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Construction and DNA immunization of human renalase eukaryotic expression vector

Sir,

Renalase is a monoamine oxidase mainly synthesized in the kidney that could reduce blood pressure and regulate cardiovascular functions by directly degrading catecholamine [1]. In order to prepare the antibody, we constructed a eukaryotic expression vector of human renalase and inoculated mice with it as a DNA vaccine.

After cleavage by restriction of endonuclease NotI or BglII polymerase chain reaction products of renalase, genes were inserted into the pBudCE4.1 vector. Constructed vectors were transformed into *Escherichia coli* (*E. coli*) DH5α, followed by amplification, purification and sequencing of the constructed plasmid. Meanwhile, the target band was identified after double digestion of a small extraction of plasmid by NotI and BglII, confirming the construction of a DNA vaccine vector. Then, the plasmids were transfected into 293T cells to ensure the expression of the constructed DNA vaccine. As a result, renalase was expressed in 293T cells. Next, the DNA vaccine was injected into BALB/c mice. Ten days following the third injection, orbital blood samples were collected. Serum from mice inoculated with the DNA vaccine was used as a primary antibody for western blot analysis. Finally, prokaryotically expressed renalase was identified, indicating the recognition of prokaryotically expressed renalase protein by serum from the DNA vaccine-inoculated mice.

Renalase is a recently discovered flavin adenine dinucleotide-dependent amine oxidase secreted by the kidney, which degrades circulating catecholamines and regulates blood pressure and cardiovascular function [2] and may be one of the basic substances of blood pressure regulation [3, 4]. A deficiency of renalase may help to explain the elevated catecholamine level, refractory hypertension and cardiovascular complications in chronic kidney disease patients. However, the recombinant renalase protein was instable. We designed renalase DNA vaccine successfully, which may pave the way for further study, such as antibody preparation and the function of renalase.

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Conflict of interest statement. None declared.

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