Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Human cytomegalovirus induces alteration of β-actin mRNA and microfilaments in human embryo fibroblast cells*

LIN Mao-fang (林茂芳), WEI Guo-qing (魏国庆)†, HUANG He (黄 河), CAI Zhen (蔡 真)

(*Department of Hematology, First Affiliated Hospital, College of Medical Science, Zhejiang University, Hangzhou 310003, China*)

†E-mail: weiguoqing2000@hotmail.com

Received Sept. 22, 2003; revision accepted Jan. 8, 2004

Abstract: Objective: To investigate the infection of human embryo fibroblast cell line HF cells by CMV as well as the effects of CMV on β-actin mRNA and microfilaments. Methods: HF cells shape was observed after the infection of CMV. RT-PCR assay was used to detect the mRNA expression of CMV immediate early (IE) gene, β-actin and GAPDH genes of HF cells infected by CMV. CMV particles and cell microfilaments were detected with electron microscope. Results: Shape of HF cell changed after the infection by CMV. HF cells infected by CMV could express IE mRNA and the expression of β-actin mRNA decreased in a time- and titer-dependent manner compared with the uninfected HF cells whose expression of GAPDH mRNA did not change much. CMV particles were found with electron microscope in the cells. Microfilaments were ruptured and shortened after the infection of CMV. Conclusion: CMV can not only infect human embryo fibroblast cells line HF cells and replicate in the cells, but can also affect the expression of β-actin mRNA and the microfilaments.

Key words: Cytomegalovirus, Human embryo fibroblast cells, Infection, β-actin, Microfilament
Document code: A **CLC number:** R373

INTRODUCTION

It is known that human embryo fibroblast cells are permissive for cytomegalovirus (CMV) replication in vitro. Data have shown that CMV infection causes a rapid, progressive disruption of the host cell cytoskeleton (CSK); and that the disruption correlates with actin depolymerization. Some experts used whole-mount (3D) electron microscopy to analyze the CSK of uninfected and CMV-infected human lung fibroblast cells; and found within 2 min of CMV infection, localized areas of cytoskeletal disruption. The disruption extended throughout the cytoplasm during the ensuing 45 to 90 min of infection and resulted in generalized cytoskeletal disorganization (Arcangeletti *et al.*, 2000; Jones *et al*., 1986). Actin appears to be the primary cytoskeletal target involved during CMV infection. But changes of actin mRNA after the infection is unknown up to now. Microfilaments comprise a very important part of cytoskeletal protein and are composed mostly of actin. Here we investigate the cell morphology and microfilaments changes as well as the effect of β-actin mRNA in human embryo fibroblast cells infected by CMV.

MATERIALS AND METHODS

Cells and viruses

HF human embryo fibroblasts and CMV str-

*Project (No. 001103058) supported by the Key Program of Science and Technology Bureau of Zhejiang Province, China

ain AD169 were donated by Professor Wang Mingli from Anhui Medical University. CMV AD169 was propagated in HF human fibroblasts. The virus was collected when cytopathic effects were >90%. Supernatants were clarified by centrifugation at 1000×g for 10 min at 4 °C and were stored at −80 °C until use. Virus titer was determined by CPE titration in HF cells according to standard procedures.

Infection of HF cells by CMV

HF cells were cultured in DMEM supplemented with 10% (v/v) fetal calf serum, 1×10^5 IU/L penicillin, 100 mg/L streptomycin and 2 mmol/L glutamine. Infections with CMV were performed at 100, 10, 1 TCID50. After 2 h at 37 °C, HF cells were washed thrice with PBS. At 48 h after infection, cells were centrifuged and detected. At 12, 24, 48 h after the infection with 100 TCID50 of CMV, the cells were centrifuged to detect the β-actin mRNA.

