

Abstract

Glutamate carboxypeptidase II (GCPII) usually called prostate specific membrane antigen (PSMA) is membrane bound metallopeptidase expressed mainly in prostate carcinoma (PCa). Agents targeting GCPII suitable for both imaging and treatment of PCa are in development and they show promising results in advanced clinical trials. Some studies showed that GCPII may serve also as PCa blood serum marker, but this has not been validated due to the lack of methods suitable for accurate detection of GCPII in human blood.

Moreover, GCPII is also expressed in brain, where it cleaves inhibitory *N*-acetyl- α -L-aspartyl-L-glutamate (NAAG) to release excitatory L-glutamate and GCPII inhibition has been shown to be neuroprotective in animal models of several neuropathies. Tight binding inhibitors of GCPII have been identified by rational design, but all have poor bioavailability and thus cannot be used in clinics. Identifying new scaffolds by 'brute force' screening methods is thus essential; however, no such method for GCPII has been developed so far.

Glutamate carboxypeptidase III (GCPIII) is also expressed in brain and cleaves NAAG. It is thus an important protein for understanding of GCPII function as well as GCPII targeting in medicine.

Here, we focused on development of novel methods for quantification of both enzymes and screening of their inhibitors. First, we developed qRT-PCR and radioenzymatic assays to quantify GCPII and GCPIII in human and mice tissues and proved lack of GCPII in murine prostate and intestine. We also developed several orthogonal assays for detection of GCPII in blood and determined GCPII blood levels in healthy and PCa individuals. Unfortunately, we showed that GCPII is not useful as a serum marker of PCa. Finally, we developed a novel method for enzyme detection (DIANA), which is based on dual recognition of the enzyme by immobilized antibody and DNA-linked inhibitor. We showed on the example of GCPII and CAIX, which is also a putative cancer marker and potential drug target, that this method is useful not only for ultrasensitive enzyme detection but also for screening of enzyme inhibitors without the need to purify the target enzyme. This makes DIANA a superior tool for biomarker detection and drug discovery.