Development of a single-chain fragment variable fused-mutant HALT-1 recombinant immunotoxin against G12V mutated KRAS colorectal cancer cells

ABSTRACT

Background: KRAS oncogenes harboring codon G12 and G13 substitutions are considered gatekeeper mutations which drive oncogenesis in many cancers. To date, there are still no target-specific vaccines or drugs available against this genotype, thus reinforcing the need towards the development of targeted therapies such as immunotoxins. Methods: This study aims to develop a recombinant anti-mKRAS scFv-fused mutant Hydra actinoporin-like-toxin-1 (mHALT-1) immunotoxin that is capable of recognizing and eradicating codon-12 mutated k-ras antigen abnormal cells. One G13D peptide mimotope (164-D) and one G12V peptide mimotope (68-V) were designed to elicit antigen specific IgG titres against mutated K-ras antigens in immunised Balb/c mice. The RNA was extracted from splenocytes following ELISA confirmation on post-immunized mice sera and was reverse transcribed into cDNA. The scFv combinatorial library was constructed from cDNA repertoire of variable regions of heavy chain (VH) and light chain (VL) fusions connected by a flexible glycine-serine linker, using splicing by overlap extension PCR (SOE-PCR). Anti-mKRAS G12V and G13D scFvs were cloned in pCANTAB5E phagemid and superinfected with helper phage. After few rounds of bio-panning, a specific mKRAS G12V and G13D scFv antibody against G12V and G13D control mimotope was identified and confirmed using ELISA without any cross-reactivity with other mimotopes or controls. Subsequently, the anti-mKRAS scFv was fused to mHALT-1 using SOE-PCR and cloned in pET22b vector. Expressed recombinant immunotoxins were analyzed for their effects on cell proliferation by the MTT assay and targeted specificity by cell-based ELISA on KRAS-positive and KRAS-negative cancer cells. Results: The VH and VL genes from spleen RNA of mice immunized with 164-D and 68-V were amplified and randomly linked together, using SOE-PCR producing band sizes about 750 bp. Anti-mKRAS G12V and G13D scFvs were constructed in phagemid pCANTAB5E vectors with a library containing 3.4 × 106 and 2.9 × 106 individual clones, respectively. After three rounds of bio-panning, the anti-mKRAS G12V-34 scFv antibody against G12V control mimotope was identified and confirmed without any cross-reactivity with other controls using ELISA. Anti-mKRAS G12V-34 scFv fragment was fused to mHALT-1 toxin and cloned in pET22b vector with expression as inclusion bodies in E. coli BL21(DE3) (molecular weight of ~46.8 kDa). After successful solubilization and refolding, the mHALT-1-scFv immunotoxin exhibited cytotoxic effects on SW-480 colorectal cancer cells with IC50 of 25.39 μg/mL, with minimal cytotoxicity effect on NHDF cells. Discussion: These results suggested that the development of such immunotoxins is potentially useful as an immunotherapeutic application against KRAS-positive malignancies.

Keyword: Cytotoxic; G12V; HALT-1; Hydra; Immunotoxin; KRAS; Phage display; Phagemid; Scfv