TREATMENT OPTIONS IN UROLOGICAL CANCER

EDITED BY

Jonathan Waxman BSc, MD, MBBS, FRCP
Department of Cancer Medicine, Imperial College of Science,
Technology and Medicine, London

Blackwell
Science

TREATMENT OPTIONS IN UROLOGICAL CANCER

TREATMENT OPTIONS IN UROLOGICAL CANCER

EDITED BY

Jonathan Waxman BSc, MD, MBBS, FRCP
Department of Cancer Medicine, Imperial College of Science,
Technology and Medicine, London

Blackwell
Science

© 2002 by Blackwell Science Ltd
a Blackwell Publishing company
Editorial Offices:
Osney Mead, Oxford OX2 0EL, UK
Tel: +44(0)1865 206206
108 Cowley Road, Oxford OX4 1JF, UK
Tel: +44(0)1865 791100
Blackwell Publishing USA, 350 Main Street, Malden, MA 02148-5018, USA
Tel: +1 781 388 8250
Iowa State Press, a Blackwell Publishing Company, 2121 State Avenue, Ames, Iowa 50014-8300, USA
Tel: +1 515 292 0140
Blackwell Munksgaard, Nørre Søgade 35, PO Box 2148, Copenhagen, DK-1016, Denmark
Tel: +45 77 33 33 33
Blackwell Publishing Asia, 54 University Street, Carlton, Victoria 3053, Australia
Tel: +61 (0)3 9347 0300
Blackwell Verlag, Kurfürstendamm 57, 10707 Berlin, Germany
Tel: +49 (0)30 32 79 060
Blackwell Publishing, 10 rue Casimir Delavigne, 75006 Paris, France
Tel: +33 1 53 10 33 10

The right of the Author to be identified as the Author of this Work has been asserted in accordance with the Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

The Blackwell Publishing logo is a trade mark of Blackwell Publishing Ltd.

First published 2002

Library of Congress Cataloging-in-Publication Data

Treatment options in urological cancer / edited by J. Waxman.
p.; cm.
Includes bibliographical references and index.
ISBN 0-632-05589-8
1. Genitourinary organs—Cancer—Treatment. I. Waxman, Jonathan.
[DNLM: 1. Urologic Neoplasms—therapy. WJ 160 T7838 2001]
RC280.U74 T735 2001
616.99′4606—dc21
2001025068

ISBN 0-632-05589-8

A catalogue record for this title is available from the British Library

Set in 10/13½ Sabon by Best-set Typesetter Ltd, Hong Kong
Printed and bound in Great Britain by MPG Books Ltd, Bodmin, Cornwall

For further information on
Blackwell Science, visit our website:
www.blackwell-science.com

Contents

Contributors, ix

Preface, xiii

Part 1 Kidney Cancer

1 The Molecular Biology of Kidney Cancer, 3
E. P. Castle, G. S. Hallman & J. B. Thrasher

2 Cytokines and Angiogenesis Inhibitor Treatments, 31
S. Chowdhury & M. Gore

3 Surgical Management of Metastatic Renal Cancer, 49
D. F. Badenoch

Kidney Cancer: Commentary, 55
J. Waxman

Part 2 Bladder Cancer

4 The Molecular Biology of Bladder Cancer, 59
P. E. Keegan, D. E. Neal & J. Lunec

5 Surgery for Advanced Bladder Cancer, 84
D. W. W. Newling

6 Chemotherapy and Bladder Cancer, 98
F. Calabrò & C. N. Sternberg

7 Treatment Options in Superficial (pTa/pT1/CIS) Bladder Cancer, 118
J. L. Ockrim & P. D. Abel

Bladder Cancer: Commentary, 137
J. Waxman

Part 3 Prostate Cancer

8 The Molecular Biology of Prostate Cancer, 141
J. Wang & J. Waxman

9 Surgical Pathology of Prostate Cancer, 160
C. S. Foster

10 Prostate Radiotherapy, 189
C. R. Lewanski & S. Stewart

11 Prostate Cancer: Immediate vs. Deferred Treatment, 206
D. Kirk

12 Hormone Therapy of Prostate Cancer, 220
R. Agarwal & J. Waxman

13 Chemotherapy in Hormone Refractory Prostate Cancer, 237
J. J. Knox & M. J. Moore

14 Radical Prostatectomy, 253
T. J. Christmas & A. P. Doherty

15 The Case against Radical Surgery for Early Prostate Cancer, 264
Leslie E. F. Moffat

Prostate Cancer: Commentary, 275
J. Waxman

Part 4 Testicular Cancer

16 Genetics of Adult Male Germ Cell Tumours, 279
J. Houldsworth, G. J. Bosl & R. S. K. Chaganti

17 Surveillance, Chemotherapy, and Radiotherapy for Stage 1 Testicular Germ Cell Tumours, 293
D. H. Palmer & M. H. Cullen

18 Chemotherapy of Testicular Cancer, 305
I. A. Burney & G. M. Mead

19 Salvage Chemotherapy Regimens for Relapsing Germ Cell Cancer, 317
M. L. Harvey & G. M. Mead

20 Surgery for Testicular Cancer, 328
W. F. Hendry

Testicular Cancer: Commentary, 350
J. Waxman

Part 5 Penile Cancer

21 Viral Agents in the Development of Penile Cancer, 353
P. H. Rajjayabun, T. R. L. Griffiths & J. K. Mellon

22 Current Concepts in the Management of Penile Cancer, 366
R. Sarin & H. B. Tongaonkar

Penile Cancer: Commentary, 382
J. Waxman

Index, 383

Colour plates fall between pp. 178 and 179

Contributors

Mr Paul D. Abel
Department of Surgical Oncology
and Technology
Faculty of Medicine
Imperial College of Science,
Technology and Medicine
Hammersmith Campus
Du Cane Road
London W12 0NN
UK

