UNIVERSITI PUTRA MALAYSIA

PROGNOSTIC VALUE OF PD-1, PD-L1, TYMS AND DCC IN COLORECTAL CARCINOMA AND ASSOCIATION WITH OVERALL AND DISEASE-FREE SURVIVAL

EBENYI EMEKA ONWE

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By

EBENYI EMEKA ONWE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

I dedicate this research work to God Almighty

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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EBENYI EMEKA ONWE

October 2019

Chairman : Associate Professor Norhafizah Mohtarrudin, PhD
Faculty : Medicine and Health Science

Some biomarkers in CRC are useful for stratifying patients more appropriately for adjuvant treatment and could be used to evaluate patients overall outcome, to monitor chances of recurrence after standard treatment. Co-expression of programmed cell death-1 (PD-1), programmed cell death- ligand 1 (PD-L1), thymidylate synthase (TYMS), and deleted in colorectal carcinoma (DCC) biomarkers are not widely studied in CRC simultaneously. This study aimed to evaluate PD-1, PD-L1, TYMS, and DCC expression in tissue blocks collected from CRC patients who attend Hospital Serdang, Selangor Malaysia. Ninety one formalin fixed paraffin embedded (FFPE) archival tumour samples from patients who underwent surgical resection, were assessed using mmunohistochemical (IHC) method. There was high expression of DCC detected in 84.6% (77/91) in most cases. TYMS expression at high level was 46.2% (42/91) and low level was 53.8% (49/91) respectively. Majority of cases showed low PD-L1 expression in 93.4% (86/91) and high expression was detected in 6.6% (6/94) of cases. PD-1 expression was low in all cases. There was a significant association between TYMS expression with gender ($P < 0.05$) with distribution of TYMS expression at high level was 76.2% in male and 23.8% in female. The Kaplan-Meier survival plot showed that overall survival (OS) mean was 94 months and disease free survival (DFS) mean was 110 months. A Log rank test showed there was no statistical significance between PD-L1, TYMS and DCC with OS and DSF patients. In conclusion, the results from this study suggest that PD-L1, TYMS and DCC expression could be used as biomarkers to predict treatment outcome in CRC, PD-L1 overexpression predict patients who could benefit from anti-PD-1 and anti-P D-L1 immunotherapy, TYMS low expression predict patients who could benefit from 5-fluorouracil therapy and DCC high expression tumours predicts a better prognosis and overall survival than DCC low expression in advanced CRC.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

NILAI PROGNOSTIK OF PD-1, PD-L1, TYMS DAN DCC DALAM KARSINOMA KOLOREKTAL DAN ASOSIASI DENGAN KESELURUHAN SURVIVAL DAN PENYAKIT PERCUMA SURVIVAL

