Jpn. J. Cancer Res. 80, 444–451, May 1989

Establishment and Characterization of Mouse Leukemia Cell Lines L615K and L7212K Derived from Transplantable Murine Leukemias Maintained in China

Shengguo You,[1,2] Sakan Maeda,[2,4] Shin-ichi Murao,[2,5] Rei Takahashi,[2,6] Jiro Ishikawa,[2] Masaaki Miyazawa,[3,7] Masato Nose[3] and Taketoshi Sugiyama[2]

[1]*Institute of Hematology, Chinese Academy of Medical Sciences, No. 288 Nanjin Road, Tianjin, China,* [2]*Department of Pathology, Kobe University School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650, Japan and* [3]*Department of Pathology, Tohoku University School of Medicine, 2-1 Seiryo-cho, Sendai 980, Japan*

Two culture cell lines L615K and L7212K were established from transplanted murine leukemias L615 and L7212, which had been established and maintained in China for years. Based on morphological, immunological and gene rearrangement analyses, L7212K cells are considered to be of T-cell origin while L615K cells might be immature T-cells. Immunofluorescence assays of viable leukemia cells and fluorescence focus assays of their culture supernate for infectious viruses suggested that recombinant mink cell focus-inducing viruses were significantly involved in both leukemic cell lines. Chromosome analysis of the L615K cells revealed a translocation t(12;17) which probably involved the c-*fos* locus on chromosome 12, since the DNA rearrangement of c-*fos* was demonstrated by Southern blot analysis with *Hind* III, and c-*fos* has been assigned to this chromosome. Although the expression of this gene was not detected by RNA Northern blot analysis, c-*myc* was slightly expressed in both L615K and L7212K cells.

Key words: Murine leukemia virus — Mouse leukemia — Oncogene — Chromosome

Two transplantable leukemias L615 and L7212[1–5] were established and maintained *in vivo* in China. L615 was a transplantable leukemia line originally derived from a spontaneous leukemia in the Kunming strain of mice (derived from domestic Chinese wild mice). A splenic cell suspension from the leukemic mouse was subcutaneously injected into a Fab mouse and a so-called reticulum cell sarcoma was obtained. A cell suspension of the induced tumor was intraperitoneally injected into a Kunming mouse, and an ascites form of reticulum cell sarcoma (ARS) was established. When the cell-free ARS extract containing C-type virus particles was subcutaneously injected into newborn Kunming mice, the injected mice regularly developed leukemia. The leukemia was associated with a marked enlargement of the liver, spleen, and lymph nodes, and was named Jin-638 leukemia. The cell-free extract of Jin-638 leukemia was then injected into newborn syngeneic mice (615 strain) and this induced the same type of leukemia. The leukemia was maintained by serial transplantation in syngeneic adult mice and named L615 by You *et al.* L7212 is a transplantable leukemia obtained by subcutaneous transplantation of cells from the spleen of a spontaneous leukemia which developed in a 615 mouse in 1972.[6,7]

Several 615 mice with transplanted L615 and L7212 cells were transported to the Department of Pathology at Kobe University and used for further analyses.

Since the leukemia lines were established in domestic Chinese mice and China had been an almost closed country for a long time after World War II, it was deemed interesting to analyze the retroviral aspects of these leukemias in order to determine the genetic and evolutionary relationship between Chinese mice and other laboratory mouse strains.

To facilitate genetic and retroviral analyses *in vitro*, permanent cultured cell lines were established from L615 and L7212 leukemias, and morphological and chromosomal studies, Southern and Northern analyses of some c-*onc*, and viral analyses of these cells were performed.

MATERIALS AND METHODS

Cell culture Cell culture was initiated from the leukemic spleen of mice transplanted with each of L615 and L7212 leukemia cells. Spleens were minced with scissors and cells were suspended in Iscove's modified Dulbecco's culture medium (IMDM) and passed through a No. 80 stainless steel mesh. The cells were cultured in IMDM (Flow Labo., Utah) supplemented with 15% fetal bovine serum (FBS) (HyClone Labo., Logan, Scotland) in 60 mm Petri dishes (Falcon, Oxnard, Calif.). At the begin-

[4] To whom communications should be addressed.
Present addresses: [5]Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA, [6]Clayton Molecular Biology Program, Children's Hospital of Los Angeles, 4650 Sunset Blvd., Los Angeles, California 90027, USA, [7]Laboratory of Persistent Viral Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana 59840, USA.

ning of cell culture, a 15% conditioned medium (CM) prepared from the thymus of a Balb/c mouse was added and half of the medium was changed every 4 days. After the establishment of the two leukemia cell lines, they were maintained in RPMI1640 medium (Flow Labo., Irvine, Scotland) supplemented with 20% FBS.

