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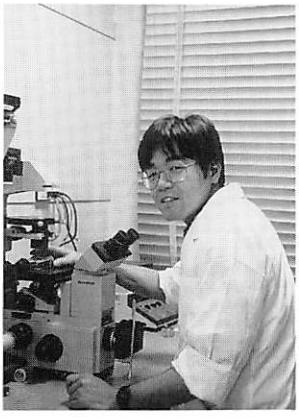
Molecular Biology





DEPARTMENT OF MOLECULAR BIOLOGY

GENERAL SUMMARY

Cancer cells are distinguished from their normal counterparts in that they express a subset of genes differently. Such changes are most likely due to the stimulation of integral programs of gene regulation that are associated with cell division and cell differentiation. It might be thought that integral programs of gene regulation are affected by transfection of oncogenes during transformation.

A majority of these oncogenes encode proteins that function in hormone signalling, for example, *sis* encodes PDGF; *erb*-B encodes the receptor for EGF; and both *src* and *ras* encode proteins that are probably involved in second signalling within the cell. A second class of oncogenes encode proteins that are thought to regulate differentiation, for example, *myc, myb* and *fos.* Therefore, it seems likely that each of oncogenes contributes to induction of malignant cell growth by affecting the regulation of a subset of other genes.

Expression of genes in any organism is highly regulated. Many different programs of gene regulation are being simultaneously carried out and are undoubtedly integrated with one another. Isolation and chracterization of molecules responsible for gene expression will permit an analysis of the programs of gene regulation. These molecules possibly play integral roles in modulation as cells proceed through the cycle, undergo diffrentiation, or express new functions. It should be possible to describe at this level how two or more oncogenes can contribute to a malignant phenotype.

We have continued our efforts in order to contribute to essential studies on the problems discussed above. More recently, we have been successful in the discovery of a novel oncogene from one specimen of human stomach cancer. The DNA fragment from the tertiary transformant having both transforming activity and human *Alu* sequence is cloned as a reconmbinant of λ vector. It is the second oncogene newly identified from human stomach cancers and has no homology to 19 known oncogenes (including *hst*). Since human stomach cancer is evidently the most important problem to be investigated and conquered among cancers occurring in Japan because of its incidence and severeness, the significance of our discovery of a new oncogene from stomach cancer must be emphasized, for the new oncogene could be an important key or a clue for the breakthrough.

In the next step of our current project, we must first attempt to determine the coding sequence required for transforming activity. And the second problem will be to find out what kind of cellular function is responsible to this gene? The third aim will be to understand what kind of subset of genes is expressed by this active oncogene during transformation.

(1) A transforming gene from a human primary stomach cencer.

K. Iida, Y. Iida, T. Kameyama and M. Mai*

We found transforming activity in DNA preparation from a human primary stomach cancer. The active transforming fragment contained no homologous sequences to v-K-*ras*, v-*raf*, v-*myc* and *hst*[1] which were reported to be active in other stomach and colon cancers. DNA fragments containing human-specific *Alu* sequences were cloned. One of the clones seemed to have transforming activity.

DNAs were prepared from 4 specimens of human primary stomach cancers (3 poorly differentiated adenocarcinomas and 1 moderately differentiated tubular adenocarcinoma). These DNAs were transfected to NIH/3T3 cells simultaneously with pSVneo DNA by the calcium method. The NIH/3T3 cells transfected were cultivated and propagated in the presence of geneticin. Tumors were developed in nude mice which received the geneticin-resistant cells, except for those cells transfected with a moderately differentiated tubular adenocarcinoma DNA. DNAs prepared from all tumors contained the human-specific *Alu* sequence.

The secondary trasformant was obtained by trasfection of DNA from a tumor (SC53 DNA transfected)[1], as shown in Fig. 1. The secondary transformant indicated tumorigenicity in nude mouse and its DNA contained *Alu* sequences by the southern hybridization technique as shown in Fig. 2. This DNA clearly indicated the absence of any homologous sequences to those oncogenes, v-K-*ras*, v-*raf* and v-*myc*. The tertiary transformant was also obtained by the transfection of DNA from the secondary transformant cells. The DNA prepared from the tertiary transformant also contained *Alu* sequences as shown in Fig. 2. The tertiary transformant also exhibited tumorigenicity in nude mouse.