Dr Roshan Agarwal
Department of Medical Oncology
Chelsea and Westminster Hospital
Fulham Road
London
UK

Mr David F. Badenoch
123 Harley Street
London W1G 6AY

Dr George J. Bosl
Department of Medicine
The Patrick M Byrne Chair in
Clinical Oncology
Memorial Sloan-Kettering Cancer
Center
1275 York Avenue
New York, New York 10021
U.S.A.

Dr Ikram A. Burney
Department of Medicine
The Aga Khan University
Stadium Road
Karachi 74800
Pakistan

Dr Fabio Calabro
Department of Medical Oncology
Vincenzo Pansadoro Foundation
Clinic Pio XI
Via Aurelia 559
Rome
Italy 00165

Dr Erik P. Castle
Section of Urology
University of Kansas Medical
Center
3901 Rainbow Boulevard
Kansas City
KS 66160-7390
USA

Dr R. S. K. Chaganti
Department of Medicine
Memorial Sloan-Kettering Cancer
Center
1275 York Avenue
New York
New York 10021
USA

Dr Simon Chowdhury
Department of Medicine
Royal Marsden Hospital
Fulham Road
London SW3 6JJ
UK

Mr Timothy J. Christmas
Department of Urology
Imperial College School of Medicine
Charing Cross Hospital
Fulham Palace Road
London W6 8RF
UK

Dr Michael H. Cullen
Cancer Centre at the Queen Elizabeth Hospital
University Hospital Birmingham NHS Trust
Birmingham B15 2TH
UK

Mr A. P. Doherty
Consultant Urologist
Queen Elizabeth Hospital
Edgbaston
Birmingham B15 2TH
UK

Professor Christopher S. Foster
Department of Cellular and Molecular Pathology
University of Liverpool
Daulby Street
Liverpool L69 3GA
UK

Dr Martin Gore
Department of Medicine
Royal Marsden Hospital
Fulham Road
London SW3 6JJ
UK

Mr T. R. L. Griffiths
University Department of Urology
Freeman Hospital
Newcastle-upon-Tyne NE7 7DN
UK

Dr Gregg S. Hallman
Cape Girardeau Urology Associates
3 Doctors Park
Cape Girardeau
MO 63701
USA

Dr M. L. Harvey
The Wessex Medical Oncology Unit
Royal South Hants Hospital
Southampton SO14 0YG
UK

Mr William F. Hendry (retired)
Department of Urology
St Bartholomew's Hospital
West Smithfield
London EC1A 7BE
UK

Dr Jane Houldsworth
Department of Medicine
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York
New York 10021
USA

Mr Phil E. Keegan
Department of Surgery and the Cancer Research Unit
The Medical School
University of Newcastle
Newcastle upon Tyne
NE2 4HH
UK

Professor David Kirk
Department of Urology
Gartnavel General Hospital
1053 Great Western Road
Glasgow G12 0YN
UK

Dr Jennifer J. Knox
Department of Medical Oncology
Princess Margaret Hospital
University Health Network
610 University Avenue
Toronto, Ontario
Canada M5G 2M9

Dr Conrad R. Lewanski
Department of Radiotherapy
Charing Cross Hospital
Fulham Palace Road
London W6 8RF
UK

Dr J. Lunec
Cancer Research Unit
The Medical School
University of Newcastle
Newcastle upon Tyne NE2 4HH
UK

Dr G. M. Mead
The Wessex Medical Oncology Unit
Royal South Hants Hospital
Southampton SO14 0YG
UK

Professor Kilian Mellon
University of Leicester
Leicester
UK

Mr Leslie E. F. Moffat
Department of Urology
Aberdeen Royal Infirmary
Aberdeen AB25 2ZN
UK

Professor Malcolm J. Moore
Department of Medicine and Pharmacology
Princess Margaret Hospital
University Health Network
University of Toronto
610 University Avenue
Toronto, Ontario
Canada M5G 2M9

Professor D. E. Neal
Department of Surgery
The Medical School
University of Newcastle
Newcastle upon Tyne NE2 4HH
UK

Professor Don W. W. Newling
Department of Urology
Free University Medical Centre
De Boelelaan 1117
1007 MB Amsterdam
The Netherlands

Mr Jeremy L. Ockrim
Imperial College School of Medicine
Department of Urology
Charing Cross Hospital
Fulham Palace Road
London W6 8RF
UK

Dr Daniel H. Palmer
CRC Institute for Cancer Studies
The Medical School
University of Birmingham
Birmingham B15 2TA
UK

Mr Paul H. Rajjayabun
Department of Surgery
The Medical School
University of Newcastle
Newcastle upon Tyne NE2 4HH
UK

Dr Rajiv Sarin
Tata Memorial hospital
Parel
Mumbai
400 012
India

Dr Cora N. Sternberg
Department of Medical Oncology
Vincenzo Pansadoro Foundation
Clinic Pio XI
Via Aurelia 559
Rome
Italy 00165

