MK・P3

★★★MK・P３細胞の歴史★★★

初代培養：1961年・Cynomolgus Monkeyの腎皮質より培養開始。

培養法：静置培養、5%牛血清+LD培地。（１）

Ｐ３系へ：1965年血清および蛋白を含まない合成培地に切り替えた。其の后、1991年からはDM-201培地、血清無添加、閉鎖培養(炭酸ガスフランキは使わない)に問題なく順応して、以后現在(2001年)まで継代を続けている。倍加時間はほぼ65時間。

UDC: 578. 085. 23 : 599. 82 : 611. 611 [Japan. J. Exp. Med. Vol. 32, 4, p. 351-368, 1962]

(1)

ID 1713

ESTABLISHMENT OF A CELL STRAIN, JTC-12, FROM CYNOMOLGUS MONKEY KIDNEY TISSUE*

clp09987.jpg

NIH90354

BY

TISSUE CULTURE WORK: Toshiko TAKAOKA and Hajim KATSUTA
Tissue Culture Laboratory, The Institute for Infectious Diseases, The University of Tokyo (Takanawa, Tokyo, Japan)

VIRUS WORK: Motoshige ENDO and Koshiro SATO
The Fourth Department of Bacteriology, The Institute for Infectious Diseases

and

CHROMOSOME WORK: Hideo OKUMURA
Department of Enteroviruses, National Institute of Health (Osaki, Tokyo, Japan)

(Received for publication, June 12, 1962)

Several strains have been established from kidney tissues of rhesus monkeys. However, few kidney strains are found originating from cynomolgus monkeys. In the present work, a cell line, designated as the strain JTC-12, was established from kidney tissue of normal cynomolgus female monkey. The culture history, some of the nutritional requirements, the chromosomal constitution and the susceptibility to type 1 poliovirus will be described.

染色体：樹立初期の検索では近い二倍体を示した。（２）

Distribution of chromosome numbers among the strain JTC-12 cells

Transfer generation	25	32	33	35	Total
40	1		1	2	4
41			2	2	4
42	1	1	1	1	4
43				1	1
44					
45				1	1
46	1		1	2	4
47			1		1
48	1		1	2	4
49				1	1
50				1	1
62		1			1
63					
64		1		1	2
65					
66					
67					
68			1		1
69				1	1
82	1		1	1	3
83		1	2		3
84		1		1	2
91				2	2
92			1		1
93					
94				1	1
>100	1		2	5	8
Total	6	5	14	25	50

Number of chromosomes

25 / 5 / 15

《テロメアとテロメラーゼ》

テロメラーゼは＋、テロメア長は4.7キロベース。

（今福先での研究）
（3－1）（3－2）：石塚稲夫・ホルモン感受性、硫脂質、イオン輸送。
（4）：久米川正好・Bt2cAMPの影響。
（5－1）（5－2）（5－3）：Eturo Ogataら。
（6）：川口辰也・ガラクトース培地による糖脂質の変化。
（7）：馬場優・肝炎ウィルスの増殖。
（8）：佐藤温重・宇宙における細胞培養。

Biochimica et Biophysica Acta, 541 (1978) 467—482
© Elsevier/North-Holland Biomedical Press

JTC-12
(3)-2
註星 高岡聡子先生 著者ら

BBA 28573

HORMONE-SPECIFIC RESPONSES AND BIOSYNTHESIS OF SULFOLIPIDS IN CELL LINES DERIVED FROM MAMMALIAN KIDNEY *

(1691)

INEO ISHIZUKA [a], KEIKO TADANO [a], NAOKAZU NAGATA [b], YUKIO NIIMURA [a] and YOSHITAKA NAGAI [b]

Department of Biochemistry, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173 and [b] Department of Biochemistry, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashi-ku, Tokyo 173 (Japan)

(Received January 16th, 1978)

Summary

The established cell lines isolated from mammalian kidney were characterized by its receptor activities against hormones and the ability to synthesize sulfolipids localized in the renal tubule.

