ

TABLE 1 GROSS PATHOLOGICAL FINDINGS (MM42)
表 1 MM42の病理所見

Premature Stillborn Fetus:	Body weight 1500 g, body length 34 cm, gestational age 33 weeks.
Cervical Vertebrae:	Spina bifida occulta with a shortening of the neck (Klippel-Feil syndrome)
Brain:	Internal hydrocephalus.
Diaphragm:	Left diaphragmatic hernia, herniation of small intestine, spleen, and part of the left lobe of the liver.
Lungs:	Marked hypoplasia of the right and left lungs, right 5 g, left 2 g.
Liver:	Abnormal shape of the left lobe with diaphragmatic hernia.
Umbilical Artery:	Single umbilical artery.

TABLE 2 IN VITRO LIFE SPAN OF FIBROBLASTS (FIBROBLAST-LIKE CELLS) PROLIFERATING FROM TISSUE OF THE LUNG, BREAST SKIN, FEMORAL BONE MARROW, KIDNEY, AND LIVER
表 2 肺臓，胸部皮膚，大腿骨骨髄，腎臓及び肝臓の組織より増殖してきた線維芽(様)細胞の試験管内寿命

Cell Origin	PDN	Culture Term in months
Lung	62.0	9.0
Breast skin	18.9	2.3
Femoral bone marrow	9.0	1.3
Kidney	7.8	1.0
Liver	13.1	3.0

Cell life span is expressed as population doubling number (PDN) of cell population before the cells cease to proliferate. Culture term shows the period during which subculture is possible. There are cells surviving for more than several months even after they cease to divide if the culture solution is regularly changed.

細胞寿命は，細胞の増殖が止まるまでの細胞集団の分裂回数(PDN)で表す．培養期間は，継代培養が可能な期間を示す．細胞の分裂が止まっても，定期的に培養液を交換してやれば，更に数か月以上も生在している細胞がある．

(Figure 1). However, as the subculture was continued, the cells gradually changed their morphology to become fibroblast-like cells. The "culture term" in Table 2 is the time required for the cells to no longer form a monolayer and not the time needed for all the cells to die. Even after they have lost their ability to divide, if the medium is changed regularly, the cells will remain attached to the dish and continue their metabolic activity for more than two months thereafter.

Distribution in the number of chromosomes in cells obtained from the lung, kidney, and skin is

ない時期から継代培養を開始した．しかし，継代培養を続けていくうちに，細胞の形態は線維芽様細胞に変わっていった．表2中の培養期間は，細胞が単層増殖を形成しなくなるまでに要した時間であり，すべての細胞が死滅するまでに要する時間ではない．細胞が分裂能力を失った後も，規則的に培地交換をしてやれば，細胞は更に2か月以上もシャーレにくっ付いて残っており，代謝活動を行っている．

