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KINETIC ANALYSIS OF BIMOLECULAR „VIRUS ‑ HOST CELL INTERACTION" BY SURFACE PLASMON RESONANCE (SPR) METHOD

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Abstract: *Since viruses are obligate intracellular parasites, they may exist and reproduce only in living host cell. Herpes simplex virus infections (HSV) are ubiquitous and widespread in the human population and represent a suitable model for study of virus-cell interactions. The aim of the present study is to detect and to evaluate the kinetics of a biomolecular "cell-host – herpes simplex virus" interaction in a condition of multi-step virus growth infectious process using the surface plasmon resonance (SPR) method at different multiplicity of infection (MOI) and time of exposure. The obtained results are compared with other widely applicable methods such as microscopic observation of structural changes of infected cells and assay for detection of cell proliferation and survival. According to the performed study, SPR method is very promising for kinetic analysis of the interaction between virus and host -cell.*

Key words: *Virus – Host-Cell, HSV-1, SPR*

INTRODUCTION

Viruses are the most common cause of infectious disease and have considerable impact on human health (Jones, K. E. et al., 2008). A thorough knowledge of the „virus – host-cell" interactions can reveal insights of infectious process and can guide to target identification of new anti-viral drugs discovery (Gulbahce, N., Yan, H., Dricot, A. et al., 2012). As obligate intracellular parasites, viruses depend on their "host-cell interactions" for the energy, macromolecular synthesis machinery, the work benches for genome replication and particle assembly and thus allow reprogramming cells for their own purposes (Dyer, M. D. et al., 2008; Weitzman, M. D. et al., 2004; Zhou, X. et al., 2018). Many "virus-cell interactions" are transient and synchronous, involving intermediate steps on the pathway, each featuring dynamic interactions between different viral and cellular components. The molecular mechanisms that harness cellular

components in order to establish a productive viral replication cycle involves the formation of specialized cellular microenvironments, where cellular and viral macromolecules are recruited ("virus factories"/replication compartments (RC)). Hence, RC may represent a control hub of "virus - host-cell interactions" that promotes efficient viral replication and simultaneously protects viral macromolecules from cellular antiviral activities (Netherton, C. L., Wileman, T., 2011; Schmid, M. et al., 2014). Viruses that replicate in the nucleus, such as Herpes simplex virus (HSV) upon infection of the cell, also have similar functional organization with distinguishable RC structures, localized on the inside of nuclei membrane and included viral subcompartments (Netherton, C. et al., 2007).

Methods that provided valuable information about morphological organization of RC structures and analyzed viral activity must encompass simple, less time consuming, low-cost, with option for direct detection of intact virions in various biological media. Recently, non-destructive methods, based on specific bimolecular biochemical interactions are of considerable current interest. Among such methods, one of the most popular is Surface Plasmon Resonance (SPR) (Homola, J., 2008).

The traditional approach for use of the SPR technique is to have receptor immobilized at the sensor surface, while an analyte remains in solution. In this case, the SPR condition depends on the effective thickness of analyte layer specifically bound to the immobilized receptor layer (human cells). The shift of SPR condition is due to variation of the parameters of cell ensemble, because of their infection with a virus. The SPR shift depends not only on the layer thickness but on the refractive index variation within the cell ensembles, caused by the morphological changes of the cells in the process of virus replication.

HSV-1 is ubiquitous infectious agent (60 – 95% of human population is infected) and one of the main model of virology, as well as one of the most promising alternatives for the development of vectors used in gene and anticancer therapy (treating of diseases that affect the central nervous system) (Latchman, D. S., 1994; Artusi, S. et al., 2018). Nevertheless, the molecular mechanisms by which HSV-1 proteins reorganize and reprogram the infected cell nuclei and cytoplasm is not completely understand. Since viral replication compartments seem to represent a regulating centre of "virus-cell interactions", a detailed study of morphological changes and bimolecular interaction and process of compartmentalization will be promising.

The aim of the current study is to detect and to evaluate the kinetics of a biomolecular "host-cell - HSV-1" interaction in a condition of one- and multi-step virus growth infectious process using the SPR method at different multiplicity of infection (MOI) and time of exposure. The results are comparing with other conventional methods (MTT – assay and microscopic observation of cell morphology) and we propose the experimental model for studying "virus- host cell interactions".

