



UNIVERSITI PUTRA MALAYSIA

***MOLECULAR CHARACTERIZATION OF HIGH-RISK HUMAN
PAPILLOMAVIRUSES IN RENAL CELL CARCINOMA***

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By

ALI FARHADI ANDAR ABI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

June 2013

DEDICATION

*Lovingly dedicated to my parents for their encouragement
and support to elham for all else*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chair: Prof. Zamberi bin Sekawi, MD, MPath, PhD

Faculty: Medicine and Health Sciences

Renal cell carcinoma (RCC) represents five percent of adult epithelial cancers and the most prevalent malignancy of the kidney. It has been estimated that known risk factors for RCC account for less than one half of the diagnosed cases. Thus, there is a need to identify additional risk factors associated with the development of RCC. While the role of high risk human papillomavirus (HR-HPV) types in cervical, non-cervical anogenital and some head and neck cancers is generally accepted by now, its oncogenic role at other anatomical sites is still debated. The aim of this study was to investigate whether HR-HPV infection is attributable to RCC or has any role in pathogenesis and development of a certain histological subtype.

Formalin fixed paraffin embedded (FFPE) tissue specimens from 127 patients with histopathologically proven RCC and their respective peritumoural tissues in

addition to 19 tissue control were available for this study. The presence of HPV-DNA was analyzed by nested PCR assays using consensus primers (MY/GP+) primers as well as type specific primers for HPV 16/18 and sequenced for different types of HPV in case of positivity for HPV-DNA. The expression of p16INK4a, p53 and Ki-67 proteins was evaluated with the use of immunohistochemistry. Catalyzed signal-amplified colorimetric *in situ* hybridization (CSAC-ISH) technique was applied to demonstrate integrated and episomal viral DNA. Detection of E6/E7 oncogene transcripts of HPV 16 and 18 genotypes was performed using nested RT-PCR assays. The prevalence of co-infection of HR-HPV with oncogenic viruses including Epstein-Barr virus, BK virus, JC virus or Human Herpesvirus 6 was determined as well. A retrospective analysis of clinicopathological characteristics between HR-HPV positive and HR-HPV negative RCC patients was also carried out.

The HPV genome was detected in 37 (30.3%) of RCC specimens and four (4.1%) of their corresponding peritumoural tissues. HPV-18 was the most common viral type identified followed by HPV-16 and 58. The prevalence of HR-HPV infection in papillary RCC was significantly higher than other histological subtypes ($p=0.007$). Nuclear and/or cytoplasmic immunoexpression of p16INK4a was detected in 24 (20.3%) cases. Data analysis showed a significant correlation between p16INK4a expression and the presence of HR-HPV DNA ($p<0.001$). The expression pattern of the proliferation factor was correlated with the nuclear grade ($p<0.001$) and HPV-

infection ($p=0.027$). Chromogenic *in situ* hybridization analysis confirmed HR-HPV infection in 18 (45%) of RCC tumours previously tested positive for HPV-DNA. Diffuse signal pattern was identified in 15 (83.3%) samples whereas a mixed pattern of diffuse and punctate signals was only detectable in three (16.7%) cases. In addition, nested RT-PCR could detect HPV-18 spliced (E6*) and unspliced E6/E7 oncogene transcripts in five cases.

In conclusion, it is proposed that human kidney tissue is susceptible to persistent HPV infections. This study indicates the association between high-risk HPV presence and renal cell carcinoma suggesting HPV infection in high-grade RCC might precede disease progression in a number of RCC tumours, particularly of papillary renal cell carcinoma subtype.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN MOLEKUL VIRUS PAPILOMA MANUSIA BERISIKO TINGGI
PADA KARSINOMA SEL RENAL**

Oleh

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Karsinoma sel renal (RCC) merangkumi 5 peratus daripada kanser epithelial di kalangan orang dewasa dan kebanyakan pembentukan kanser di buah pinggang. Faktor risiko yang diketahui adalah dianggarkan kurang daripada satu setengah kes yang dikesan. Maka, pengesanan untuk faktor risiko tambahan yang berhubung kait pembentukan RCC. Sementara itu, peranan virus papilloma manusia jenis berisiko tinggi (HR-HPV) dalam serviks, anogenital bukan serviks dan beberapa kanser kepala dan leher adalah diketahui umum, namun peranan onkogenik dari segi anatomi yang lain masih didebatkan. Tujuan penyelidikan ini adalah untuk mengkaji sama ada jangkitan HR-HPV menyumbang kepada RCC atau mempunyai ianya peranan dalam patogenesis dan pembentukan jenis histologikal tertentu.

Spesimen tisu formalin-tetap atau parafin tertanam (FFPE) daripada 127 orang pesakit digunakan dalam penyelidikan ini dengan histologinya telah dibuktikan mempunyai RCC dan peritumoral tisu, 19 daripada sampel tersebut adalah kontrol. DNA bagi virus papilloma dikesan melalui analisis nested-PCR menggunakan primers spesifik (MY/GP+); dan ini juga membolehkan jenis HPV 16/18 dapat dikenalpasti kemudian; kajian diteruskan dengan proses jujukan bagi kes yang menunjukkan HPV-DNA positif. Protein p16INK4a, p53 dan Ki-67 yang telah menghasilkan tindak balas telah dinilai dengan menggunakan immunohistokimia. Teknik isyarat gandaan kalorimetri hibridisasi in situ (CSAC-ISH) telah digunakan untuk menunjukkan percantuman DNA virus dengan isyarat penanda bintik dalam nukleus and episom DNA virus melalui isyarat resapan dalam keseluruhan nukleus. Pengesanan transkripsi gen E6/E7 oleh genotip HPV 16 dan 18 telah dijalankan dengan menggunakan nested-PCR. Kadar kelaziman jangkitan sampingan virus papilloma dengan virus lain yang boleh menyebabkan pembentukan kanser seperti virus Epstein-Barr (EBV), virus BK, virus JC atau virus herpes 6 (HHV-6) juga telah ditentukan. Analisis retrospektif telah dilakukan tentang ciri-ciri pathologi klinikal antara kes yang positif dan negatif juga telah dijalankan.

