GENE EXPRESSION STUDY ON TUMOUR SUPPRESSOR GENE RASSFI ISOFORMS AND SELECTED INFLAMMATORY CYTOKINES IN BLADDER CARCINOMA

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GENE EXPRESSION STUDY ON TUMOUR SUPPRESSOR GENE RASSF1 ISOFORMS AND SELECTED INFLAMMATORY CYTOKINES IN BLADDER CARCINOMA

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DECLARATION

I hereby declare that no portion of the work referred to this thesis has been submitted in support of an application for another degree or qualification to this or any other university or institution of higher learning

(Nur Diana Anuar)

Date:
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ABSTRACT

Bladder carcinoma (BC) arises as a result of deregulation in oncogene and tumour suppressor gene. Loss of heterozygosity at chromosome region 3p has become common in most cancer development. A tumour suppressor gene, RASSF1 located at this particular region has been reported to be hypermethylated in most cancer including the bladder and has been a major targeted for tumour suppressor. However, this gene has known to have 7 alternative isoforms which might have different function in bladder tumourigenicity. In this study we aimed to explore the expression status of RASSF1 isoforms in BC biopsy as an effort to explore its role in BC progression. Besides the deregulation of tumour suppressor gene, BC can also be caused by inflammation. The role of inflammation in the etiology of squamous cell carcinoma of the bladder is well-established in a number of developing countries. However, the association of inflammation in transitional cell carcinoma (TCC) of the bladder which is a predominant subtype in Malaysia and other developed countries is less clear. In this aspect we aimed to study the expression level of pro- and anti-inflammatory cytokines simultaneously in order to identify any association of inflammation in TCC subtype of BC. In this study, 25 normal and tumour bladder biopsied tissues were taken from the Faculty of Medicine and Health Science, UNIMAS and Sarawak General Hospital. Total RNA were isolated and RT-PCR was utilized to examine the expression of RASSF1 isoforms and the inflammatory genes. The expression distribution of the genes was analyzed with statistical analysis using Least Significant Different (LSD). In the first aspect of this study, our study revealed that RASSF1A and RASSF1D expression is not only reduced, but they are also upregulated especially
RASSF1C which persistently overexpressed in BC cases suggesting potential role other than tumour suppression. In the second aspect, our results showed that proinflammatory cytokines, particularly IL-8 has the most significant overexpression than the anti-inflammatory cytokines, TGF-β1. The overexpression profile of proinflammatory cytokines has suggested that inflammation is a plausible event in the tumourigenicity of TCC. The expression profile has also led us to a possible antagonism activity between pro- and anti-inflammatory and this imbalance could increase the risk of bladder cancer progression. Understanding the role of different isoforms of a gene and other mechanism that could also contribute to bladder cancer, may provide important insights into prevention, early detection and effective treatment.
ABSTRAK

Kajian Ekspresi Gen ke atas Gen Penindas Tumor, RASSFI Serta Isoformnya, dan Sitokin-Sitokin Keradangan Dalam Karsinoma Pundi Kencing.

UNIMAS serta dari Hospital Umum Sarawak. Seterusnya, RNA dari sampel tisu tersebut dipencilkan dan pendekatan RT-PCR telah digunakan untuk memeriksa kadar ekspresi gen-gen isorm RASSF1 dan gen-gen keradangan. Kemudian, kadar ekspresi yang diperolehi telah dianalisa menggunakan ujian statistik Least Significant Different (LSD). Dalam aspek pertama dari kajian ini, kajian kami mendedahkan bahawa ekspresi isoform RASSFIA dan ekspresi RASSFID menurun, manakala ekspresi isoform RASSFIC meningkat secara konsisten dalam kes-kes karsinoma pundi kencing. Ini telah mendorong kami untuk menyarankan peranan RASSF1C yang berpotensi dalam peranan lain, selain sebagai gen penindas tumor. Pada aspek kedua, keputusan kami menunjukkan bahawa sitokin pro-inflamasi, khususnya IL-8 mengalami peningkatan ekspresi yang lebih tinggi dari sitokin anti-inflamasi, TGF-β1. Profil ini menyarankan bahawa peradangan adalah proses yang berkaitan dengan karsinoma sel transisional kanser pundi kencing. Profil ekspresi ini juga telah menunjukkan aktiviti antagonisme yang mungkin berlaku di antara sitokin pro- dan anti-inflamasi dan ketidakseimbangan ini boleh meningkatkan risiko pembangunan kanser pundi kencing. Memahami peranan isoform berbeza daripada ahli keluarga sesebuah gen dan mekanisme lain yang boleh juga menyumbang kepada pembangunan kanser pundi kencing, dapat memberikan kefahaman yang mendalam dalam usaha pencegahan, pengesanan awal dan rawatan yang berkesan bagi karsinoma ini.
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<td>BC</td>
<td>Bladder carcinoma</td>
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<tr>
<td>TCC</td>
<td>Transitional cell carcinoma</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>kb</td>
<td>Kilobase pairs</td>
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<tr>
<td>µg</td>
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<td>µl</td>
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<td>mg</td>
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<td>ml</td>
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<tr>
<td>ng</td>
<td>nanogram</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>Rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>°C</td>
<td>Degree Celsius</td>
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CHAPTER ONE

