UNIVERSITI PUTRA MALAYSIA

HEDGEHOG PATHWAY PROTEINS AND THEIR ASSOCIATION WITH CLINICOPATHOLOGICAL PARAMETERS OF BLADDER CANCER

KHAIRUNNISA BINTI MOHD ARIFFIN

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By

KHAIRUNNISA BINTI MOHD ARIFFIN

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

August 2016
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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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August 2016

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Hedgehog pathway is important for growth and patterning during embryonic development. Previous studies have shown that constitutive activation of Hedgehog pathway can lead to various types of malignancies including medulloblastoma, basal cell carcinoma, gastrointestinal, breast and prostate cancer. The purpose of this study was to investigate the expression of Hedgehog pathway proteins in bladder cancer and determine their association with clinicopathological parameters. The expression of Sonic hedgehog (SHH), its receptor Patched (PTCH1), downstream transcription factor GLI1 and its signal transducer SMO in 112 bladder cancer tissues from Hospital Kuala Lumpur were determined by immunohistochemistry. SHH was expressed in 108 (96.4%) cases, GLI1 in 104 (92.9%) cases, PTCH1 in 111 (99.1%) cases and SMO in all cases. The relationship between the expression of these four proteins and its clinicopathological parameters were analysed statistically. Immunohistochemical staining results showed SHH, GLI1 and SMO proteins were mainly located in the cytoplasm of tumour cells, whereas PTCH1 was mainly located in the nucleus of tumour cells. Positive expression of SHH, PTCH1, GLI1 and SMO proteins were correlated with a few variables which include grade and stage of bladder cancer, lymph node metastasis and distant metastasis (Mx). Among all of the clinicopathological parameters, SMO was found to be the only protein that showed statistically significant association with higher grade (p=0.001) and higher stage (p=0.042) of bladder cancer. SMO expression also showed borderline association with lymph node metastasis (p=0.056). These findings indicate that SMO expression may be a poor prognostic marker in bladder cancer.

Keywords: Hedgehog signalling pathway, Sonic hedgehog, Gli1, Patched, Smo, bladder cancer.
LALUAN HEDGEHOG PROTEIN DAN HUBUNGKAITNYA DENGAN PARAMETER PATOLOGI KLINIKAL KANSER PUNDI KENCING

Oleh

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Laluan hedgehog penting untuk pertumbuhan dan corak semasa perkembangan embrio. Kajian sebelum ini menunjukkan bahawa pengaktifan berterusan laluan hedgehog boleh membawa kepada pelbagai jenis tumor termasuk medulloblastoma, karsinoma sel basal, kanser gastrousus, kanser payudara dan kanser prostat. Kajian ini bertujuan untuk menyiasat ekspresi protein laluan Hedgehog dalam kanser pundi kencing dan menentukan hubungkaitnya dengan parameter patologi klinikal. Ekspresi Sonic hedgehog (SHH), penerima (PTCH), faktor transkripsi, (GLI1) dan isyarat transduser, (SMO) bagi 112 tisu kanser pundi kencing daripada Hospital Kuala Lumpur telah ditentukan dengan teknik immunohistokimia. SHH menunjukkan ekspresi dalam 108 (96.4%) kes, GLI1 dalam 104 (92.9%) kes, PTCH1 dalam 111 (99.1%) kes dan SMO dalam semua kes. Hubungan ekspresi untuk keempat-empat protein dan parameter patologiklinikal telah dianalisa secara statistik. Keputusan pewarnaan imunohistokimia menunjukkan protein SHH, GLI1 dan SMO kebanyakannya terletak di sitoplasma sel-sel tumor, manakala PTCH1 kebanyakannya terletak di dalam nukleus sel-sel tumor. Ekspresi positif daripada SHH, GLI1, PTCH1 dan SMO protein telah dikorelasi dengan beberapa pembolehubah yang merangkumi gred dan peringkat kanser pundi kencing, metastasis nodus limfa dan metastasis lebih jauh. Antara semua parameter patologi klinikal, SMO protein sahaja yang menunjukkan hubungkait yang signifikan secara statistik dengan gred tertinggi \( p=0.001 \) dan peringkat tertinggi \( p=0.042 \) kanser pundi kencing. Ekspresi SMO juga menunjukkan garis batas berkait dengan nodus limfa \( p=0.056 \). Keputusan ini menunjukkan bahawa ekspresi SMO mungkin merupakan petanda prognostik yang lemah untuk kanser pundi kencing.

