

Dasar pengembangan vaksin kontrasepsi pria kloning dan ekspresi protein rekombinan hVDAC3 (human voltage dependent anion channel 3) untuk produksi antibodi poliklonal = A Basis for development of male contraceptive vaccine cloning and expression of hVDAC3 human voltage dependent anion channel 3 recombinant protein to produce its polyclonal antibodies

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Abstrak

ABSTRAK

Pendahuluan. VDAC merupakan protein kanal ion yang bertanggung jawab atas aliran ion Ca²⁺ dan ATP dalam flagela spermatozoa. Defisiensi gen VDAC3 pada mencit dan mutasi gen VDAC3 pada manusia menyebabkan penurunan motilitas spermatozoa, sehingga VDAC3 dapat dijadikan antigen potensial untuk pengembangan vaksin kontrasepsi laki-laki. Tujuan penelitian ini adalah memproduksi protein rekombinan hVDAC3 dari gen hVDAC3 ekson 5-8 spesifik spermatozoa, dan digunakan sebagai antigen untuk produksi antibodi poliklonal pada kelinci.

Metode. Gen hVDAC3 ekson 5-8 spermatozoa diperoleh melalui RT PCR, gen disisipkan ke plasmid pET100/D-TOPO dan diklonasi dalam E coli TOP 10. Analisis gen sisipan dengan PCR, enzim restriksi dan sekuensing DNA. Protein rekombinan hVDAC3 diekspresikan dalam E coli BL21 StarTM (DE3).

Karakterisasi protein dilakukan dengan uji Bradford, SDS PAGE, western blot dan purifikasi protein dengan resin Ni-NTA. Antibodi poliklonal diperoleh dengan cara imunisasi protein rekombinan hVDAC3 ke kelinci dan diukur dengan indirect ELISA. Determinasi lokasi hVDAC3 di spermatozoa dengan metode immunofluorescence.

Hasil. Amplifikasi PCR gen hVDAC3 ekson 5-8 berukuran 435 pb dan analisis BLAST menunjukkan 100% identik dengan gen VDAC3 manusia dari bank gen. Vektor rekombinan berukuran 6195 pb mengekspresikan protein rekombinan hVDAC3 berukuran 20 kDa. Antibodi poliklonal telah diproduksi kelinci secara bermakna (p<0.05) dengan titer 2.817, dan antibodi dapat berikatan dengan protein hVDAC3 di kepala dan flagela spermatozoa. Selanjutnya, antibodi poliklonal ini akan digunakan dalam pengembangan vaksin kontrasepsi pada laki-laki.

<i>ABSTRACT</i>

Introduction. Voltage dependent anion channels (VDAC), also known as mitochondrial porins, are group of proteins in mitochondrial outer membrane that allow the passage of metabolites across the mitochondrial outer membrane, and are involved in ions and ATP transport in sperm flagella. Deficiency and mutation of VDAC3 may cause abnormality in structure and motility of human spermatozoa. VDAC3 could be a potential target to develop non hormonal male contraceptive vaccine. The objective of the study was to produce hVDAC3 recombinant proteins from exon 5 to 8 of human sperm VDAC3 specific gene.

This recombinant protein was subsequently used as an antigen to produce polyclonal antibodies in rabbits.

Methods. hVDAC3 sperm gene obtained by RT PCR, this gene was inserted into plasmid pET 100/D-TOPO and cloned in E coli TOP 10. The gene was analyzed by PCR method, restriction enzymes and DNA sequencing. The proteins expressed in E coli BL21 StarTM (DE3). Characterization of proteins was evaluated by Bradford method, SDS PAGE and western blot. The recombinant protein was purified with NTA resin. Polyclonal antibodies were obtained by immunization of hVDAC3 recombinant protein into rabbits. Indirect ELISA was done to analyze the antibody. Localization of the VDAC3 recombinant protein in human spermatozoa was evaluated by immunofluorescence method.

Result. By doing PCR amplification and BLAST analysis, the study showed that the hVDAC3 gene had 100% identical to hVDAC3 genes in data bank. E coli BL21 StarTM (DE3) containing recombinant vector (6195 bp) expressed the recombinant protein of hVDAC3 in 20 kDa. This protein produced polyclonal antibodies that bound VDAC3 protein on the head and flagella of human spermatozoa.