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Site-Directed Mutagenesis for the Production of Mutant *TP53* Gene and Analysis of Its Tumor Suppressor Activity

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Abstract The p53 therapy based approach is a good candidate for treatment of *TP53*-defect cancer. However, this treatment is unsuitable for some cancer cases especially for that caused by dominant-negative activity of the mutant p53 protein.Thus, more effective treatment is needed to overcome this problem and DNA vaccination may be the suitable candidate where mutant p53 may become a suitable candidate as an antigen for the DNA vaccination strategy. Therefore, the research aims to produce mutant *TP53R248Q* through PCR site–directed mutagenesis and to confirm its tumor suppression ability. The mutation of R248Q was generated via Polymerase Chain Reaction (PCR) site-directed mutagenesis to pCMVp53 plasmid. Following that, these constructed *TP53R248Q* was transfected into the *TP53*-null H1299 cell lines in order to investigate its tumor suppression ability, by studying its phenotype and genotype expression. Phenotype study was conducted by using colony formation assay while quantification of *TP53* downstream target gene, $p21^{waf1}$ was conducted for the genotype study. The transfection with exogenous *TP53R248Q* resulted in massive colony proliferation and downregulation of $p21^{waf1}$, thus confirmed that the generated mutant *TP53* has lost its ability to restrain cells growth. These data therefore confirmed that the PCR site-directed mutagenesis technique has been successfully carried out to induce the desired mutation in the *TP53* gene. Thus, this technique may become an interesting option to generate novel recombinant proteins, especially in the development of specifically designed DNA vaccine as a gene therapy in the future.

Keywords: dominant-negative activity, p53, PCR site-directed mutagenesis