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Genomic profiling of papillary renal cell tumours identifies small regions of DNA alterations: a possible role of HNF1B in tumour development

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Abbreviations: RCT, renal cell tumour; BAC, bacterial artificial chromosome; MTSCC, mucinous tubular and spindle cell carcinoma; MA, metanephrogenic adenoma; RCC, renal cell carcinoma; CGH, comparative genomic hybridization; TMA, tissue microarray

Abstract

Aims: Papillary renal cell tumours (RCT) are characterized by specific trisomies. The aim of this study was to identify small regions of duplications marking putative tumour genes.

Methods and results: Full-tiling path BAC array hybridisation of 20 papillary RCTs confirmed the duplication of chromosome 7 and 17 and also displayed smaller regions of gains/amplifications of 1.3 to 13.1 Mb in size. Detailed analysis showed a microamplification of BAC clones containing the *MET* at the 7q31.2 and also amplification of a DNA segment harbouring the transcription factor *HNF1B* at chromosome 17q12. Nuclear expression of *HNF1B* protein was detected in 38 of 67 papillary RCTs, in 5 of 5 mucinous tubular and spindle cell carcinomas (MTSCC) and 5 of 5 metanephric adenomas by immunohistochemistry. Moreover, all the 9 nephrogenic rests containing tubular differentiated structures and all 14 and 5 precursor lesions associated with papillary RCTs and MTSCCs, respectively, showed a strong nuclear positivity when compared to the expressions level in proximal tubuli of the corresponding normal kidney.

Conclusions: Our finding indicates a role of *HNF1B* in association with the high frequency of chromosome 17q duplication in the development of papillary RCTs and MTSCCs as well as in their precursor lesions.

Introduction

Papillary renal cell tumours (RCT) make up approximately 10-15% of RCTs in surgical series. Trisomies or tetrasomies of entire chromosomes 7 and 17 occur in 85% and 92% of the cases, respectively.¹⁻⁴ The fact that small papillary RCTs of 1-2 mm in size display combined trisomy of chromosome 7 and 17 indicates that alteration of genes at both chromosomes are involved in the early stage of tumour development.² Partial trisomies of chromosome 7 and 17 have been described in some of the papillary RCTs.³⁻⁷ Karyotyping, microsatellite allelotyping and chromosomal comparative genomic hybridization (CGH) studies delineated partial trisomies of chromosomes 3q, 8q, 12q, 16q and 20 in 25-50% of the cases.³ Loss of the Y chromosome has been detected in 85% of the papillary RCTs arising in male patients.⁸ However, the chromosomal loci of genes involved in the development and progression of papillary RCTs, with exception of the *MET* oncogene at chromosome 7q31, have not yet been identified.⁹ This might be due to the fact that the resolution of karyotyping or chromosomal CGH is limited to 5-10 Mb, or the genetic alterations in papillary RCTs simply encounter only trisomies of entire chromosomes or large chromosomal regions of several Mb in size. The new developments in the molecular cytogenetic techniques opened the way to detect genetic imbalances at higher resolution in a single genome-wide assay using CGH to arrays containing bacterial artificial chromosomes (BAC) and commercially available microarray platforms, such as oligonucleotide and SNP-arrays. In this study we have applied a full tiling path CGH-array, which contains around 32K overlapping BAC clones, to detect small DNA segments carrying genes associated with the development and progression of papillary RCTs.

Materials and methods

TUMOUR SAMPLES

Fresh tumour and corresponding normal parenchymal tissues were obtained immediately after nephrectomy. A piece of the tumour was snap-frozen in liquid nitrogen and another part was used for cell culture as described earlier.¹ The tumours used in this study represent all developmental and clinical stages of papillary RCTs (Table 1). Samples 3a, 3b, 3c were obtained from a patient with hereditary papillary RCTs showing a germ line mutation of the *MET* oncogene.¹⁰ All other cases were without a familial history. The papillary RCTs 7a and 7b represent two distinct neoplasms of the same patient. **The 20 papillary RCTs analysed in detail in this study was included in a previous publication showing the correlation between chromosome 1q duplication and tumour progression.**¹¹ The collection and use of all tissue samples for this study was approved by the Ethics Committee of the University of Heidelberg.

DNA ISOLATION

To avoid a contamination with normal cells, especially macrophages, DNA was extracted from tumour cells growing in short-term culture as described earlier¹ and from the corresponding normal kidney tissues by phenol/chloroform treatment after proteinase K digestion (Sigma Aldrich, Taufkirchen, Germany), precipitated in ethanol and dissolved in a TE buffer. For array CGH analysis, 15 µg of high molecular genomic DNA was cleaned using QIAamp purification kit according to manufacturers' instruction (QIAGEN, Hilden, Germany). DNA integrity was electrophoretically examined on a 2% agarose gel and its concentration and quality was assessed using the NanoDropTMND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Thereafter 100 µg of DNA was sonicated by the

Soniprep150 (MSE, London, UK) and separated on a 1.2% (w/v) agarose gel. Size of the DNA was estimated by comparison with λ marker running with the samples. Only samples with approximately the same fragments length were taken for further analysis.