DNA from the tertiary transformant was digested with *Bam* Hl and ligated to the arms of EMBL 3. The resulting library was screened with *Alu* sequence and eight λ-phage clones were selected. Five of them were the same clones, so named 50α-2. Complete digestion of 50α-2 with *Bam* Hl resulted giving 10.0 kbp and 4.0 kbp fragments containing the human-specific *Alu* sequence and no fragment without *Alu* as shown in Fig. 3.

DNA of the tertiary transformant and 50α-2 had no homology to *hst*, which is the transforming gene found recently in human stomach cancer[2]. the homology of 50α-2 DNA was examined in comparison with the 18 oncogenes; namely v-*abl*, v-*erb*A, v-*erb*B, *erb*B-2, v-*fes*, v-*fgr*, v-*fos*, v-*mos*, v-*myc*, N-*myc*, v-*myb*, v-*raf*, v-H-*ras*, v-K-*ras*, N-*ras*, v-*rel*, v-*sis* and v-*src*. No homologous sequences to those oncogenes were found in at all 50α-2.

As a consequence, we have now succeeded in isolating and molecular cloning a novel transforming gene from a human stomach cancer. Our efforts are now concerned with determination of the structure and the function of a discrete DNA segment responsible for transforming activity.

* Department of Surgery

1) SC53: a poorly differentiated adenocarcinoma as indicated in the following summary.

2) Sakamoto, H. et al., Proc. Natl. Acad. Sci., 83, 3997 (1986).

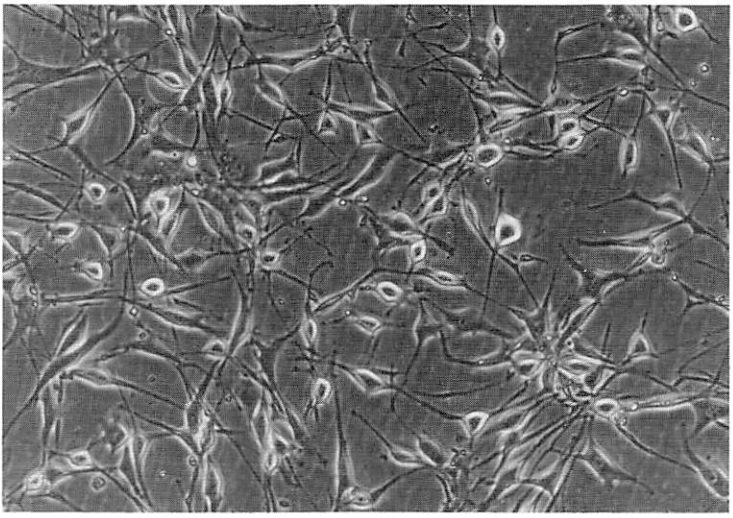


Fig. 1. The secondary transformant cells obtained by transfection with DNA of the primary transformant tumor.

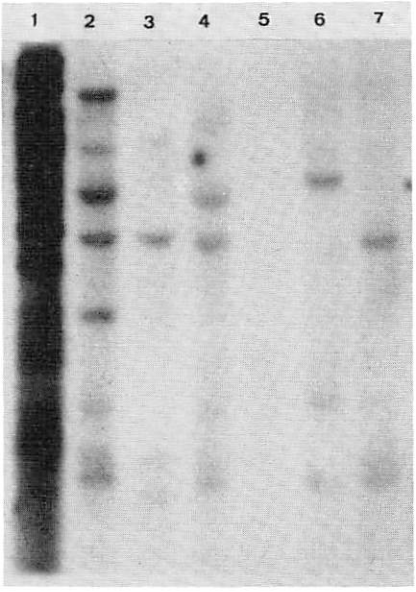


Fig. 2. Presence of human-specific *Alu* sequences in the primary, secondary and tertiary transformants. Primary transformant; # 1, secondary transformant; # 2 and tertiary transfromants; # 3, 4, 5, 6 and 7.