Colony formation in soft agar Cultured cells were seeded in 0.3% soft agar on 0.5% hard base agar. The number of colonies was counted on days 10–13.

Chromosome analysis Metaphase spreads were prepared from cultured cells by adding colcemid for 2 h. Trypsin-Giemsa-banding was carried out and chromosomes were identified; the nomenclature is that used for banded mouse chromosomes.[8]

Immunofluorescence (IF) assays To characterize phenotypic markers of the leukemic cell lines, IF studies were performed using FITC-conjugated monoclonal anti-Thy 1.2, anti-Lyt 1, anti-Lyt 2 antibodies (Becton Dickinson Immunocytochemistry Systems, Mountain View, Calif.), anti-L3T4 (kindly supplied by Dr. T. Tokuhisa) and the monoclonal anti-Mac 1 antibody (clone M1/70, Bioproducts for Science, Inc., Indianapolis, Ind.).

Detection of cell surface retroviral antigens and titration of infectious retroviruses Expression of murine leukemia viral (MuLV) structural proteins on the surface of the leukemia cell lines was analyzed using a panel of anti-MuLV monoclonal antibodies.[9] The culture supernatant from the leukemia cells was titrated for infectious ecotropic, xenotropic, and mink cell focus-inducing (MCF) viruses by a fluorescent focus assay using several type-specific monoclonal antibodies.[10] Infections ecotropic and xenotropic retroviruses were also titrated by XC-plaque assay[11] and MiCll focus induction assays,[12] respectively.

Electronmicroscopy The transplanted subcutaneous tumors and spleen, and cultured cells were fixed in 5% glutaraldehyde, washed in 0.2 *M* phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols, cleared in propylene oxide and embedded in an epoxy-resin mixture. The ultrathin sections were examined with a 1200EX electron microscope (Japan Electron Microscopy Co.) after heavy metal staining.

Southern blot analysis High-molecular genomic DNA was extracted from subcutaneous tumors and cultured L615K and L7212K cells. DNA was digested with either *Eco*RI, *Bam*HI, or *Hind* III, or on the other hand, either *Eco*RI, *Hind* III, or *Pvu* II for immunoglobulin and T-cell receptor β-chain probes, subjected to electrophoresis in a 0.7% agarose gel and transferred to Nylon membranes (Pall, Biodyne, N.Y.) by Southern blotting.[13] The membranes were baked at 80°C for 2 h and hybridized with ^{32}P-labeled probes in buffer (5×SSPE, 10×Denhardt's, 0.2% SDS and 0.1 mg/ml of denatured salmon sperm DNA) at 65°C for 14–20 h. After hybridization, filters were washed in 5 m*M* NaH_2PO_4, 1 m*M* EDTA, 0.2% SDS (pH 7.0) at room temperature. Autoradiography was performed using X-ray film (Fuji RX, Tokyo) and an intensifying screen (Du Pont, Boston, Mass.) at −80°C overnight.

RNA blot hybridization Total cytoplasmic RNA was extracted from cultured cells by the guanidine thiocyanate method.[14] Total RNA (20 μg in each lane) was run in a gel in 1.0% phosphate buffer after glyoxal treatment, transferred[15] to a Nylon membrane by the method of Southern, and hybridized with the labeled probe DNAs.

Radioisotope labeling of probes Ha-*ras* (0.55 kb), p-fos-1 (1.3 kb), pMYC3 (1.5 kb), pABSubIII (2.3 kb), mouse T-cell receptor β-chain probe (RBL-5 cDNA)[16] and J region probe of mouse immunoglobulin heavy chain (IgJ_H4) were used for labeling. They were labeled with ^{32}P-dCTP (3000 Ci/mmol, Amersham PB10205) by either the nick translation[17] or the primer extension (Amersham Multiprime Kit) method. The specific activity of labeled DNA was 2–5×10^8 cpm/μg.

RESULTS

Establishment of cultured cell lines The L615 leukemia cells started to grow without CM 4 weeks after the start