Kata kunci: Laluan hedgehog protein, Sonic hedgehog, GLI1, PTCH1, SMO, kanser pundi kencing.
Firstly, I would like to thank to Allah who gave me such a great strength and courage throughout this journey and without His blessing I do not think I could complete this thesis successfully.

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I certify that a Thesis Examination Committee has met on 3 August 2016 to conduct the final examination of Khairunnisa binti Mohd Ariffin on her thesis entitled "Hedgehog Pathway Proteins and their Association with Clinicopathological Parameters of Bladder Cancer" 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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LIST OF ABBREVIATIONS

WHO   World Health Organization
TCC   Transitional cell carcinoma
HH    Hedgehog
Shh   Sonic hedgehog
PTCH1 Patched homologue 1
SMO   Smoothened homologue
GLI1  GLI family zinc finger 1
mL    millilitre
G1    well differentiated
G2    moderately differentiated
G3    poorly differentiated
WHO/ISUP World Health Organization/International Society of Urological Pathology
PUNLMP papillary urothelial neoplasms of low malignant
TNM   Tumour nodes metastasis
AJCC  American Joint Committee on Cancer
Ihh   Indian Hedgehog
Dhh   Desert Hedgehog
SUFU  Suppressor of fused
DNA   Deoxyribonucleic acid
GliR  GLI family zinc finger repressor
GliA  GLI family zinc finger activator
BCC   Basal cell carcinomas
MB    Medulloblastoma
CSCs  Cancer stem cells
FFPE  Formalin-fixed paraffin embedded
H&E   Hematoxylin & Eosin
DPX   Di-N-Butyle Phthalate in Xylene
Ag-Ab Antigen antibody
ABC   Avidin-biotin complex
LSAB  Labelled streptavidin-biotin
PAP   Peroxidase-antiperoxidase
mM   mili molar
PBS   Phosphate buffered saline
TBST  Tris Buffered Saline with Tween 20
HRP   Horse radish peroxidase
µl    microlitre
DAB   Diaminobenzidine
SPSS  Statistical Package for the Social Sciences
Mx    Distant metastasis
CHAPTER 1

INTRODUCTION

1.1 General Introduction

Cancer is one of the common health problems worldwide. In the United States, one in four deaths is due to cancer (Siegel, Naishadham, & Jemal, 2013). In year 2007, based on Ministry of Health Malaysia Hospitals after Heart Diseases & Diseases of Pulmonary Circulation and Septicaemia, cancer was the third common cause of death in Malaysia. A total of 18,219 new cancer cases were diagnosed in 2007 and registered at the National Cancer Registry. Among the cases reported, there were 8,123 (44.6%) males and 10,096 (55.4%) females (Zainal & Nor Saleha, 2011). Worldwide, bladder cancer ranks eleventh most common malignancy. It is the 7th most common cancer in men and the 19th most common cancer in women (Jacques Ferlay, Isabelle Soerjomataram, Rajesh Dikshit, 2012). The disease is more common in whites than blacks, and the average age at diagnosis is 65 years. In addition, it has been recommended that the stage survival of bladder cancer in female is worse than in male (Kirkali et al., 2005).

Approximately 90% of bladder tumours arise from the urothelial lining, and transitional cell carcinoma (TCC) is the most common histological type in cases arising in the United States. TCC or as known as urothelial carcinoma starts in the cells lining of the bladder and later will spread to the nearby organ or other body parts if it is not treated at early stage. Smoking habit, chemical exposure, age, sex and chronic bladder inflammation are risk factors that can contribute to the development of bladder cancer (Jemal et al., 2011). However, the pathogenesis of bladder cancer still largely remains unknown. Bladder cancer is treated based on the tumour stage and grade. Besides the treatment protocols, the incidence of therapeutic resistance and failure are still present.

Malignant cells aberrantly reactivate oncogenic developmental pathways such as Hedgehog, Wnt and Notch to provide growth and survival advantage to tumour cells (Ingham, Mcmahon, Ingham, & Mcmahon, 2001). Emerging evidence clearly suggesting the activation of Hedgehog pathway has been implicated in the tumour development of a large number of human cancers (Beachy, Karhadkar, & Berman, 2004).

The exact genetic events leading to urothelial transformation involve the activation and inactivation of oncogenes, inactivation or loss of tumour suppressor genes and alterations in the apoptotic gene products (Sandberg and Berger, 1994). Study shows that there are a lot of genetic abnormalities but the specific genes involved are still unidentified. This is because there are a lot of gene mutations detectable in bladder cancer (Cantile et al., 2003).