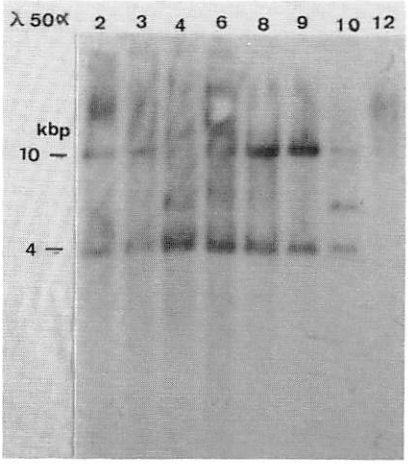


Fig. 3. Presence of human-specific *Alu* sequences in clones. Number indicates clones.

(2) Amplification of c-oncogenes in human stomach cancer.

Y. Iida, K. Iida, M. Mai*, Y. Takahashi* and T. Kameyama

In tumor development, oncogenes appear to be activated from their prototypes by point mutation, gene amplification, promoter insertion and DNA rearrangement. We examined human stomach cancers and found some cases which carry amplification of oncogenes. Eleven cases of stomach cancer examined were pathologically classified and abbreviated as follows:

A. Primary stomach cancer
  Poorly differentiated adenocarcinoma;
    SC31, SC32, SC53, SC54, SC55
  Moderately differentiated tubular adenocarcinoma;
    SC33
B. Stomach cancer transplanted in nude mouse
  Poorly differentiated adenocarcinoma;
    TYS, IFS
  Moderately differentiated tubular adenocarcinoma;
    OSS
  Papillary adenocarcinoma;
    TTS

The total cellular DNAs were prepared from tumor tissues, digested with the restriction endonuclease *Eco*RI and analyzed by the Southern blot method. Specific DNA fragments of oncogenes (most of them derived from virus, except c-*erb*B-2, N-*ras* and N-*myc*) were nick-translated and used as probes. Table 1 shows the results from 16 oncogenes which were at normal or amplified level in our examined DNAs. The most outstanding result was amplification of the c-*erb*B-2 gene in SC33. The degree of amplification was about 100-fold as shown in Fig. 1 and Fig. 2(a). Similar results for amplification of the c-*erb*B-2 gene in tumors, especially adenocarcinomas, have been reported by others.[1] The c-*sis* gene was amplified in SC31 and SC53 about 10 times more than that of the normal human tissue as shown in Fig. 2(b). This amplification of the c-*sis* gene in tumors is the first case we know. The c-*myc* gene was amplified 10-fold in SC55 and several fold in SC31, SC33 and SC53. Curiously, many oncogenes were amplified several fold in SC53, namely *abl, raf,* H-*ras,* K-*ras, myc,* N-*myc, fos* and *sis* genes. As shown in the previous abstaract, we have succeeded in isolating NIH/3T3 transformants by SC53 DNA transfection. Among stomach cancers transplanted into nude mouse, the N-*ras* gene was amplified several fold in TYS, TTS and OSS.

It seemes to us that these multiplied oncogenes are closely concerned with cause or effect of stomach cancers.

* Department of Surgery

1) Semba, K., Kamata, N., Toyoshima, K. and Yamamoto T., Proc. Natl. Acad. Sci., 82, 6497 (1985).

Table 1. Amplification of oncogenes in 11 human stomach cancer DNAs. + shows normal level and ++ to +++++, low to high levels of oncogenes.

Oncongenes	31	32	33	52	53	54	55	TYS	TTS	OSS	IFS
1. *src*	+	+	++	+	+	+	+	+	+	+	+
2. *fes*	+	+	+	+	+	+	+	+	+	+	+
3. *erb*B-1	+	+	+	+	+	+	+	+	++	++	++
*erb*B-2	+	+	+++++ ×100	+	+	+	+	+	++	+	+
4. *abI*	+	+	+	+	+++	+	+	+	+	++	++
5. *mos*	+	+	+	+	+	+	+	+	+	+	+
6. *raf*	+	+	+	+	++	+	+	+	+	+	++
7. H-*ras*	+	+	+	+	++	+	+	+	+	+	+
8. K-*ras*	++	++	++	+	++	+	+	++	++	++	++
9. N-*ras*	+	+	+	+	+	+	+	+++	+++	+++	+
10. *myc*	++	+	+++	+	++	+	+++++ ×10	+	+	+++	+
11. N-*myc*	+	+	+	++	++	+	+	+	+	+	+
12. *fos*	+	+	++	+	+++	+	+	+	+	+	+
13. *myb*	+	+	+	+	+	+	+	+	+	+	+
14. *sis*	+++	+	++	+	++++ ×10	+	+	++	++	+	+
15. *erb*A	+	+	+	+	+	+	+	+	+	+	+