肺臓，腎臓及び皮膚由来細胞の染色体数分布を表3

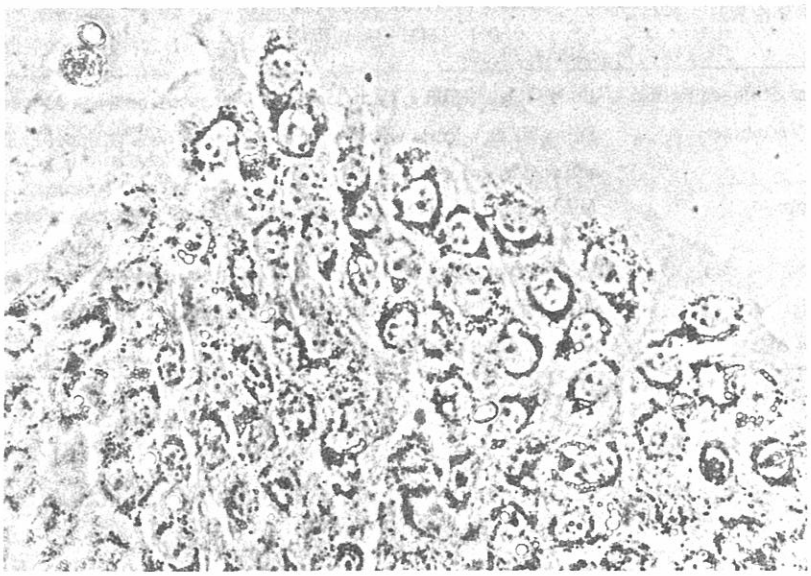

Figure 1. Phase microscopic photograph of epithelial-cells proliferating from skin tissue section (×400). Proliferation of epithelial-cells stops soon and proliferation of fibroblasts begins from the outer edge.

図1　皮膚組織片から増殖してきた上皮性細胞の位相差顕微鏡写真（×400）．上皮性細胞の増殖はすぐに止まり，外縁から線維芽細胞が増殖してくる．

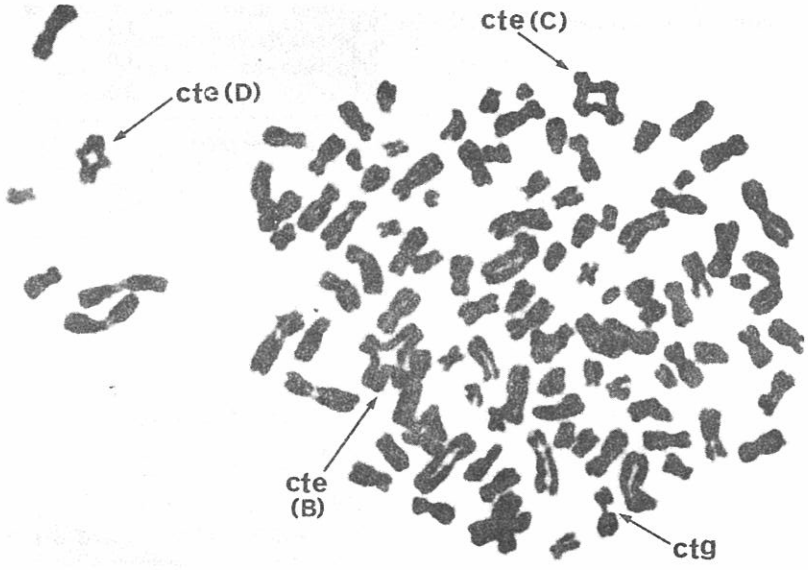

Figure 2. A case with three chromatid exchanges found in a hypertetraploid cell derived from the kidney. These chromatid exchanges occur between B group chromosomes, C group chromosomes, and D group chromosomes.[27]

図2　腎臓由来のhypertetraploid細胞で見つかった3個のchromatid exchangeをもつ1例．各々のchromatid exchangeはBグループ染色体間，Cグループ染色体間，Dグループ染色体間で成っている．[27]

TABLE 3 CHROMOSOME NUMBERS IN CELLS DERIVED FROM THE LUNG, SKIN, AND KIDNEY

表3　肺臓，皮膚及び腎臓由来細胞についての染色体数

Cell Origin	Metaphases Scored	Chromosome Counts				
		44	45	46	47	70+
Lung	50	1	1	45	1	2
Skin	50	0	0	48	0	2
Kidney	50	0	6	37	0	7

TABLE 4 CHROMOSOME ABERRATIONS IN CELLS WITH 44-47 CHROMOSOMES

表4　44－47本の染色体をもつ細胞についての染色体異常

Cell Origin	Metaphases	Aberrant Cells			
		Single Chromatid			Isochromatid
		Gaps	Breaks	Deletions	Breaks
Lung	48	1 (Bq)	0	0	0
Skin	48	1 (Bq)	1 (Cp)	1	1
Kidney	43	8	0	0	6

Position of aberration in parentheses.

shown in Table 3. Over 90% of lung cells and skin cells had 46 chromosomes, but a large proportion of kidney cells were hypoploid (45 chromosomes) and hyperploid (70-120 chromosomes). Table 4 shows data from analysis in the type of chromosome aberrations in the cells with 44-47 chromosomes shown in Table 3. In kidney cells, there were 8 metaphase cells with a total of 9 gaps and 6 metaphase cells with one isochromatid break. Further, among the 7 hyperploidy cells, there was one which had 3 chromatid exchanges (Figure 2). The chromatid exchanges occurred between B group chromosomes, C group chromosomes, and D group chromosomes.