Dr Simon Stewart
Department of Radiotherapy
Charing Cross Hospital
Fulham Palace Road
London W6 8RF
UK

Professor J. Brantley Thrasher
Section of Urology
University of Kansas Medical Center
3901 Rainbow Boulevard
Kansas City
KS 66160-7390
USA

Professor Herman B. Tongaonkar
Professor and Surgeon
Department of Urologic Oncology
Tata Memorial Hospital
Parel
Mumbai 400 012
India

Dr Jayson Wang
Imperial Cancer Research Fund
44 Lincoln's Inn Fields
London WC2A 3PX
UK

Professor Jonathan Waxman
Department of Cancer Medicine
Faculty of Medicine
Imperial College School of Science,
Technology and Medicine
Hammersmith Campus
Du Cane Road
London W12 0NN
UK

Preface

Urological malignancies are a significant part of both urological and oncological practice. There have been changes in the epidemiology of urological malignancy so that these diseases have become important for all of us and our government.

There have been significant changes in our understanding of the biology of this group of tumours, and these changes are very much at the forefront of molecular biology. This science has been applied to urological tumours, and we are at a point where we are close to understanding the chromosomal change in testicular and renal cancers and the causes for androgen independence in prostate cancer.

There have also been significant changes in the treatment of urological malignancies. New hormonal therapies have become available for prostate cancer, cytokines have been developed for the treatment of renal tumours and new chemotherapy programmes have become available for testicular and bladder cancer.

A combined approach to the management of malignancy is never more important than in urological oncology, where oncologists, radiotherapists and surgeons together have brought forward practice and improved upon survival. But this is an area where we need to do better—an area where the multidisciplinary approach really does have to kick in in order to facilitate advances in research and survival. This ambition is, I hope, signposted in this book, which brings together the science and treatment of urological cancer.

Part 1
Kidney Cancer

1: The Molecular Biology of Kidney Cancer

E. P. Castle, G. S. Hallman & J. B. Thrasher

Introduction

Renal cell carcinoma (RCC) accounts for approximately 3% of adult malignancies [1]. It is the most common primary renal malignancy, accounting for 30 000 cases annually in the United States [2,3]. RCC affects males twice as frequently as females, is more common in those from urban settings and most commonly affects individuals between the ages of 50 and 70. The aetiology of this disease has been studied extensively, and the strongest candidate that has been identified is cigarette smoking [2,4–6]. In addition, obesity, prescription diuretic use and occupational exposures such as leather finishing products and asbestos may lead to RCC [2,4,5,7–10]. An increased incidence is found in patients with end-stage renal disease, especially in those with acquired renal cystic disease, and in individuals with congenital disorders such as von Hippel–Lindau disease, tuberous sclerosis or autosomal dominant polycystic kidney disease [2,4,11–15]. The clear cell histopathological type (Fig. 1.1) is the most common, accounting for 80–85% of RCC, while papillary histopathology is seen in 5–10% of tumours [4,16].

RCC occurs in sporadic and familial forms. The familial forms of disease are typified by the tendency for development of multifocal and bilateral tumours and earlier age of onset [4,17]. Four different types of familial RCC have been identified: RCC in von Hippel–Lindau disease, hereditary clear cell renal carcinoma, hereditary papillary renal carcinoma and hereditary renal oncocytoma [4]. With the identification of the familial nature of some renal carcinomas, intense investigation into the genetic alterations leading to tumour formation has ensued. Promising genetic loci have been identified and characterized in each of the familial forms of RCC, and investigation continues in order to further delineate the specific functions of these genes. Herein, we will discuss in detail the molecular genetics of renal carcinomas as well as molecular markers that may play an important role in the future with respect to the diagnosis and management of these tumours.

Von Hippel–Lindau disease

Von Hippel–Lindau (VHL) disease is a hereditary cancer syndrome character-

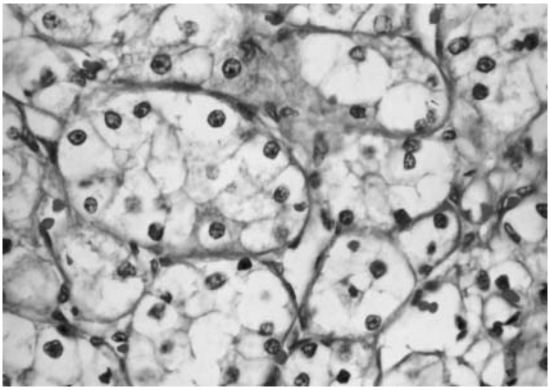

Figure 1.1 Renal cell carcinoma—clear cell variant.

ized by the presence of benign and malignant tumour development in multiple organ systems, including the eyes, cerebellum, spine, inner ear, pancreas, adrenal gland and kidneys [4]. Retinal angiomas, cerebellar and spinal haemangioblastomas and renal cell carcinomas are the hallmark lesions of this disease. Renal cysts, pancreatic cysts and carcinomas, phaeochromocytomas, epididymal and broad ligament cystadenomas and endolymphatic sac tumours may also manifest in affected individuals. The incidence of VHL is estimated to be 1/36 000 live births [18,19]. It is inherited in an autosomal dominant fashion and has an estimated penetrance of 80–90% by the age of 65 [18,19]. RCC eventually develops in 28–45% of those affected with VHL [4]. These tumours are frequently multicentric and bilateral and are predominantly of the clear cell variety [4,20–24] (Fig. 1.2). The management of RCC in these patients involves nephron-sparing surgery to help maintain renal function as long as possible and to reduce the risk of metastatic disease [4,20,25–29].