The level of 3′:5′-cyclic AMP in JTC-12.P3 (monkey kidney) cells increased in 2 min as much as 2.5—5-fold on activation with 1.0 unit/ml of bovine parathyroid hormone or 1.9 units/ml of synthetic parathyroid hormone (1—34) resulting in intracellular cyclic AMP concentration of more than 40 pmol/mg protein. Prostaglandin E_1 (14 μM) and isopropylnorepinephrine (10 μM) were also found to increase the concentration of cyclic AMP by more than 30- and 7-fold, respectively. Addition in medium of calcitonin, arginine vasopressin, adrenocorticotropic hormone and glucagon caused no significant changes of cyclic AMP level in the cell.

In contrast, MDCK, a cell line isolated from canine kidney, reacted to arginine vasopressin, isopropylnorepinephrine and prostaglandin E_1 and only slightly to parathyroid hormone. MDBK cell line derived from bovine kidney or fibroblast cell lines from rat lung and guinea pig kidney did not react to any of the hormones specific to kidney, i.e. arginine vasopressin, calcitonin or parathyroid hormone in the presence of theophylline. However, in the presence of 2 mM isobutylmethylxanthine, small but significant elevation of cellular cyclic AMP levels in response to calcitonin, arginine vasopressin, isopropylnorepinephrine and prostaglandin E_1 was observed.

哺乳動物腎の生化学的研究

—培養細胞株を用いて—

(3)-1

石　塚　稲　夫*

Biochemistry of Renal Tubules

—Studies with established cell lines isolated from mammalian kidney epithelium—

Ineo Ishizuka

Department of Biochemistry Teikyo University School of Medicine

The cellular population in the kidney is very heterogeneous. Epithelial cells in the kidney have each specific biochemical character and function inherent to the portion of nephron. Thus, it is usually difficult to obtain meaningful knowledge by analyzing whole perfused kidney, slice from cortex or medulla or even isolated renal tubules. One approach to solve this problem is to examine the homogeneous kidney cell lines retaining physiological and biochemical features of the cells originated from nephron.

The established cell lines derived from mammalian kidney were characterized by their receptor activities against hormones and the ability to synthesize sulfolipids localized in the renal tubule. The results suggested that JTC-12 (from cortex of monkey kidney), MDCK (canine kidney) and MDBK (bovine kidney) cell lines are of epithelial origin and also JTC-12 and MDCK originated most probably from proximal convoluted tubule and medullary collecting tubule, respectively. These cell lines may serve as a good model to study various aspects of kidney, especially concerned with specific function of nephron.

5530 — 5534

Effects of Dibutyryl Adenosine 3′:5′-Cyclic Monophosphate and Other Agents on Induction of Alkaline Phosphatase Activity in Monkey Kidney Cells

5534

Takao YORA,* Yoshikatsu SAKAGISHI,* Yohtalou TASHIMA,** and Masayoshi KUMEGAWA***

*Department of Biochemistry, Saitama Medical School, Moroyama-machi, Iruma-gun, Saitama 350-04, **Department of Biochemistry, School of Medicine, Akita University, Akita 010, and ***Department of Oral Anatomy, Josai Dental University, Sakado, Saitama 350-02

(4)