Detection of CMV immediate early (IE) gene mRNA, β-actin mRNA and GAPDH gene mRNA

(1) mRNA extraction. For total RNA extraction, TRIZOL (GIBCO) was used. Briefly, cells were homogenized with 0.5 ml of TRIZOL; 0.1 ml of chloroform was added to the homogenate, and the mixture was held on ice for 5 min, followed by sedimentation at 12000 g at 4 °C. RNA was precipitated by combining the aqueous phase with an equal volume of isopropanol; the precipitate was recovered by centrifugation (12000 g at 4 °C), washed once in 75% ethanol, and solubilized in diethylpyrocarbonate (DEPC)-treated water. RNA concentrations were measured with RNA/DNA calculator (Pharmacia, genequant), $A_{260}/A_{280}\geq1.8$.

(2) Reverse transcription. Extracted RNA (3 μg) was diluted in 5× amplification buffer (Songon) with 10 mmol/L dNTPs, 20 mmol/L dithiothreitol, 200 U/ml reverse transcriptase (Songon), and 100 pmol/ml random primers (Promega) and brought to a final volume of 40 μl with DEPC-treated water. The mixture was incubated at 42 °C for 60 min and then held at 70 °C for 15 min.

(3) Polymerase chain reaction (PCR). cDNA sequences generated by reverse transcription were amplified by PCR. Reverse transcription products 2 μl were added to a reaction tube containing 2.5 μl of l0× amplification buffer, 0.5 μl of 10 mmol/L dNTPs, 1.0 U of Taq polymerase (Promega), 0.5 μl of a 25 μmol/L solution of each primer, and DEPC-treated water, to a total volume of 25 μl. Primers sequences and PCR conditions are shown in Table 1. After amplification, the products were visualized by electrophoresis through a 2% agarose gel, staining with 1 μg/ml ethidium bromide, and illumination with a UV lamp.

Electron microscope detection

HF cells infected with 10 TCID50 of CMV were fixed with 2.5% of glutaral, then dehydrated, embedded, sliced up, dyed with uranium acetic acid and lead nitrates according to the routine procedure; then CMV particles and microfilaments were detected with electron microscope (Philips, Tecnai-10).

RESULTS

Effects of CMV on HF cells morphology

HF cells infected by CMV changed from thin shuttle shape to round and thick ball shape, even es-

Table 1 Primers and PCR conditions for detection of CMV IE, β-actin and GAPDH mRNA

mRNA	Primers (5'→3')	Product size (bp)	PCR condition
IE	U: CCAAGGCCACGACGTTCCTGCAGACTA	390	95 °C/4 min; (94 °C/60 s; 60 °C/30 s;
	D: TGCTCCTTGATTCTATGCCGCACCA		72 °C/60 s)×35; 72 °C/10 min
β-actin	U: CGCTGCGCTGGTCGTCGACA	612	94 °C/4 min; (94 °C/30 s; 54 °C/45 s;
	D: GTCACGCACGATTTCCCGCT		72 °C/60 s)×25; 72 °C/10 min
GAPDH	U: CGACCACTTTGTCAAGCTCA	240	94 °C/5 min; (94 °C/30 s; 58 °C/60 s;
	D: AGGGGTCTACATGGCAACTG		72 °C/30 s)×32; 72 °C/10 min

caping from wall. Cell shape changed more obviously with increased CMV titer. See Fig.1 and Fig.2.

Expression of IE, β-actin and GAPDH mRNA of HF cells infected by CMV

The ratio of IE and GAPDH or β-actin and GAPDH were regarded as the expression of IE and β-actin mRNA. Our result showed that HF cells infected by CMV could express IE mRNA; and that the expression of β-actin mRNA decreased (1.24 ±0.14, 0.77±0.10, 0.48±0.08, 0.29±0.06, F=52.306, P<0.001) in a titer-dependent manner compared with the uninfected HF cells while the expression of GAPDH mRNA did not change much. See Fig.3.

Kinetics of the change of β-actin mRNA

At 0, 12, 24, 48 h after the infection with 100 TCID50 of CMV, β-actin mRNA were detected. Data showed that β-actin mRNA were decreased in a time dependent manner after the infection (1.24 ±0.14, 0.66±0.12, 0.44±0.10, 0.29±0.06, F=44.836, P<0.001). See Fig.4.