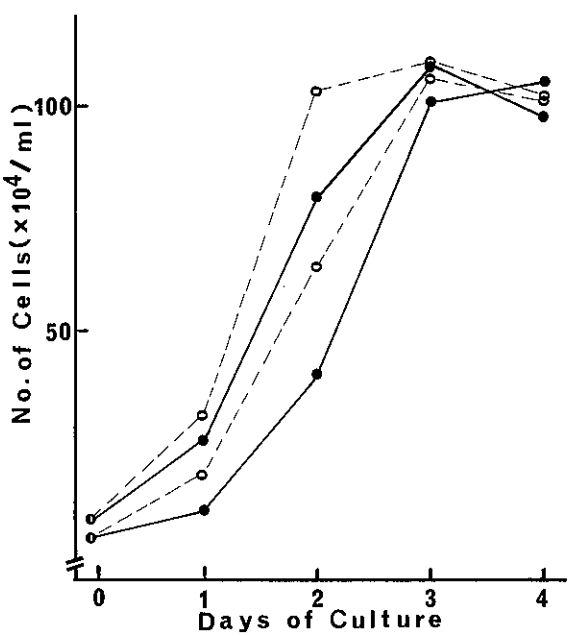

Fig. 1. Growth curves of L615K (○) and L7212K (●) cultured cells. Different numbers of both cell lines were seeded in 60 mm Petri dishes and cell numbers were counted every day. The data are the averages of three different experiments.

of the culture. On the other hand, the L7212 cells did not become CM-independent for a long time. The CM-independence was induced by gradually decreasing the CM. After 4 months, L7212 cells started to grow without CM. The established leukemia cell lines, distinguished from the original transplantable leukemia cell lines and designated as L615K and L7212K, respectively, were maintained in RPMI1640 medium supplemented with 10–15% FBS. The L615K cells grew mostly as single cell suspensions, and about 20% were spindle-shaped cells attached to Petri dishes. The L7212K cells grew only as single cell suspensions. The growth curves of the L615K and L7212K cells are shown in Fig. 1. When 8×10^4 cells per ml were seeded, the doubling time of L615K was 16.3 h and that of L7212K was 21.9 h.

Macroscopic and microscopic findings of L615K and L7212K The macroscopic manifestations of the leukemia caused by the injection of these two cell lines were essentially identical. Marked hepatosplenomegaly and lymph node enlargement were found in leukemic mice within several weeks after transplantation. No thymic enlargement was observed in any of the animals. Hematoxylin-eosin staining of sections from involved tissues showed proliferation of medium-sized, round or polymorphic cells. Many mitoses indicated rapid proliferation of leukemia cells. Most organs in recipient mice were markedly infiltrated with leukemia cells within 6 days after transplantation, indicating direct migration of the injected leukemia cells. Both cells were positive for nonspecific esterase activity using α-naphthyl acetate as

Table I. Colony Formation in Soft Agar without CM

No. of cells seeded (plate)	Colony No. (plating efficiency %)	
	L615K	L7212K
$\times10^3$	117 (11.7)	86 (8.6)
$\times10^2$	19 (19.0)	13 (13.0)

Cells were seeded in soft agar (0.3%) onto hard base agar (0.5%) in 60 mm Petri dishes (Falcon). Colony numbers are averages of three independent experiments.

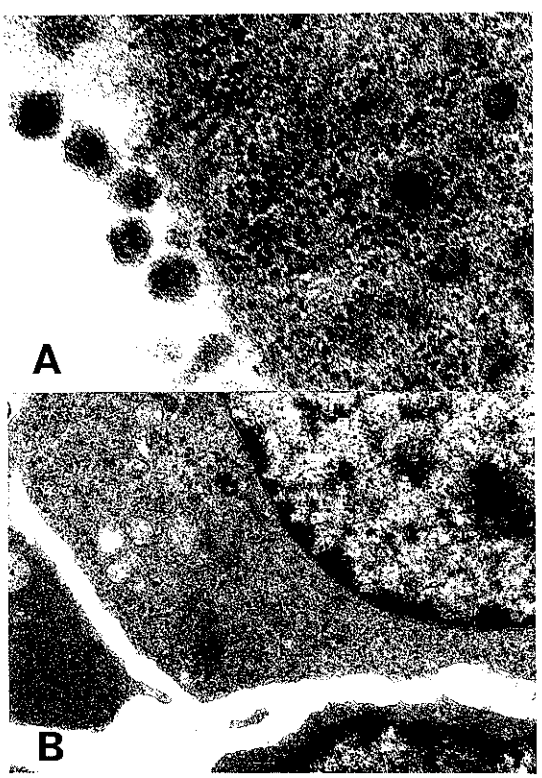

Fig. 2. Electronmicroscopic examination of cultured L615K (A) and L7212K (B) cells. A: Numerous C-type virus particles were observed in the extracellular space in the L615K cell line, ×48,000. B: No virus particle was found in the L7212K cell line, ×6,000.