Received for publication, July 19, 1983

The induction of alkaline phosphatase (ALP) by dibutyryl adenosine 3′:5′-cyclic monophosphate (Bt_2cAMP) was investigated in strain JTC-12·P3 cells derived from monkey (*Macaca irus*) kidney cortex. ALP activity was increased by Bt_2cAMP in a dose-dependent manner, reaching a plateau at concentrations higher than 5 mM with the activity being about 4 times that of the controls. The concentration of Bt_2cAMP required for half-maximal induction of ALP activity was about 0.8 mM. ALP activity was increased rapidly by Bt_2cAMP for the first 5 days and then continued to increase gradually towards a plateau level. Removal of Bt_2cAMP from the medium caused a rapid decrease in the activity, suggesting that the induction of ALP activity by Bt_2cAMP is reversible. ALP activity was induced synergistically in the presence of 1 mM sodium butyrate together with Bt_2cAMP at concentrations from 0.01 to 1 mM. It was also found that in the presence of 1 mM Bt_2cAMP, sodium butyrate increased ALP activity in the same manner as Bt_2cAMP did in the presence of 1 mM sodium butyrate. Although dexamethasone, a potent glucocorticoid, had no effect on ALP activity in control cells, the hormone suppressed the ALP activity induced by Bt_2cAMP in a dose-dependent manner. At concentrations above 0.2 mM, two xanthine derivatives, theophylline and 3-isobutyl-1-methyl-xanthine (IBMX), also inhibited the induction of ALP activity by 1 mM Bt_2cAMP. Inhibitors of protein synthesis, cycloheximide (1.5 μg/ml) and pactamycin (10 μg/ml), as well as inhibitors of RNA synthesis, actinomycin D (2 μg/ml) and α-amanitin (50 μg/ml), suppressed the induction of ALP activity. Hydroxyurea markedly enhanced the induction of ALP activity by 1 mM Bt_2cAMP in the range of dosages examined (5 to 80 μg/ml), while it did not cause any change in ALP activity in control cells. 5-Bromodeoxyuridine (BrdU) did not affect the level of ALP activity in control cells or in cells treated with 1 mM Bt_2cAMP. Thus, the level of ALP activity in JTC-12·P3 cells was found to be influenced by several agents such as Bt_2cAMP, sodium butyrate, dexamethasone, xanthine derivatives, and hydroxyurea.

宇宙における細胞培養

Cell Culture in Space Life Science

(8)

佐藤　温重*

はじめに

1992年9月12日に打ち上げられ，8日間の宇宙飛行を行ったスペースシャトル・エンデバーには日本人初の宇宙飛行士（搭乗科学者）毛利衛博士が搭乗し，日本初の大規模な宇宙実験が行われたことはよく知られていることである。この宇宙実験は第一次材料実験（FMPT，ふわっと'92）と称され，34の実験テーマが実施されたが，その中の12テーマはライフサイエンスの実験であり，宇宙医学，細胞生物学，放射線生物学，バイオテクノロジー，時間生物学など多様なテーマの実験が実施された。L-6の実験は筆者らの研究グループの細胞培養の実験であった。L-6実験の成果の一端[1]と宇宙実験により最近明らかになりつつある細胞の重力応答の機序について述べることにする。

1. 宇宙実験に用いられた培養細胞

動植物の培養細胞は微生物などと同様に比較的単純な生命維持装置で培養が可能であり，宇宙飛行実験において要求されるきわめて厳格な搭載条件を比較的容易に合格できたため，培養細胞は宇宙実験の初期から宇宙飛行に供されている（表1）。実験の目的は主として細胞の生存，増殖，維代，生理機能に及ぼす宇宙環境の影響を調べることであった。宇宙実験でいう宇宙環境とは宇宙環境因子のうち，宇宙実験室内にその作用が及ぶ無重力，宇宙放射線などをさしている。これまでの動植物培養細胞を用いた実験は無重力の影響に主眼がおかれている。しかし，培養細胞は宇宙飛行中に無重力に曝露されるだけでなく，宇宙放射線あるいは打ち上げ時や帰還時の加速等に曝露されているので，飛行中に1Gを負荷した飛行1G対照との対比がなされた実験以外は，無重力の影響を含む宇宙飛行の影響というのが正確である。

2. L-6実験の経過

筆者の研究グループが提案した実験はL-6A「哺乳類培養細胞の超微構造と機能に及ぼす微小重力の影響に関する研究」である。この研究の目的は①サル腎細胞の細胞内骨格の再配列，②同細胞のブドウ糖消費量，ウロキナーゼ産生に及ぼす無重力の影響，ならびに③宇宙における細胞培養法の確立などであった。