BAC-ARRAY CGH

The details for the production and processing of the 32K BAC arrays are described in detail elsewhere.^{12,13} Labeling of test and reference DNA, hybridization, washing, and scanning of microarrays were performed at the Nijmegen Centre for Molecular Life Science as previously described.¹¹ Spot identification and two-color fluorescence intensity measurements were acquired using the GenePix 5.0 image analysis software and the data were uploaded into the "Nijmegen Micro-Array Server" for subsequent analysis and storage. The log₂ transformed tumor/reference values were then plotted at whole-genome view scale, as well as per chromosome, with cytogenetic annotation and physical mapping positions. Regions with three or more consecutive BACs with log₂>0.27 or <-0.27 were defined as gained or deleted, respectively. A graphical viewing tool that plots all clones according to their chromosomal positions was used for the visualization of results. The gene mapping information was obtained from public database (<http://genome.ucsc.edu/>) and the NCBI hg18 assembly (Build 36.1, March 2006).

QUANTITATIVE RT-PCR

Frozen tumour and normal kidney tissue samples were homogenized in TRIzol reagent (Invitrogen, Karlsruhe, Germany) and RNA was extracted according to the manufacturer's instructions. Two μ g of total RNA was reverse transcribed with the SuperScript II reverse transcriptase (Invitrogen) and the cDNA was amplified with 0.5 μ M of each forward and reverse primer and the Platinum SYBR Green qPCR SuperMix UDG kit (Invitrogen). Detection of product amplification, melting curve analysis and semi-quantification was performed by DNA Engine Opticon system (MJ Research, Inc., Watertown, USA). Relative quantity was calculated by dividing the gene specific expression with the expression of GAPDH. All reactions were carried out in duplicates and the results were averaged using statistical analysis tools of MS Excel. Primer sequences are available upon request.

IMMUNOHISTOCHEMISTRY

Original paraffin blocks of fetal and adult kidneys, Wilms' tumours and distinct types of RCTs as well as kidneys containing precursor lesions were obtained from our tissue bank. Tissue microarrays (TMA) was constructed from paraffin embedded material after marking the areas of interest on H&E stained slides by one of the authors (GK) with a Manual Tissue Arrayer (MTA1, Beecher Instruments, Inc., Sun Prairie, USA) using 0,6 mm core biopsies. The 4 μ m sections were deparaffinized in xylene, and rehydrated in gradient ethanol. Antigen retrieval was performed in 10mM citrate buffer (pH 6.0) using the Biocare pressure cooker. The non-specific binding was blocked with 10% goat serum (Dako, Hamburg, Germany) in 1% BSA-TBS by incubation for 2h at room temperature. Primary antibodies diluted in 1%BSA-TBS, anti-PCDH11X (1:500; HPA000432, Atlas Antibodies, Stockholm, Sweden) and anti-HNF1B (1:40, HPA002083, Atlas Antibodies), were applied and incubation was performed overnight at 4°C. Endogenous peroxidase activity was quenched for 15 min with 0.3% hydrogen peroxide, following by incubation with anti-rabbit RPH-conjugated secondary antibody (Dako) for 1h at room temperature. Visualization of the antigen antibody immunoreaction was completed using AEC (Dako). The tissue sections were counterstained by immersing in Mayer's haemalaun solution (Merck, Darmstadt, Germany), mounted with

aqueous permanent mounting medium Ultramount (Dako) and cover-slipped with Pertex (Medite GmbH, Burgdorf, Germany). The slides were evaluated twice by one of the authors (GK).

Results

COPY NUMBER CHANGES AT CHROMOSOME 7 AND 17

Our full tiling path BAC-array CGH analysis confirmed the duplication of specific chromosomes in papillary RCTs (Figure 1). An increased DNA copy number level at the chromosomal regions 7p22.3-p15.1, 7p22.3-p14.2 and 7p22.3-p22.1 was seen each one time, marking the smallest overlapping duplication of approximately 7 Mb at 7p22.3-p22.1 (Table 2). Overlapping gains at chromosome 7q in two and three tumours mark the 7q11.23 and 7q22.1 DNA segments of 5.5 and 5.8 Mb in size, respectively (Table 2). One of the tumours showed the gain of signal at 7q22.1-q36.1 corresponding to at least four copies of this chromosomal segment (Figure 2A). Within this chromosomal region the amplification of around 1 Mb DNA sequences (115.684.553-116.646.410 bp) including the *MET* locus and another small amplified region of approx. 1 Mb (129.818.275-130.808.479 bp) including the *MEST* gene locus was seen (Figure 2A). Selective amplification of the *MET* locus was also detected in another papillary RCT as well.

