Construction of a HPV-16 fusion protein for the detection of HPV
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Background: Human papillomavirus (HPV) infection has been associated with a subset of oral cancer and is believed to be one of the causal agents of oral cancer. However, the ability to detect the presence of HPV in oral cancer easily and reliably has been a major challenge in determining the true involvement of this virus in oral carcinogenesis. Currently there is a lack of serological assays for the detection of the HPV. The HPV E6 viral oncoprotein is known to play crucial role in tumorigenesis, therefore it could be a good biomarker for HPV infection. In this study, we constructed a HPV 16 E6 fusion protein which would be used for the detection of HPV using an Enzyme-linked immunosorbent assay (ELISA) assay.

Materials and methods: We modified the pGEX plasmid containing glutathione-s-transferase (GST) and HPV 16 E6 gene, (from Peter Howley, The President and Fellows of Harvard College) to contain a detection sequence (KT3 Tag; amino acid sequence KPPTPPPEPET). This constructed plasmid was transformed into Escherichia coli strain BL 21 for the production of the fusion protein. The fusion protein was harvested through centrifugation and freeze-thaw cell lysis and confirmed by western blot analysis.

Result: We successfully constructed the pGEX HPV 16 E6-Tag plasmid and the correct sequence was validated through sequencing. The fusion protein was overexpressed in E. coli as shown by western blot.

Conclusion: This production of the E6 protein KT3 tag facilitates easy detection to ensure that the right protein has been produced. In addition, the presence of the GST allows for its direct use in the ELISA without further extensive purification and renaturation of the proteins. The fusion protein produced in this study will be ready for use in the ELISA assay to detect HPV from serum samples once the optimization stage is completed.
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