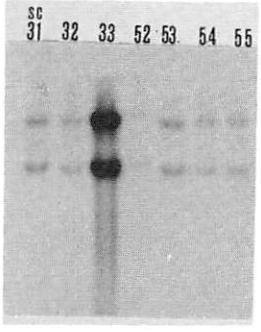
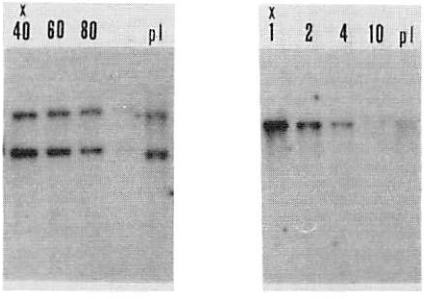


Fig. 1. Southern blot analysis of DNAs from human stomach cancers. 10 μg of DNA were digested with *Eco*RI, electrophoresed, transferred to a nitrocellulose membrane and hybridized with ^{32}P-labeled c-*erb*B-2 (KX fragment; kindly provided by Dr. T. Yamamoto)



(a) (b)

Fig. 2. Caluculation of the degree of (a) c-*erb*B-2 and (b) c-*sis* gene amplification. 10 μg of DNA from (a) SC33 and (b) SC53 were serially diluted with a buffer and the intensity of the bands was compared with that of 10 μg of human placental DNA (in the pl lane).

(3) Suppression of tumorigenicity in PCC4-azal teratocarcinoma without antigenic alteration.

T. Nomura and T. Kameyama

We have already shown that the embryoid body cells of strain 129 mouse teratocarcinoma OTT6050 can proliferate well in diffusion chambers implanted into mouse peritoneal cavities[1]. These embryoid body cells grown in diffusion chambers showed a marked reduction in tumorigenicity without antigenic alteration[2]. These results may be explained by a) differential growht of non-tumorigenic minority cells, b) the conversion of cells from tumorigenic to non-tumorigenic without accompanying differentiation, or c) the loss of tumorigenicity coupled with cell differentiation. In order to distinguish among these possibilities and also to determine the generality of the previous results, we carried out diffusion chamber experiments with the established cell line PCC4-azal.

The PCC4-azal cells, derived from OTT6050 teratocarcinoma, were given by the Pasteur Institute. This EC (embryonal carcinoma) cell line is multipotent in differentiation and has a strong tumorigenic activity. Cells were cultured in diffusion chambers which implanted into mouse intraperitoneally. The grown cells by this unique technique were examined for their tumorigenic activity and antigenicity. Tumorigenicity was estimated by the survival time of the syngeneic 129 mice after receiving 1×10^6 living cells. Experiments were terminated after 140 days and the animals that died without carrying tumors were not included in the analytical data. The antigenicity tested here were F9 and H-2. For the determination of antigenicity, a quantitative absorption method was adopted and a complement dependent cytotoxicity test was carried out for the detection of anti-serum activity. F9 cells and 129 spleen cells were used as cytotoxicity test targets for the specific anti-F9 and anti-129 serum respectively. Animals, receiving in vitro grown PCC4-azal cells, started to die after the 19th day of injection and within the following 15 days, 95% of the tested mice died with tumors (Fig. 1). On the contrary, the tumorigenic activity of the PCC4-azal cultured in diffusion chambers was reduced markedly. The onset of death of mice was delayed and, more importantly, these mice also lived much longer compared with the control mice treated with the same number of in vitro grown cells (fig. 2). On the other hand, all of the PCC4-azal cells tested failed to absorb anti-129 serum activity irrespective of thier cell numbers and culture conditions (Fig. 3) whereas all the tested cells, in vitro and also in diffusion chamber cultured PCC4-azal and control F9, absorbed anti-F9 serum activity in a cell number dependent way (Fig. 4). In order to examine the cellular alteration about differentiation ability by diffusion chamber culture, the cells recovered from chambers were transferred again on plastic and re-cultured in vitro. Morphologically differentiated cells could be detected only when the cells were re-cultured on plastic dishes for several days.