EXPOSITION

Materials and Methods

1.1. Cell culture and viruses

Lep (human embryonal lung fibroblasts) cell line was obtained from National Center of Infectious and Parasitic Diseases (NCIPD), Bulgaria. The cell line was cultured in Dulbecco's Modified Eagle's medium (DMEM, Sigma) with L-glutamine, supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% streptomycin (100 μg/ml) and penicillin (100 IU/ml) (Gibco, USA). The culture was incubated at 37ºC in 5% CO_2 atmosphere and trypsinization was used for cell maintenance. The herpes simplex virus type 1 (HSV-1), strain Victoria (from viral collection at NCIPD) was used. In the antiviral experiments, the growth medium was changed with 2% FBS. For the production of the viral suspensions, 1 mL of HSV-1 was inoculated into a cell culture flask with a confluent Lep monolayer. After viral infection, when lysis was observed in 70% of the cell monolayer, the infected flask was exposed to freezing (-80°C) and thawing cycles (ambient temperature), in order to release the viral particles retained inside the cells. Virus titer was obtained by the limit-dilution method and expressed as 50% tissue culture infections dose/mL ($TCID_{50}$/mL)

(Reed, L. J. & Muench, H., 1938). The viral suspension stock was then aliquoted and stored at -80°C.

1.2. Virus infection kinetic study

Human embryonal lung fibroblasts were seeded at a density of 3 x 10^3 cells/mL and infected simultaneously with HSV-1, strain Victoria with variety multiplicity of infection (MOI) (0.03, 0.05, 0.1, 0.2) for different periods (hours) (Shabani, M. et al., 2019). Samples to determine the viral titer were taken after 2 h and then every 6 h and 12 h until a total incubation time of 72 h. Additionally, the cell density of an infected cell culture is compared to an equally seeded non infected cells (cell control).

1.3. MTT-assay

The measurement of optical density (OD) values by MTT-assay have used as a sensitive method to quantify the density of an infected cell suspension (Meerloo, V. J. et al., 2011). The MTT assay is an established colorimetric method based on the determination of cell viability, as opposed to cell cytopathology, utilizing the reaction of a tetrazolium salt (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT) with the mitochondria of metabolically active cells (Matza-Porges, S. et al., 2014). The absorbance (OD values) was measure at a wavelength of 540 nm using a spectrophotometer, which reflects the concentration of viable cells in a tissue culture plate (Takeuchi, H. et al., 1991; Chattopadhyay, D. et al., 2009).

1.4. Cell morphology evaluation by inverted light microscopy

Determination of the cytopathic effect (CPE) of HSV-1 on adherent cells was observed daily for microscopically detectable morphological alterations, such as loss of confluency, cell rounding and shrinking, formation of syncytia, cytoplasm granulation, vacuolization and some nuclear changes (Alvarez, A. L. et al., 2011).

1.5. SPR measurement

At SPR sensor surface were cultivated Lep cells (3 x 10^3 cells/mL). The reference SPR conditions are established for non-infected cells. After simultaneously infection of human embryonal fibroblasts with HSV-1, strain Victoria with variety MOI, SPR conditions are measured at different time intervals between 2 – 72 h. Then SPR shift is evaluated according the reference SPR conditions for non-infected cells.

RESULTS AND DISCUSSION

As described in the Introduction, viral and cellular proteins that modulate and participate in viral replication processing, as well as components of the cellular antiviral response are associated with specific morphological changes in infected cells. In order to analyze in more detail the cellular response against viral exposure we used different methods and proposed the protocol for study of this complementation.

Since HSV-1 causes strong cytopathic effects, there are a variety of assay methods, such as measuring the number of plaque-forming units (pfu) by plaque-titration (Juarez, D., Long, K. C., Aguilar, P., Kochel, T. J. & Halsey, E., 2013), colorimetric assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) (Meerloo J., Kaspers, G. J., Cloos, J., 2011) and other can be used as a monitoring tool to determine cell growth and viability of infected and non-infected cells in a condition of one- or multi-step growth curve. These traditional systems lack accuracy and high sensitivity in studying cellular changes resulting from infection of an intracellular pathogen. Conventional methods for such an are time consuming, laborious and lacks economic feasibility. In addition, a combination of two or more methods/techniques is often required to perform a complete analysis (Griffiths, P. D., 2009).

The newly applied SPR method was further verified and validated with conventional assays, such as MTT and optical microscopic analysis of cytopathic effect (CPE).

The results of performed measurements from SPR showed similar trend as was achieved with standard methods, but with a variation in sensitivity (the change of the optical properties of the cell monolayer) and with options for quantitative assessment. The replication cycle stages - adsorption, penetration of the virus in the cell, changes in cell metabolism, as well as the assembly and release of daughter progeny, causes such changes and respectively, generate SPR shift. The results of performed measurements are shown in Figure 1a, b.