A papillary RCT showed a gain of the entire long arm of the chromosome 17 with a higher signal level at q23.1-qter corresponding to four or more copies of the DNA segment. A gain corresponding to at least tetrasomy at 17q21.31-qter was detected in another papillary RCT as well (Figure 2B). We also found a higher copy number of an approximately 1.3 Mb DNA segment at 17q12 (32.424.242-33.731.730 bp) in the latter case. This small chromosomal region harbors 10 genes including the *HNF1B* gene.

COPY NUMBER CHANGES AT OTHER AUTOSOMAL CHROMOSOMES

Recurrent gains of entire chromosomes together with partial duplications marking the smallest overlapping regions were seen at chromosomal regions 3q22.1-q26.31 (30%), 8q24.3 (30%), 12q13.12-qter (40%), 16q22.1 and 16q24.1-24.2 (45%), and chromosome 20p (60%). We have observed the loss of chromosome 1p and gain of chromosome 1q in 5 and 6 cases, respectively. The gain at 1q21.3-q23.3 and 1q31.3-q32.1 mark the smallest overlapping regions at chromosome 1q, which are associated with the deadly outcome of the disease.¹² Aside gains at the specific chromosomes we found an overlapping loss of the signal at chromosome 9p21.3 in three cases, and gain or loss of DNA at 9q34.2-34.3 in 5 tumors. Gains or losses were also detected at chromosomal regions 10q11.2 and 10q26.3 each in 5 cases. Gains of the smallest overlapping region at chromosome 11p13 was seen in two papillary RCTs.

LOSS OF Xq21.2-Yp11.2 HOMOLOGOUS REGION

Ten of the 13 male papillary RCTs showed loss of the entire Y chromosome (Table 1), whereas one of the two X chromosomes was lost in two of the seven female tumours. The loss of a small region at Xq21.31 including the X-Y homologous block was seen in three additional female cases, and the representative profile of the X chromosome is shown in Figure 2C. Loss of the same region of Xq21.31 was also detected in 9 papillary RCTs obtained from males. Altogether, a hemizygous or homozygous loss of the approximately 3.8

Mb including the *PCDH11X/Y* and *PABPC5* genes was seen at the homologous region Xq21.31-Yp11.2 in 14 of the 20 cases.

EXPRESSION OF *HNF1B*

Real-time PCR analysis revealed an increased expression of the *MET* gene in all papillary RCTs as well as in some of the conventional and chromophobe RCCs and renal oncocytomas. **The expression of *MEST* gene was not correlated with any type of RCTs.** A lack or decreased expression of the *PCDH11XY* gene was observed in papillary RCTs in comparison to the corresponding normal kidney tissues. We did not get specific signal in paraffin embedded material with the commercially available antibody produced against the *PCDH11XY* protein. The quantitative RT-PCR analysis showed an increased expression of the *HNF1B* gene in papillary RCTs in comparison to conventional RCCs, renal oncocytomas and chromophobe RCCs as well as to normal kidneys (Figure 3). Only low level of expression was seen in Wilms' tumours. Immunohistochemical analysis revealed a strong nuclear expression of the *HNF1B* protein in 38 of the 67 papillary RCTs (Figure 4 A,B). Rarely occurring RCTs such as metanephric adenoma (MA) and mucinous tubular and spindle cell carcinoma (MTSCC), each of 5 cases showed also a strong positive staining (Figure 4 C,D). A scattered positivity was seen in the nuclei of 7 of the 98 conventional RCCs (Figure 4 E,F). Only one of the 18 renal oncocytomas but none of the 21 chromophobe RCCs displayed a positive staining (Figure 4 G,H).

As the *HNF1B* expressed preferentially in adult RCTs of embryonal rest origin, we have stained fetal kidneys and Wilms' tumours as well as precursor lesions (nephrogenic rests) associated with clinically recognized papillary RCT, MTSCC, or Wilms' tumour. In fetal kidneys the blastemal cells, S-shape bodies and primitive tubuli were negative whereas differentiating tubuli especially proximal tubuli displayed a weak to strong nuclear positivity (Figure 5 A). Similar staining pattern was seen in Wilms' tumours (Figure 5 B,C). All the 9 nephrogenic rests containing tubular differentiated structures and also all the 14 and 5 precursor lesions associated with papillary RCTs and MTSCCs, respectively, showed a strong nuclear positivity when compared to the expressions level in proximal tubuli of the corresponding normal kidney (Figure 5 D-F).