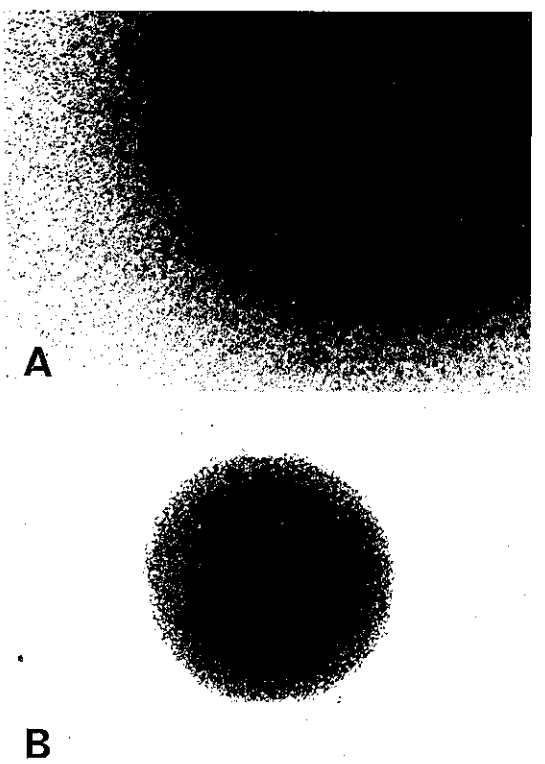

Fig. 3. Different types of colonies formed by L615K (A) and L7212K (B) in soft agar. A: L615K cells formed disperse-type colonies probably due to the locomotion of cells at the periphery, ×135. B: L7212K cells formed compact-type colonies, ×30.

the substrate and negative for peroxidase staining and non-specific esterase activity using naphthyl butyrate as the substrate.

Electronmicroscopy Many C-type virus particles were found electronmicroscopically around the L615K cells and many A-type particles within the cytoplasm (Fig. 2 A). The cells were considered to be lymphoid cells because of the lack in cisternae of rough endoplasmic reticulum and the presence of many free ribosomes and glycogen particles within the cytoplasm. On the other hand, no virus particle was observed in or around the L7212K cells (Fig. 2B).

Colony formation in soft agar The two lines showed different characteristics in cell growth, cell attachment and colony structure when they were seeded in soft agar. The cloning efficiency of both cell lines is shown in Table I. L615K cells formed a dispersed colony (Fig. 3A) probably due to cell movement, while L7212K cells formed a compact colony (Fig. 3B). The dispersed colonies were smeared on glass and stained with Giemsa solution. Although cells from dispersed colonies usually showed differentiation towards myeloid cells, their morphology was that of undifferentiated blast cells, similar to the mass-cultured L615K cells.

Cell surface markers Most of the L7212K cells were positive for Thy 1.2, while 40% of L615K cells were positive for this antigen in IF assays. The lower expression of Thy 1.2 with L615K cells was due to the heterogeneity of the latter cell line because Thy 1.2-positive and negative sublines could be established by cloning of the

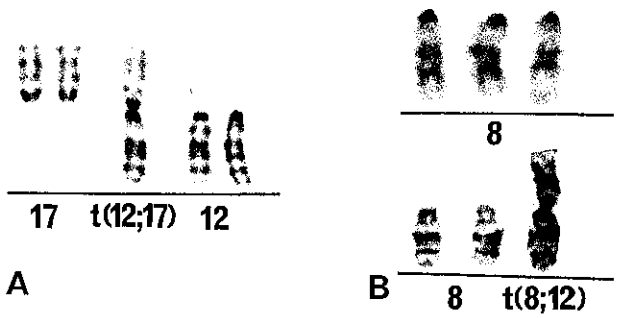

Fig. 5. Partial karyotypes (G-banding) of L615K and L7212K cells. Figure 5A shows the partially deleted #12 chromosome translocated to #17 chromosome. Figure 5B shows #8 trisomy (upper lane) and t(8;12) marker chromosome (lower lane) observed in L7212K cells.