The dose-survival response of cells derived from the lung and kidney after irradiation with various doses of UV was determined by the colony formation method (Figure 3). The plating efficiencies of unirradiated cells were 4.1% to 16.2% for lung cells and 3.1% to 3.2% for kidney cells. There were no differences between

に示す．肺細胞及び皮膚細胞では90%以上が46本の染色体数をもつが，腎細胞では hypoploid（45染色体）と hyperploid（70－120染色体）の割合が非常に多かった．表3の44－47本の染色体をもつ細胞について，染色体異常の型を調べたのが表4である．腎細胞では，8個の分裂中期細胞に合計9個の gap があり，6個の分裂中期細胞に1個ずつの isochromatid break があった．更に，7個の多倍体細胞の中，3個の chromatid exchanges を含む一つの細胞があった（図2）．chromatid exchanges はBグループ同士，Cグループ同士，Dグループ同士の間でできている．

肺臓及び腎臓由来の細胞に種々の線量の UV を照射後の生残率を，コロニー形成法でみたのが図3である．紫外線照射しなかった細胞のコロニー形成率は，肺由来細胞で4.1%－16.2%，腎由来細胞で3.1%－3.2%であった．肺細胞を用いて3回，腎細胞を用いて

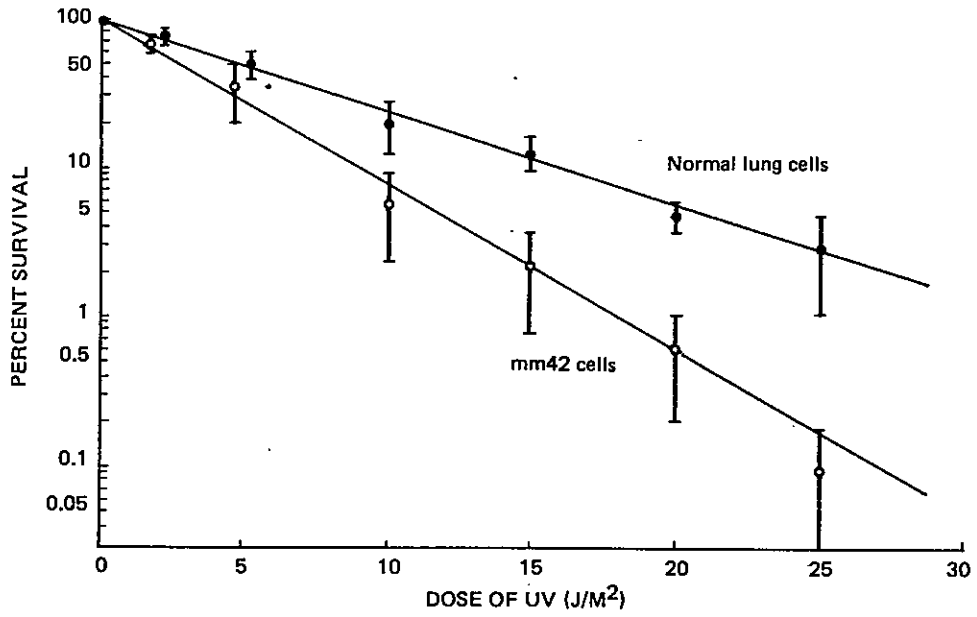

Figure 3. Dose-survival response of MM42 cells after exposure to various doses of UV. The bars indicate standard deviation of the mean for three independent experiments using cells derived from the lung and of two independent experiments using cells derived from the kidney. The curves are exponential with D_0 = 3.8 J/m^2, n = 1.0. Control experiment was made using cells obtained from normal lung tissues. UV dose-survival response of control cells had no shoulder and a D_0 of 7.0 J/m^2.