Discussion

By applying full tiling path BAC-arrays we confirmed the high specificity of chromosomal duplications in papillary RCTs, which were revealed nearly 20 years ago by classical karyotyping.^{1,2} In addition, we have delineated smaller regions of losses/amplifications of 1.3 to 13.1 Mb in size (and also three large regions of 27.4-80.7 Mb) at these chromosomes. However, these regions with exception of chromosome 7q31 and 17q12 amplification and Xq21.31-Yp11.2 loss, harbor 25 to over 100 genes, which makes it difficult to identify the gene(s) responsible for the tumorigenesis.

Until now, only one gene located at the specific chromosomes has been associated with papillary RCTs. The *MET* gene at chromosome 7q31 was identified by linkage analysis of families with hereditary papillary RCTs.⁹ Although germline mutation in the tyrosine kinase domain of the *MET* oncogene is segregated with the development of multiple papillary RCTs and the mutated *MET* allele is consequently duplicated in the cancer cells, mutation of the *MET* was seen in only 5-13% of the sporadic tumours.^{10,14,15} In this study, we detected a selective amplification of the *MET* locus in two papillary RCTs and also a duplication of the *MET* locus (and the locus of its ligand *HGF*) in 80% of the cases. Duplication/amplification of the *MET* locus may explain by its dosage effect the increased expression of the gene in papillary RCTs.^{10,15,16} The specific expression of the *MET* in papillary RCTs has also been confirmed by immunohistochemical analysis.¹⁷ The *HGF/MET* mediates morphogenic,

mitogenic, angiogenic and motogenic signals and plays a crucial role in the embryonal kidney development.¹⁸ Thus, the selective amplification of the *MET* loci and its overexpression strongly suggest its role in the development of sporadic papillary RCTs as well.

Our study now confirmed the specific duplication of the large chromosome 17q21.31-qter region which was previously reported and specified to an overlapping duplication at the chromosome band 17q21.32 by fluorescent microsatellite analysis.^{3,5-7} **In addition, we found the duplication/amplification of chromosome 17q12 region containing the hepatocyte nuclear factor 1 beta (*HNF1B*) gene.**

The *HNF1B* is expressed at early stages of organ development and restricted only to polarized epithelial cells in adult.¹⁹ The renal tubular system develops from mesodermal blastem, which becomes polarized during induction by the ureteric bud. It was shown that overexpression of the dominant negative form or inactivation of the *HNF1B* leads to cystic changes of the renal tubules.^{20,21} The loss of *HNF1B* function may affect development of kidneys and urinary tract.²² Mutation or altered expression of the *HNF1B* depending on the expression of its coactivators may lead to restricted or delayed tubulogenesis in early stages of nephrogenesis, whereas disruption of the interactions after the renal tubulogenesis is completed may rise to cystic changes.^{19,23,24} We showed in this study that *HNF1B* is expressed first in differentiating tubular cells in embryonic kidneys. Wilms' tumor recapitulates the embryonal kidney development and may composed of blastemal, tubular and stromal cells. The nephrogenic rests associated with Wilms' tumour also display blastemal cells as well as partially differentiated tubular cells. As we showed here, the *HNF1B* protein is expressed only in tubular differentiated cells of nephrogenic rests and Wilms' tumours.

We have suggested previously that papillary RCTs develop from embryonal rest, which persist during life.^{25,26} We showed in this study that **all precursor lesions for papillary RCTs and MTSCCs as well as all MTSCCs and MAs and 56% of papillary RCTs are consequently positive for *HNF1B* staining.** Our data suggest that constitutive overexpression of the *HNF1B* in papillary RCTs, MTSCCs and MAs and their precursor lesions is associated with the maintenance of delayed tubular differentiation. It is not yet known, whether the precursor lesions in kidneys with Wilms' tumour and also with papillary RCT and MTSCC share common genetic alterations or their cellular differentiation corresponds to distinct stages of nephrogenesis. The high *HNF1B* expression in all precursor lesions in our series suggests that a delayed tubular differentiation **may play** a role in the development of each type of precursors **and hence in the development of papillary RCTs and related tumours of the kidney.** Heterozygous mutation of the *HNF1B* leads to a special form of type 2 diabetes mellitus, so-called maturity-onset diabetes of the young, type 5 (MODY5) and associates with dysplastic kidneys.²⁷ Mutation of the *HNF1B* has been described in two chromophobe RCCs that developed in MODY5 patients.²⁸ The germline mutation and loss of heterozygosity around the *HNF1B* locus at chromosome 17q12 resulted in biallelic inactivation of the gene in tumour cells. Therefore, the authors suggested that the *HNF1B* may function as a tumour suppressor gene in chromophobe RCCs. However, MODY5 is associated with a germline mutation of *HNF1B* and LOH occurs along the entire chromosome 17 in 90% of chromophobe RCCs.^{29,30} Therefore, the occurrence of mutation and LOH in these cases seems to be coincidental and not instrumental.