Epidemiological data suggest that tumour-suppressor gene inactivation is responsible for the development of VHL [18,30]. According to Knudson's 'two-hit' hypothesis, the carriers of mutations in a tumour-suppressor gene have a germline mutation in one allele of the gene, and a somatic mutation occurs in the homologous normal allele, which leads to tumour formation [18,31]. The germline mutation is transmitted in an autosomal dominant fashion with each offspring having a 50% risk of inheriting the mutated allele. These genes normally inhibit tumour development through regulation of cell proliferation and differentiation, and their inactivation predisposes an individual to cancer through loss of these regulatory processes.

Researchers sought to confirm that the VHL gene was indeed a tumour-suppressor gene. Tory *et al.* [32] used restriction fragment length polymorphism (RFLP) analysis to evaluate RCC from VHL patients and showed that the wild-type chromosome 3p allele, which was inherited from the unaffected

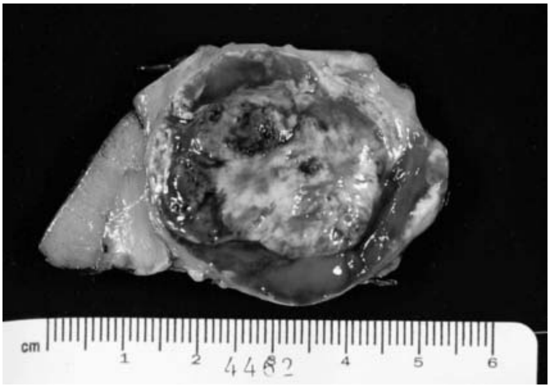

Figure 1.2 VHL tumour—gross specimen.

parent, was lost. Thus, these patients retained only the abnormal germline copy of the gene inherited from the affected parent. Lubensky *et al.* [33] subsequently demonstrated loss of the wild-type 3p allele with maintenance of the inherited, mutated allele in 25 of 26 renal lesions from individuals with VHL. This loss of heterozygosity (LOH) was detectable in atypical renal cysts and cysts with RCC *in situ*. Therefore, the VHL gene was indeed believed to be a tumour-suppressor gene, and loss of function of both gene copies appeared to be an important early step towards tumour formation.

The VHL gene was mapped to the short arm of chromosome 3, sub-band 25 (3p25) [20,34,35]. Seizinger *et al.* [34] used genetic linkage analysis to study nine families with VHL disease, which included 71 affected individuals, and found that the VHL gene linked to the RAF1 oncogene at 3p25. The linkage to RAF1 was confirmed by Hosoe *et al.* [36], who also reported linkage of the VHL gene to D3S18, a polymorphic DNA marker located at 3p26. Richards *et al.* [37] then demonstrated tight linkage of the VHL gene to the DNA probe D3S601, which was located in the region between RAF1 and D3S18. The VHL gene was subsequently identified by Latif *et al.* in 1993 through the use of yeast artificial chromosomes and cosmid-phage contigs [35]. It was found to be a single-copy gene with evolutionary conservation across several species, thus pointing to a role in essential cellular processes [2].

The VHL gene contains three exons with an open reading frame of 852 nucleotides that encode a protein of 213 amino acids [2,4]. Several hundred germline mutations have been recognized in VHL kindreds. These include microdeletions/insertions, large deletions and mis-sense and nonsense mutations. Chen *et al.* [38] studied 114 VHL families and identified mutations throughout the coding region, but clustering occurred at the 3′ end of exon 1 and the 5′ end of exon 3 with a paucity of mutations in exon 2. Specific mutations have since been correlated with certain phenotypic characteristics in

VHL patients. VHL type I families (without phaeochromocytoma) most frequently have large deletions, microdeletions/insertions or nonsense mutations, whereas in VHL type II families (with phaeochromocytoma) 96% of the mutations are mis-sense [18,38,39]. Gnarra *et al.* [40] evaluated sporadic clear cell renal carcinoma and found VHL gene mutations in 57% of the cancers with LOH of the gene in 98%. The mutations clustered at the 3′ end of exon 1 and at the 5′ end of exon 3; however, exon 2 also had a high frequency of mutations (45%). The high number of mutations as well as splice site mutations that would eliminate its translation suggested that exon 2 may have a role in the function of the protein product.

The functions of the VHL gene protein product have been difficult to predict as there is no important homology to other proteins [18,35]. Further characterization has been performed through cellular localization studies. Immunofluorescence microscopy demonstrated that the protein product is located primarily in the cytoplasm but can also be found in the nucleus [4,18,20,41–44]. Co-immunoprecipitation of the VHL protein with two proteins of 9 and 16 kDa was also identified. These two proteins were subsequently identified as Elongin C and Elongin B, respectively [4,45–47]. This protein interaction was very weak or nonexistent when certain mis-sense mutations of the VHL gene occurred [4,44]. This relationship of the normal VHL protein and loss of this association with certain mutations has led several investigators to study protein–protein interactions. Many important interactions have subsequently been identified.