サル腎由来のJTC-12細胞は4個の培養容器（ACC 1, 2, 3, 4）に植込み，2日間前培養されたのちにスペースシャトルに搭載され，打ち上げられた。シャトルが軌道に達したMET（宇宙経過時間 Mission Elapsed Time）0日から37℃のインキュベーター内で培養が開始された。毛利衛搭乗科学者によってMET 2日，3日および5日に位相差顕微鏡写真が撮影された。ACC 1および2の細胞はMET 2日にトリプシン処理をして再培養し，ACC 1の細胞は2時間後に，またACC 2の細胞は24時間後にグルタルアルデヒドで固定し冷蔵して帰還した。ACC 4の細胞はMET 2日から24時間無血清培地で培養し，その培地を採取し，冷蔵し

* Atsushige Sato：東京医科歯科大学歯学部歯科理工学第二講座 Department of Biomaterials Science, Faculty of Dentistry, Tokyo Medical

DIFFERENTIATED PROPERTIES CHARACTERISTIC OF RENAL PROXIMAL EPITHELIUM IN A CELL LINE DERIVED FROM A NORMAL MONKEY KIDNEY (JTC-12)

(5)-1

YOH TAKUWA[1] AND ETSURO OGATA

Fourth Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo 112, Japan

(Received 6 November 1984; accepted 22 April 1985)

SUMMARY

The JTC-12 cell, an established cell line derived from a normal monkey kidney, was studied in an attempt to characterize the epithelial qualities. Phase contrast microscopy showed dome formation in confluent monolayers and electron microscopic examinations revealed the presence of numerous microvilli on the apical membranes and desmosome between cells. Sonicated cells showed activities of γ-glutamyl transpeptidase, leucine aminopeptidase, alkaline phosphatase, and trehalase, marker enzymes of renal proximal epithelium. Alkaline phosphatase activity exhibited the characteristics of a renal type isozyme. Furthermore, confluent JTC-12 monolayers exhibited Na^+-dependent transport of hexose, amino acid as well as inorganic phosphate. These findings indicate that JTC-12 cells in monolayer culture maintain ultrastructural, biochemical, and physiological properties of renal proximal epithelial cells. This cell line will be useful for further studies on cellular functions of renal proximal epithelium.

Characterization of Na^+-dependent phosphate uptake in cultured kidney cells (JTC-12) from monkey

(5)-2

Yoh TAKUWA* and Etsuro OGATA

Fourth Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo 112, Japan

(*Received 18 March 1985/13 May 1985; accepted 30 May 1985*)

Phosphate uptake was studied in confluent monolayers of an epithelial-cell line (JTC-12) derived from monkey kidney. Phosphate uptake consisted of a saturable, Na^+-dependent, component, which accounted for about 80% of the uptake, and a non-saturable, Na^+-independent, component. The saturable component was specifically dependent on the presence of extracellular Na^+ and has an apparent K_m value for phosphate of 0.12 mM at 137-mM-Na^+, which is close to those reported in the brush-border membranes in mammalian kidneys. The presence of Na^+ in the uptake solution decreased the K_m for phosphate without affecting the V_{max}. Phosphate uptake was inhibited by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and ouabain, suggesting that phosphate transport is an active, energy-dependent, process and is dependent on an Na^+ gradient across cell membranes. With respect to the effect of external Na^+ concentration, a sigmoid relation was seen between the initial velocity of phosphate uptake and Na^+ concentrations, and Hill analysis gave a Hill coefficient of 1.8. In the pH range 6.6–7.4, phosphate uptake declined with increasing pH. Phosphate uptake was stimulated when cells were cultured in the presence of insulin, and was also affected by changes in phosphate concentrations in cultured medium. These results indicate that JTC-12 cells have an Na^+-dependent phosphate-transport system with many of the features of phosphate transport in the proximal tubule.

Regulation of sodium-coupled phosphate transport by extracellular phosphate in cultured kidney cells (JTC-12)

(5)-3

YOH TAKUWA, YASUHIRO TAKEUCHI AND ETSURO OGATA

Fourth Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo, Japan

(*Received 4 February 1986; accepted 11 April 1986*)

Summary

1. Cultured monkey kidney cells (JTC-12) have a Na^+-dependent phosphate (P_i) transport system with characteristics similar to that of the renal proximal tubule. Na^+-dependent P_i uptake in JTC-12 cells is affected by P_i concentrations in the culture medium. In this investigation, further characterization of this phenomenon was carried out.