Therefore, it seems plausible that the conversion of cells from tumorigenic to non-tumorigenic may occur without any major visible differentiation in this diffusion chamber culture. However, it still remains to be shown whether the loss of tumorigenicity is the first step, or an independent event, in the differentiation of teratocarcinoma cells.

1) Satoh, N., T. Nomura and T. Kameyama, Develop. Growth & Differ., 19, 249, (1977).
2) Nomura, T., N. Satoh and T. Kameyama, Exp. Cell Res., 153, 506, (1984).
3) Nomura, T. and T. Kameyama, Cancer Letters, 34, 21, (1987).

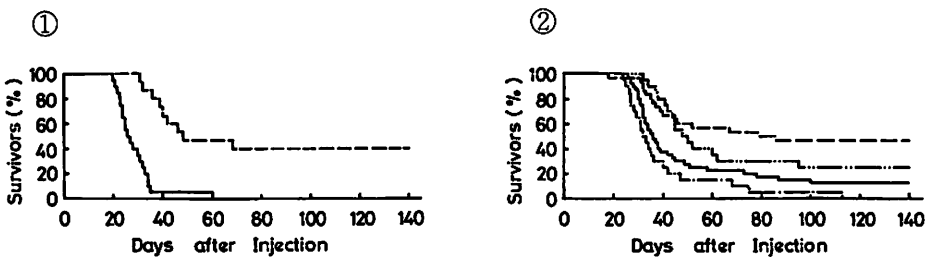


Fig. 1. Survival profiles of mice after i.p. injection of in vitro cultured PCC4-azal cells. (a) $1X10^6$ cells/mouse (20 mice) (————); (b) $1X10^4$ cells/mouse (15 mice) (— — —).

Fig. 2. Tumorigenicity of differently conditioned PCC4-azal cells. $1X10^6$ viable cells were injected into a mouse intraperitoneally. (a) 25 days cultured in diffusion chambers (40 mice) (————); (b) 80 days cultured in diffusion chambers (30 mice) (— — — —); (c) maintained for 80 days in diffusion chambers, transferred about every 20 days (20 mice) (—·— ·—); (d) treated as in (c) with additional 80 days of continuous culture in diffusion chamber (20 mice) (—··——··—).

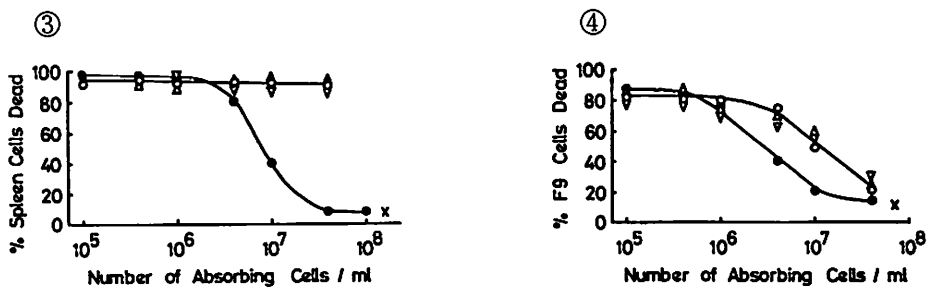


Fig. 3. Residual cytotoxicity of anti-129 serum after quantitative absorption by differently conditioned cells. ●, 129 spleen cells; ○, in vitro PCC4-azal cells; △, PCC4-azal cells cultured in diffusion chambers for 30 days and ▽, PCC4-azal cells cultured in diffusion chambers for 70 days; x, complement control.

Fig. 4. Residual cytotoxicity of anti-F9 serum after quantitative absorption by differently conditioned cells. ●, in vitro F9 cells; ○, in vitro PCC4-azal cells; △, PCC4-azal cells cultured in diffusion chambers for 30 days and ▽, PCC4-azal cells cultured in diffusion chambers for 70 days; x, complement control.

(4) Studies on U series small nuclear ribonucleoprotein particles from mouse teratocarcinoma cells.

M. Matsuda, T. Yamada*, T. Nomura and T. Kameyama

One area of current interest in the field on gene regulation and cell metabolism is the role played by snRNAs and snRNPs. And mouse teratocarcinoma cells are considered to be useul as a model system for studies on early mammalian development and cell differentiation. It is important, therefore, to characterize the snRNAs and snRNPs in this system for the understanding of gene regulation during the differentiation. So the U-snRNP particles from the mouse teratocarcinoma embryoid bodies (OTT-6050) and F9 cells were studied.