The VHL protein product normally binds tightly to elongin B and C, which are regulatory subunits of elongin (SIII), while it does not bind to elongin A [4,45,48–50]. Elongin (SIII) is known to hasten DNA transcriptional elongation by RNA polymerase II by inhibiting temporary pausing of polymerase at certain DNA sites and by controlling its release from DNA [4,18,51,52]. With binding of elongin B and C, the VHL protein is able to abort the formation of the active heterotrimeric protein elongin (SIII) [4,45]. The transcription of certain genes may be downregulated as a result of these binding sequences [18,49]. As mentioned previously, a number of VHL proteins with mis-sense gene mutations have been found to complex minimally or not at all with the Elongin regulatory subunits [4,44]. This inability to inhibit the formation of elongin may lead to the loss of regulation of transcription rates of genes important in tumour suppression [18,44,50].

The association of VHL with elongin B and C may function to promote tumour suppression in ways other than by influencing transcription rates by RNA polymerase. Cullin-2 (Cul-2), a member of the Cdc53 family of proteins, has been found to bind to VHL–elongin B/C, forming a stable tetrameric complex [4,53]. Cul-2 is involved in targeting specific proteins for ubiquitination and proteasome degradation. After being tagged with ubiquitin, proteins are destroyed by a proteasome [42,48]. VHL may therefore play a crucial role in this process by helping direct specific cellular proteins to proteasomes for degradation [48,54]. For example, HIF1-alpha, a transcription factor which enhances the transcription of hypoxia-inducible genes, undergoes ubiquitination and degradation under normoxic conditions [20,55,56]. Wild-type VHL appears to be critical for the series of steps leading to HIF1-alpha destruction [20,55]. Mutant forms of VHL theoretically could alter this relationship and cause enhanced hypoxia-inducible gene expression in normoxic conditions with neovascularization and resultant malignant growth.

Renal cancers associated with VHL are typically hypervascular. Several stimuli are believed to induce neoangiogenesis in tumours, including hypoxia and polypeptide growth factors [18,57]. Vascular endothelial growth factor (VEGF) is a polypeptide growth factor that assists in the migration, proliferation and differentiation of vascular endothelial cells [2]. This protein is normally expressed in the brain and kidney as well as other tissues, and it is markedly overexpressed in sporadic and VHL-associated RCC [2,58–61]. The VHL gene is believed to regulate VEGF [2,58,62–65]. Cell lines that lack wild-type VHL produce increased levels of hypoxia-inducible messenger RNAs such as VEGF mRNA under both hypoxic and normoxic conditions [18,58,62,63]. The restoration of the hypoxia-inducible profile of these mRNAs is possible with the reintroduction of the wild-type VHL protein into these VHL (–/–) cells, and VEGF production under normoxic conditions is prevented [2,58,62,63]. Evidence suggests that the VHL protein may regulate stabilization of these hypoxia-inducible mRNAs: VEGF mRNA stability is prolonged four times in cells without wild-type VHL compared with those in which wild-type VHL is reintroduced [2,66]. Therefore, it seems that regulation of VEGF mRNA stabilization is another important function of the VHL protein product.

Mukhopadhyay *et al.* [67] have shown that a direct interaction between the VHL gene product and the ubiquitous transcriptional activator Sp1 leads to transcriptional repression of the VEGF promoter. They found that Sp1 interacts with a specific isoform of protein kinase C in RCC causing transcriptional promotion of VEGF [64]. In the presence of the wild-type VHL protein product, Sp1 and protein kinase C interaction is inhibited, resulting in lower levels of VEGF. There may also be direct complexation of protein kinase C by the wild-type VHL protein leading to inhibited VEGF expression. Also, the VEGF receptors KDR and Flt-1 are overexpressed in sporadic and VHL-associated RCC [2,68]. The VHL protein may therefore have an important role in the control of these proteins' genes.

Another polypeptide growth factor, transforming growth factor β1 (TGF-β1), appears to be regulated by the VHL protein. This factor seems to function in a proliferative fashion through a paracrine mechanism to promote RCC formation [2,20,69,70]. Tumour development is suppressed with reintroduction of the wild-type VHL protein product which inhibits TGF-β1 production or through administration of anti-TGF-β1 antibodies [20,69].

In recent investigations, Ivanov and colleagues [4,71] have shown that the VHL gene downregulates the cell membrane spanning proteins carbonic anhydrases 9 and 12. They identified increased expression of these two proteins in two RCC cell lines which were without the wild-type VHL gene. These enzymes regulate extracellular pH and cell membrane ion channels, and evidence suggests that extracellular pH may affect invasiveness of cancer cells [20].

The VHL protein product has also been found to bind to fibronectin, an extracellular glycoprotein involved in extracellular matrix cell signalling through integrins [48,72–74]. Extracellular fibronectin matrix assembly is altered in VHL (–/–) cells, and this alteration is corrected with the reintroduction of the wild-type VHL protein [20,72]. In mutated cells, neovascularity parallels the changes in the extracellular matrix [20,75]. Tumour suppression may therefore be a result of appropriate fibronectin matrix assembly with regulation of neoangiogenesis.

VHL disease has provided investigators with a large framework to expand our knowledge of the basic molecular abnormalities leading to the development of RCC. The VHL gene and its general function as a tumour-suppressor gene have been identified, and several functions of the VHL protein product have been delineated. Mutations of the gene leading to inactivation have also been characterized and associated with specific clinical phenotypic results. Intense research continues in this disease to further understand the gene's multiple functions so that therapeutic targets might be established.