INTRODUCTION

1.0 Introduction

Bladder carcinoma (BC) is one of the common urothelial cancers that has become a major health problem in Malaysia, being the sixth most frequent cancer in men. The incidence of BC is 4.7 times more common in men than women (Omar et al., 2006). According to the National Cancer Registry of Malaysia (2006) urinary bladder cancer is more prevalent in Malay than in Chinese and Indians.

Seventy five percent of BC is presented as epithelial carcinoma to the mucosa (stage Ta and Tis) and lamina propria layers (stage T1) (Jung and Messing, 2000). There are 2 main histologic subtypes of bladder cancer, transitional and squamous cell carcinoma (Michaud, 2007). Most bladder cancers presented as transitional cell carcinoma which originated from either polyclonal or metastasize from a single clone (Arisan, 2003). This indicates the heterogeneous nature and malignant potential of transitional cell carcinoma of the bladder (Jung and Messing, 2000). Recurrent disease remains the major cause of mortality in patients with BC. It is reported that 10 to 30 percent of superficial BC will metastasize to invade muscle tissue which has a poor prognosis (Jung and Messing, 2000). Currently, the common non-invasive detection of BC relies on the conventional urine cytology (Arisan, 2003). However the sensitivity of this method is low especially in low grade (Ta) transitional cell carcinoma of the bladder (Arisan, 2003).
to identify reliable biomarkers because the current molecular models of BC are unable to explain the complexity of the disease. Although reports on disease-associated molecular markers are widely published, there are still very few on validated markers. Research has shown that genomic biomarkers should have a much stronger association with the disease outcome, if it is to provide a basis of early diagnosis or prediction in individual patients (Novelli et al., 2008).

Loss of heterozygosity at chromosome region 3p has become common in most cancer development including the bladder. A tumour suppressor gene, \textit{RASSF1} located at this particular region has been reported to be hypermethylated in most cancer including the bladder and has been a major targeted for tumour suppressing function (Lee et al., 2001). In this study, we aimed at studying a tumour suppressor gene, \textit{RASSF1} and its isoforms in local BC cases. The loss of expression of this gene appears to be closely related to the tumourigenicity of the bladder. However, an association of aberrant gene expression to a particular disease is not direct because of the variability in sensitivity and susceptibility in different individual (Ryan et al., 2007). Therefore, validation of expression is still necessary. In this study we aimed to identify the expression pattern of this gene and its isoforms in local bladder biopsies by reverse transcriptase polymerase chain reaction (RT-PCR) approach in order to support \textit{RASSF1} gene as tumour suppressor gene in BC.

Besides a defined sequence that includes the activation of oncogene and deactivation of tumour suppressor gene, tumour formation and progression is also highly associate with inflammation and infection (Coussen and Werb, 2001; Lin and Karin 2007). There are strong evidences that associated inflammation in
squamous cell carcinoma of the bladder (Michaud, 2007). However, the role of inflammation in the etiology of transitional cell carcinoma which is the predominant type in most developed countries such as Malaysia is less clear and still to be elucidated. Therefore to determine the role of inflammation in initiation or progression of our local BC cases, genetic susceptibility to inflammatory pathways needs to be understood. If inflammation does play a role in the progression of our local BC cases, we proposed that as the bladder tumour progress, pro-inflammatory cytokines are expressed higher than anti-inflammatory cytokines. We aimed to evaluate the quantitative relations of the targeted transcripts simultaneously by using GeneXPTM Human Cytokine assay kit and further confirm the expression pattern by RT-PCR test.

