MOLECULAR CLONING AND HOMOLOGY MODELLING OF HUMAN CYCLIN DEPENDENT KINASE 3 (CDK3)

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A dissertation submitted in partial fulfilment of the
requirements for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
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DECEMBER 2017

To my family

ACKNOWLEDGEMENT

Praise be to ALLAH for HIS merciful and gracious. For the hands that reached out for me in the time of need, I sincerely in debt on this.

I gratefully acknowledge the support and guidance from my supervisor, Dr HARYATI BINTI JAMALUDDIN. Without her endless guidance, advices, thoughtful encouragement and careful supervisions, this thesis would never have taken shape.

I also gratefully acknowledge the guidance and help from NURUL FARAHANA. She taught me great laboratory skills and assisted me throughout my thesis. May Allah bless her and grant her success in this life and in the hereafter.

ABDULRAHMAN NABIL M.SH. SHAWISH

19 DECEMBER 2017

ABSTRACT

Cancer comprises of a set of over 100 diseases that each developed in time while involving the unrestrained division of body cells. It disrupts the cell cycle in which it forces the cells to propagate irrepressibly. Cyclin Dependent Kinases (CDKs), a group of over more than 20 members are referred to as “master regulators” of progression of cell cycle, molecular engines that activate cell cycle transitions. CDK3, a member of this family, is an important component of cell cycle regulation and is also a part of the cell cycle transitions of G0 G1 and G1 S stages. Over-expression of CDK3 in many cancer cell lines, indicated that it may have an important role in malignant transformation and cell proliferation. The human CDK3 gene codes for a 307-amino acid protein. In this study, the gene coding for CDK3 was amplified from MCF-7 bBreast cancer cell line cDNA and cloned into cloning plasmid pGEM T-easy. The amplified gene has a size of 915 base pairs. The sequence was verified by Sanger sequencing and pairwise sequence alignment with Uniprot reference sequence (Q00526). It was then cloned into pGEM®-T EASY cloning vector by direct ligation of PCR product with linearized 3’-A overhangs. The three-dimensional (3D) structure of Cyclin Dependent Kinase 3 (CDK3) was modelled based on the crystal structure of Cyclin Dependent Kinase 2 (CDK2). Prediction of 3-D structures of CDK3 is a stepping stone towards further analysis of its function and potential. It shed light on various possible actions of this enzyme based on the predicted folding of the model, especially in terms of binding affinities, paving the path for further favorable modifications. This study clearly predicted that the conserved catalytic residues and domains characteristic which provides a preliminary insight into the specific function of this enzyme. Further experimental studies are required to characterize and analyze the actual functional capabilities of this enzyme.

ABSTRAK

Kanser terdiri daripada lebih 100 penyakit yang masing-masingnya berkembang pada masa yang melibatkan sel-sel badan tidak terkawal. Ia mengganggu kitaran sel yang mana ia memaksa sel-sel untuk menyebarkan secara tidak teratur. Cyclin Dependent Kinases (CDKs), merupakan sekumpulan 20 lebih ahli yang dirujuk sebagai "pengawal selia induk" bagi perkembangan kitaran sel, enjin molekul yang mengaktifkan peralihan kitaran sel. CDK3, ahli keluarga ini, merupakan komponen penting dalam peraturan kitaran sel dan juga merupakan sebahagian daripada peralihan kitaran G0-G1 dan G1-S. Lebih ekspresi bagi CDK3 dalam banyak bar sel kanser, telah menunjukkan bahawa ia mungkin mempunyai peranan penting dalam transformasi ganas dan percambahan sel. Kod CDK3 gen manusia adalah untuk protein asid amino-307. Dalam kajian ini, pengekodan gen untuk CDK3 berjaya diamplifikasi dari MCF-7 bar sel kanser payudara cDNA dan diklonkan ke dalam plasmid pengklonan mudah-pGEM T. Gen yang diperkuat mempunyai saiz 915 pasangan asas. Urutan ini disahkan oleh penyelarasan urutan Sanger dan penjajaran urutan berpasangan dengan urutan rujukan Uniprot (Q00526). Ia kemudiannya diklonkan ke dalam vektor pengklonan pGEM®-T EASY dengan ligation langsung produk PCR dengan keterlebihan 3'-A yang dilinearisasi. Struktur tiga dimensi (Cyclin Dependent Kinase 3) (CDK3) dimodelkan berdasarkan struktur kristal Cyclin Dependent Kinase 2 (CDK2). Ramalan struktur 3-D CDK3 adalah batu loncatan ke arah analisis selanjutnya terhadap fungsi dan potensinya. Ia memberi penerangan tentang pelbagai kemungkinan enzim ini berdasarkan model liputan yang dijangkakan, terutamanya dari segi pertalian yang kukuh dan membuka laluan untuk pengubahsuaian yang lebih baik. Kajian ini dengan jelas meramalkan bahawa ciri-ciri residu dan dominan pemangkin yang dipelihara yang memberikan persepsi awal ke dalam fungsi spesifik enzim ini. Kajian eksperimen selanjutnya diperlukan untuk mencirikan dan menganalisis keupayaan fungsi sebenar enzim ini.