In summary, we found the gain/amplification of the chromosome 17q12 sequences containing the *HNF1B* locus and showed that the *HNF1B* protein is expressed only in differentiating tubuli of fetal kidney and Wilms' tumours, and overexpressed in adult tumours of embryonal origin, such as papillary RCTs, MAs and MTSCCs and in their precursor lesions. Papillary RCTs display a combined trisomy of chromosome 7 and 17 as the only karyotype change in an early stage of development. Therefore, we suggest that in addition to gain/amplification and overexpression of the *MET* gene at chromosome 7q21, the gain/amplification and overexpression of the *HNF1B* gene at chromosome 17q12 is associated with the delayed

tubular differentiation in precursor lesions. Better understanding the signalling pathway how the *HNF1B* controls the renal tubulogenesis may help to understand the molecular basis of delayed cellular differentiation in precursor lesions, which persist during life and give to rise to MAs, MTSCCs and papillary RCTs.

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Legends

Figure 1. Representative BAC array CGH profile of papillary RCT. Gain of chromosome 3, 7, 12, 16, 17 and 20 as well as loss of the Y chromosome are shown as horizontal bars upon and under a zero line, respectively.

Figure 2. Array CGH profile of chromosome 7, 17 and X. (A) Line right from 0 marks the DNA profile along the chromosome 7 indicating the gains at the regions 7p22.3-p15.3; 7p12.3-q11.22; 7q11.23-q21.11; 7q21.3-q36.1. (B) Line right from 0 shows duplication of 17q arm with the 17q23.1-qter corresponding to four copies of the DNA segment. (C) Line left from 0 indicates loss at the Xq21.31-q21.32 region.

Figure 3. Relative expression of the *HNF1B* in RCTs by real-time PCR. Transcript copy number of *HNF1B* was normalised to the copy number of the reference *GAPDH*. Copy numbers for the individual groups of tumours were summarised and the standard error of the mean was calculated. (cRCC - conventional RCC, WT - Wilms' tumour, RO - renal oncocytoma, chRCC - chromophobe RCC, NK - normal kidney)

Figure 4. Immunohistochemical analysis of *HNF1B* in RCTs. (A,B) Positive nuclear staining reaction was seen in papillary RCTs irrespectively of the cellular differentiation. (C,D) Strong nuclear positivity in MA and MTSCC, respectively. (E) No staining for *HNF1B* in a conventional RCC. (F) Occasionally, positive reaction was seen in conventional RCCs as shown here. (G,H) Renal oncocytomas and chromophobe RCCs were negative for *HNF1B* staining.

Figure 5. Immunohistochemical analysis of *HNF1B* in fetal kidneys, Wilms' tumours and precursor lesions (nephrogenic rests). (A) Nuclear positivity was only seen in differentiating tubuli in fetal kidneys. (B) Negative *HNF1B* staining in Wilms' tumour but nuclear positivity in differentiating tubuli of surrounding nephrogenic rest and in normal tubular cells. (C) Positive *HNF1B* immunoreactivity in tubular differentiating cells but no staining in stromal and blastemal cells of a Wilms' tumour. (D) Although the less differentiated part of nephrogenic rest associated with Wilms' tumor was negative, some of the differentiating tubuli were strongly positive. (E,F) Precursor lesions associated with papillary RCTs and MTSCCs displayed a strong nuclear positivity in comparison to normal tubular cells.

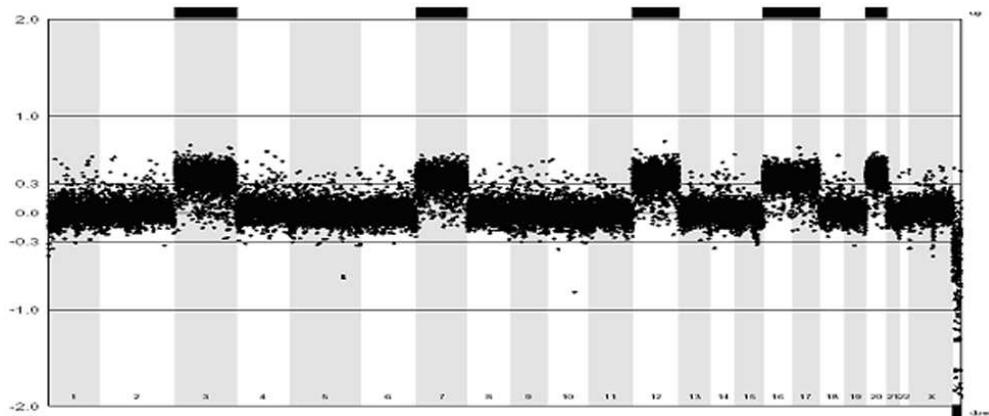


Figure 1. Representative BAC array CGH profile of papillary RCT. Gain of chromosome 3, 7, 12, 16, 17 and 20 as well as loss of the Y chromosome are shown as horizontal bars upon and under a zero line, respectively.
82x34mm (300 x 300 DPI)