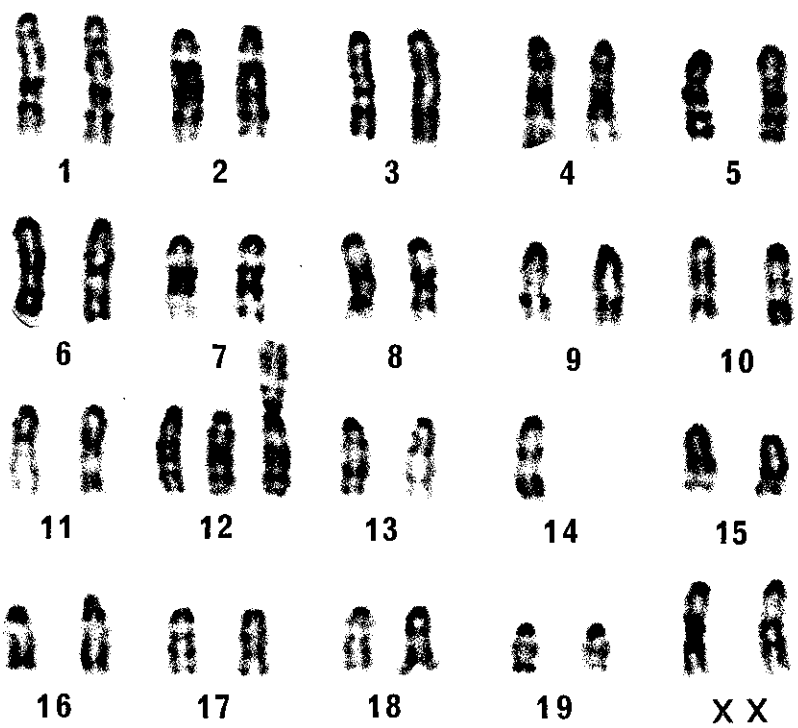

Fig. 4. The G-banded karyotype of L615K, showing 40XY, +t(12;17), −14. A metacentric marker chromosome is characteristic.

Table II. Reactivities of Several Type-specific Anti-MuLV Monoclonal Antibodies to Live L615K and L7212K Cells

Antibody	Virus specificity	Immunofluorescence[a]		Reference
		L615K	L7212K	
18-6	Xenotropic	−	−	(9)
24-6	Xenotropic	−	−	(9)
603	Xenotropic	−	−	(19)
613	Xenotropic	−	−	(19)
24-8	Endogenous ecotropic	−	NT	(9)
48	Exogenous Friend ecotropic	−	NT	(18)
500	Exogenous Friend-Moloney-Rausher ecotropic	−	NT	(18)
502	MCF[b]	+	+	(18)
514	MCF	+	+	(18)

a) +, positive immunofluorescence; −, no detectable immunofluorescence; NT, not tested.
b) Mink cell-focus inducing virus.

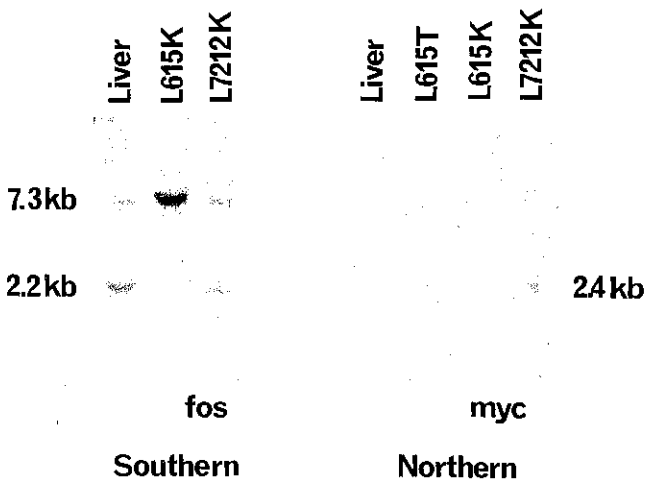

Fig. 6. Southern and Northern blot analyses of the L615K and L7212K cultured cells. The 2.2 kb band was deleted and the intensity of the 7.3 kb band increased in L615K cells relative to mouse liver (control) and L7212K using v-*fos*-specific probe. The increased c-*myc* expression was observed in both L615K and L7212K cultured leukemia cells in the Northern blot. L615T was obtained from a transplantable subcutaneous tumor.

original L615K cells. Most of the L7212K and L615K cells were positive for L3T4. On the other hand, no expression of Lyt 1, Lyt 2, Mac 1, or immunoglobulins was detectable on the surface of cells of both leukemia lines.

Chromosomal changes Chromosome numbers were distributed from 37 to 42 in the L615K line and from 38 to 42 in L7212K line. The modal chromosome number in the L615K line (50% metaphases) was 40 and that in the 7212K line (60% metaphases) was 41. According to the G-band analysis of chromosomes, the karyotype of L615K was 40,XY,+t(12;inv.17),−14 (Figs. 4 and 5A). One of the trisomic #12 chromosomes was translocated to chromosome #17, and the proximal segment of the tanslocated segment around the centromere was inverted. On the other hand, L7212K showed mosaicism of 41,XX,+8 (Fig. 5B, upper lane) and 40XX,t(8;12),−12 (Fig. 5B, lower lane) with the rate of the former karyotype being 77%.

Expression of MuLV structural proteins and production of infectious MuLVs Most of the L615K and L7212K cells expressed the envelope antigen of MCF virus on their surface, and this was detectable with type-specific monoclonal antibodies 502 and 514.[18] They also expressed the envelope antigen specific to ecotropic MuLV but not the envelope antigens of common endogenous ecotropic (N-tropic) MuLVs, xenotropic MuLVs, and exogenous Friend-Moloney-Rausher viruses as shown by IF assays (Table II).

