ABSTRACT

The aim of this study was to create a monoclonal antibody against VP1 protein BK virus for study of individual steps of its replication cycle. BK virus is human polyomavirus which causes serious disease - polyomavirus associated nephropathy in immunocompromised patients after renal transplantation or hemorrhagic cystitis after bone-marrow transplantation. Virions of BK virus have capsids with icosahedral symmetry consisting of 360 molecules of the major capsid protein VP1 and two minor structure proteins VP2 and VP3, which are not exposed on the capsid surface. VP1 is highly immunogenic what gives us possibility to produce antibodies capable of BK virus monitoring by capsid protein detection. Empty BK virus particles produced in insect cells from a recombinant baculovirus were used as antigen to immunize mice for preparing hybridomas. Cloning of hybridomas created by fusion of myeloma cells with B-lymphocytes from immunized mouse gave us series of primary hybridoma clones. One of them, 5/G10/A5/B2/8, was cloned to homogenity and monoclonal antibody against VP1 BK virus was purified from hybridomas medium by affinity chromatography. The analysis of antibody properties revealed that it recognizes a conformation epitope and can be used for VP1 protein and virion detection in cells by indirect immunofluorescence. Antibody does not detect VP1 of related SV40 virus.

Polyomavirus minor proteins, VP2 and VP3, bind intracellular membranes and perforate them. It was suggested that this ability could be used to release virions from the endoplasmic reticulum to cytoplasm on their trafficking to cell nucleus. It is postulated that molecules of VP3 oligomerise at the membrane and create an aperture for partially disassembled virion escape. We decided to prove the ability of VP3 oligomerization by BiFC (bimolecular fluorescence complemantation). For this we designed series of oligonucleotide primers for constructing 4 plasmids for expression of VP3 protein fused with one or other part of yellow fluorescent protein in both orientations. Sequencing of all four constructs proved two of them – pcDNA3-NYFP-VP3 which encodes fusion protein consisting of N-terminal part YFP linked to the N-terminus of VP3 and pcDNA3-VP3-NYFP which encodes fusion protein consisting of N-terminal part of YFP linked to the C-terminus of VP3 protein to be correct. They are now ready to use. Unfortunately, vectors carrying gene for 8 C-terminal part of YFP fused with VP3 in both orientation exhibited several sequence defects. Therefore, we were not able to prove VP3 protein oligomerization by complementation of YFP - BiFC yet.

Key words: polyomavirus, BKV, VP1, antigen, VP2, VP3, oligomerization, BiFC