Acta Medica Okayama

Volume 41, *Issue* 3 1987 *Article* 6

JUNE 1987

Establishment and characterization of a virus-free chick cell line.

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Abstract

A line of chick embryo cells (CEC) was obtained from CEC treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The cells, designated CHCC-OU2, were contact-inhibited, formed no colony in soft agar and did not produce tumors when inoculated into syngeneic chickens. The electron microscopic examination and reverse transcriptase assay showed no virus production from the cells. Subgroup A avian sarcoma virus (ASV) and Newcastle disease virus replicated well in the cells of this cell line.

KEYWORDS: chick cell line, MNNG, contact inhibited, no virus production

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*PMID: 3115063 [PubMed - indexed for MEDLINE]

Acta Med Okayama 41 (3) 141-143 (1987)

— Brief Note—

Establishment and Characterization of a Virus-free Chick Cell Line

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A line of chick embryo cells (CEC) was obtained from CEC treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The cells, designated CHCC-OU2, were contact-inhibited, formed no colony in soft agar and did not produce tumors when inoculated into syngeneic chickens. The electron microscopic examination and reverse transcriptase assay showed no virus production from the cells. Subgroup A avian sarcoma virus (ASV) and Newcastle disease virus replicated well in the cells of this cell line.

Key werds : chick cell line, MNNG, contact inhibited, no virus production

Since Akiyama *et al.* established chick cell line from Marek's disease virus-induced lymphomas (1), the establishment of several avian cell lines has been reported (2-6). We have also reported the establishment of two chick embryo cell lines (7). But neither of these lines was suited to the culture of viruses, because they produced endogenous avian retroviruses. In the present paper, we report the establishment of a virus-free cell line derived from chick embryo cells (CEC) treated with MNNG.

Embryonate eggs of the C/O and C/CE phenotypes, which are chick helper factor-negative and specific pathogen-free, were obtained from Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University, Kanonji. The viruses used were standard ASVs of the SRA strain (subgroup A), SRB strain (subgroup B), BH-RSV (RAV-7) strain (subgroup C), SRD strain (subgroup D) and QV2f strain (subgroup E), and the Italian strain of Newcastle disease virus. The whole tissue of 11-day-old embryos were digested with trypsin, and the cells were cultured in minimal essential medium (Nissui Co., Japan) supplemented with 1% heat-inactivated chicken serum (Flow Laboratories, USA), 5% calf serum (Difco Laboratories, USA) and 10% tryptose phosphate broth (Difco Laboratories, USA) in an incubator at 41°C under 5% CO_2 atmosphere.

The secondary culture of CEC of the C/CE phenotype was treated with 10 μg/ml of MNNG for 60 min, washed 3 times and maintained in culture medium. After treatment with MNNG, the cells showed a moderate cytopathic effects. The surviving cells were subcultured. The growth of the cells was exponential until about the 25th passage, when the growth deteriorated gradually. The cells stopped growing at the 28th passage. The aging-released CEC showed exponential growth again from the 35th passage. The cells are currently in the 300th passage, over 3 years since the beginning of culture, and are still growing exponentially. Thus the cells were considered to be an established cell line and were designated as CHCC-

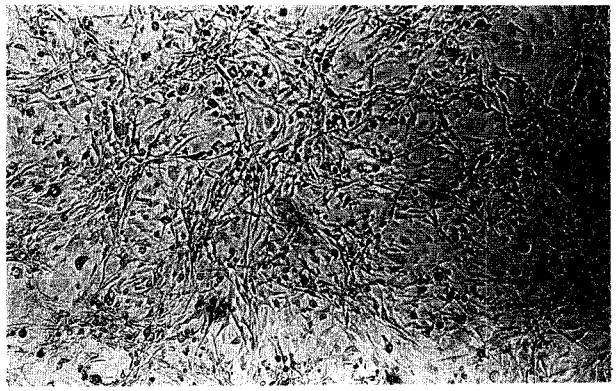

Fig. 1 Micrograph of CHCC-OU2 cells in a semiconfluent culture state. Cells are fibroblastic and contact-inhibited.
Unstained. ×120.

OU2 according to the proposed method for naming avian cell lines (8). Untreated CEC ceased growing after 20-27 passages (2.5-3 months) and were lost completely.

The CHCC-OU2 cells were fibroblastic, contact-inhibited (Fig. 1) and did not form colonies in soft agar. Five syngeneic chickens were inoculated into wing webs with the cells (5×10^7 cells per chicken) and observed for one month. Tumors did not form, indicating that CHCC-OU2 cells were not malignantly transformed, despite the treatment with MNNG.

To examine whether the cells produce endogenous avian retroviruses, the culture supernatants were concentrated at several passages, and the total activity of magnesium-dependent exogenous reverse transcriptase was determined by measuring the incorporation of the radioactive precursor thymidine monophosphate (^{3}H-TMP) into the acid-precipitable fractions as described previously (9). No reverse transcriptase activity was detected. At the 173rd passage, ultrathin sections of CHCC-OU2 cells were made and observed electron microscopically. No virus particles were detected.

To determine the susceptibility of CHCC-OU2 cells to avian retroviruses, they were infected with ASVs of subgroups A, B, C, D and E. The culture supernatants of ASV-inoculated CHCC-OU2 cells were then titrated by focus assay on CEC of the C/O phenotype. As shown in Table 1, subgroup A ASV replicated well in CHCC-OU2 cells,

Table 1 Titer of ASV in the culture supernatant of ASV-inoculated CHCC-OU2 cells

ASV strain used	Subgroup	Focus forming units/ml of supernatant
SRA	A	1×10^6
SRB	B	6
BH-RSV(RAV-7)	C	0
SRD	D	5
QV2f	E	0

CHCC-OU2 cells were inoculated with ASV and maintained at 37°C for 7 days. The supernatant was inoculated onto the secondary culture of C/O phenotype CEC, and focuses were counted on the 7th day.

whereas those of subgroups B and D replicated only slightly and those of subgroups C and E did not replicate at all. Newcastle disease virus also replicated well in CHCC-OU2 cells (up to about 1×10^7 plaque-forming units per ml.

In the present study, a cell line, which appeared to be non malignant, was established from cells treated with MNNG. The actual role of MNNG treatment in the establishment of the cell line is not known at the present. It is also not known why the cells were only slightly permissive to ASVs of subgroups B and D which replicated well in the primary culture of C/CE phenotype CEC. To our knowledge, the present cell line

is the first virus-free chick cell line. The line should prove useful for various aspects of virus research.

Acknowledgment. We thank Dr. Y. Yabe for his support and for a critical reading of the manuscript.

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Received : January 30, 1987
Accepted : March 25, 1987

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