ABSTRACT

Goal of the thesis: The main aim of this thesis was to introduce the in vitro cytomegalovirus (HCMV) infection method of the VR-1590 strain, followed by study of the in vitro HCMV infection by xCelligence RTCA methodology and to use ionizing radiation to provide immunosuppression in host fibroblasts. Detection and analysis of selected signaling pathways of immunosuppressed host cells using 1D electrophoresis, Western blot, immunodetection and antibody PathScan technology.

Methods: In this work, HCMV infection of VR-1590 and human embryonic fibroblasts of the MRC-5 line was performed. Basic laboratory techniques, as well as ionizing gamma rays produced by the enclosed ^{60}Co radiator, were used as a factor for immunosuppression. Changes in signaling pathways in host fibroblasts were monitored by PathScan antibody technology and further verified by one-dimensional Western blot electrophoresis techniques.

Results: Cellular proliferation of the host cells was verified in real time. Specimens were developed using modeling situations using optimized dose of HCMV infection and subsequent induction of immunosuppression or model with induced immunosuppression. Expression of detected signal molecules (p53, Bad, Caspase 3, Stat1, Stat3, ERK1/2 and others) indicates that cells affected by ionizing radiation apparently disappear via apoptosis because proapoptotic markers exhibit increased expression with increasing radiation doses. Our results show that the line of fibroblasts without the influence of infection is extensively radioactive, corresponding to changes in the expression of anti-apoptotic markers.

Conclusions: CMV modulates the early stages of signal pathway infection within both natural and adaptive immunity. Due to ionizing radiation, proliferation of pro-apoptotic proteins has been demonstrated to drive the NF-κB signaling pathway, which controls the gene by expressing the anti-inflammatory cytokines necessary to functionally synchronize the immune system cells, but also to reduce interferon production, which is of course required to reduce viral replication infected cells and to inhibit DNA replication.

Key words: human cytomegalovirus; ionizing radiation; apoptosis; immunosuppression.