Dual Effect of Pseudorabies Virus Growth Factor (PRGF) Displayed on Actin Cytoskeleton

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Abstract. Pseudorabies virus growth factor (PRGF) was shown to possess transforming activity as well as transformation repressing activity in *in vitro* systems In order to better understand these phenomena we studied actin cytoskeleton and its alterations induced by PRGF using normal human fibroblasts VH-10 and transformed cell line HeLa For specific detection of filamentous actin cells were stained with phalloidin conjugated with fluorescein isothiocyanate (FITC)-phalloidin PRGF was applied to VH-10 cells for various length of time from 10 min up to 48 h The effect was very fast and changes in actin filament composition could be detected already after 10 min In comparison to untreated cells the staining of treated cells was more diffuse and a number of actin microfilaments in individual stress fibers became reduced After 30 min thick short actin bundles appeared in the perinuclear region A 24-h exposure resulted in a large reduction of actin bundles After additional 24 h a partial restoration of actin cytoskeleton in cells was observed In transformed HeLa cells PRGF induced opposite process than in normal cells the number of filamentous actin structures increased We hypothesise that PRGF may act as a transcription-like factor and may initiate changes in gene expression which consequently result in actin cytoskeleton alterations

Key words: Pseudorabies virus growth factor — Actin cytoskeleton — *In vitro* cell lines — Fluorescence microscopy

Introduction

Pseudorabies virus growth factor (PRGF) was detected and isolated from mammalian cells infected by pseudorabies virus (Golais et al 1988, 1990) It consists of two subunits $PRGF_A$ and $PRGF_B$, both subunits with a molecular weight less than

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1 kD (Gašperík et al. 1994) Each of the subunits alone is sufficient to transform normal cells *in vitro*. However, for suppression of transformed phenotype (inhibition of growth in soft agar, restoring contact inhibition) of malignant cells, the presence of both units, i. e. the whole PRGF complex was necessary (Gašperík et al. 1994). *In vivo* experiments in mammals showed that PRGF facilitated growth and postnatal development of newborn mice and rats (Csabayová et al. 1995). A dual effect of PRGF has been demonstrated in aquatic animals; high concentrations of PRGF caused retardation of fish development as well as growth of their weight and length, low concentrations of PRGF increased the weight and length of exposed fish (Kovrižnych et al. 1998).

Actin cytoskeleton is known to be involved in many essential cellular processes, such as proliferation, signal transduction, cytokinesis, etc. The importance of the actin cytoskeleton in viral transformation was clearly demonstrated by Pollack et al (1975). In order to investigate phenomena induced by PRGF on subcellular level we studied actin cytoskeleton microfilaments after the influence of PRGF By fluorescence microscopy we were able to detect changes of F-actin organisation within the individual cells. We showed that in normal fibroblasts, PRGF induced actin cytoskeleton disintegration, while in transformed cells it increased filamentous structures.

Materials and Methods

Cell Culture. Normal human foreskin fibroblasts VH-10 were cultured in MEM medium (Biocom, Czech Republic) supplemented with 10% fetal calf serum (Biocom, Czech Republic) at 37 °C with 5% CO_2 without antibiotics Transformed human cell line HeLa was cultured in the same conditions except the serum, with 10 % bovine serum used instead of fetal serum For fluorescence microscopy experiments cells were seeded at a density of 3×10^4 cells per ml one day before the treatment to allow cells to attach on the cover glass and to spread sufficiently Cells were exposed to PRGF dissolved in serum-free medium for 10 min up to 48 h Control cells were kept in serum-free medium for the same period of time

Fluorescence microscopy Visualisation of filamentous actin was done according to Urbančíková with some modifications (Urbančíková and Grófová 1990) After the exposure to PRGF, the cells were washed with PBS and fixed in 4% paraformaldehyde for 20 min at room temperature Then, the cells were permeabilized with 0 2% Triton X-100 for 3 min and thoroughly washed with PBS The cells were subsequently incubated with 1 μmol/l phalloidin conjugated with fluorescein isothiocyanate (FITC-phalloidin) (Sigma, USA) diluted in phosphate buffer saline (PBS) for 30 min in the dark The washed cells were mounted on microscope slides with elvanol (polyvinylalcohol, Serva, Germany) Stained cells were examined under an Opton Axiophot fluorescence microscope, and photographed on DX 400 Fomapan film (Foma Bohemia, Czech Republic)

