Preparation of monoclonal antibodies and expression plasmids for studies of properties of BK virus structural proteins

ABSTRACT:

The new aim of our laboratory is to solve mechanisms of individual steps of infection of human polyomavirus BK (BKV). BKV is a nonenveloped DNA virus that asymptomatically infects 60 to 90 % of the human population. However, BKV is the primary etiological agent of polyomavirus-associated nephropathy, which causes irreversible graft loss in a part of kidney transplant patients. Moreover, recent results point toward a role for BKV in early prostate cancer progression. BKV is composed of the major structural protein VP1, minor structural proteins VP2 and VP3 and a nucleocore. Functions of the minor proteins, VP2 and VP3 in BKV life cycle are not clear but they might play roles in each step of BKV replication cycle. The major structural protein, VP1 is responsible for receptor binding but it may have other, yet unknown functions.

The aims of this study were: i) to prepare monoclonal antibodies against BKV structural proteins and ii) to construct recombinant expression plasmids ensuring production of EGFP-fused VP3 in mammalian cells for investigation of VP3 interactions with host cell structures.

Recombinant baculovirus for production VP2 BKV fused with HIS-tag in insect cells was constructed. Isolation of VP2 from insect cells by several methods failed, because of insolubility of VP2. As an antigen for mice immunization protein VP3 isolated from VLPs composed of VP1 and VP3 was finally used. VP3 was isolated by SDS-PAAGE of VLP lysate and by electroelution of separated VP3 from the gel.

Selected hybridomas producing monoclonal antibodies against VP3 appeared to be unstable and stopped production of anti VP3 antibody during passaging. Surprisingly, a stable hybridoma cell line secreting a monoclonal antibody against VP1 BKV was obtained, probably due to contamination of VP3 antigen with a degradation product of VP1. Anti VP1 monoclonal antibody recognizes denatured VP1 on western blots as well as native VP1 in cells by indirect immunofluorescence method.

Plasmids carrying sequence for VP3 fused with EGFP (VP3-EGFP and EGFP-VP3) were constructed. Production of fusion proteins in mammalian cells was verified. The constructs were used for monitoring of VP3 localisation in simian and mouse cells. It was shown, that both fused VP3 proteins were predominantly targeted into the cell nucleus below nuclear lamina. In the cell nucleus, fused proteins colocalised with cellular DNA cumulated in nucleus periphery. The lamina seemed to be demaged in VP3 producing cells.

Key words: BK virus, minor structural proteins VP2 and VP3, major structural protein VP1, baculovirus expression system, monoclonal antibody.

Klíčová slova: BK virus, minoritní strukturní proteiny VP2 a VP3, hlavní strukturní protein VP1, bakulovirový expresní systém, monoklonální protilátka.