Oleh

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Oktober 2019

Pengerusi : Profesor Madya Norhafizah Mohtarrudin, PhD
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Sesetengah biomarker di CRC berguna untuk menstratkan pesakit lebih sesuai untuk rawatan pesakit dan boleh digunakan untuk menilai pesakit keseluruhan hasil, untuk memantau peluang berulang selepas rawatan standard. Programmed cell death-1 (PD-1), programmed cell death-ligand 1 (PD-L1), thymidylate synthase (TYMS) dan deleted in colorectal carcinoma (DCC) tidak dikaji secara meluas dalam CRC secara serentak. Kajian ini bertujuan untuk menilai ekspresi PD-1, PD-L1, TYMS, dan DCC dalam blok tisu yang dikumpulkan dari pesakit CRC yang hadir Hospital Serdang, Selangor Malaysia. Sembilan puluh satu formalin yang telah ditetapkan parafin terbenam (FFPE) sampel tumor arkib dari pesakit yang menjalani reseksi pembedahan, telah dinilai menggunakan kaedah mmunohistokimia (IHC). Terdapat ungkapan tinggi DCC yang dikesan dalam 84.6% (77/91) dalam kebanyakan kes. Ekspresi TYMS pada tahap tinggi adalah 46.2% (42/91) dan tahap rendah masing-masing 53.8% (49/91). Majoriti kes menunjukkan ungkapan PD-L1 yang rendah dalam 93.4% (86/91) dan ungkapan tinggi dikesan dalam 6.6% (6/94) kes. Ungkapan PD-1 rendah dalam semua kes. Terdapat persamaan yang signifikan antara ekspresi TYMS dengan jantina ($P < 0.05$) dengan pengedaran ekspresi TYMS pada tahap tinggi adalah 76.2% pada lelaki dan 23.8% pada wanita. Plot kelok jangka hayat Kaplan-Meier menunjukkan bahawa keseluruhan hidup (OS) bermakna 94 bulan dan bermakna kelok jangka hayat penyakit (DFS) adalah 110 bulan. Ujian pangkat Log menunjukkan tiada statistik statistik antara PD-L1, TYMS dan DCC dengan pesakit OS dan DSF. Kesimpulannya, hasil dari kajian ini menunjukkan bahawa PD-L1, TYMS dan DCC boleh digunakan sebagai biomarker untuk meramalkan hasil rawatan dalam CRC, PD-L1 ekspresi tinggi meramalkan pesakit yang boleh mendapat manfaat daripada anti-PD-1 dan anti-PD-L1 imunoterapi, ekspresi rendah TYMS meramalkan pesakit yang boleh mendapat manfaat daripada terapi 5-fluorouracil dan tumor tinggi DCC meramalkan prognosis yang lebih baik dan kelok jangka hayat keseluruhan daripada tumor DCC-negatif dalam CRC maju.

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I certify that a Thesis Examination Committee has met on 23 October 2019 to conduct the final examination of Ebenyi Emeka Onwe on his thesis entitled "Prognostic Value of PD-1, PD-L1, TYMS and DCC in Colorectal Carcinoma and Association with Overall and Disease-Free Survival" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Name of Member of Supervisory Committee: Associate Professor Dr. Maha Abdullah

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

According to World Health Organization (WHO), colorectal carcinoma (CRC) is defined as a malignancy of epithelial origin in the large bowel. The carcinoma for tumours of the colon and rectum can be considered adenocarcinoma by the invasion of malignant glands through muscularis mucosae and beyond (C. C. Compton & Greene, 2009). CRC prevalence is one of the most serious health burdens in developing and developed countries of the world. The incidence and mortality of CRC has been a rapid rise in this episode in many Asia countries during the few decades including Malaysia. In Malaysia, CRC is the second most common cancer, which occurs higher in male than females (Wen et al., 2012). Despite its management, many CRC patients present at a late stage with poor prognosis (Rashid et al., 2009). The following biomarkers of interest were immunohistochemically assessed, they include; programmed cell death 1(PD-1), programmed cell death ligand-1 (PD-L1), thymidylate synthase (TYMS), and deleted in colorectal carcinoma (DCC).

PD-1 is an immune checkpoint receptor and one of the most important inhibitory co-receptors expressed by T cells (Kawasaki et al., 2007, Keir, Butte, Freeman, & Sharpe, 2008). The major work of PD-1 is to inhibit effector T-cell activity and enhance the function and development of regulatory T-cells, which inhibit T-cell responses and prevent overstimulation of immune responses in peripheral tissues (Ritprajak & Azuma, 2015). However, inhibition of PD-1 promotes cancer growth by direct recognition of PD-L1 in tumour cells which inhibits its T-cells function (Carter et al., 2002, Collins et al., 2002). Futhermore, upregulation of PD-L1 allows cancer cells to evade the immune system undetected. The overexpression of PD-L1 predicts response rate and overall survival (Pardoll, 2012).

TYMS is an enzyme that is involved in DNA synthesis and repair; and clinically significant because of its target for chemotherapy (Salonga et al., 2000). TYMS as an oncogene plays a novel role as an essential DNA synthesis enzyme (Rahman et al., 2004) and impaired TYMS enzyme is associated with chromosome damage and fragile site induction which promotes carcinogenesis. TYMS inhibitors have been used clinically and experimentally to inhibit TYMS (Hammond, Swaika, & Mody, 2016).