In this context, the lytic stage of HSV-1 infection occurs as a tree-stage program. As a general rule, the increase of SPR shift is caused by enhance of the refractive index of cell layer and vice versa. Such an increase is observed in the intervals 6 – 12 h and 24 – 48 h, while SPR shift decreases in the time intervals 2 - 6 h, 12 - 24 h and 48 - 72 h.

During the first interval (6 – 12 h), in which is observed a global shutoff of host gene expression, capsid maturation, formation of replication compartments, and potential egress of viral capsids from the nucleus into the cytoplasm (Scott, E. S. & O'Hare, P., 2001) are occur first morphological modification and reorganisation of host-cell nuclei (Simpson-Holley, M. et al., 2005). This process increases the nucleus density and compact viral chromatin structure, which lead to the increase of refractive index. In this period, the MTT-assay measured the optical density changes as a result of stimulation of HSV infection in host-cells by clustering of mitochondria, migration of virus structure proteins to a site around the nucleus and maintains function until at least the middle stage of infection (Murata T. et al., 2000).

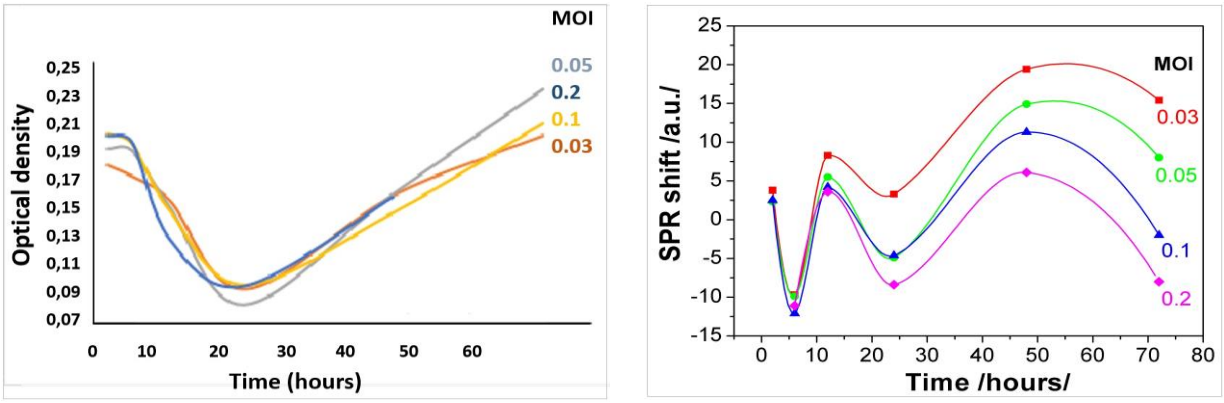

Fig. 1 Measurements provided by: a/ MTT method: optical density vs time; b/ SPR method

During the second interval (12 – 24 h) the virus assembly takes place into the cytoplasm, which coincides with the end of the first replication cycle of HSV-1. On that period conventional and SPR methods indicated reducing the density of cell monolayer and proliferation rate, as well as and decreasing of intercellular distance. MTT method also identifies this point since around 24 h the quantity of leaving cells is minimal what correspo

nd to the minimum of optical density. This point (around 24 h) is clearly observed for all MOI. (Fig. 1b).

During the interval 24 - 48 h the new virions produced in the host cell, infect adjacent cells. The adsorption of virus on newly infected cells increases thickness of cell's layer what increases the SPR shift.

At the beginning of the time interval 48 – 72 h the cytopathic effect (CPE) is observed. Herpesviruses characteristically produce cell fusion (syncytium formation) and rarely other forms of CPE. With this CPE, the plasma membranes of four or more host cells fuse and produce an enlarged cell with at least four nuclei. Although large cell fusions are sometimes visible without staining. This process take place to the end of this period and leads to drastic reduction of cells packing density, low proliferation rate, as well as and decreasing of intercellular distance. The relatively large yield of virus obtained per cell by about 30 h is the result of multiple cycles of replication in newly infected cells.

CONCLUSION

Performed study shows that SPR method possesses high sensitivity in all steps of virus infection in the model of one- or multiple-step growth curves. Hence, SPR method represent a novel system to study morphological changes, which occur in infected cells through the different stages of viral replication cycle. This was experimentally proven by studying the effects of interaction with HSV - 1, after which they were compared with standard test methods - MTT and morphological observations of the monolayer under a light microscope. we propose the experimental model for studying "virus- host cell interactions".