A polyclonal SLE anti-Sm antibody column was useful to purify teratocarcinoma Sm snRNPs, which contain Ula, Ulb, U2, U4, U5, U6 and X snRNAs, from both cellular and nuclear extract (Fig. 1). The high proportion of Ula-snRNP to Ulb-snRNP was reproduced in the population of Ul-snRNP from teratocarcinoma cells. The X snRNA could not be detected in the Sm snRNP fraction from human KB cells and 129 male mouse liver nuclei. An anti-2, 2, 7-trimetylguanosine antibody column was also useful for fractionation of U-snRNPs from embryoid bodies. Only Ula, Ulb and U2 snRNPs were retained by and eluted from the column. However, when the deproteinized RNA was loaded onto the column, other U snRNAs (U4 and U5) were also retained and eluted as well as the previous three. By fractionation with a DEAE Sepharose column, partially purified Ul-snRNP (s) was eluted at approximately 0.18 M NH_4Cl and U2 + U4 + U5, as well as Ul-snRNP (s), were eluted at approximately 0.3 M NH_4Cl. The U6 and U7/U10-snRNPs were also eluted at approximately 0.35 M NH_4Cl. An RNA component, whose migration properties correspond to that of U8 snRNA, was also detected in the U1-snRNP fraction. Fractionation of the nuclear extract by sucrose gradients at physiological ionic strength revealed that U-snRNPs sediment into three different regions. It was also suggested that the snRNP species show differences in their molecular weights and/or in their situations with the counterpart particles.

These observations and other available information suggest more diverse molecular forms and species of U-snRNPs in embryonal states than in differentiated states.

*Lab. of Internal Medicine II, Azabu University School of Veterinary Medicine.

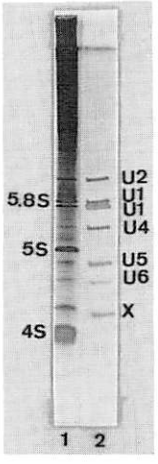


Fig. 1. Analysis of RNA eluted from the polyclonal SLE anti-Sm antibody column. Cytoplasmic RNA of embryoid bodies (1) and U-snRNA from F9 cell (2).

(5) Asymmetric development in chick embryonic gonads.

H. Kawakami

In chick, at the 7-8th embryonic day an indifferent gonad differentiates into an ovary and a testis respectively after the genetic sex. Its morphological appearance is marked by the asymmetric development of a female gonad in contrast to the symmetric one of a male gonad. The synthetic estrogen, diethylstilbestrol dipropionate (DES) artificially causes the asymmetric development of a male. In other words, it is a feminization of a genetic male. This feminization has occured effectively through DES treatment by the 4th day as previously reported. It also occured in a similar way by DES treatment after the divergence from indifferent to the testis began. In 14 day-embryos treated at the 9th day, a male gonad had a morphological and histological structure similar to that seen when treated at 4th day. The left gonad was covered by a thick cortex containing many germinal cords. Primordial germ cells (P. G. C.) were recognized in a peripheral layer and the germinal cords, and under the cortex layer a medulla developed. That is an ovotestis. On the other hand, the right gonad was still retained, covered by a few thin flattened germinal epithelia under which, in the medulla, the male cords developed containing P.G.C. DES treatment at the 11th day, when the solid male cords had become distinct in the medulla, brought a layer constituted from a few cubic cells among which P.G C. were embedded shortly after 2 days. On the next day after treatment, even at the 13th day, the cubic cells appeared in areas among the flattened germinal epithelia. Here, it was shown that an artificial transformation of a differentiated male into a female (this case is an ovotestis) occurred asymmetrically between the left and the right as an indifferent male.

Male chicks have mullerian ducts that have regressed completely by the 12-13th embryonic day, while females have left mullerian ducts that are persistant and right ones that have partially regressed. It was observed that the feminization of male by DES caused not only the alteration of a testis to an ovotestis, but also the reappearance of the mullerian ducts. In the 4-day's treated male the mullerian ducts had almost regressed by the 11-12 days and then became slender and thin like a membrane, but the clear duct became evident by the 13th day, when the extent of asymmetricity between the left and the right gonad was greater. These were certainly not the same as the female ducts in their macroscopical aspects, at least under these conditions. The asymmetricity between the left and the right was partial, though the right was a little shorter than the left. Some treated male embryos had only the mark of the duct like a string. In such a case, an embryo had a less feminized testis. A search for more effective conditions continues. Many workers suggest that in chick embryonic development, the regression of the female right ovary occurs by a mechanism different from that of the right mullerian duct.