Sporadic clear cell renal carcinoma

Researchers have extensively evaluated chromosome 3 in sporadic RCC. Cytogenetic evaluation identified abnormalities of 3p in as many as 95% of renal tumours [2,76–80]. RFLP analysis for loss of heterozygosity showed consistent segmental loss on the short arm of chromosome 3 [4]. Subsequently, an area of deletion in the 3p21–26 region in clear cell renal carcinomas was described by Anglard *et al.* [81]. As this genetic locus for RCC was being further investigated and defined, Seizinger *et al.* mapped VHL disease to the short arm of chromosome 3 in the same genetic region [34]. With the identification of the VHL gene in 1993 by Latif *et al.* [35] its role in sporadic RCC formation was questioned.

Strong evidence accumulated suggesting that a single tumour-suppressor gene, the VHL gene, was responsible for both VHL-associated and sporadic clear cell RCC. Latif *et al.* showed VHL gene mutations in five sporadic RCC cell lines [35]. Gnarra *et al.* [40], through tumour evaluation from 108 patients, detected LOH at the VHL gene locus in 98% of clear cell RCC and found mutations of the remaining copy of the VHL gene in 57%. As was discussed earlier, mutations were identified in all three exons, but the majority involved exon 2 (45%), indicating that this coding region may impart an important function to the protein. No VHL gene mutations were found in 12 papillary renal carcinomas. Furthermore, Shuin *et al.* [2,82] analysed 47 sporadic RCCs and identified VHL mutations in 56% and LOH at the VHL locus in 84% of clear cell carcinomas. The same team also found no VHL gene mutations in eight non-clear cell carcinomas.

The finding that VHL gene mutation is associated with sporadic clear cell renal carcinoma provided further evidence that this gene plays a critical role in the development of clear cell renal carcinoma in general. Other genetic loci such as 3p13–14, 17p, and the p53 tumour-suppressor gene may be involved in the pathogenesis of sporadic clear cell renal carcinoma, but the data are much more convincing for the VHL gene in the development of this disease [2,83–85].

Hereditary clear cell renal carcinoma

Early studies attempting to identify the location of a gene responsible for renal carcinoma involved the analysis of families with multiple affected members. In 1979, Cohen *et al.* [86] published data on 10 family members with clear cell renal carcinoma from three generations. There was an autosomal dominant inheritance pattern present, and 8 of the 10 individuals had a balanced constitutional translocation of 3p to 8q. None of the family members with a normal karyotype developed RCC. Other investigators subsequently reported familial clear cell RCC with the development of early onset, multiple, bilateral tumours. Karyotypic analyses in these families consistently revealed translocations involving chromosome 3p and either chromosomes 2 or 6 [4,87,88].

Studies of these familial tumours has provided further evidence of the importance of VHL gene mutations in clear cell tumours. The tumours from family members with the balanced translocations t(3;8) and t(3;2) revealed deletion of one VHL allele in the chromosomal product [4,88,89]. Somatic mutations of the remaining VHL gene were identified in the majority of tumours (6/9) from these families [88,90]. Thus, the inactivation of both VHL alleles occurred leading to the development of RCC. Hereditary clear cell renal carcinomas appear to form as a result of a variation of the events leading to sporadic clear cell RCC [4,91].

Tuberous sclerosis

Tuberous sclerosis (TS) is an autosomal dominant inherited disease in 15–25% of cases, while 60–70% of cases result from *de novo* germline mutations [92,93]. The disease has been classically described as a triad of epilepsy, mental retardation and adenoma sebaceum. The birth incidence varies between 1 in 9000 and 1 in 170 000 [92,94]. Multiple hamartomas typically form in patients, including facial angiofibromas, periungual fibromas, calcified retinal hamartomas, multiple cortical tubers and renal angiomyolipomas [20]. RCC may also form in TS and can be found in approximately 2% of afflicted individuals [92]. TS-associated RCC may be single or multiple and unilateral or bilateral, and clear cell histology is the predominant type [95,96]. Two genes, tuberous sclerosis complex 1 (TSC1) on chromosome 9 and tuberous sclerosis complex 2 (TSC2) on chromosome 16, have been identified as being responsible for TS development [97].

TSC1 maps to 9q34 and contains 23 exons, and TSC2 maps to 16p13 and has 42 exons [20,98,99]. Mutations that have been reported to date in TSC1 are predicted to lead to a truncated protein product and are found mostly in familial forms of the disease [100]. Germline mutations of TSC2 include deletions, nonsense mutations and mis-sense mutations [101]. Tumours associated with TS have LOH at the TSC1 or TSC2 genetic locus as a result of inactivation of the wild-type allele [20,102,103]. This suggests that these genes are tumour-suppressor genes.

The TSC2 gene encodes a protein of 1807 amino acids [98]. With loss of the wild-type TSC2 allele, the functional protein (tuberin) is lost and tumour formation occurs. Tumour growth is suppressed *in vitro* through the introduction of wild-type TSC2 protein in TSC2 –/– RCC [20,104]. The TSC2 protein has significant homology to the catalytic domain of human guanosine triphosphatase (GTPase) activating protein 3 (GAP3) [98]. GAP proteins stimulate GTPases, and this interaction has negative regulatory effects on Ras/Ras-like protein GTP complexes [105]. Loss of function of tuberin may lead to activation of Rap1a and Rab5, which are Ras-like proteins involved in cellular signalling via growth factor receptors [20]. Hamartin is the 1164 amino acid protein product of the TSC1 gene, and it interacts with tuberin *in vivo* [106]. It may function by helping to control the same pathway as tuberin.