In initial assays using XC-plaque and MiCll focus induction techniques, we could detect only a very low titer (600 PFU/ml) of infectious ecotropic viruses and a similar amount (600 PFU/ml) infectious xenotropic viruses in the culture supernate of the L615K cells, but not in that of the L7212K cells. Further titration by fluorescent focus assays using type-specific monoclonal antibodies revealed the production of a large amount (5,000 to 17,000 PFU/ml) of infectious MCF viruses from L615K cells which could be detected almost exclusively on *Mus dunni* cells.[20] However, even with these sensitive focus assays no infectious MCF viruses were detectable from L7212K cells.

Southern blot analysis The *Hin*d III fragments of 7.3 and 2.2 kb size were normally hybridized with v-*fos*-specific probe in Southern blot analysis. The 2.2 kb band was deleted and the 7.3 kb band increased in intensity with L615 cells (Fig. 6, Southern). No other band abnormality was observed after digestion with other restriction enzymes.

The rearrangement of T-cell receptor β-chain gene in both cell lines was observed by digestion with *Eco*RI, *Hin*d III, and *Puv* II, relative to the control liver. On the other hand, immunoglobulin heavy chain gene was not rearranged in either of the cell lines (data not shown).

RNA blot analysis The expression of the c-*myc* gene was also detected by Northern blot hybridization with total RNA of the L615K and L7212K cells (Fig. 6, Northern). C-*fos* transcript was not detected by blot analysis of

total RNA (data not shown). Transcripts of other c-*oncs* such as c-Ha-*ras* and c-*abl* were also not detected (data not shown).

DISCUSSION

For the establishment of cultured lines, hematopoiesis-inducing microenvironments play an important role.[21] We added CM at the beginning of cell culture and the CM-independence of L7212K cells was induced by gradually decreasing the CM.

In terms of expression of Thy 1.2, cell attachment to culture dishes, and colony morphology, the L615K and L7212K cells revealed different characteristics. Although neither cell line expressed a detectable amount of Lyt 1 or Lyt 2 markers, it is reasonable to conclude that both cell lines are derived from a T-cell lineage because they were Thy 1.2 and L3T4 positive, Mac 1 negative, and showed rearrangement of T-cell receptor β-chain.

Originally, the Bj chromosome or Rob(6;7) was recognized in L615 cells.[2] None of these was observed, but a new marker t(12;17) appeared in L615K cells. It is possible, therefore, that a minor cell population with t(12;17) was selected and became predominant during the long-term, *in vivo* passage. L7212K showed 41XX, +8 as previously reported in China.[5] The marker t(12;17) probably involves c-*fos* since the mouse c-*fos* is assigned to chromosome #12, as shown by somatic hybridization analysis.[22] This gene is often related to the differentiation of hemopoietic cells.[23]

From the evidence of DNA rearrangement of c-*fos* in L615K cells shown by Southern blot analysis, the rearrangement of c-*fos* by t(12;17) is suspected, although a secondary rearrangment of c-*fos* cannot be excluded from the evidence of an associated chromosomal inversion. Although both cell lines had a marker chromosome related to chromosome 12 and the immunoglobulin heavy chain gene is known to be localized on chromosome 12, those markers consisted of centric fusion but not reciprocal translocation. So these chromosome abnormalities might not imply the activation of c-*myc* or rearrangement of immunoglobulin heavy chain gene. There are several reports concerning the relationship between viral integration and c-*onc* activation.[24, 25] The c-*onc* activation in L615K could also be caused by the integration of the proviral genome near the c-*onc* loci. Moloney MuLV induces murine leukemia by integration adjacent to the c-*myc* sequence.[24] Integration in the Pim-1 region has been reported in the case of MCF viruses.[26] In thymic lymphoma induced by gamma irradiation or intraperitoneal injection of n-nitrosomethylurea, the preferential activation of K-*ras* gene was reported.[27]

Mouse nucleolar organizer regions (NOR) having ribosomal cistrons are located in the juxtacentromeric region of the short arm of the 3–5 pairs of chromosomes 12, 15, 16, 17, 18, and 19. The distribution of NOR depends on mouse strains, and the involvement of NOR in translocations and c-*onc* activation has been reported.[28] *In situ* hybridization, using labeled ribosomal RNA gene as a probe is required to investigate the role of NOR in this translocation.