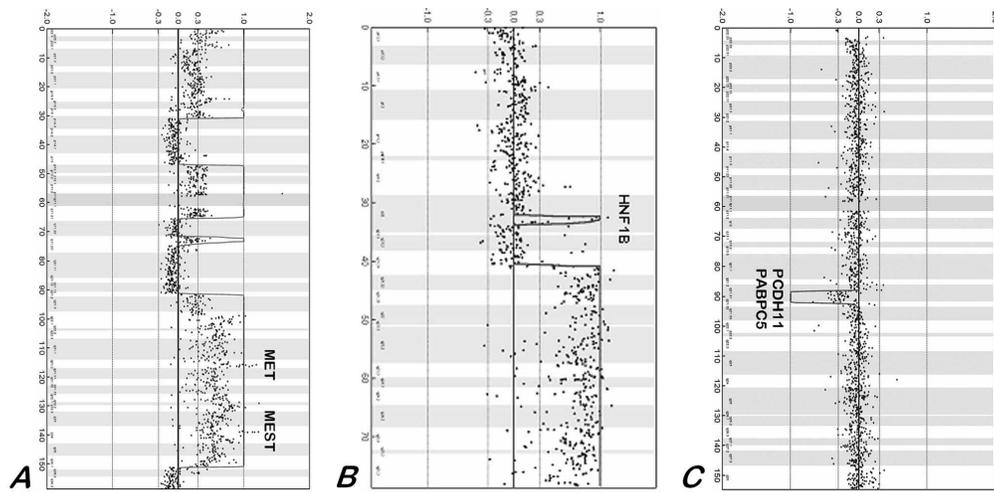


Figure 2. Array CGH profile of chromosome 7, 17 and X. (A) Line right from 0 marks the DNA profile along the chromosome 7 indicating the gains at the regions 7p22.3-p15.3; 7p12.3-q11.22; 7q11.23-q21.11; 7q21.3-q36.1. (B) Line right from 0 shows duplication of 17q arm with the 17q23.1-qter corresponding to four copies of the DNA segment. (C) Line left from 0 indicates loss at the Xq21.31-q21.32 region.
170x82mm (300 x 300 DPI)

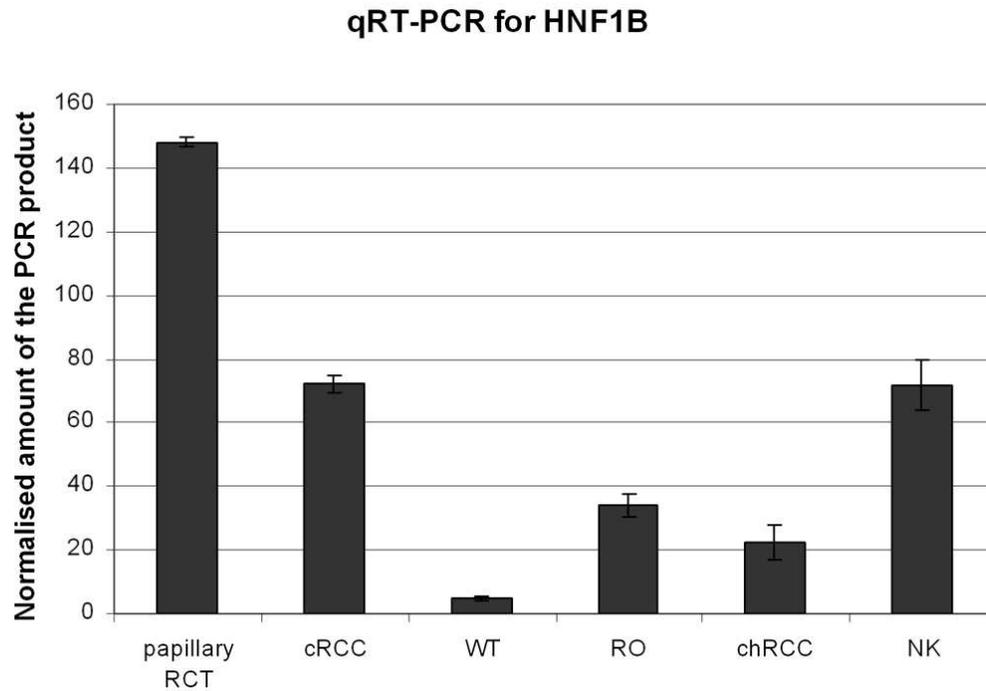


Figure 3. Relative expression of the HNF1B in RCTs by real-time PCR. Transcript copy number of HNF1B was normalised to the copy number of the reference GAPDH. Copy numbers for the individual groups of tumours were summarised and the standard error of the mean was calculated. (cRCC - conventional RCC, WT - Wilms' tumour, RO - renal oncocytoma, chRCC - chromophobe RCC, NK - normal kidney
85x59mm (300 x 300 DPI)