CMV particles and microfilaments detection with electron microscope

With electron microscope, many CMV particles were found in the nucleus and plasma of HF cells infected by CMV. See Fig.5. Microfilaments in HF cells infected by CMV were short and ruptured, arranged turbulently. While microfilaments in HF cells uninfected by CMV were arranged in bundles. See Fig.6.

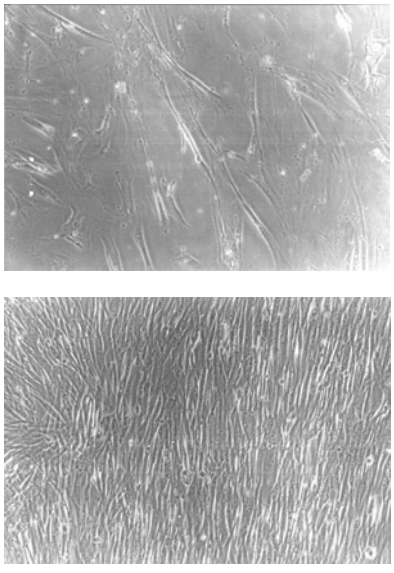

Fig.1 HF cells uninfected by CMV(100×)

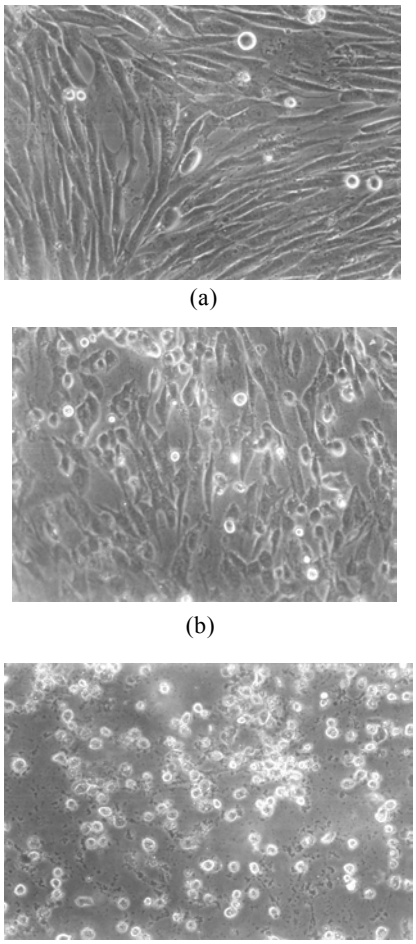

Fig.2 HF cells infected by CMV(100×)

(a) HF cells infected with 1 TCID50 of CMV; (b) HF cells infected with 10 TCID50 of CMV; (c) HF cells infected with 100 TCID50 of CMV

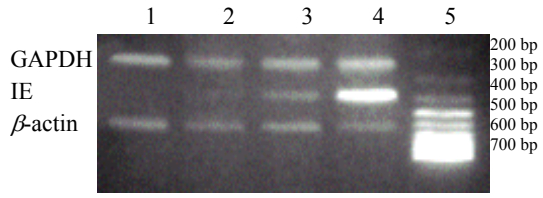

Fig.3 GAPDH, IE and β-actin mRNA expression in HF cells infected by CMV

1: HF cells; 2: HF cells infected with 1 TCID50 of CMV; 3: HF cells infected with 10 TCID50 of CMV; 4: HF cells infected with 100 TCID50 of CMV; 5: Marker

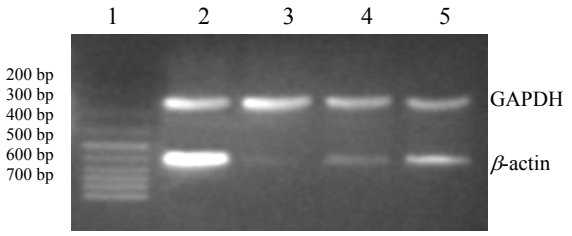

Fig.4 The kinetics of the change of β-actin mRNA after the infection with 100 TCID50 of CMV
1: Marker; 2: HF cells; 3: At 48 h after the infection of CMV; 4: At 24 h after the infection of CMV; 5: At 12 h after the infection of CMV