(6) Partial simulation of physiological constraints on tumor growth by in vitro culture.

T. Nomura

When a large number (1×10^5 or more) of transformed BALB/3T3 cells is inoculated into nude mice subcutaneously, they consistently produce rapidly growing sarcomas within around 2 weeks. When a smaller number of cells (1×10^3 – 1×10^4) is inoculated, however, the tumor growth is much slower so that in some cases no tumors appear until 2 months or more after the inoculation. In these cases the growth rates and also the latent periods of each tumor vary widely. Fewer than 1×10^3 cells rarely result in tumor formation, whereas the original cultured cells used for the inoculation increase in number from 2000 to 4000 fold per week in vitro. It is apparent that the transformed cells multiply much more slowly in vivo than in vitro. One of the possible explanations of this growth difference is the nutrient. Cells in the animal body receive their nutrients from the interstitial fluid, which is drained from the lymphatic system. In order to understand the in vivo constraints on the tumor development of cell growth, we simulated the in vivo nutritional condition in vitro by using the lymph as a macromolecular supply.

The cells used in this work were 14g2c and 21k, both derived from BALB/3T3 clone A31. The 14g2c is a spontaneously transformed cell line and is recloned in vitro after twice passage in nude mice where they produce sarcomas. On the contrary, the 21k formed neither colonies in agar nor tumors in nude mice. These cells were cultured on plastic tissue culture plates in concentrations of lymph up to 100%. Calf lymph was less effective in supporting multiplication than calf serum up to about 50%. As the concentration of each fluid increased above 50%, the cell multiplication decreased progressively. But this decrease or lower effectiveness of up to 100% serum or lymph was cancelled by addition of the nutrients of synthetic medium MCDB402. Chromatographic analysis revealed that the concentrations of essential amino acids are much lower in biological fluids than that in MCDB402. The most striking difference was for cystine, which was not detectable in either fluid but was present at 200 μM in MCDB402; and for glutamine, present 150-300 μM in serum and lymph and at 5000 μM in synthetic medium. Supplementation of cystine and glutamine was essential for growth enhancement on the plastic (Table 1). However, when the cells were cultured in agar, lymph was much less effective than serum in promoting colony formation, even when supplemented with both cystine and glutamine or with all the constituents of the synthetic medium (Table 2). The low efficiency of tumor production and the reduced growth rate of transformed cells in mice could be the result of a combination of 1) the shortage of growth factors in the interstitial fluid, 2) the remarkable reduction in the concentration of essential amino acids encountered by the cells in passing from culture into mice, 3) the space in subcutane might not be so beneficial for cell attachment and multiplication as on a solid substratum. Other factors such as the growth inhibiting effects of direct contact with quiescent muscle and connective tissue cells still remain to be evaluated.

This work has been done with Professor Harry Rubin at the Department of Molecular Biology and Virus Laboratory, University of California, Berkeley.

1) Rubin, H. and T. Nomura, Cancer Res., In press.

Table 1 Supplementation of undiluted calf serum and lymph with MCDB402 organic nutrients at concentrations present in the synthetic medium.

% biological fluid	Addition	N_4/N_0 [a]	
		Serum	Lymph
10	Regular MCDB402, 90%	23.2	N.D.
40	Regular MCDB402, 60%	N.D.	27.8
100	None	0.5 [b]	2.9
100	All MCDB402 nutrients except major salts	17.5	28.8
100	All MCDB402 nutrients except major salts and glutamine	1.9	N.D.
100	All 18 amino acids of MCDB402	7.6	16.2
100	All 18 amino acids of MCDB402 except glutamine	N.D.	1.3
100	13 essential amino acids	9.2	14.7
100	5 nonessential amino acids	0.4 [b]	3.2
100	Cystine, 200 μM	2.3	4.7
100	Glutamine, 5000μM	0.25 [b]	3.2
100	Cystine, 200μM and Glutamine 5000μM	7.5	9.8

a N_4/N_0; $\frac{\text{number of cells on day 4}}{\text{number of cells on day 0}}$

b cell damaged and detachment.