Evidence has been accumulating showing that recombinant MCF viruses play a critical role in leukemogenesis in mice.[29, 30] As found by IF assays, both leukemia cell lines expressed MCF viral envelope antigen on their surfaces. Since it has been suggested that production of the MuLV envelope antigen itself may potentially cause immortalization and/or transformation of T-lymphocyte lineage cells,[31] it may be an important observation that even L7212K cells lacking production of infectious MCF viruses still continuously express the envelope antigen on their surfaces. Leukemogenicity of the infectious MCF virus isolated from L615K cells is currently under study. The precise localization of c-*fos* on chromosome 12, shown by *in situ* hybridization and genomic cloning of this region in the above marker chromosome, may clarify the mechanism and the role of this translocation in L615K cells, since chromosomal translocations are often related to the activation of c-*oncs* in various cells.[32–34] Thus, the L615K cell line is a new tool to investigate this problem. Further studies are necessary to elucidate the role of viruses, chromosomal changes, and c-*onc* activation in these cells.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Education, Science and Culture of Japan. We are grateful to Dr. T. Honjo for providing J region probe of mouse immunoglobulin heavy chain through the Japanese Cancer Research Resources Bank.

(Received February 10, 1989/Accepted March 23, 1989)

REFERENCES

1) You, S., Guang, Q., Jinghua, W., Jianxin, C., Jianling, L., Huanting, X., Shuling, Q., Baozhang, F., Yuchu, Y. and Yuhua, S. Laboratory studies on a transplantable mouse leukemia (L615). *Acta Genet. Sin.*, **1**, 81–97 (1974).

2) Yihua, N., Huipeng, W., Lianrui, L., Shang, F., Xinquan, M., Bin, W. and Hongguong, L. A marker chromosome of Chinese transplantable leukemia (L615) has been found. *Acta Genet. Sin.*, **3**, 231–234 (1976) (in Chinese).

