Summary:

Designing a high affinity binder to viral protein E6

By: Nakash Shetty

Cervical cancer is the second largest cause of cancer related deaths in woman. It can be described as the malignant cell growth in the area of lower uterus. The leading cause of cervical cancer is the infection by Human Papilloma Virus (HPV). HPV type 16, 18, 31 are the high risk variants of HPV, these strains are responsible for more than 90 % cases of cervical cancer. We shall concentrate on the type HPV 16 for the purpose of the project. Gene products from oncogenes E6, E7 interact with tumour suppressors and keep the cells in the stage suitable for virus production. E6 is known to interact with the synapse associated protein SAP 97, which is a tumour suppressor.

The E6 protein is 150 amino acids long. The HPV 16 E6 has 2 zinc binding motifs one in N terminal and the other in C terminal. SAP 97 consists of 3 PDZ domains, a SH3 domain and one guanylate kinase like domain. The C terminal tail of E6 interacts with the PDZ 2 domain of SAP 97. The viral protein E6 in association with E6AP brings about degradation of SAP97 with the 26 S proteasome.

A possible cure for cervical cancer would be to obtain a binder to the E6 which would bind to the E6 stronger than wild type PDZ domain. A mutant PDZ 2 was obtained from PDZ 2 domain of SAP 97 by phage display method. The mutant PDZ 2 protein was expressed in *E coli* BL 21 cells. It was purified using immobilized metal affinity chromatography and ion exchange chromatography. The identity of the protein was confirmed with mass spectroscopy. HPV 16 E6 was also expressed and purified for the purpose of binding studies. Stopped flow spectrophotometer and spectrofluorimeter were utilized to check the binding between mutant PDZ and HPV 16 E6. There was no binding seen between the mutant PDZ and the E6.