Characterization and site-specific bio-functionalization of nanobodies for the early detection of ovarian cancer biomarkers

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Abstract

Epithelial ovarian cancer (EOC), of which the incidence increases with age, ranks fifth in cancer deaths among women[1]. The common symptoms of EOC are indistinct and similar to other benign observations[2]. Most women are diagnosed at advanced stage III or IV of the disease, at which the 5-year relative survival rate is low (around 39% for stage III and only 17% for stage IV)[2,3]. EOC can be successfully treated (with rate 92%) if it is detected early but the diagnosis of EOC at early stages is difficult since there are no symptoms and no screening test has proven to be effective (only 15% of all ovarian cancers are found at the early stage). In this study, different nanobodies[4] targeting EOC markers such as Human epididymis protein 4 (HE4)[2], Secretory leukocyte protease inhibitor (SLPI)[3] and Progranulin (PGRN)[3] were selected based on their expression level as well as their target binding affinity using enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) methods. The binding properties of the selected nanobodies were also determined by epitope mapping. The nanobodies with high expression level (almost 8 mg/ mL, 21 mg/ mL and 11 mg/ mL for HE4, SLPI and PGRN, respectively) and high binding affinity (K_D value around 10^{-10}– 10^{-8} M) were selected as best candidates for the development of multi-array biosensors that are capable of detecting multiple targets rapidly and combine high selectivity, high sensitivity and specificity for the detection of EOC in early stage. The selected nanobodies were successfully site-specifically alkynated at their C-terminus using the Expressed protein ligation (EPL) technique and were coupled to an azidified PEG counterpart using “click”-chemistry[5]. The attachment of a click-chemistry functionality at the C-terminus of the nanobodies would pave the way to sensor platforms at which all nanobodies are covalently coupled with a unique and uniform orientation, allowing optimal target binding and resulting in improved sensitivity and selectivity since all nanobodies will have their active region accessible for the target binding.

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