2. Lowering the concentration of extracellular P_i (3.0 mmol/l to 0.3 mmol/l) induced an increase in Na^+-dependent P_i uptake compared with that in control cells maintained in 3.0 mmol/l P_i, whereas Na^+-dependent transport of hexose and amino acid was not altered. This response was first evident at 4 h after the extracellular P_i concentration was reduced and slowly developed over the subsequent 24 h.

3. Kinetic analysis showed an increase in the V_{max} without a change in the apparent K_m for P_i in cells cultured in the low P_i concentration compared with control cells.

4. The response of P_i uptake was only partially prevented by cycloheximide, suggesting that both protein synthesis-dependent and -independent mechanisms are involved in the development of the response. Insulin, which has a stimulatory effect on P_i uptake in JTC-12 cells, did not affect this response.

5. These data indicate that JTC-12 cells respond to changes in extracellular P_i concentration by changing the Na^+-dependent P_i uptake system. This response has a number of properties typical of the phenomenon of adaptation of renal P_i transport *in vivo* to dietary phosphorus load.

Key words: adaptation, extracellular phosphate, kidney cells, sodium-dependent phosphate transport.

Correspondence: Dr Yoh Takuwa, Division of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, U.S.A.

A New Approach to the Modification of Cell Membrane Glycosphingolipids: Ganglioside Composition of JTC-12 P3 Cells Altered by Feeding with Galactose as a Sole Carbohydrate Source in Protein- and Lipid-Free Synthetic Medium

(6)

TATSUYA KAWAGUCHI,*·† TOSHIKO TAKAOKA,* EIKO YOSHIDA,* MASAO IWAMORI,*·‡ KIYOSHI TAKATSUKI,† and YOSHITAKA NAGAI*·‡·[1]

**Department of Biochemistry, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, †Second Department of Internal Medicine, Kumamoto University Medical School, 2-2-1 Honjyo, Kumamoto 860, and ‡Department of Neurobiology, Brain Research Institute, Niigata University, Asahi-machi, Niigata 951, Japan*

A significant difference in the glycosphingolipid composition of JTC-12 P3 cells established from monkey kidney tissue was observed when cells cultured in a protein- and lipid-free synthetic medium containing glucose (DM-160) as a sole carbohydrate source were transferred and cultured in the same medium containing galactose and pyruvic acid (DM-170) in place of glucose. In particular, the amounts of gangliosides GM3, GM2, and GD3 in the cells cultured in DM-170 were 5.3-, 17.8-, and more than 8-fold those in the cells cultured in DM-160, respectively, indicating that anabolism of gangliosides is greatly enhanced in cells cultured in the presence of galactose and pyruvic acid, as compared with cells cultured in the presence of glucose. In fact, after cultivation of cells in the medium with *N*-acetyl-D-[^{14}C]mannosamine for 96 h, the radioactivity incorporated into the gangliosides of the cells in DM-170 was 10-fold that of the cells in DM-160. Among the gangliosides of the cells in DM-170, highly sialylated molecules such as GD3, GD1a, GD1b, and GT1b were preferentially labeled, indicating that the sialyltransferases responsible for the synthesis of gangliosides are significantly more activated in cells cultured in DM-170 than in DM-160. These observations reveal that the glycosphingolipid composition of the plasma membrane can be modified epigenetically under well-defined conditions and provide important clues for clarifying the roles of glycosphingolipids associated with particular cell functions.