Finally, the characterization and validation of potential biomarkers as well as other mechanism(s) that may also contribute to bladder carcinogenesis will efficiently provide important insights into prevention, early detection and effective treatment.
1.2 Objectives

There are two specific objectives in this study:

a) To validate \textit{RASSF1} gene and its isoforms down regulation in local BC cases.

b) To identify the association of inflammation in the TCC subtype of bladder cancer.
CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy and histology of the bladder

The bladder is a hollow muscular organ located in the pelvic cavity with 2 main functions 1) as low pressure storage of urine and 2) high pressure expulsion of urine at an appropriate time and place. Urine is produced by the kidney carried to the bladder by the ureter and discharged from the bladder through the urethra (Figure 2.1).

As explained in Patel and Chapple (2009), the bladder is composed of 3 distinct layers, namely serosa (an outer adventitial connective tissue layer),
detrusor muscle (a middle smooth muscle layer, comprising a syncytium of interlacing muscle bundles with fibres running randomly in all directions) and urothelium (an innermost lining, composed of transitional cell epithelium providing an elastic of barrier that is impervious to urine (Figure 2.1). Beneath this lie the suburothelial layer, the urothelium and suburothelium that have a high metabolic rate and a rich nervous innervation and are involved in sensing the fullness of the bladder and modulating bladder function (Patel and Chapple, 2009)

2.2 Bladder carcinoma (BC)

BC accounts for approximately 90 percent of cancers of the urinary tract. BC usually originates in the bladder lining, which consists of a mucous layer of surface cells that expand and deflate (transitional epithelial cells), smooth muscle, and a fibrous layer (Sheridan, 2005). Bladder cancers are likely to occur in two principal forms namely low grade superficial tumours involving mucosa and lamina propria and high grade invasive cancer involving superficial or deep muscle layers of the bladder. Meanwhile, carcinoma in situ (CIS) of the bladder is a flat, superficial lesion which may act as the most common precursor to invasive bladder cancer (Saran et al., 1996). Approximately 90% of bladder cancer consists of transitional cell carcinoma and exhibits a spectrum of biological aggressiveness from superficial low-grade papillary to highly malignant solid anaplastic tumours (Saran et al., 1996). The traditional tumour node metastases (TNM) classification or the WHO classification system for the urinary bladder cancer relies on the pattern recognition and nomenclature for
reporting biopsies, the interpretation is rather subjective and this morphological information is unable to specify the risk of progression and the response to treatment for an individual with BC.

Neoplasm of the bladder encompasses a spectrum of histologic types in which any of its cellular elements composing the bladder wall and lining is able to undergo malignant transformation (Theodorescu and See, cited in Droller, 2001). The histologic variants comprise of transitional cell carcinoma, squamous cell carcinoma, adenocarcinoma and sarcoma (Theodorescu and See, cited in Droller, 2001). The most common histologic variants occur in Malaysia and in this study (of cases in Sarawak) is the transitional cell carcinoma type.

The common diagnostic tools in detection of BC are conventional urine cytology, intravenous urography and cystoscopy, and biopsy (Jung et al., cited in Droller 2001). In microscopic urine cytology, the detection of malignant cells is subjective to cytopathologist, particularly in the interpretation of specimens with atypical (abnormal) or dysplastic (precancerous) cells (Jung et al., cited in Droller 2001). Thus, it was suggested that this method is only sensitive in patients with high-grade tumours. In urography on the other hand, all patients are subjected to the investigation for hematuria. This method is useful in examining the upper urinary tract for tumours or obstruction (Jung et al., cited in Droller 2001). In spite of these two low sensitivity non-invasive methods, cystoscopy and biopsy still remain the gold standard for diagnosing new or recurrent bladder tumours (Jung et al., cited in Droller 2001). Nonetheless, even the gold standard cystoscopy is increasingly being demonstrated to miss both Ta and papillary bladder cancer (Dolicanin et al., 2007).