Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 21
Sofia ● 2015

Comparative Study on Cell Lines Established from Retrovirus-Induced Transplantable Chicken Liver Cancer

R. Alexandrova[*], T. Zhivkova[*], L. Dyakova[**], I. Dankov[,***], P. Jordanova[*], V. Altanerova[****], C. Altaner[****]*

[*]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, Sofia, Bulgaria;
[**]Institute of Neurobiology, Bulgarian Academy of Sciences;
[***]Faculty of Medicine, Sofia University St. Kl. Ohridski
[****]Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia

The cell lines LSCC-PR2-Mc29 and LSCC-SF-Mc29 were established from a transplantable liver cancer in chicken induced by the myelocytomatosis virus Mc29. The aim of the present study was to evaluate comparatively some biological characteristics of these cells. Both cell lines have been found to grow as monolayer cultures and form 3D colonies in semi-solid medium, express v-myc (gag-myc) gene; are sensitive to the cytotoxic/cytostatic effects of the widely used commercial anticancer drug cisplatin. On the other hand, these cells differ in their tumorigenic potential *in vivo*.

Key words: myelocytomatosis virus Mc29, transplantable chicken liver cancer, cell line.

Introduction

Avian myelocytomatosis virus Mc29 was isolated in Bulgaria in 1961 from a Rhode Island Red chicken with spontaneous mielocytomatosis [3]. It belongs to the group of defective avian leukemia retroviruses (ALVs). It has been shown that all three genes essential for the replication of ALVs, i.e. *gag*, *pol* and *env*, are defective in Mc29 virus (gene *pol* is completely missing). The virus possesses a specific oncogene – *v-myc* [5, 8]. *In vitro* the virus Mc29 induces transformation of fibroblasts, epithelial cells and macrophages. *In vivo* transmission causes primarily myelocytomatosis and myelocytomas in chickens but it is also responsible for a broad spectrum of leukemias and tumor growths, including endothelioma, mesothelioma sarcoma and erythroblastosis. Of particular interests are some epithelial cell tumors in the kidney, pancreas and especially in the liver [4, 6, 8]. LSCC-PR2-Mc29 [9] and LSCC-SF-Mc29 [1] are cell lines derived from Mc29 virus-induced transplantable chicken liver cancer. The aim of the present study was to compare some biological characteristics of these cell lines.

Materials and Methods

The cells were grown as monolayer cultures in D-MEM medium, supplemented with 5-10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. The cultures were maintained at 37 °C in a humidified CO_2 incubator. For routine passages adherent cells were detached using a mixture of 0.05% trypsin – 0.02% ethylendiaminotetraacetic acid (EDTA). The investigations were carried out by colony-forming method [2], karyological analysis and polymerase chain reaction (PCR). The influence of cisplatin on cell viability and proliferation was evaluated by thiazolyl blue tetrazolium bromide test (MTT) [7]. *In vivo* experiments were performed using inbred 15I White leghorn chickens.

Chromosome analysis is performed according to the standard procedure. Briefly, the cells have been arrested in metaphase with 0.02 μg/ml Colcemide for 2 h. Chromosome spreads are prepared by hypotonic treatment, fixation and Giemsa staining. Fifty well spread metaphases have been analyzed following the standard nomenclature for chicken chromosomes.

The DNA isolation was carried out by the method of phenol: chloroform: isoamyl alcohol extraction. PCR was performed in DNA thermal cycler (Perkin Elmer). Primers specific for chicken Mc29 virus gag-myc gene were designed: 5'-GAC GGG GGG AAC GGA CTA ACT T-3' (forward) and 5'-TTC CAG ATG TCC TCG GAC GG-3' (reverse). The resulted PCR product was 535 base pairs (bp) in length and contained region from 1707 to 2241 nucleotide positions. An initial step for denaturation of dsDNA was performed at 94 °C for 2 min. Amplification was carried out for 35 cycles consisting of: denaturation at 94 °C for 40 sec, annealing at 66 °C for 1 min, synthesis at 72 °C for 50 sec and prolongation of DNA synthesis at 72 °C for 7 min. Then reaction mixture was cooled to 4 °C. Separation of PCR products was provided by electrophoresis in 1% agarose gel. Smart ladder (Eurogentech) was used as a molecular weight marker.