Results

The majority of normal human fibroblasts VH-10 possessed well developed numerous stress fibres (Fig. 1*A*). PRGF was applied at 10^8 U/ml for 10, 20, 30, 60 and 120 min, 24 h and 48 h. The effect of PRGF on actin filament composition was observed soon after the application of the factor. In comparison to untreated VH-10

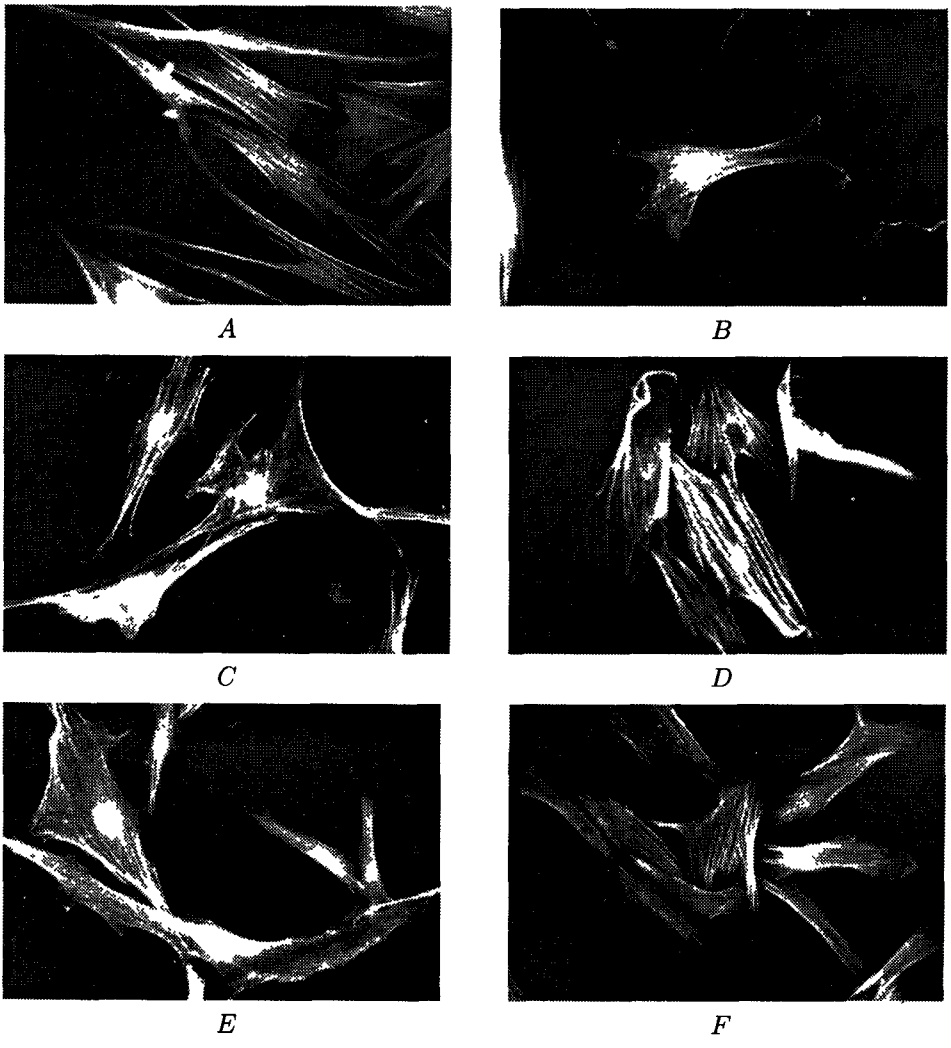
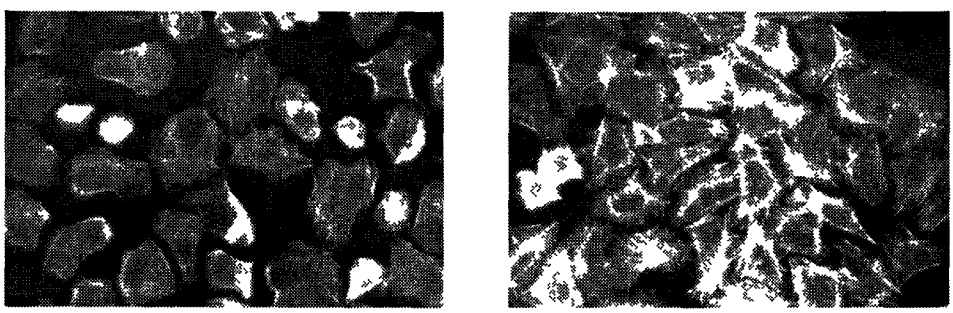