3) Yihua, N., Huipeng, W., Lianrui, L., Shang, F., Xinquan, M., Bin, W. and Hongguong, L. The establishment of leukemia cell line of mouse L615. *Acta Genet. Sin.*, **9**, 1–7 (1982).
4) You, S. A study on tumor transplantation antigen of mouse leukemia L615. *Chin. J. Oncol.*, **7**, 189–193 (1985).
5) You, S. Studies of surface markers in mouse leukemia L615 cells. *Clin. Oncol.*, **12**, 52–55 (1985) (in Chinese).
6) Jianxin, C., Shuling, Q., Jianling, L., You, S., Yuchu, Y., Yuhua, S. and Yifen, Q. Establishment and properties of a transplantable mouse leukemia L7212. *Chin. J. Oncol.*, **3**, 287–291 (1981) (in Chinese).
7) Lihua, L., Shaowu, M. and Min, W. Trisomy of chromosome 8 of a transplantation of lymphocytic leukemia L7212 in mice. *Acta Genet. Sin.*, **9**, 105–108 (1982).
8) Nesbitt, M. N. and Franke, U. A system of nomenclature for band patterns of mouse chromosomes. *Chromosoma*, **41**, 145–158 (1973).
9) Portis, J., McAtee, F. J. and Cloyd, M. W. Monoclonal antibodies to xenotropic and MCF murine leukemia viruses derived during the graft-versus-host reaction. *Virology*, **118**, 181–190 (1982).
10) Sitbon, M., Nishio, J., Wehrly, K., Lodmell, D. and Chesebro, B. Use of a focal immunofluorescence assay on live cells for quantitation of retroviruses: distinction of host range classes in virus mixtures and biological cloning of dual-tropic murine leukemia viruses. *Virology*, **141**, 110–118 (1985).
11) Rowe, W. P., Pugh, W. E. and Hartley, J. W. Plaque assay technique for murine leukemia viruses from mouse embryo cells. *Virology*, **42**, 1136–1139 (1970).
12) Peebles, P. T. An *in vitro* focus-induction assay for xenotropic murine leukemia virus, feline leukemia virus C, and the feline-primate viruses RD-144/CCC/M-7. *Virology*, **67**, 288–291 (1975).
13) Southern, E. M. Detection of specific sequences among DNA fragments separated by gel electrophoreses. *J. Mol. Biol.*, **98**, 503–519 (1975).
14) Deeley, R. G., Gorden, J. I., Burns, A. T. H., Mallinix, K. P., Baltimore, M. and Goldbergest, R. F. Primary activation of the vitellogenin gene in the rooster. *J. Biol. Chem.*, **252**, 8310–8317 (1977).
15) Thomas, P. S. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA*, **77**, 5201–5205 (1980).
16) Caccia, N., Kronenberg, M., Saxe, D., Haars, R., Bruns, G., Goverman, J., Malissen, M., Willard, H., Somon, M., Hood, L. and Mak, T. W. The β T cell receptor genes are located on chromosome 7 in humans and chromosome 6 in mice. *Cell*, **37**, 1091-1099 (1984).
17) Rigby, P. W., Dieckmann, M., Rhodes, C. and Berg, P. Labeling deoxyribonucleic acid to high specific activity *in vivo* by nick translation with DNA polymerase I. *J. Mol. Biol.*, **113**, 237–251 (1977).
18) Chesebro, B., Wehrly, K., Cloyd, M., Britt, W., Portis, J., Collins, J. and Nishio, J. Characterization of monoclonal antibodies specific for Friend murine leukemia virus-induced erythroleukemia cells: Friend-specific and FMR-specific antigens. *Virology*, **112**, 131–138 (1981).
19) Portis, J. L. and McAtee, F. J. Monoclonal antibodies derived during graft-versus-host reaction. II. Antibodies detect unique determinants common to many MCF viruses. *Virology*, **126**, 96–105 (1983).
20) Lander, M. R. and Chattopadhyay, S. K. A *Mus dunni* cell line that lacks sequences closely related to endogenous murine leukemia viruses and can be infected by ecotropic, amphotropic, xenotropic, and mink cell focus-forming viruses. *J. Virol.*, **52**, 695–698 (1981).
21) Hiai, H., Nishi, Y., Miyazawa, T., Matsudaira, Y. and Nishizuka, Y. Mouse lymphoid leukemias: symbiotic complexes of neoplastic lymphocytes and their microenvironments. *J. Natl. Cancer Inst.*, **66**, 713–722 (1981).
22) Human Gene Mapping 8. *Cytogenet. Cell Genet.*, **40**, 552 (1985).
23) Gonda, T. J. and Metcalf, D. Expression of *myb* and *fos* proto-oncogenes during the differentiation of a murine myeloid leukemia. *Nature*, **310**, 249–251 (1984).
24) Steffen, D. Proviruses are adjacent to c-*myc* in some murine leukemia virus-induced lymphomas. *Proc. Natl. Acad. Sci. USA*, **81**, 2097–2101 (1984).
25) Li, Y., Halland, C. A., Hartley, J. W. and Hopkins, N. Viral integration near c-*myc* in 10–20% of MCF 247-induced AKR lymphomas. *Proc. Natl. Acad. Sci. USA*, **81**, 6808–6811 (1984).
26) Cuypers, H. T., Selten, G., Wuint, W., Zijlstra, M., Maandag, R., Boelens, W., Wezenbeek, P. V., Melief, C. and Berns, A. Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. *Cell*, **37**, 141–150 (1984).
27) Diamond, E. L., Gurrero, I. and Pellicer, A. Concomitant K- and N-*ras* gene point mutation in clonal murine lymphoma. *Mol. Cell. Biol.*, **8**, 2233–2236 (1988).
28) Takahashi, R., Horita, Y., Chen, H-L., Maeda, S., Murao, S., Shisa, H., Chattopadhay, S. C. and Sugiyama, T. Molecular and cytogenetical studies on nucleolar cistrons (rDNA) in mouse leukemia cells. *Cancer Genet. Cytogenet.*, **29**, 109–118 (1987).
29) Cloyd, M. W., Hartley, J. W. and Rowe, W. P. Lymphomatogenicity of recombinant mink focus-inducing murine leukemia viruses. *J. Exp. Med.*, **151**, 542–552 (1980).
30) Elder, J. H. On the role of recombinant retroviruses in murine leukemia. *In* "Concepts in Viral Pathogenesis," ed. A. L. Notokins and M. B. A. Oldstone, pp. 86–96 (1984). Springer-Verlag, New York.
31) McGrath, M. S. and Weissman, I. L. The role of immunospecific receptors in retrovirus-induced lymphomatogenesis. *In* "Concepts in Viral Pathogenesis," ed. A. L. Notokins and M. B. A. Oldstone, pp. 216–224 (1984). Springer-Verlag, New York.
32) Chen, H-L., Maeda, S., Takahashi, R. and Sugiyama, T. Chromosome marker and enhanced expression of c-Ha-*ras*

in a DMBA-induced erythroleukemia cell line (D5A1). *Cancer Genet. Cytogenet.*, **28**, 301–309 (1987).
33) Leder, P., Battery, J., Lenoir, G., Moulding, C., Murphy, W., Pottrt, H., Stewart, T. and Taub, R. Translocation among antibody gene in human cancer. *Science*, **222**, 765–771 (1983).
34) Takahashi, R., Mihara, K., Maeda, S., Yamaguchi, T., Chen, H-L., Aoyama, N., Murao, S., Hatanaka, M. and Sugiyama, T. Secondary activation of c-*abl* may be related to translocation to the nucleolar organizer region in an *in vitro* cultured rat leukemia cell line (K3D) *Proc. Natl. Acad. Sci. USA*, **83**, 1079–1083 (1986).