Papillary renal carcinoma

Papillary carcinoma is the second most common malignant tumour of the kidney. It is responsible for 10–15% of renal carcinomas [107,108]. It has a male predominance of 5–8:1. It has a better prognosis associated with it than clear cell carcinoma as the 5-year survival rate may be as high as 87–100% for stage I disease [109]. It also appears to be more common in patients on chronic dialysis for end-stage renal disease. The tumours tend to be multifocal and arise independently of one another [4,20,110] (Fig. 1.3). There are two broad classifications of this tumour: sporadic and hereditary. Papillary carcinoma is not associated with 3p mutations as found in clear cell carcinomas. Instead it is associated with a proto-oncogene, MET, found on chromosome 7. Studies have shown that the MET allele and the c-MET receptor have tumorigenic properties consisting of increased proliferation, motility, extracellular invasion and tubule formation [20,111]. Selective studies that have provided insight into the cytogenetic function of this tumour and its associated oncogene are discussed below.

Sporadic papillary carcinoma of the kidney is associated with more than one chromosomal abnormality. Roughly 80% of sporadic papillary tumours possess polysomies [110]. Tumour tissue from patients with this tumour has revealed trisomies of chromosomes 7, 16 and 17 [4,20,110,112]. Kovacs *et al.* [113] proposed that papillary carcinomas arise from papillary adenomas. Papillary adenomas are characterized by polysomies of chromosomes 7 and 17. They reported trisomy 17 in all adenomas and 80% in grade I, 50% in grade II and none in grade III carcinomas. Other chromosomal abnormalities reported include LOH and somatic translocation. Thrash-Bingham *et al.* [114] detected LOH on chromosomes 9q, 11q, 14q, 21q and 6p. Somatic translocation of chromosomes 1 and X [t(X;1)(p11;q21)] has been reported [115–117]. The transcription factor TEF3 at the breakpoint Xp11.2 has been considered to play a role in the development of this subgroup of papillary neoplasms. Fusion of the gene at 1q21.2 with the TEF3 gene seems to result in loss of transcriptional control in these tumours [117,118]. Loss of the Y chromosome in 80–90% of males with papillary renal carcinoma has also been found [113,119–121].

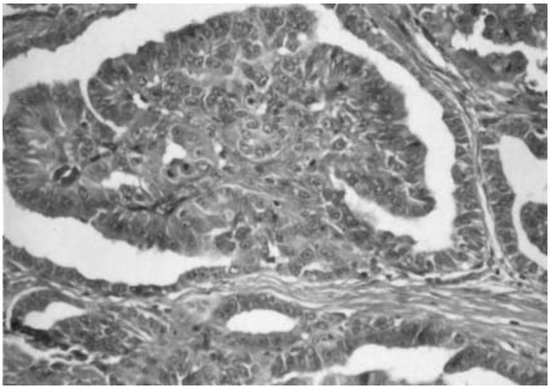

Figure 1.3 Renal cell carcinoma—papillary variant.

The high association of sporadic papillary renal carcinomas with abnormalities of chromosome 7 has led to the finding of the MET proto-oncogene, which is strongly associated with the hereditary form of this neoplasm. The gene was localized to 7q31.1–34 and is in the same supergene family (receptor tyrosine kinase) as RET, a proto-oncogene found in MEN II [4]. The MET gene is found in a high percentage of hereditary papillary renal carcinoma family members and in a subset of sporadic papillary renal carcinomas. The gene contains 21 exons spanning a 100-kb genomic region [20,122]. The gene is found in several different tissues, including neuronal tissue, endothelial cells, haematopoietic precursors, adult epithelial cells and the kidney [20,111]. Hereditary papillary renal cell carcinoma (HPRCC) is characterized by an autosomal dominant inheritance pattern and is associated with bilateral and multifocal renal tumours [4,122]. Zbar *et al.* [123–125] performed multigenerational studies of HPRCC. HPRCC family members were found to have germline mis-sense mutations of the tyrosine kinase portion of the MET gene. Another study by Schmidt *et al.* [126,127] evaluated two North American families with HPRCC. Both families possessed identical mutations in the MET region which were reportedly associated with a low penetrance: 19% at 40 years. HPRCC is clearly linked to genotypic mutations of the MET gene on chromosome 7.

Additional studies of the MET gene and c-MET receptor support its role as a proto-oncogene and not as a tumour suppressor. MET mutations studied by fluorescence *in situ* hybridization (FISH) reveal a non-random duplication of chromosome 7 [128,129]. Oncogenic properties of this gene come from studies of its amplification and mutations that result in the activation of its encoded protein. Function of the c-MET receptor is of special interest. It is a tyrosine kinase receptor involved in motility, proliferation and morphogenic signals [20]. Hepatocyte growth factor/scatter factor (HGF/SF) is its ligand [130]. Normal organogenesis is dependent on the MET receptor–HGF/SF pathway. Activation of cells possessing HGF/SF causes increased proliferation, increased motility, extracellular invasion and polarization and tubule formation [111]. Studies performed on mice with increased levels of tyrosine phosphorylation and enhanced kinase activity revealed a correlation between tumorigenesis and biological activity of this pathway [131]. Furthermore, ‘knock-out’ mice lacking HGF/SF die *in utero* associated with faulty organogenesis [20]. The C-terminal is the docking site of this receptor. Once activated by ligand binding, the receptor is upregulated by autocatalytic pathways ultimately increasing enzymatic and biological activity of the receptor [132,133]. It is this upregulation of the c-MET receptor that is suspected to result in the development of papillary renal cell carcinoma of patients with both the sporadic and the hereditary forms of this neoplasm.