PROPAGATION OF HEPATITIS A VIRUS IN A RENAL CELL LINE JTC-12.P3 OF CYNOMOLGUS MONKEY ORIGIN

(7)

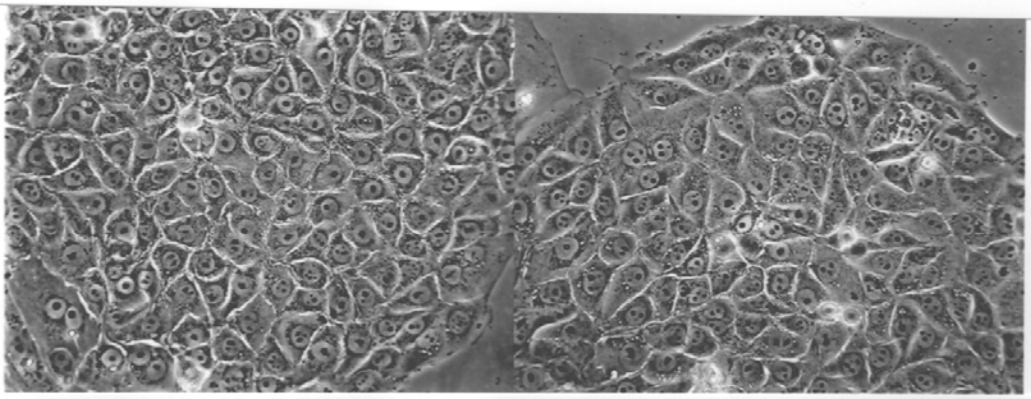
M. BABA, M. TAKEGAWA, M. KAITO, K. MIYAMOTO, S. SUZUKI

Third Department of Internal Medicine, Mie University School of Medicine 2-174, Edobashi, Tsu City, Mie Prefecture, Japan

Received May 27, 1992; revised January 8, 1993

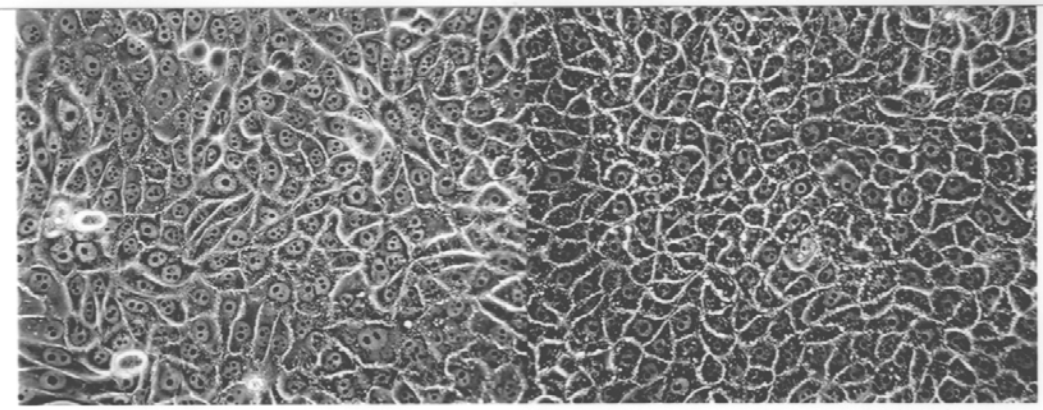
Summary. – Human hepatitis A virus (HAV) derived from 10 % HAV infected marmoset liver homogenate and faeces from acute hepatitis A was successfully propagated *in vitro* in a new cell line, JTC-12.P3. The cell line originated from the renal cortex of cynomolgus monkey which was adapted to growth in a serum free, protein free, chemically defined synthetic medium. Replication of the virus was followed by solid phase RIA, immunofluorescent staining, and immunoelectron microscopy. The propagation of HAV occurred over several passages, with the 1st and 2nd passages requiring at least 8 weeks each. However, with the increasing serial passage of virus, the period needed to detect it was shortened, suggesting the adaptation of HAV to the cells. The identity of the newly synthetized virus particles with HAV was established by immunoelectron microscopy and immunofluorescent blocking effect with human convalescent serum. The HAV propagated in JTC-12.P3 cells banded predominantly at a density of 1.32 g/cm^3 in CsCl gradient. The infected cells showed no specific signs of CPE. Ultrastructurally, clusters of virus particles 27 nm in diameter were observed mainly in the lysosomal vesicles and freely in crystalline array in the cytoplasm, too. Addition of 0.1 % of various anti-HAV negative sera or of prostaglandin E_1 to the culture medium caused accelerated propagation of HAV.

TC4B



MK-P3

TC 9B



MK-P3