Results and Discussion

Both cell lines have been found to share the following common characteristics: they grow as monolayer cultures and form 3D colonies in semi-solid medium, express v-myc (gag-myc) gene; they are sensitive to the cytotoxic/cytostatic effects of the widely used commercial anticancer drug cisplatin. On the other hand, these cells differ in their tumorigenic potential in vivo. The results obtained are summarized in **Tables 1** and **2**.

Table 1. LSCC-SF-Mc29 and LSCC-PR2-Mc29 chicken liver cancer cell lines – comparison of some biological characteristics

Cell line	LSCC-SF-Mc29	LSCC-PR2-Mc29
Establishment of the cell line	After trypsinization of tumor explants*	After culturing of tumor explants**
Karyotype	Diploid and threeploid	Hypodiploid**
Formation of 3D colonies in semi-solid medium	Yes	Yes
Presence of *v-myc* (*gag-myc*) gene	Yes	Yes
Sensitivity to cis-platin (CC_{50}, μg/ml)	Yes (< 0.7)	Yes (< 0.7)
Tumor growth at the site of s.c. cancer cell inoculation	Yes	No
Tumor growth outside the place of s.c. cancer cell transplantation	Rare	Yes

* - According to Alexandrova, Ogneva [1]; ** - According to Sovova et al. [9];
CC_{50} – concentration of the compound that reduces the number of viable cells by 50% as compared to the untreated control. CC_{50} was determined by MTT test after 24 and 48 h treatment.

In order to investigate the *in vivo* tumorigenic potential of LSCC-SF-Mc29 and LSCC-PR2-Mc29 cell lines, the viable cancer cells (in 0.5 ml phosphate saline buffer) were transplanted s.c. into the knee flexure of 7- to 14-day-old 15I White leghorn chickens (**Table 2**).

Table 2. Tumorigenicity in vivo of cell lines LSCC-SF-Mc29 and LSCC-PR2-Mc29 in 15I White Leghorn chickens

Cell line	Age at the time of inoculation (days)	Number of inoculated cells	Latent period (days)	Number of tumor-bearing chickens***/ total number of inoculated chickens
LSCC-SF-Mc29	7	5.0×10^6	6–9	5/7 (71.4%)
	8	7.0×10^6	5–8	7/7 (100%)
	11	2.5×10^6	12–14	3/5 (60%)
	11	5.0×10^6	12	5/5 (100%)
	12	7.5×10^6	9–10	4/5 (80%)
	13-14	5.0×10^6	10–12	5/5 (100%)
LSCC-PR2-Mc29	7	2.5×10^6	–	0/5 (0 %)
	7	5.0×10^6	–	0/4 (0 %)
	7	7.5×10^6	–	0/5 (0 %)
	7	10.0×10^6	–	0/4 (0 %)
	7	20.0×10^6	–	0/2 (0 %)
	14	7.5×10^6	–	0/4 (0 %)
	14	10.0×10^6	–	0/4 (0 %)
	14	20.0×10^6	–	0/2 (0 %)

*** - The tumors observed are at the site of cancer cell inoculation.

In the case of LSCC-SF-Mc29 cell line, tumor growth was observed at the site of cancer cell inoculation in 60-100% of the transplanted chickens. In one of the chickens (transplanted with 2.5×10^6 cells) tumor growth was observed also in the liver.

The situation with LSCC-PR2-Mc29 cell line was different. Tumor growth at the site of transplantation was not detected in anyone of the cases. Part of the chickens (19/30) were euthanized and autopsied 14-18 days after implantation of cancer cells – no significant visible changes were noticed in tissues and organs of these chickens. The rest (11/30) of the chickens were monitored over a longer period of time: ≥ 34 days. In part (9/11) of them solid tumors were found in the thymus (tightly elastic consistency, measuring 1-4 mm in diameter, sometimes bilateral). In one chicken tumor growth (two nodes with weight of 1.5 g and 18.8 g) was observed also in one kidney.