Table 2 Growth of substratum-attached versus agar-suspended cells in supplemented lymph and serum.

Cells (5×10^3) were seeded in multiwells for attachment in 2% serum, and 100 cells were seeded in agar in the indicated medium. The medium of the attached cells was changed the next day to the indicated medium, and the cells were counted 3 days later. Agar colonies were counted at 14 days.

Medium	Substratum attached cells, N_4/N_1	Agar suspended cells, CEFag (%)
100% lymph + cystine (200 μM) glutamine (5000μM)	5.5	2
100% lymph + all the constituents of MCDB402	7.6	18
90% lymph + 10% serum + cystine (200μM) and glutamine (5000μM)	9.8	40
90% lymph + 10% serum + all the constituents of MCDB402	14.8	72
100% serum + cystine (200μM) and glutamine (5000μM)	13.6	76
100% serum + all the constituents of MCDB402	16.9	73
10% serum in lymph-like MCDB402 [b]	11.0	53
10% serum in standard MCDB402	16.2	76

a N_4/N_1; $\frac{\text{number of cells on day 4}}{\text{number of cells on day 1}}$

b MCDB402 but has lymph like amino acid components.

(7) Amino acid assessment of transformed and non-transformed cell multiplication in vitro.

T. Nomura

Transformed and non-transformed BALB/3T3 cells multiply poorly or not at all in undiluted calf serum or lymph unless the essential amino acids were enriched[1]. Then the quantitative amino acid assessment studies were carried out on cell multiplication in vitro by using the biological fluids. The cells used were transformed 14g2c and non-transformed 21k as described in the previous abstract.

Transformed cells show a higher requirement for glutamine than non-transformed cells do in conventional culture. Because of their faster multiplication, the rapid depletion of glutamine in transformed cell culture may cause their higher requirement of this amino acid. The growth rate of 14g2c cells was reduced to the level of 21k cells by controlling the serum concentration. Then the growth of the two were compared directly in adequate glutamine concentration (Fig. 1). The growth rates of both cells in high glutamine were approximately the same. The 14g2c cells, however, failed to multiply in low glutamine, whereas the 21k cells did so. Then it becomes clear that the transformed cells have a higher glutamine requirement than the non-transformed cells. By contrast, 21k cells showed a slightly higher requirement for cystine than 14g2c cells. Decreasing the concentration of the other essential amino acids to the physiological level caused the reduction of 14g2c cell multiplication at all concentrations of serum and lymph. But this was not so drastic as by the shortage of the glutamine and cystine.

The calf lymph was much less effective than serum for 14g2c cells and ineffective for 21k cells. Indeed lymph has no or very little growth factors which may enhance the utilization of amino acids through the cell multiplication. So the tumor cells have a higher requirement for glutamine than their counterpart of normal tissues. And the lymph may prove useful medium for testing the growth factors.

This work has been done with Professor Harry Rubin at the Department of Molecular Biology and Virus Laboratory, University of California, Berkeley.

1) Rubin, H. and T. Nomura, Cancer Res., In press.

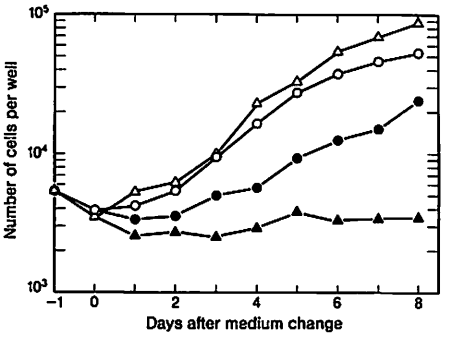


Fig. 1. Direct comparison of the glutamine requirments for transformed and non-transformed cells.

One day after seeding at 5.4×10^3 cell/well, media were changed to experimental ones with 10% calf serum for the non-transformed 21k cells and with 1% calf serum for the transformed 14g2c cells. The media were changed daily through the 7th culture day.

○——○, 21k cells with 1000μM glutamine;
●——●, 21k cells with 27 μ M glutamine;
△——△, 14g2c cells with 1000 μM glutamine;
▲——▲, 14g2c cells with 27 μM glutamine.