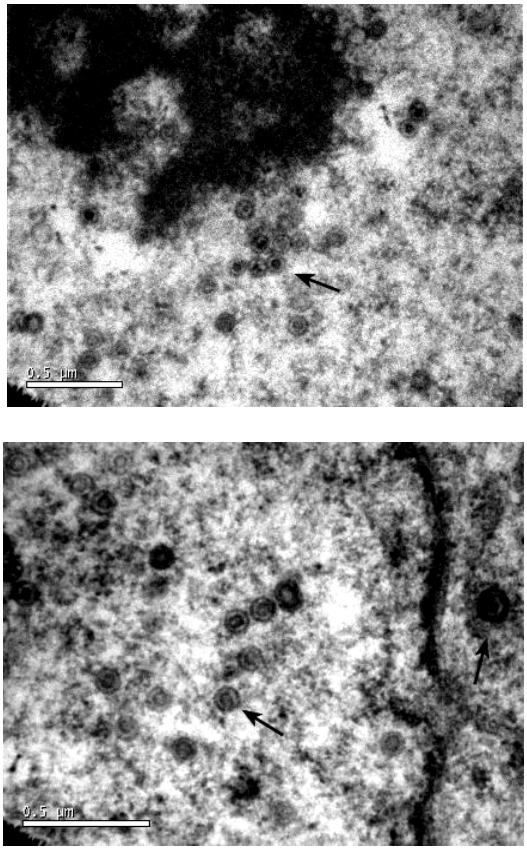

Fig.5 CMV particles in HF cells (10 TCID50)

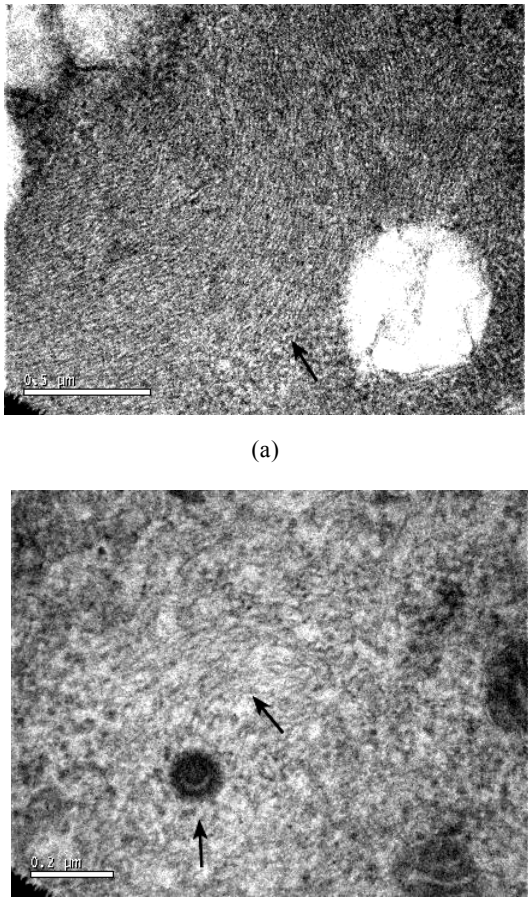

Fig.6 Microfilament in HF cells infected by CMV (10 TCID50)
(a) HF cells uninfected by CMV (Microfilament in the middle); (b) HF cells infected by CMV (CMV particle and microfilament in the middle)

DISCUSSION

The CSK is mainly composed of three distinctive cytoplasmic fibrous structures, namely, microfilaments, intermediate filaments and microtubules. The fundamental function of the CSK is to provide structural framework for the cytoplasm. Through such framework the CSK is involved in various cellular organizations and activities such as cell shaping, distribution of cell organelles, cell motility, membrane phenomena, and metabolism. Microfilaments, the slenderest in the three cytoskeletal structures, are mainly composed of actin protein. Actin proteins are very important in supporting the cell structure and function. Several studies indicated that viruses could induce different cytoskeletal modifications. Gupta *et al.*(1998) reported that the cellular cytoskeletal protein actin, in its polymeric form, plays a role in the transcription of human parainfluenza virus type 3 (HPIV3) in vitro, strongly suggesting that actin microfilaments