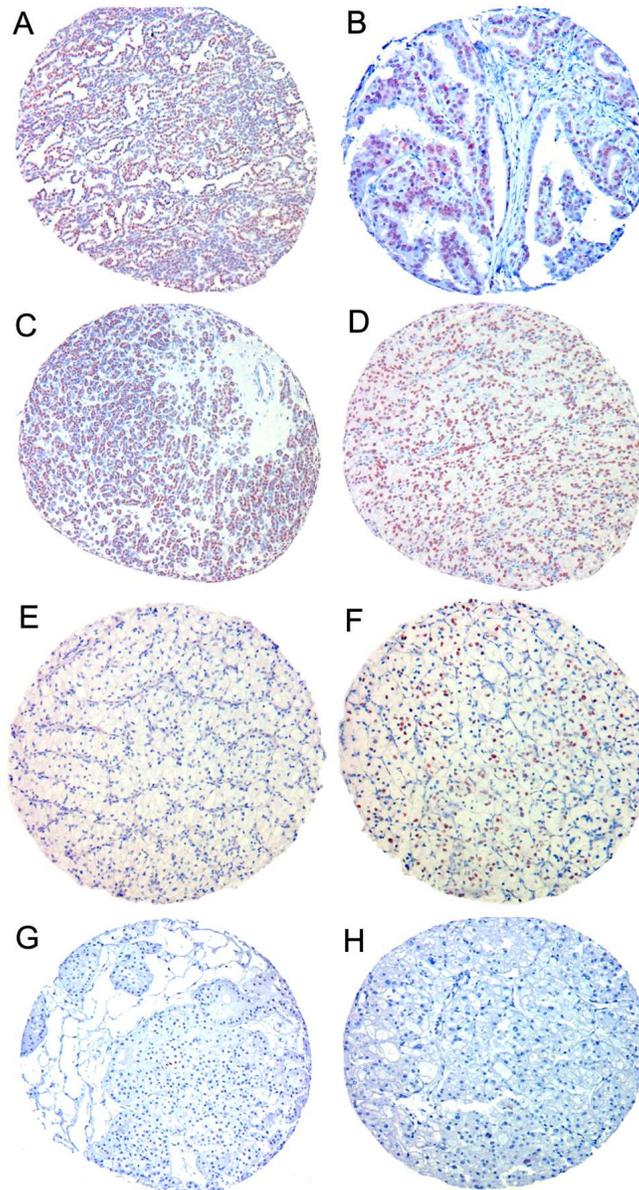


Figure 4. Immunohistochemical analysis of HNF1B in RCTs. (A,B) Positive nuclear staining reaction was seen in papillary RCTs irrespectively of the cellular differentiation. (C,D) Strong nuclear positivity in MA and MTSCC, respectively. (E) No staining for HNF1B in a conventional RCC. (F) Occasionally, positive reaction was seen in conventional RCCs as shown here. (G,H) Renal oncocytomas and chromophobe RCCs were negative for HNF1B staining.
82x151mm (300 x 300 DPI)

