Abstract

Prostate cancer is one of the most common human malignancies and, consequently it is critical to develop appropriate diagnostic and therapeutic tools. Glutamate carboxypeptidase II (GCPII) is currently being considered one of the most important prostate cancer markers due to its tissue-specific expression. Whereas in healthy prostatic tissue the expression levels of GCPII are low, the transformation into the tumor is associated with the substantial increase of GCPII expression, with the highest levels observed in androgen-independent metastatic tumors. GCPII is thus considered a promising marker for early phase as well as advanced metastatic stages of prostate cancer. Current research is focused on the development of highly sensitive and specific reagents that allow detection of small amounts of GCPII, for example in early stages of cancer. Antibody derivatives are promising molecules for this purpose because they have high affinity and specificity and minimum negative side effects. Protein engineering is a prefered approach for preparation of various antibody molecules that differ in size, binding properties, stability, solubility, and production means. Different types of derivatives are being developed for medical needs such as in vitro diagnosis, therapy, and in vivo imagingSmall molecular weight reagents such as single chain fragment (scFv) or diabodies are favored for *in vivo* imaging. This diploma thesis describes production and characterization of several variants of GCPII-specific scFv fragments.

This study is aimed at the production and characterization of scFv fragments derived from the 5D3 monoclonal antibody (mAb) that reveals high affinity and specificity for GCPII. 5D3 mAb was developed in our laboratory. Expression vectors encoding several scFv 5D3 variants were prepared and expression of scFvs was evaluated in several heterologous expression systems including *E. coli*, *K. lactis* and insect S2 cells. Purified scFv fragments were characterized in detail by ELISA, flow cytometry and immunofluorescence microscopy and their characteristics compared to the parent 5D3 mAb. This study thus provided an optimized protocol for production of functional, highly purified scFv fragments 5D3 that could be used for *in vivo* imaging of GCPII-positive tissues in the future.

Key words: prostate cancer, glutamate carboxypeptidase II, antibodies, recombinant antibody fragment, single chain scFv fragment, *in vivo* imaging, diagnostics