図3　MM42細胞の，種々の線量のUV照射に対する生残率関係．肺由来細胞を用いての3回の実験と，腎由来細胞を用いての2回の実験で得られた結果の平均値を示す．D_0 =3.8J/m^2，n =1.0の指数対数的減少がみられる．対照実験は，正常肺組織より得られた細胞を用いて行った．D_0 =7.0J/m^2，n =1の線量一生残率関係がみられた．

the results of three experiments using lung cells and two experiments using kidney cells. These cells showed a survival response with a shoulder extrapolation number (n) of 1.0 and an exponential slope (D_0) of 3.85 J/m^2. Control experiments were undertaken using cells obtained at autopsy from the lungs of four children 0-4 years old and one male 66 years old. The plating efficiency of unirradiated cells was 8.1% to 20.7%. The mean values for the results of two or more experiments for each cell type are shown in the figure. The dose vs survival response of control cells had no shoulder and a D_0 of 7.0 J/m^2.

2回の実験結果の間に差異はなく，D_0 =3.85J/m^2，n =1.0の指数対数的な線量一生残率関係が得られた．対照実験は，0－4歳の児童4体及び66歳の男性1体の剖検時に得られた肺由来細胞を用いて行った．紫外線照射しなかった細胞のコロニー形成率は8.1%－20.7%であった．各々の対照細胞について，2回以上の実験結果の平均値を図示すると，D_0 =7.0J/m^2，n = 1の指数対数的な線量一生残率関係がみられた．

DISCUSSION

In culture systems, normal human diploid cells are in a state in which they repeatedly divide. However, there is a limit to the ability of such cells to divide, and proliferation eventually stops.[1,2] Of course, all cells in a population do not necessarily have a fixed division potential.[18-21] There are cells that lose their ability to divide and drop out of the culture system immediately after establishment of the cell strain. That is, the frequency of division of a population can be said to be the mean value of the various division potentials of the individual cells.[22] In addition, because the mean value is very stable, the hypothesis that the life span of human diploid cells can be determined by the frequency of division of the cell population has been widely accepted.

It is well known that mammalian cells which have secured infinite proliferative ability in culture systems in general are aneuploid. However, diploidy is firmly maintained in most normal human cells and it is difficult to transform them by chemical or physical means.[22-25] Indeed, it can be said that there is a close relationship between securing infinite proliferative ability in the culture system and disappearance of diploidy.[26]

In the current study, however, it is of interest to study the in vitro life span of cells with aneuploidy but without infinite proliferative capacity. The source of these cells (MM42), encountered by chance among the autopsies performed at RERF, was a premature stillborn fetus with malformations in various organs. The karyotypes, the proliferative life span, and the UV sensitivity of fibroblasts and fibroblast-like cells established from several organs were studied. Analysis of cells derived from the lung, skin, and kidney showed some chromosome aberrations, but particularly frequent in the kidney cells. Only a few abnormalities were seen in cells derived from the lung and skin, but there was a large difference in the in vitro life span between the two. We have reported that there was little difference in life span between fibroblasts from the lung and fibroblasts from the skin of a normal human fetus.[14] The reason is unknown why the life span of cells from the skin was short in this experiment. It may be because the cells having proliferated from all the skin tissue sections in the first generation culture were epithelial-type cells. That is, there may be

考　察

培養系においては，正常ヒト二倍体細胞は分裂，増殖を重ねるような状態におかれている．しかし，その細胞の分裂能には限りがあり，やがては増殖を停止する．[1,2] もちろん，集団内のすべての細胞が一定の分裂能をもっているわけではない．[18-21] 細胞株の樹立直後から分裂能を失い，培養系から脱落していく細胞もみられる．すなわち，集団分裂回数は個々の細胞の多様な分裂能の平均値であるといえよう．[22] しかし，その平均値が非常に安定しているために，ヒト二倍体細胞の寿命は，細胞集団の分裂回数で規定することができるとする仮説が広く受け入れられている．

