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Immunogenicity of B-cell epitope predicted peptide of the Fap2 protein of the *Fusobacterium nucleatum* and its potential use in the detection of colorectal cancer

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recent studies in the development of colorectal cancer, the third most common cancer in the world, revealed the role of KFusobacterium nucleatum, particularly the Fap2 protein, in the immune evasion of tumor cells. Fap2 is an outer membrane protein which has the ability to bind to the tumor-expressed Gal-GalNAc causing the inhibition of the binding of the T-cell immunoreceptor with Ig and ITIM domains (TIGIT) of the human natural killer cells. This inhibition of TIGIT binding to colorectal tumor cells allows the continuous growth and proliferation of tumor cells. With this role of the Fap2 protein in the development and progression of colorectal cancer and in the absence of reported immunogenic epitopes of Fap2 protein of Fusobacterium nucleatum, our group aimed to search for immunogenic epitopes of the Fap2 protein and determine the presence of the anti-Fap2 immunogenic epitope antibodies in colorectal cancer (CRC) patients' sera which may potentially be used in developing a diagnostic method or early detection for CRC. Selection of candidate immunogenic epitope was done using the B-cell epitope prediction function of the Immune Epitope Database (IEDB). IEDB-predicted peptide was synthesized by Genscript Corporation (New York, USA) and was used as mimotope for immunization and antibody detection. Peptideconjugated to bovine serum albumin and emulsified with Freund's complete adjuvant were used in the immunization of white New Zealand rabbits. Four booster immunizations using the BSA-conjugated peptide emulsified with Freund's incomplete adjuvant were also given at 14 days interval. Immunogenicity of the peptide was assessed by determining the development of the anti-peptide antibody in the rabbit sera by indirect enzyme-linked immunosorbent assay (ELISA). Reactivity of sera from CRC patients (n=37) and case-matched control was also determined by indirect ELISA. Production of antibody against the peptide was observed in rabbits. This indicates that the IEDB B-cell epitope prediction predicted immunogenic epitope of the Fap2 protein of Fusobacterium nucleatum is immunogenic. It was also observed that 100% the sera of CRC patients showed reactivity to the peptide while only 32% among the case-matched controls were reactive. Fischer's exact probability test revealed association of the presence of the anti-Fap2 immunogenic epitope antibodies to colorectal cancer (p-value=<0.001). This observation shows the potential of anti-Fap2 immunogenic epitope antibodies as a biomarker for the diagnosis of colorectal cancer.

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