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LIST OF ABBREVIATIONS

µL	-	Microliter
ASR	-	Age-standardised rate
ATF1	-	Activating Transcription Factor 1
ATP	-	Adenosine triphosphate
BFF	-	Basic Fibroblast Factor
BRCA1	-	Breast cancer Gene 1
CAKs	-	CDK activating kinases
CCDS	-	Consensus Coding Sequence
CDK	-	Cyclin-Dependent Kinase
CDK2	-	Cyclin Dependent Kinase 2
CDK3	-	Cyclin Dependent Kinase 3
CIP	-	Cyclin-dependent kinase inhibitor proteins
CKI	-	Cyclin-Dependent Kinase Inhibitors
CTD	-	Carboxy-Terminal Domain
dH_2O	-	Distilled water
Dh5α	-	Escherichia coli bacterial strain
DMSO	-	Dimethyl sulfoxide
DNA	-	Deoxyribonucleic acid
dNTPs	-	Nucleoside triphosphate
ERα	-	Estrogen receptor α
INK	-	Inhibitor of Cyclin-Dependent Kinase
IPTG	-	isopropyl 3-D-thiogalacto-pyranoside
kDa	-	Kilodalton
KIP	-	Kinase Inhibitor p27
LB	-	Luria-bertani
MCF 7	-	Michigan Cancer Foundation-7 Breast cancer cells

miR-873	-	microRNA-873
mL	-	Milliliter
NCR	-	The National Cancer Registry
ng	-	Nanogram
NPC	-	Nasopharyngeal cancer
PCR	-	Polymerase Chain Reaction
PGEM-TEASY	-	PGEM-T Vector systems
pH	-	Hydrogen Concentration
RCF	-	Relative Centrifugal Force
RNA	-	Ribonucleic acid
SDS gel	-	Sodiumdodecylsulphate polyacrylamide gel
TAE	-	Tris base, acetic acid and EDTA
UTR	-	Untranslated Region

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CHAPTER 1

INTRODUCTION

1.1 Introduction

In 2012, breast, lung, prostate, colorectal, liver and stomach cancers accounted for 55% of the worldwide occurrences (Ferlay *et al.*, 2015). According to a The National Cancer Registry (NCR) report for 2007–11, in Malaysia, the age-standardised rate (ASR) of acquiring cancer for males is 89.6 per 100,000, and for females is 89.0 per 100,000. The rate is different in the case of Chinese (males (112.9), females (126.2), per 100,000, also the highest), Indians (males (70.4), females (110.1), per 100,000) and Malays (males (70.7), females (81.9), per 100,000). The Penang Cancer Registry report for 2004–08 indicates the incidence to be 48 per 100,0000). Among males, the most prevailed types of cancer are, colorectal, lung, nasopharynx, lymphoma and prostate. While on the other hand, most types of cancer that are widespread among females are, breast, colorectal, cervix uteri, ovary and lung (Azizah *et al.*, 2016).

Tumour-related errors in cell cycles are frequently mediated by modifications of cyclin-dependent kinase (CDK) activities. Deregulation of these kinases due to their overexpression leads to proliferation of cancer cells (Peyressatre *et al.*, 2015).

The CDK3 was initially categorised as part of the CDK family through its high sequence identity of □74% with both as well as *CDK2* (Perez et al., 2009).

CDK3 underlies the regulation of cell cycles and is entailed in both G0-G1 and G1-S staged cell cycle transitions (Miyata *et al.*, 2010; Ren and Rollins, 2004).

1.2 Problem Statement

The emergence of CDK3 as a key regulator in cell cycle proliferation and cancer mutagenesis has provoked a great interest to study its structure and function . in targeting overactivation CDK3 as potential anti-cancer treatment. However, a major limiting factor is the low number of studies conducted on this gene. Production of recombinant CDK3 will facilitate in the functional and structural characterization.

1.3 Research Objectives

This research will be conducted in accordance to the objectives below:

1. To amplify the *CDK3* cDNA from breast cancer cells (MCF7)
2. To clone the amplified *CDK3* gene into the cloning vector (PGEM-TEASY).
3. To perform *in silico* modeling and analysis of CDK3 protein structure using RAPTOR-X to determine important amino acid and structural regions involved in catalysis.

1.4 Research Scope

This research was conducted at Structural Biology Laboratory, Block T02, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia for the duration of 10 months. The methodology included amplification of the *CDK3* gene from cDNA of breast cancer cells (MCF7) provided by Cancer Research Malaysia (CRM). Molecular cloning of the amplified *CDK3* gene into the cloning

vector (PGEM-TEASY) to be used for sequencing of *CDK3* gene. Finally, *in silico* modeling and analysis of CDK3 protein structure to further analyze CDK3 model.

1.5 Significance to Knowledge

Cancer is a major concern worldwide, the conventional cancer treatment such as chemotherapy could treat the cancer cells but simultaneously harm the healthy cells. Therefore, understanding the causes, effects, and possible approaches of treating cancer is important to guarantee a better life for humans. The focus of this research is to amplify and characterize the *CDK3* gene from cancer cell lines for the purpose of producing recombinant form of CDK3 for further functional and structural characterization. The homology modelling of CDK3 is of a great importance as it shows the structure of CDK3 which can take much time to be elucidated through wet laboratory experimental work. Furthermore, structural understanding of the catalytic mechanism of CDK3 will be useful for prediction of inhibitor sites that can be developed as therapeutic cancer drugs.

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