play an important role in the replication of HPIV3. Ravkov *et al.*(1998) reported that cytoskeletal proteins are involved in the morphogenesis of Black Creek Canal virus (BCCV), indicating that actin filaments may play an important role in hantavirus assembly and/or release. As for CMV, Jones *et al.*(1986) and Arcangeletti *et al.*(2000) used whole-mount (3D) electron microscopy to analyze the CSK of uninfected and CMV-infected human lung fibroblast cells, and found that CMV infection causes a rapid, progressive disruption of the host cell CSK that correlates with actin depolymerization. In our study, cell shape changes were observed very early during the CMV replicative cycle, we also got the same result as that of Jones with electron microscopy: Microfilaments were not only short and ruptured, but also arranged turbulently after the infection by CMV. On the other hand, RT-PCR assay was used in our study to detect the changes of β-actin mRNA. Data showed that β-actin mRNA of HF cells infected by CMV decreased in a time- and titer-dependent manner compared with the uninfected cells. This suggested that down-regulation of actin genes might be involved in microfilament rearrangements.

Although there is no definitive evidence of the association of CMV infection with human cancer, the oncogenic potential of CMV has been well established by in vitro studies demonstrating the ability of UV-irradiated or infectious virus to transform a variety of cells. Some reported results of analysis of CSK composition would be useful in diagnosis of clinical specimens and aid in studies of lineage relationships of neoplasms. Researchers also found a loss of microfilaments in the transformed cells (Liotta and Steler, 1991). Similar changes of the microfilaments could be seen in our study when we infected HF cells with CMV. So it is deduceded that the change of cytoskeletal structure is one of the mechanisms with which CMV induces normal cells to transformed cells. CMV IE proteins were recently shown to block apoptosis probably by suppressing the ability of the antioncogene p53 to activate a reporter gene. The interactions of CMV with tumor suppressor proteins such as p53 or retinoblastoma (Rb) susceptibility protein are reminiscent of those mediated by the oncoproteins of DNA tumor viruses (Billstrom *et al.*, 2002; Castillo and Kowalik, 2002). The acquisition of a fully malignant phenotype by normal cells is thought to require several mutations in a number of cellular genes. In this connection, CMV may play the role of a nonobligate either direct or indirect cofactor for tumor genesis, e.g. by blocking apoptosis, which may be an essential requirement for tumor progression. Due to the stimulation of growth factors and/or inhibition of antioncogenes by its gene products, CMV may modulate the malignant potential for tumor cells.

In addition, since infection of CMV could affect the expression of β-actin mRNA in the cells, β-actin mRNA should not be the reference for common PCR; while GAPDH mRNA could be if needed.

References

Arcangeletti, M.C., Pinardi, F., Medici, M.C., 2000. Cytoskeleton involvement during human cytomegalovirus replicative cycle in human embryo fibroblasts. *New Microbiol*, **23**:241-256.

Billstrom, S.M., Christensen, R., Worthen, G.S., 2002. Human cytomegalovirus protects endothelial cells from apoptosis induced by growth factor withdrawal. *J Clin Virol*, **25**(supl.):149-157.

Castillo, J.P., Kowalik, T.F., 2002. Human cytomegalovirus immediate early proteins and cell growth control. *Gene*, **290**:19-34.

Gupta, S., De, B.P., Drazba, J.A., 1998. Involvement of actin microfilaments in the replication of human parainfluenza virus type 3. *J Virol*, **72**:2655-2662.

Jones, N.L., Lewis, J.C., Kilpatrick, B.A., 1986. Cytoskeletal disruption during human cytomegalovirus infection of human lung fibroblasts. *Eur J Cell Biol*, **41**: 304-312.

Liotta, L.A., Steler, W.G., 1991. Tumor invasion and metastasis: an imbalance of positive and negative regulation. *Cancer Res*, **51**:5054.

Ravkov, E.V., Nichol, S.T., Peters, C.J., 1998. Role of actin microfilaments in Black Creek Canal virus morphogenesis. *J Virol*, **72**:2865-2870.