UNIVERSITI TEKNOLOGI MARA

AMPLIFICATION OF HUMAN *VKOR* GENE USING PCR FOR USE IN CLONING & EXPRESSION

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ABSTRACT

Mutations of human VKOR gene leading to warfarin resistance in certain patients and polymorphism which is associated with a significantly higher risk of aortic calcification in certain patients have been identified. By identification of VKOR gene defects, prevention or treatment inititative such as gene therapy may be initiated for affected patients. PCR is a technique to amplify specific DNA sequences in vitro. PCR is able to rapidly amplify a specific region of a single DNA molecule to yield sufficient quantities that can be cloned, sequenced, or analysed by sequences mapping . The aim of this study is to amplify human VKOR gene using PCR for use in cloning and expression. In this study, specific primers flanking the complete VKOR coding region was designed. The specificity of the primers were evaluated using Oligo Explorer 1.2 Software. The PCR was then performed to detect the VKOR gene. The PCR conditions were optimized by varying the PCR temperature and time. In order to prove the target PCR products were obtained after amplification, the PCR products were separated by gel electrophoresis and visualized under UV transilluminator. The band of interest which was 492 base pairs in size were obtained after human VKOR gene amplification was performed. The amplicon can then be used for cloning and expression of VKOR enzyme. However, more studies need to be performed for more specific amplification without non-specific co-amplification.

CHAPTER 1

INTRODUCTION

Vitamin K is essential for blood clotting but must be enzymatically activated. This enzymatically activated form of vitamin K is a reduced form required for the carboxylation of glutamic acid residues in some blood-clotting proteins. The product of this gene encodes the enzyme which is vitamin K epoxide reductase that is responsible for reducing vitamin K 2,3-epoxide to the enzymatically activated form. (Tie *et. al.*, 2008). Fatal bleeding can be caused by vitamin K deficiency and by the vitamin K antagonist warfarin, and it is the product of this gene that is sensitive to warfarin.

In humans, mutations in VKOR gene can be associated with deficiencies in vitamin-K-dependent clotting factors and, in humans and rats, with warfarin resistance. Two pseudogenes have been identified on chromosome 1 and the X chromosome. Two alternatively spliced transcripts encoding different isoforms have been described. Mutations such as human VKOR gene defects are related to warfarin resistance in certain patient. There was also research evidence stated that polymorphism in VKOR gene is associated with a significantly higher risk of aortic calcification in white people (Teichert *et. al.*, 2008). By identification of VKOR gene defects, prevention or treatment inititative such as gene therapy may be initiated for affected patients.