Abstract

Dipeptidyl peptidase IV (DPP-IV) is a serine protease, which executes its proteolytic activity by cleaving X-Pro dipeptides from the N-termini of its substrates. Furthermore, DPP-IV exhibits many biological functions independent of its enzymatic activity. Previous studies in our laboratory proved increased expression of DPP-IV in high-grade astrocytic tumours. To evaluate the enzymatic and non-enzymatic functions of DPP-IV in a glioma model, clones of astrocytic cell line U373MG transfected by enzymatically inactive, mutated DPP-IV (mutDPP-IV) and enzymatically active, wild type DPP-IV (wtDPP-IV), were prepared. Enzymatically inactive mutDPP-IV was prepared using point mutation the active site serine residue. Cells U373MG were transfected using a doxycycline inducible Tet-On® system. For further analysis of the transgenic forms of DPP-IV, methods were used for verification of protein expression, enzymatic activity and subcellular localization. Doxycycline induced U373MG mutDPP-IV and U373MG wtDPP-IV cells, expressing mutated and wild type DPP-IV, respectively, exhibited increased expression of transgenic DPP-IV in a concentration and time dependent manner. Doxycycline induced U373MG wtDPP-IV cells exhibited both increased expression and enzymatic activity of DPP-IV. In contrast, DPP-IV enzymatic activity in doxycycline induced U373MG mutDPP-IV cells corresponded to the activity of endogenous DPP-IV in untransfected U373MG cells. Western blot analysis revealed two transgenic DPP-IV forms with MW 178 kDa and 183 kDa, which likely represent dimeric DPP-IV. Possible difference in glycosylation was analysed according to deglycosylation by β-N acetylglucosaminidase H (endo H), previously used to differentiate mature and immature forms of DPP-IV. Surprisingly, exposure of DPP-IV to endo H did not influence its MW, which indicates that the two MW forms are not produced by different glycosylation of DPP-IV. Thus other posttraslational modifications may be involved.

On the basis of biochemical and immunochemical analysis, two stable clones U373MG mutDPP-IV and U373MG wtDPP-IV expressing transgenic forms of DPP-IV under the control of doxycycline were selected for further biological studies.