DCC is a tumour suppressor gene and has potential as an independent prognostic biomarker for CRC. The expression of DCC in CRC was a strong positive predictive factor for survival in both stage II and stage III (Ruppert et al., 2006). Evaluation of DCC in CRC identifies patients with stage II who could benefit from adjuvant therapy. Although, some investigations on these biomarkers expression have not been clearly identified in CRC. Therefore, the aim of this study is to evaluate PD-1, PD-L1, TYMS, and DCC expression in CRC.

1.2 Statement of the Problem

The burden of CRC is expected to increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030, worldwide (Arnold et al., 2017). There is an increasing trend in CRC incidence globally and there has been a rapid rise in its incidence in many Asia countries during the past few decades including in Malaysia. Moreover, there are some controversial issues in selecting prognostic and predictive biomarkers for patient care. Researches have shown that PD-1, PD-L1, TYMS and DCC biomarkers have prognostic and therapeutic potential in CRC. However, these four biomarkers expression in CRC have shown some prognostic and predictive discrepancies with clinocopathologic and prognostic survival among CRC cases.

Therefore, this research will address the research question, what is the correlation of the PD-1, PD-L1, TYMS, and DCC expression with the overall survival (OS) and disease free survival (DSF) among CRC cases.

1.3 Objective of the Study

1.3.1 General Objective

The main objective of this study is to evaluate the PD-1, PD-L1, TYMS, and DCC expression and prognostic survival in CRC.

1.3.2 Specific Objectives

i. To determine the expression of PD-1, PD-L1, TYMS, and DCC in CRC using immunohistochemical stains.
ii. To compare the relationship between the four biomarkers.
iii. To determine the association between the expression of PD-1, PD-L1, TYMS, and DCC with the clinicopathologic (grade, stage, tumour sites) and demographic (age, gender, race) parameters of CRC patients.
iv. To correlate the expression of PD-I, PD-L1, TYMS and DCC with Overall Survival (OS) and Disease-Free Survival (DFS) among CRC cases.

1.4 Study Hypothesis

i. PD-1, PD-L1, TYMS, and DCC are overexpressed in CRC cases.
ii. PD-1, PD-L1, TYMS and DCC expression showed significant association with each other.
iii. PD-1, PD-L1, TYMS and DCC are associated with age, gender, race and clinicopathologic parameters.

iv. PD-I, PD-L1, TYMS and DCC expression showed significant correlation with Overall Survival (OS) and Disease-Free Survival (DFS).

1.5 Justification of the Study

CRC is most common among men and the second most common among women in Malaysia at a prevalent rate of 13.2 percent, as reported in the Malaysian National Cancer Registry Report (2007 – 2011). The figures also revealed that the mortality rate for males was 1.42 times higher as compared to females. Statistics showed that the prevalence of CRC is highest among the Chinese, followed by Malays and Indians. Moreover, genetics, alcoholic consumption, lack of exercise and smoking are the contributing risk factors to CRC prevalence. Although, so many works have been done on the epidemiological survey and public awareness on how to reduce the menace of CRC in Malaysia, yet there is a paucity of reports on the prognostic and diagnostic biomarkers to measure the progress of the disease in patients. Therefore, the expression levels of TYMS, DCC, PD-1 and PD-L1 biomarkers will open a frontier for researchers and pathologist on the characterization of CRC stages, progression, and a novel option for treatment.

1.6 Significance of the Study

i. Findings from this study have the potential as baseline information for eliciting an immune response in CRC patients using prognostic biomarkers.
ii. Data generated from this study will identify gaps in the molecular target in CRC, which will be able to comprehend the barriers that stifle the progress towards creating successful immunotherapy/chemotherapy for CRC, since few studies have looked into these barriers in the local population in Malaysia.
iii. Data generated from this study will boost the literature for further researches.
iv. This study will complement the use of other investigative approaches in identifying suitable CRC patients for directed therapy and determine the prognosis of the patients.