REFERENCES

Alvarez, A. L., Habtemariam, S., Juan-Badaturuge, M., Jackson, C. & Parra, F. (2011). In vitro anti HSV-1 and HSV-2 activity of Tanacetum vulgare extracts and isolated compounds: an approach to their mechanisms of action. *Phytother Res.,* 25, 296–301.

Artusi, S., Miyagawa, Y., Goins, W. F., Cohen, J. B., Glorioso J. C. (2018). Herpes Simplex Virus Vectors for Gene Transfer to the Central Nervous System. *Diseases,* 14, 6(3), 74.

Chattopadhyay, D., Sarkar, C. M., Chatterjee, T., Dey, R. & Chakrabarty, P. S. (2009). MTH: Recent advancements for the evaluation of Antiviral activities of natural products. *New Biotechnology,* 25 (5), 347-368.

Dyer, M. D. et al. (2008). The landscape of human proteins interacting with viruses and other pathogens. *PLOS Pathogens,* 4 (2), 1–14.

Griffiths**,** P**.** D. (2009). Herpesviruses. *Medicine***,** 37**,** 668-72.

Gulbahce, N., Yan, H., Dricot, A. et al. (2012). Viral perturbations of host networks reflect disease etiology. *PLOS Computational Biology,* 8 (6).

Homola, J. Surface plasmon resonance sensors for detection of chemical and biological species (2008). *Chem. Rev.,* 108, 462–493.

Jones, K. E. et al. (2008). Global trends in emerging infectious diseases. *Nature,* 451 (7181), 990–993.

Juarez, D., Long, K. C., Aguilar, P., Kochel, T. J. & Halsey, E. S. (2013). Assessment of plaque assay methods for alphaviruses. *J. Virol. Methods*., 187, 185-189.

Latchman, D. S. (1994). Herpes simplex virus vectors for gene therapy. *Mol Biotechnol.*, 2, 179–195**.**

Matza-Porges, S., Eisen, K., Haberman, I. H., Fridlender, A. B. & Joseph, G. (2014). A new antiviral screening method that simultaneously detects viral replication, cell viability, and cell toxicity. *J Virol Methods.*, 208, 138-143.

Meerloo, V. J., Kaspers, G. J., Cloos, J. (2011). Cell sensitivity assays: the MTT assay. *Methods Mol Biol.*, 731, 237-245.

Netherton, C. L., Wileman, T. (2011). Virus factories, double membrane vesicles and viroplasm generated in animal cells. *Curr Opin Virol*., 1, 381–387.

Netherton, C., Moffat, K., Brooks, E., Wileman, T. (2007). A guide to viral inclusions, membrane rearrangements, factories, and viroplasm produced during virus replication. *Adv Virus Res*, 70, 101–182.

Reed, L. J. & Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology*, 27(3), 493–497.

Schmid, M., Speiseder, T., Dobner, T., Gonzalez, R. A. (2014). DNA virus replication compartments. *J Virol*, 88, 1404–1420.

Scott, E. S. and O'Hare, P. (2001). Fate of the inner nuclear membrane protein lamin B receptor and nuclear lamins in herpes simplex virus type 1 infection. *J. Virol.,* 75, 8818-8830.

Simpson-Holley, M., Colgrove, R. C., Nalepa, G., Harper, J. W. and Knipe, D. M. (2005). Identification and functional evaluation of cellular and viral factors involved in the alteration of nuclear architecture during herpes simplex virus 1 infection. *J. Virol.*, 79, 12840-12851.

Shabani, M., Esfahani, N. B., Ehdaei, S. B., Moghim, S., Mirzaei, A., Sharifi, M. & Mouhebat, L. (2019). Inhibition of herpes simplex virus type 1 replication by novel hsa-miR-7704 in vitro. *Res Pharma Sci*., 14, 167-174.

Murata T., Goshima F., Daikoku T., Inagaki-Ohara K., Takakuwa H., Kato K., Nishiyama Y. (2000). Mitochondrial distribution and function in herpes simplex virus-infected cells. *J Gen Virol*., 81(Pt 2), 401-406.

Takeuchi, H., Baba, M. & Shigeta, S. (1991). An application of tetrazolium (MTT) colorimetric assay for the screening of anti-herpes simplex virus compounds. *J. Virol. Methods*, 33, 61–71.

Weitzman, M. D. et al. (2004). Interactions of viruses with the cellular DNA repair machinery. *DNA Repair*, 3(8), 1165–1173.

Zhou, X. et al. (2018). A generalized approach to predicting protein-protein interactions between virus and host. *BMC genomics*, 19(6), 568.