World Journal of Gastroenterology

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DOI: 10.3748/wjg.v6.iSuppl3.117

World J Gastroenterol 2000 September 15; 6(Suppl 3): 117
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Construction, expression and characterization of double-copy genes of truncated form of human insulin-like growth factor-Ⅰ

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Author contributions: All authors contributed equally to the work.

Supported by the grants from Science Foundation of Shanxi No. 951029 and Reback Science Foundation No. 94124

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Received: February 13, 2000
Revised: April 5, 2000
Accepted: May 10, 2000
Published online: September 15, 2000

Abstract

AIM: To increase the production of recombinant des (1-3) IGF-Ⅰ by increasing the copy number of gene carried on an expression vector, and to partially purify the expressed des (1-3) IGF-Ⅰ, as well as compare its bio-activity with standard IGF-Ⅰ.

METHODS: Second copy of des (1-3) IGF-Ⅰ gene was inserted into pExSec1/IGF-Ⅰ expression vector constructed by our previous work and carryed already one des (1-3) IGF-Ⅰ gene, to form PExSec1/2 (IGF-Ⅰ) expression plasmid, which carried two copies of tandem des (1-3) IGF-Ⅰ gene. This plasmid was transformed into a protease deficient *E. coli* strain BL21 (DE_3). The engineered bacteria was cultured and induced at low temperature. The expressed product was purified through ultra-filtration and gel-filtration. The bio-activity of partially purified protein was tested by MTT method and compared with standard IGF-Ⅰ.

RESULTS: The amount of des (1-3) IGF-Ⅰ expressed by pExSec1/2 (IGF-Ⅰ) reached up to 19%-22% of the total soluble bacterial protein, which is about 7% higher than that of des (1-3) IGF-Ⅰ expressed by pExSec1/IGF-Ⅰ. The purity of recombinant des (1-3) IGF-Ⅰ reached 49% and 82% respectively after the treatments by ultra filtration and gel-filtration. The result of MTT assay showed that the bio-activity of des (1-3) IGF-Ⅰ after gel-filtration was about 77% of that of standard IGF-Ⅰ at the same concentration.

CONCLUSION: The yield of recombinant des (1-3) IGF-Ⅰ was increased about 7% by construction of expression plasmid with two copies of des (1-3) IGF-Ⅰ gene, compared with only one copy of gene, preliminarily purified des (1-3) IGF-Ⅰ showed relatively high biological activity, which was about 77% of that of standard IGF-Ⅰ.

Key words: Insulin-like growth factor Ⅰ; Gene expression; Ultrafiltration; Chromatography, gel

Sun HY, Liu XH, Liang SW. Construction, expression and characterization of double-copy genes of truncated form of human insulin-like growth factor-Ⅰ. *World J Gastroenterology* 2000; 6(Suppl 3): 117 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/iSuppl3/117.htm DOI: http://dx.doi.org/10.3748/wjg.v3.iSuppl3.117

E- Editor: Hu S

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