Genom HPV telah dikesan dalam 37 (30.3%) specimen RCC dan 4 (4.1%) tisu peritumoural yang sama. HPV-18 adalah jenis yang paling biasa virus yang dikenal pasti diikuti oleh HPV-16 dan 58. Kadar kelaziman jangkitan HR-HPV dalam

papilari RCC adalah lebih tinggi daripada subjenis histological ($p=0.007$). Immunoekspresi nuklear dan/atau sitoplasma p16INK4a dikesan dalam 24 (20.3%) kes. Analisis data menunjukkan terdapat hubungan yang signifikan antara ekspresi p16INK4a dan kehadiran HR-HPV ($p<0.001$). Corak tindak balas yang dipunyai oleh faktor proliferasi adalah berhubungkait dengan gred nuklear ($p<0.001$) dan jangkitan HPV ($p=0.027$). Jangkitan HR-HPV pada 18 (45%) tumor-tumor RCC yang mana sebelumnya didapati positif HPV-DNA telah disahkan oleh analisis kromogenik hibridisasi in situ. Pola isyarat resapan telah dapat dikenalpasti dalam 15 (83.3%) sampel sementara campuran pola resapan dan isyarat penanda bintik hanya dapat dikenalpasti dalam 3 (16.7%) kes. Di samping itu, nested RT-PCR dapat mengesan HPV-18 transkrip onkogen disambungkan (E6*) dan tidak disambungkan E6/E7 dalam 5 kes.

Kesimpulannya, kajian semasa menunjukkan bahawa tisu buah pinggang manusia adalah mudah terdedah oleh jangkitan HPV secara berterusan. Hasil kajian menunjukkan terdapat perkaitan dengan jenis virus papilloma (HPV) yang berisiko tinggi dengan sel renal karsinoma dan jangkitan HPV ini akan menyebabkan penambahan jumlah tumor RCC, terutamanya di bahagian sel subjenis karsinoma renal papilari.

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I certify that a Thesis Examination Committee has met on 4 June 2013 to conduct the final examination of Ali Farhadi Andar Abi on his thesis entitled "Molecular Characterization of High-Risk Human Papillomaviruses in Renal Cell Carcinoma" in accordance with Universities and University College 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 Doctor of Philosophy.

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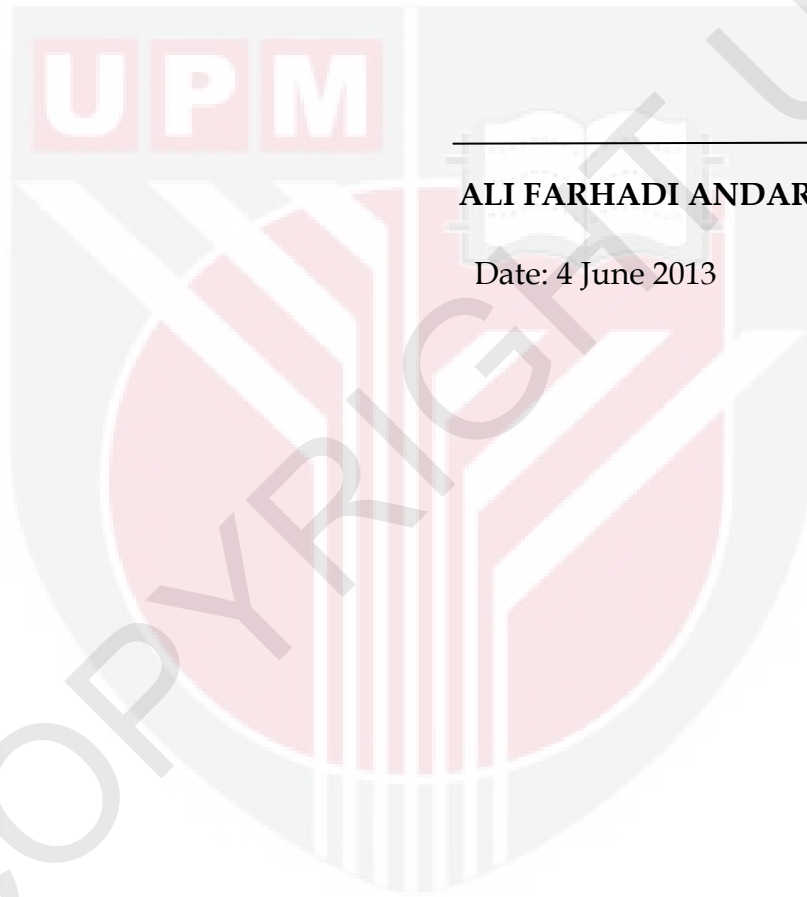
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institution.



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Date: 4 June 2013

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