In 2010, Chen et al. have genotyped 177 single-nucleotide polymorphisms (SNP) in 11 SHH pathway genes in a study including 803 bladder cancer cases and 803 controls. They also analysed SNP associations with cancer risk and clinical outcomes in 318
cases of muscle-invasive and 419 cases of non-muscle-invasive bladder cancer and metastatic bladder cancer. As their findings, it has been confirmed that germ-line genetic variations in the SHH pathway could predict the clinical outcomes of non-muscle-invasive bladder cancer patients received transurethral resection and Bacillus Calmette-Guérin.

Hedgehog signalling pathway operates in organogenesis, control of proliferation, and the differentiation of embryonic adult stem cells, thus, not surprisingly it has been linked with several different tumours (Sverrisson et al., 2014) including gastrointestinal cancers and is frequently activated in esophageal (Ingham et al., 2001), pancreatic (Morton et al., 2007) and gastric cancer (Merchant, Saqui-Salces, & El-Zaatari, 2010). On the other hand, the role of hedgehog signalling pathway has been less investigated in the development of bladder cancer and its involvement to the progression of transitional cell carcinoma of bladder is not clear (Haraguchi et al., 2007). However, He et al. successfully proved that the positive expression of SHH, GLI1 and PTCH1 proteins in bladder cancer were significantly higher than in adjacent normal bladder. Ha, Y. S. et al. (2007) has shown that SMO is closely related with the differentiation and progression of bladder cancer. Therefore, SHH, GLI1, PTCH1 and SMO may play important roles during development of bladder cancer (He et al., 2012). This study is necessary to contribute the knowledge in understanding the role of HH pathway proteins in bladder cancer.

1.2 Problem statement

Bladder cancer is generally diagnosed by cystoscopy and biopsy. It has a very high frequency of recurrence and therefore requires follow up cystoscopy as well as urine cytology as periodic surveillance to identify early recurrence. Failure to treat this cancer at an early stage will lead to advance stage which may further complicate the management of the patient. Currently, radical cystectomy is the treatment for urothelial carcinoma. As this cancer needs long-term follow up and surveillance procedure to monitor for tumour recurrence, many of the patients tend to default their follow up. The role of hedgehog signalling pathway in bladder development is well recognized but its role in human bladder cancer progression is uncertain and has been quite controversial. So, this study was done to determine the association of hedgehog pathway protein expression with the progression of bladder cancer so that it can be treated at an early stage with more effective treatments.

1.3 Significance of the study

The findings from this study will contribute knowledge in understanding the role of SHH, GLI1, PTCH1 and SMO in bladder cancer. This study will also offer overview on the expression of these proteins in bladder cancer to better evaluate the function of these proteins in tumour progression. It also provides fundamental background for further investigations on clinical use of related antibodies for personalised cancer management.
1.4 Research objectives

1.4.1 General objective

- To study the HH signalling pathway in bladder cancer.

1.4.2 Specific objectives

1) To determine the demographic distribution of bladder cancer in Hospital Kuala Lumpur from 2008 to 2013.
2) To analyse the immunohistochemical expression of SHH, GLI1, PTCH1 and SMO in bladder cancer.
3) To study the correlation in the immunohistochemical expression between Hedgehog pathway proteins (SHH, GLI1, PTCH1 and SMO) in bladder cancer.
4) To study the association of demographic factors (age, gender, ethnicity) and clinicopathological parameters (grade, stage, lymph node metastasis, distant metastasis) of bladder cancer with the expression of hedgehog pathway protein.

1.5 Research hypothesis

1) There is high expression of hedgehog pathway proteins (SHH, GLI1, PTCH1 and SMO) in bladder cancer.
2) There is significant correlation among the expression of hedgehog signalling proteins (SHH, GLI1, PTCH1 and SMO).
3) There is significant association between demographic factor and clinicopathological parameters of bladder cancer with the expression of hedgehog pathway proteins (SHH, GLI1, PTCH1 and SMO).
1.6 Conceptual Framework

Immunohistochemical staining of bladder cancer

Expression of SHH
Expression of GLI1
Expression of PTCH1
Expression of SMO

Demographic Factors
- Gender
- Age
- Ethnic

Clinicopathological Parameters
- Grade
- Stage
- Lymph node metastasis
- Distant metastasis
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