

In silico-guided sequence modifications of K-ras epitopes improve immunological outcome against G12V and G13D mutant KRAS antigens

ABSTRACT

Background: Somatic point substitution mutations in the KRAS proto-oncogene primarily affect codons 12/13 where glycine is converted into other amino acids, and are highly prevalent in pancreatic, colorectal, and non-small cell lung cancers. These cohorts are non-responsive to anti-EGFR treatments, and are left with non-specific chemotherapy regimens as their sole treatment options. In the past, the development of peptide vaccines for cancer treatment was reported to have poor AT properties when inducing immune responses. Utilization of bioinformatics tools have since become an interesting approach in improving the design of peptide vaccines based on T- and B-cell epitope predictions. Methods: In this study, the region spanning exon 2 from the 4th to 18th codon within the peptide sequence of wtKRAS was chosen for sequence manipulation. Mutated G12V and G13D K-ras controls were generated in silico, along with additional single amino acid substitutions flanking the original codon 12/13 mutations. IEDB was used for assessing human and mouse MHC class I/II epitope predictions, as well as linear B-cell epitopes predictions, while RNA secondary structure prediction was performed via CENTROIDFOLD. A scoring and ranking system was established in order to shortlist top mimotopes whereby normalized and reducing weighted scores were assigned to peptide sequences based on seven immunological parameters. Among the top 20 ranked peptide sequences, peptides of three mimotopes were synthesized and subjected to in vitro and in vivo immunoassays. Mice PBMCs were treated in vitro and subjected to cytokine assessment using CBA assay. Thereafter, mice were immunized and sera were subjected to IgG-based ELISA. Results: In silico immunogenicity prediction using IEDB tools shortlisted one G12V mimotope (68-V) and two G13D mimotopes (164-D, 224-D) from a total of 1,680 candidates. Shortlisted mimotopes were predicted to promote high MHC-II and -I affinities with optimized B-cell epitopes. CBA assay indicated that: 224-D induced secretions of IL-4, IL-5, IL-10, IL-12p70, and IL-21; 164-D triggered IL-10 and TNF- α ; while 68-V showed no immunological responses. Specific-IgG sera titers against mutated K-ras antigens from 164-D immunized Balb/c mice were also elevated post first and second boosters compared to wild-type and G12/G13 controls. Discussion: In silico-guided predictions of mutated K-ras T- and B-cell epitopes were successful in identifying two immunogens with high predictive scores, Th-bias cytokine induction and IgG-specific stimulation. Developments of such immunogens are potentially useful for future immunotherapeutic and diagnostic applications against KRAS(+) malignancies, monoclonal antibody production, and various other research and development initiatives.

Keyword: Mimotope; KRAS; In silico prediction; Immunogen; IEDB; Peptide vaccine