Our data confirm the results published by Sovova et al. [9] – after inoculation of 5×10^6 or 5×10^7 LSCC-PR2-Mc29 cells into the wing web of 1-day-old Brown leghorn chickens, the authors did not usually find tumors at the site of inoculation. While Sovova and collaborators [9] found tumor growths mainly in the mesentery and liver of Brown leghorn chickens transplanted with LSCC-PR2-Mc29 cells, the main location of tumors observed in 15I White leghorn chickens implanted with the same cells in our experiments was thymus.

It can be suggested that the differences between these cell lines (LSCC-SF-Mc29 and LSCC-PR2-Mc29) could be explained at least partially by:

1. A different approach applied for the preparation of cell lines – with and without chemical (enzyme) treatment – which can result in the isolation and selection of different cell populations;

2. Both lines have been established independently of each other at different times and in different laboratories from transplantable chicken liver cancers that undergone large number of *in vivo* passages. During these passages under the influence of various factors (such as tumor-host relationships) may have become a selection of different cell subpopulations;

3. The high mutation capacity of cancer cells and the tumor cell heterogeneity phenomenon which converts any malignant neoplasm (resp. tumor cell line) in a unique system.

In conclusion, both cell lines have their specific characteristics and can be used for various scientific and biotechnological purposes. The presence of v-myc gene makes them valuable experimental models – the disturbed regulation of its cellular analogues (c-myc, L-myc, N-myc) is involved in pathogenesis of > 80% of human cancers.

Acknowledgement: Tanya Zhivkova is supported by Grant No BG051PO001-3.3.06-0048 from 04.10.2012; European Social Fund and Republic of Bulgaria, Operational Programme "Human Resources Development", 2007-2013 framework.

References

1. **Alexandrova, R., V. Ogneva.** Transplantable chicken hepatoma as a model for tumor heterogeneity investigations. – In: The Proceedings of the First Joint Meeting of Departments of Animal Sciences of the Balkan Countries. BALNIMALCON, 2-8 June 2001, Tekirdag, Turkey, 127-131**.**
2. **Alexandrova, R., A. Vacheva, M. Kirilova**, **G. Miloshev, E.-M. Mosoarca, R. Tudose, O. Costisor.** Investigations on cytotoxic and antiproliferative effects in vitro of a newly synthesized mixed ligand copper (II) complex. – Acta Morphol. Anthropol., **12**, 2007, 72-78.
3. **Ivanov, X., Z. Mladenov, S. Nedyalkov, T. Todorov, M. Yakimov.** – Bulletin de l'Institute de Pathologie Comparee des Animaux, **10**, 1964, 5-38 (In Bulgarian).
4. **Law, W., M. L. Linial.** Transforming ability of Gag-Myc fusion proteins correlates with Gag-Myc protein stability and transcriptional repression. – Oncogene, **20**(9), 2001, 1118-1127.
5. **Lee, C. M., E. P. Reddy.** The v-myc oncogene. – Oncogene, **18**, 1999, 2997-3003.
6. **Mladenov, Z.** Comparative Pathology of Avian Leukoses. – Sofia, Publishing House of the Bulgarian Academy of Sciences, 1974 (In Russian).
7. **Mossman, T.** Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. – J. Immunol. Meth., **65**(1), 1983, 55-63.
8. **Payne, L. N.** Biology of avian Retroviruses. – In: Levy, (Ed. A. Jay), New York and London, The Retroviridae. Plenum Press, **1**, 1992, pp. 299-404.
9. **Sovova, V., I. Hlozanek, H. Cerna, V. Dostalova, H. Sainerova.** Analysis of a cell line derived from a chicken hepatoma induced by virus Mc29. – Avian Pathol., **10**, 1981, 461-469.