Abstract

Glutamate carboxypeptidase II (GCPII) is a metalloprotease responsible for cleaving the neurotransmitter N-acetyl-aspartyl-glutamate in the central nervous system to N-acetyl aspartate and glutamate. At the same time, in the human small intestine, it facilitates folate absorption by cleaving γ-linked glutamate from folyl-poly-γ-glutamate. In humans, GCPII is also expressed in a number of other organs (e.g., kidney and prostate) and tumors, where its physiological function is unknown. In an attempt to characterize the physiological function of the enzyme, we first characterized the commercially available monoclonal antibodies against GCPII. Further, we developed a fully synthetic replacement based on a hydrophilic polymer with bound GCPII inhibitors. We evaluated the suitability of using a murine biomodel to study GCPII function *in vivo*. We found the difference in GCPII expression profile in mouse and human. We did not observe GCPII in either the mouse prostate or small intestine. To assess physiological and pathophysiological functions of the enzyme we analyzed a GCPII-deficient mouse model. Apart from the observation of enlarged seminal vesicles in older males, we did not detect any other obvious phenotype. Similarly, we confirmed that GCPII cannot cleave amyloid peptides ($A\beta_{1-40}$ and $A\beta_{1-42}$). Thus, it appears that the lack of GCPII activity in the organism has no obvious negative effects. It can be therefore assumed that pharmacological inhibition of GCPII will not lead to significant adverse effects either and the clinical use of GCPII inhibitors will be likely safe. In the context of drug targeting, we have shown that the inhibitor-GCPII interaction allows specific targeting of different nanostructures to tumor cells, while the optimization of the nanoparticle bio-nano interface is important to reduce non-specific binding.