Preparation of constructs for transgenic expression of DPP-IV and FAP

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Abstract:

DASH (Dipeptidyl peptidase-IV Activity and/or Structure Homologues) protein group involves multi-functional molecules typically bearing enzymatic activity similar to the Dipeptidyl peptidase-IV (DPP-IV, EC 3.4.14.5, identical with lymphocyte differentiation antigen CD26). In general, they cleave multiple regulatory as well as structural peptides and proteins, possessing proline residue on the penultimate position from the N-terminus. We focused on two members of this group: canonical DPP-IV and Fibroblast activation protein alpha (FAP-α). Both are typically type II plasma membrane proteins with specific tissue distribution. Soluble extracellular forms have also been identified. Available knowledge suggest important roles of these proteins in oncogenesis, executed by their enzymatic activity but also by non-proteolytic interactions.

To study their role in gliomagenesis we designed several experimental models exploiting astrocytoma cell lines with defined DPP-IV or FAP-α phenotype. Enzymatically inactive forms and analogues with different subcellular distribution will also be included. These models will allow to assess the impact of DPP-IV and FAP-α on the glial tumor development and the importance of their enzymatic activities and localization in these processes. Experiments using inducible expression DPP-IV and FAP-α and their isoforms will be performed in vitro as well as in vivo using stereotactic grafting into the immunodeficient mice.

Here we present the first part of these studies – preparation of constructs for expression of different forms DPP-IV and FAP-α. Three groups of constructs were prepared: for native forms, for cytosolic forms with transmembrane segment deletion, and for fusion forms with signal peptides at the N-terminus for extracellular secretion.

(In Czech)