Abstract

The role of glutamate carboxypeptidase II in mammalian organism is already known quite well but only little is known about its homologue glutamate carboxypeptidase III.

For structural and functional characterization of any protein, a large amount of protein is required. Protein could be obtained by expression in tissue culture. Properties of the protein may be affected by post translational modifications, where different organisms create different modifications. Therefore, we set to develop a system for recombinant expression of GCPIII in mammalian cells.

First the mammalian expression system HEK 293–6E was introduced as a substitute for the current insect expression system. The advantage of this mammalian expression system is its option of transient transfection and that it is easy to cultivate cells under suspension conditions. Further, transfection conditions for this system were optimized by green fluorescent protein expression, for easy detection by flow cytometry.

DNA encoding the extracellular part of mouse GCPIII (mEXSTII) was cloned into five expression plasmids with His or Fc tags attached to N– or C–termini. Cells were transfected with prepared plasmids. The presence of mEXSTII in media was tested using Western blot and subsequently the activity of GCPIII was tested by cleaving its specific substrate β–citrylglutamate.

The protein was expressed in all cases but plasmid pYD11, coding for a large C–terminal Fc tag, produced significantly lower amount of the protein with negligible actvity. We conclude taht in order to express large amount of the mEXSTII, it is advisable to use plasmid pTT22SSP4 that produce the protein with N–terminal His tag followed by a protease cleavage site for the tag removal or plasmid pYD5 that produces the protein with N–terminal Fc tag followed by thee same protease cleavage site.