E.COLI DERIVED CAMELID ANTIBODIES AS A SENSOR FOR P53 IN SALIVA

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Introduction. Oral squamous cell carcinoma (OSCC) is a malignant tumor with 640,000 new cases annually in the world [1]. Saliva testing is non-invasive procedure that is capable to detect potential biomarkers for OSCC. It was shown that elevated level of p53 protein was identified in OSCC patients at different stages of the disease (ibid). Camelid antibodies containing only variable regions, nanobodies (VHH) and single-chain variable regions (scFv) with VH and VL, are becoming popular in many biological studies including diagnostic applications. It was identified that VL region alone showed higher affinity to p53 than VHH, and dimerization of VL region with another one increases the affinity up to 10 folds [2]. Camelid antibodies have similar affinity to its substrate as human antibodies and can be conjugated to other proteins without functional lose. They can be expressed and secreted in many organisms including *E.Coli* in high amount, which reduces the cost of antibodies production. Thus, the aim of this project is to design a biosensor, based on available sequence of antibodies, to detect p53 in saliva samples for OSCC diagnosis.

Materials and methods. We transformed *E. coli Dh5* alpha with pVDL9.3 vector encoding HlyB and HlyD genes and then with with pUC57 vector encoding Vhhp53 gene conjugated to leucine zipper, red fluorescent protein and alpha-hemolysin (HlyA). Then we induced the expression of the protein with 1uM IPTG and collected both lysate of the cells and the medium to check the photometric intensity of protein expression with spectrophotometer. We purified proteins with Ni/NTA columns and checked the size on SDS PAGE gel. Furthermore we mausered the concentration of the purified proteins with nanodrop ND8000.

Results and discussion. We obtained a single band of the expected size 71 kDa which accounts for the size of single chain Vhhp53 antibody. We did not observe the band for dimerized protein, but we are planning to check whether the dimerization occurs or not on native SDS PAGE in the future.

Conclusions. Vhp53 nanobody was purified from both medium and bacterial lysate. We plan to test the affinity of this nanobody for cancer biomarker p53. In future, this protein-nanobody interaction can be used to design a biosensor, based on available sequence of antibodies, in particular, to detect p53 in saliva samples for OSCC diagnosis

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