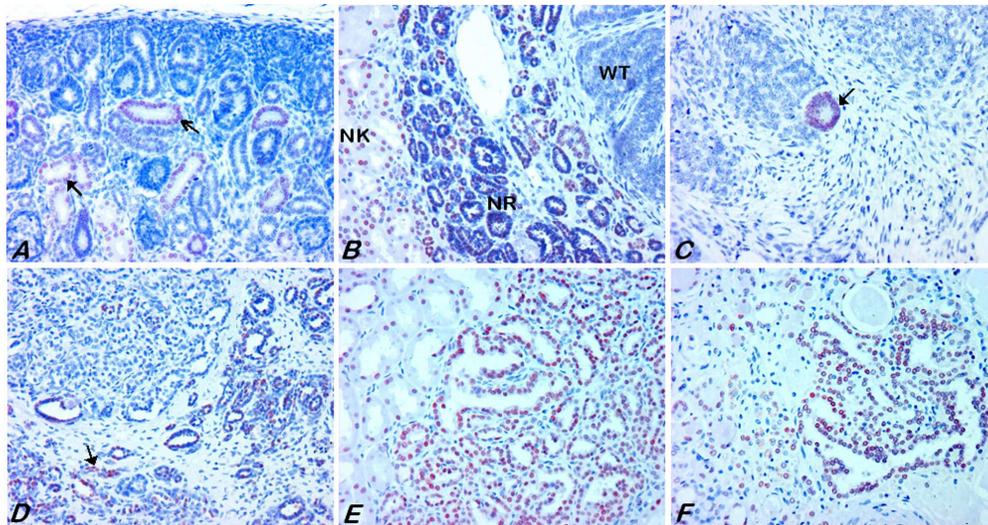


Figure 5. Immunohistochemical analysis of HNF1B in fetal kidneys, Wilms' tumours and precursor lesions (nephrogenic rests). (A) Nuclear positivity was only seen in differentiating tubuli in fetal kidneys. (B) Negative HNF1B staining in Wilms' tumour but nuclear positivity in differentiating tubuli of surrounding nephrogenic rest and in normal tubular cells. (C) Positive HNF1B immunoreactivity in tubular differentiating cells but no staining in stromal and blastemal cells of a Wilms' tumour. (D) Although the less differentiated part of nephrogenic rest associated with Wilms' tumor was negative, some of the differentiating tubuli were strongly positive. (E,F) Precursor lesions associated with papillary RCTs and MTSCCs displayed a strong nuclear positivity in comparison to normal tubular cells.

170x89mm (300 x 300 DPI)

Table 1. Pertinent clinicopathological and genetic data of papillary renal cell tumours

NR.	AGE SEX	STAGE	GRADE	SIZE [mm]	AUTOSOMES		SEX CHROMOSOMES		
					GAIN	LOSS	LOSS Xq21.3	Y	
1	41 M	T1,NX,MX	G1	26	7*, 17, 20, 21				
2	62 M	T1,N0,MX	G1	65	7, 12, 17, 20, 21			yes	yes
3a	37 F	T1,N0,M0	G2	21	7, 8q*, 16*, 17, 19, 20, 22			yes	
3b	37 F	T1,N0,M0	G1	23	7, 17			yes	
3c	37 F	T1,N0,M0	G1	20	7, 17			yes	
4	75 F	T1,N0,MX	G1	38	3, 7, 12, 16, 17, 20			yes	
5	80 M	T1,N0,M0	G2	35	3, 7, 8, 16, 17, 20			yes	yes
6	72 M	T1,N0,M0	G2	48	4q, 5*, 7, 12, 16, 17, 19				yes
7a	64 M	T1,N0,M1	G2	25	3, 7, 12, 16, 17, 20			yes	yes
8	33 F	T1,N0,MX	G1	40	7, 17	18			
9	74 M	T1,N0,M0	G2	65	7, 8, 17	16, 19		yes	yes
10	55 F	T2,N0,M0	G2	75	16, 17, 20				
11	47 M	T1,N0,M0	G1	17	3q, 7, 12q*, 17, 20			yes	yes
12	52 M	T1,N2,MX	G2	55	1q, 5, 9q*, 17q, 19, 22	1p, 9p*, 9q*, 12, 15, 17p			
13	74 M	T3a,N2,M1	G3	105	1q, 16, 20	1p, 6, 22		yes	yes
14	67 M	T1,N0,M1	G2	25	7, 12, 16, 17, 20	11q*, 21		yes	yes
7b	64 M	T2,N0,M0	G2	150	1q	1p		yes	yes
15	59 F	T4,NX,M1	G1,G3	130	1q, 7, 8q*, 12, 17, 18p, 20	5p, 8p, 9p		yes	
16	74 M	T3a,NX,MX	G3	160	1q, 3q*, 7p, 17q*	1p, 4, 6q, 10p, 16q, 22			
17	57 M	T4,N2,M1	G3	95	1q, 7, 8q, 16, 20	1p, 8p*, 11, 15, 21, 22		yes	yes

* indicates a partial gains or loss of chromosomal arms

Table 2. Smallest overlapping copy number changes at chromosomal regions of **gains** and **losses**.

Change	Cytoband	BAC position (start)	Size [Mb]	Known genes
Loss	1p36.33-p36.31	2,177,963-5,601,878	3.4	25
Loss	1p36.11-p34.3	24,033,566-35,911,112	11.8	>100
Gain	1q21.3-q23.3	144,209,246-157,382,646	13.1	>100
Gain	1q31.3-q32.1	195,131,525-204,963,741	9.8	87
Gain	3q22.1-q26.31	132,016,257-175,224,751	43.2	>100
Gain	7p22.3-p22.1	103,198-7,142,251	7.1	62
Gain	7q11.23	71,938,022-77,424,475	5.5	74
Gain	7q22.1	97,607,500-103,430,677	5.8	90
Gain	8q24.3	143,839,800-146,172,071	2.3	69
Gain	12q13.12-qter	51,555,670-132,309,499	80.7	>100
Gain	16q22.1	64,781,994-69,027,246	4.2	81
Gain	16q24.1-q24.2	82,481,483-85,999,642	3.5	30
Gain	17q12	32,424,242-33,731,730	1.3	10
Gain	17q21.31-qter	40,683,734-78,359,502	27.4	>100
Loss	Xq21.31	88,140,433-92,212,179	3.8	3

The localisation and size of aberrations within the genome was estimated from the position of an individual BAC clone according to the UCSG Human Browser on Mar 2006 Assembly. Abbreviations: BAC-bacterial artificial chromosome; Mb-megabase