培養系において無限増殖能を獲得した哺乳動物細胞では，異数性を示すことはよく知られている．しかし，正常ヒト二倍体細胞を化学物質あるいは物理的要因によって形質転換させることは極めて困難であり，二倍体性は強固に維持されている．[22-25] すなわち，培養系における無限増殖能の獲得と培数性の消失には密接な関係があるといえる．[26]

こういった意味からも，癌化していなくて，異数性をもつ細胞の試験管内寿命を調べることは興味のあるところである．放影研で手掛けた剖検の中で，たまたま出会った材料（MM42）は各主要臓器に奇形をもつ早死産児であった．各種臓器より樹立した線維芽細胞及び線維芽様細胞の染色体型と細胞寿命，及びUV感受性を調べた．肺，皮膚及び腎由来細胞について染色体分析を行ったが，腎由来細胞に非常に多くの染色体異常がみられた．肺及び皮膚由来細胞にはわずかの異常しかみられなかったが，両者の試験管内寿命には大きな差が認められた．我々は，正常ヒト胎児の肺及び皮膚由来の線維芽細胞の間には，寿命の差がほとんど認められなかったことを既に報告している．[14] 今回の実験で，皮膚由来細胞の寿命が短かかった原因はわからない．初代培養において，すべての皮膚組織片から増殖してきた細胞が上皮様細胞であったことが原因かもしれない．すなわち，

a difference in origin between the previously reported cells derived from the skin and those of the present study. Or, the life span of the skin cells may have been shortened due to chromosome aberrations.

先の報告の皮膚由来細胞と今回の皮膚由来細胞では，細胞の起源が異なるのかもしれない．あるいは，染色体異常が関与して，皮膚細胞の寿命が短かくなっているのかもしれない．

The life span of fibroblast-like cells obtained from the bone marrow, kidney, and liver was shorter compared with that of cells derived from the lung. The cause of this difference is not clear, but may include:

骨髄，腎臓，肝臓由来線維芽様細胞の寿命は，肺臓由来細胞のそれと比べて非常に短かかった．この差が何によるのかは，明確ではない．その原因として次のことが考えられる．

The life span of cells is related to the extent of chromosome aberrations.

細胞寿命の長さは染色体異常の多少に関与している．

Tissue specificity is involved in the life span of cells.

細胞寿命には，組織特異性がある．

Mutation has occurred in the bone marrow, kidney, and liver, and this has shortened the life span of cells derived from these organs.

骨髄，腎臓，肝臓に突然変異があり，したがって，これら臓器由来細胞の寿命が短かくなっている．

There was no difference in dose-survival response relationship between lung and kidney cells. After irradiation with UV, both were approximately twice as sensitive as normal cells. This suggests that the UV sensitivity does not differ between the cells by the length of their life span, extent of chromosome aberrations, or origin. We have made a study using cells obtained from patients with xeroderma pigmentosum which show high sensitivity to UV, ataxia telangiectasia which show high sensitivity to gamma rays, and Werner's syndrome which show premature senility, and reported that the UV sensitivity does not vary with aging in vitro.[14] The present data are in accord with our hypothesis.

肺臓及び腎臓由来の細胞に UV を照射した場合，両者の線量一生残率関係の間に差はなく，いずれも正常細胞の約2倍の高感受性を示す．すなわち，両者の細胞間の細胞寿命の長短，染色体異常の多少あるいは細胞の由来によって，UV に対する感受性が変わることはないことを意味する．我々は，UV に高い感受性を示す色素性乾皮症，γ線に高い感受性を示す ataxia telangiectasia，あるいは早老症である Werner's syndrome の疾患をもつ患者由来の細胞を用いて，UV感受性が老化に伴って変動することはないことを報告している．[14] 今回の報告は，この我々の仮説を更に支持することになると思われる．

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