Figure 1. Effect of PRGF on actin cytoskeleton in VH-10 cells stained with FITC-phalloidin and examined by fluorescence microscopy. *A*, control untreated cells; *B*, 10 min exposure to PRGF, *C*, 20 min exposure to PRGF, *D*, 2 h exposure to PRGF; *E*, 24 h exposure to PRGF, *F*, 48 h exposure to PRGF

cells, the staining of the cells after 10 min treatment with PRGF was more diffuse, the numbers of filaments in stress fibres became reduced as the fibres were much thinner (Fig. 1*B*). The most obvious difference was the heterogeneous staining of the nuclei. We assume that there might be visualisation of the nucleoli in the nucleus. Many cells were of irregular shape, some of them created long thin axon-like protrusions. After 20 min thick short actin bundles appeared in the region of the nucleus (Fig. 1*C*). A very similar pattern was observed after 30 min of exposure.

In comparison to 10 min exposure, the staining of the nuclei was in most cases less heterogeneous, i.e. nucleoli were not well visualised. After two hours of exposure to PRGF, some cells expressed very thick bundles, much thicker than those in control cells (Fig. 1*D*). In this case, an interesting phenomenon was observed: some cells had stained nucleus and there were also cells with no nucleus staining on the same preparation. It remains unclear whether this is significant with respect to the *in vitro* action of PRGF. Twenty-four hours of PRGF exposure resulted in a large reduction of actin bundles (Fig. 1*E*). Within the next 24 h, i.e. after a total of 48 h exposure to PRGF, a partial restoration of the cytoskeleton was observed in some cells (Fig. 1*F*). In the remaining cells the actin cytoskeleton composition remained the same as 24 h earlier.

HeLa cells were exposed to different concentrations of PRGF (10^5, 10^7, 10^9, 10^{11}, 10^{13} U/ml) in serum-free medium for short periods up to 1 h and 24 h. After short periods of exposure no distinct differences in actin cytoskeleton composition were observed in comparison to control untreated HeLa cells. 24-h treatment resulted in distinct rearrangement of the actin cytoskeleton. Untreated HeLa cells are characterized by reduced actin microfilament bundles which, in our case, were localised mainly in the cytoplasm close to the periphery of the cells (Fig. 2*A*). Cells exposed to PRGF contained a greater number of actin stress fibres. The largest increase in filamentous structures was observed at PRGF concentration of 10^{11} U/ml (Fig. 2*B*).



A *B*

Figure 2. Effect of PRGF on actin cytoskeleton in HeLa cells stained with FITC-phalloidin and examined by fluorescence microscopy *A*, control untreated HeLa cells, *B*, HeLa cells exposed to 10^{11} PRGF U/ml

Discussion

Viral infection is usually accompanied by a rearrangement of the actin cytoskeleton. During their life cycle, viruses are known to be able to interact with the actin cytoskeleton of the host cell (Way 1998). However, little is known about this process. Recently, it was shown that similarly to *Listeria*, viruses are able to recruit and exploit actin to facilitate their spreading from cell to cell (Cudmore et al. 1995).

Decrease of F-actin observed after rabies virus infection was ascribed as a possible indirect action of rabies virus on actin-binding proteins, such as synapsin I (Ceccaldi et al. 1997).

PRGF isolated form cells infected with pseudorabies virus (PRV) was shown to be synthesized when production of infection virus was reduced or inhibited, e g. during a latent period or in semi-permissive or non-permissive cells (Golais et al. 1992). Alterations of actin cytoskeleton observed in HeLa cells exposed to PRGF are in good correlation with previous results obtained in the same cells by analysing morphological and some functional parameters (Golais et al. 1990), provided that restoration of actin cytoskeletal structures is considered an event of the non-transformed phenomenon.

We could show that, on the cellular level, PRGF interfered with actin structures. Whether this interaction was direct or through the regulation proteins of the cytoskeleton assembly or on the level of gene expression remains to be answered Results of *in vivo* studies in mice, rats and fish indicate that PRGF might act as a transcription-like factor as it promoted development and growth of animals; moreover, in some cases it induced malformations (Csabayová et al. 1995; Kovrižnych et al 1998).

Acknowledgements. This research was supported by Ministy of Health of the Slovak Republic's grant 049207 Authors would like to thank Mrs Eva Dobríková for her excellent technical assistance

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