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INSERTION OF HUMAN INTERLEUKIN-15 INTO pJET VECTOR FOR ANTI-CANCER VACCINE DEVELOPMENT

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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan projek yang bertajuk "INSERTION OF HUMAN INTERLEUKIN-15 INTO pJET VECTOR FOR ANTI-CANCER VACCINE DEVELOPMENT" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh NG HUAY SHIN sebagai memenuhi syarat untuk kursus BMY 4999.

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ABSTRACT

Cancer is claimed to be the first killer for human beings nowadays. Tumours have high capability to escape from host immune responses, thus currently there are still no effective immunotherapeutic strategies that can be used to cure cancer. Today, cytokines are highly involving in cancer immunotherapy. The interleukin-15 (IL-15) possess several properties in cancer treatment including the regulation of natural killer (NK) cells activities and the triggering and stimulation of B- and T-lymphocytes proliferation. IL-15 appears to be a better option for treating cancer as it is less toxic when compared to IL-2. In order to develop an effective vaccine against cancer, the human IL-15 (hIL-15) gene was cloned as a cDNA and inserted into the pJET cloning vector. The recombinant plasmid was transformed into chemically competent *Escherichia coli* TOP10 cells and was analyzed by agarose gel electrophoresis. The restriction enzyme used for digestion of this recombinant plasmid was the *Nhe*I to confirm the presence of hIL-15 gene in the pJET vector. The hIL-15 gene was proven to be inserted into the pJET vector after digestion with *Nhe*I restriction enzyme which showed an insert size of 0.5 kb. The recombinant plasmid was sent for sequencing to determine the nucleotide sequence of the inserted gene. BLAST was performed and the result confirmed the 100% similarity of the sequence homology to the published hIL-15 gene. The recombinant plasmid could be used for further analysis for the development of anti-cancer vaccine.

ABSTRAK

Kanser ialah pembunuh pertama untuk manusia pada masa kini. Tumor mempunyai keupayaan tinggi untuk mengelakkan diri daripada sistem imun badan manusia, dengan itu sekarang masih kekurangan strategi rawatan yang berkesan untuk melawan kanser. Pada hari ini, sitokin memang memainkan peranan yang penting dalam rawatan kanser. Interleukin-15 (IL-15) mempunyai ciri-ciri untuk merawat kanser termasuk pengawalan aktiviti sel-sel pembunuh semulajadi (NK) dan sebagai pencetus dalam pertumbuhan dan percambahan limfosit B dan T. IL-15 boleh menjadi pilihan yang lebih baik untuk melawan kanser jika berbanding dengan IL-2 sebab kekurangan tosiknya. Gen IL-15 manusia telah diklonkan sebagai cDNA dan dimasukkan ke dalam vektor pJET dalam usaha untuk mencipta vaksin yang berkesan terhadap kanser. Plasmid rekombinan telah ditransformasikan ke dalam sel kompeten kimia *Escherichia coli* TOP10 dan dianalisis dengan elektroforesis gel agarosa. Enzim penyekat yang digunakan untuk pemotongan plasmid rekombinan ini adalah *Nhe*I untuk mengesahkan kewujudan gen IL-15 manusia dalam vektor pJET itu. Gen IL-15 manusia telah dipastikan berjaya diklonkan ke dalam vektor pJET selepas ditindak oleh enzim penyekat *Nhe*I, dengan menunjukkan saiz 0.5 kb. Plasmid rekombinan telah dihantar untuk penjujukan bagi menentukan turutan nukleotida gen yang telah diklonkan. BLAST telah dijalankan dan keputusannya telah memaparkan persamaan homologi sebanyak 100% dengan gen IL-15 manusia yang diterbitkan. Plasmid rekombinasi boleh digunakan untuk analisasi selanjutnya bagi penciptaan vaksin anti-kanser.

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TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

α	Alpha
β	Beta
℃	Degree Celsius
γ	Gamma
µg	Microgram
µL	Microliter
µm	Micrometer
BLAST	Basic Local Alignment Search Tool
bp	Base pair
$CaCl_2$	Calcium chloride
cDNA	Complementary deoxyribonucleic acid
cds	Coding sequence
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
K562	Human erythromyeloblastoid leukemia cell line
kb	Kilobase
M	Molar
mg	Milligram
$MgCl_2$	Magnesium chloride

mL	Milliliter
mM	Millimolar
mRNA	Messenger RNA
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
NEB	New England Biolabs
ng	Nanogram
NH_4	Ammonium
nm	Nanometer
OD	Optical density
RNA	Ribonucleic acid
RNase A	Ribonuclease A
rpm	Revolutions per minute
SDS	Sodium dodecyl sulfate
TM	Trademark
Tris-Cl	Tris hydrochloride
U	Unit
USA	United States of America
UV	Ultraviolet
w/v	Weight/volume

CHAPTER 1

INTRODUCTION

Cancer is claimed to be the main reason of mortality and morbidity over the world today. According to Salminen *et al.* (2005), the worldwide incidence of cancer is expected to grow, with 15 million new cases and 12 million mortality cases forecasted in 2020. Viruses had been introduced in 1920s to be used as oncolytic agents (Sinkovics and Horvath, 1993). By performing oncolytic activity, Newcastle disease virus (NDV) was claimed as a uniquely promising agent for cancer virotherapy (Schirrmacher and Fournier, 2009). However, the effectiveness of NDV in killing the cancer cells is still hampered by the host immune response.

Interleukin-15 (IL-15) has been discovered in 1994 (Burton *et al.*, 1994). It is sharing many biological activities with IL-2 (Bamford *et al.*, 1994). Although interleukin that is currently being tested to enhance the effectiveness of NDV is IL-2, but IL-15 is said to be less toxic if compared to IL-2 due to the capability of IL-15 to trigger the T lymphocytes and natural killer (NK) cells, to block the activation-induced cell death (AICD) and also to prolong the CD8 memory T lymphocytes (Zhang *et al.*, 2009). According to Niu *et al.* (2014), IL-15 was proved to suppress the tumour recurrence and metastasis by enhancing the cytotoxic T lymphocytes and NK cells activities where these two cells are actively involving in the roles of fighting cancer.

1.1 HYPOTHESIS

Successful subcloning of hIL-15 gene together with appropriate restriction enzyme site into a pJET cloning vector can be achieved by using T4 DNA Ligase and this will allow the gene to further cloned into the M-F intergenic region towards the development of Newcastle disease virus cancer vaccine.

1.2 OBJECTIVE

The main objective of this study was to clone the hIL-15 gene into the pJET cloning vector which can be further developed as an anti-cancer vaccine.

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