The identification of the MET proto-oncogene and the receptor it encodes, c-MET, has given researchers some understanding of the formation of papillary renal carcinoma. These tumours can be either sporadic or familial. Although the exact function of the c-MET receptor has not been delineated, it clearly is important in normal organogenesis and possesses tumorigenic properties if amplified. In addition to the MET gene, other chromosomal abnormalities have been reported. Consequently, additional studies need to be performed in order to obtain a genuine understanding of the development of papillary renal carcinoma.

Wilms' tumour

Wilms' tumour is the most common malignant neoplasm of the urinary tract in children and one of the most common solid tumours of children. First described in 1814 by Rance and subsequently characterized by Max Wilms in 1899, Wilms' tumour has become an excellent model for the link between cancer and development [1]. The tumour occurs with a frequency of about 1 in 10 000 live births and approximately 350 new cases occur per year in the United States [1,134]. The peak incidence is between the third and fourth years of life, with 90% of patients presenting before the age of seven. There does not appear to be a sex predominance. Several other congenital abnormalities have been found in patients with Wilms' tumour, including aniridia, hemihypertrophy, musculoskeletal abnormalities, neurofibromatosis and second malignant neoplasms (sarcomas, adenocarcinomas and leukaemias). Wilms' tumour has also been associated with other congenital syndromes including Beckwith–Wiedemann, Denys–Drash, WAGR, Perlman and Bloom syndrome [1,135].

The biology of this tumour has been studied extensively and it is thought that its formation is due to aberrant expression of the normal developmental programme. Abnormal proliferation of metanephric blastemal tissue that lacks maturation and normal differentiation is felt to be the source of this tumour. Normal nephrogenesis is the product of controlled proliferation, differentiation and apoptosis. Wilms' tumour is likely to be due to the interruption of normal signalling, proliferation and controlled apoptosis during development [135]. Multiple chromosomal regions have been identified as playing a role in the development of Wilms' tumours; however, only the Wilms' tumour gene, WT1, has been clearly proven to play a significant role. Studies of normal gene function of WT1 suggest that its roles as a tumour suppressor and developmental regulator are crucial to normal nephrogenic development [134]. Furthermore, the presence of nephrogenic rests in Wilms' tumours supports the association of this tumour with immature blastemal tissue [135].

The WT1 gene maps to 11p13 and is expressed in normal developing nephrogenic tissue. Point mutations and LOH for alleles on 11p have been found in Wilms' tumours. Approximately 40% of cases of Wilms' tumour have been found to have LOH at the 11p alleles. The two-hit model by Knudson and Strong was based on studies of Wilms' tumour [136,137]. This model was also applied to retinoblastoma. Although the clinical observations associated with retinoblastoma fit the two-hit model appropriately, the development of Wilms' tumour appears to be more complex. The rarity of familial Wilms' tumour suggests that multiple gene abnormalities are involved [135].

The WT1 locus encodes four different proteins. It contains 10 exons and spans nearly 50 kb [134,138–141]. The gene has a zinc finger in which mutations are found. Alternative RNA splicing results in inclusion or exclusion of different exons resulting in as many as 16 WT1 isoforms [134]. It is the complex functions of these multiple splice variants that are involved in cellular development. Expression of the WT1 gene and function of its encoded proteins have been studied in the attempt to determine how WT1 regulates cellular maturation.

The exact function of WT1 is unknown but it is felt that its expression results in coordinated apoptosis, differentiation and proliferation. Haber *et al.* [142] have shown that wild-type WT1 can induce apoptosis in embryonal tumour cell lines. Englert *et al.* [143] created cell lines with WT1 expression which resulted in EGF-receptor downregulation and cell apoptosis. Studies such as these support the role of WT1 as a tumour suppressor. In addition, the development by Kreidberg *et al.* [144] of knock-out mice with a WT1 null mutation resulted in the failure of normal kidney development, suggesting WT1's role as a regulator of cell proliferation and differentiation. *In vitro* studies by Menke *et al.* [134] looked at the function of WT1 splice variants. They found that multiple WT1 isoforms may act as post-transcriptional regulators, with each having different functions and likely to be cell dependent. The specific role of WT1 as a transcriptional regulator is not clear as the physiologic ratio of splice variants and necessary cellular environment are all unknown. In all, one can conclude that the WT1 locus encodes data required by nephrogenic tissue to achieve normal developmental maturation.

Nephrogenic rests in kidneys removed for Wilms' tumour have been found to have mutations at WT1. Nephrogenic rests are microscopic residues of renal blastemal tissue, found in about 1 in 200–300 infant autopsies [135]. This immature blastemal tissue is rarely found after infancy except in kidneys removed for Wilms' tumour. It is felt that these rests are precursors for Wilms' tumour. Nephrogenic rests also occur in other syndromes associated with an increased incidence of Wilms' tumour. There are two