1.7 Framework of the Study

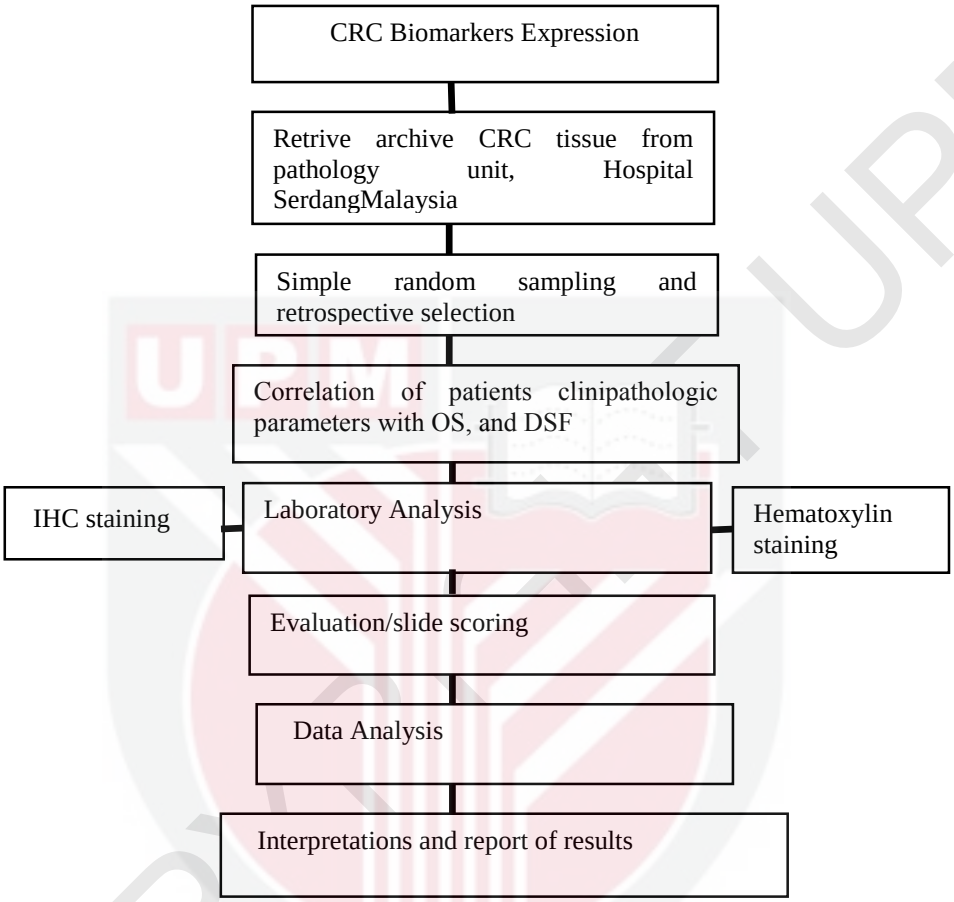

Figure 1.1 : Conceptual framework of the study

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LIST OF PUBLICATIONS

Ebenyi Emeka Onwe, Fauzah Abd Ghani, Reena Rehavu Zin, Maha Abdullah, Norhafizah Mohtarrudin. (2019). Programmed cell death-ligand 1, thymidylate synthase and deleted in colorectal carcinoma biomarkers in colorectal carcinoma Malaysian Journal of Medicine and Health Sciences Vol.15 Supp 8, November 2019.

Ebenyi Emeka Onwe, Fauzah Abd Ghani, Reena Rehavu Zin, Maha Abdullah, Norhafizah Mohtarrudin. Predictive potential of PD-L1, TYMS and DCC expressions in treatment outcome of colorectal carcinoma. BMRAT-2019-11-154 was accepted for publication in the Biomedical Research and Therapy.

Seminars and Workshops

Real Talk: Be a smart autoclave user organized by FC-BIOS SON BHD. 19th April 2018

3D Adherent Cell Culturing For Bioprocessing organized by Esco Biological Institute. 7th August 2019.

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