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Expression of the vitamin D receptor, 25-hydroxylases, 1α-hydroxylase and 24-hydroxylase in the human kidney and renal clear cell cancer☆

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ABSTRACT

Background: The vitamin D receptor (VDR), CYP27B1 and CYP24A1 are expressed in the human kidney, but the segmental expression of the 25-hydroxylases is unknown. A comprehensive analysis of CYP2R1, CYP27A1, CYP27B1, VDR and CYP24A1 expression in normal kidney and renal clear cell cancer (CCc) would reveal the segmental location of expression, and clarify whether the reported loss of VDR in CCc is coincident with alterations of vitamin D metabolism.
Materials and methods: Tissue was obtained from nine patients (eight CCc and one atrophic kidney), mRNAs were detected with RT-PCR and *in situ* hybridisation (ISH), and expression of proteins determined by immunohistochemistry and western blotting.
Results: We detected expression of VDR and the vitamin D metabolising enzymes in normal kidney. VDR and CYP27B1 were strongly expressed in proximal tubules, while CYP2R1 and CYP27A1 had a marked expression in distal tubules. In CCc expression was lost for VDR and all the enzymes, except for very few cells expressing all the investigated proteins.
Conclusion: This study shows that VDR and all the vitamin D metabolising enzymes are expressed in the normal kidney. During the malignant transformation to CCc, expression of VDR and the metabolising enzymes is lost, however the implications of this loss are unknown.

1. Introduction

The systemic activation of vitamin D is a tightly controlled process, which follows conversion of 7-dehydrocholesterol to cholecalciferol by UVB radiation in the skin. Cholecalciferol is metabolised by the 25-hydroxylases (CYP2R1 and CYP27A1) in the liver, before 25-hydroxycholecalciferol ($25(OH)D_3$) relocates to the circulation. After glomerular filtration cellular uptake of $25(OH)D_3$ is facilitated by megalin and cubilin in the proximal tubules (PT) [1] and the 1α-hydroxylase (CYP27B1) subsequently converts the intracellular $25(OH)D_3$ to the active $1,25(OH)_2D_3$ (calcitriol). Calcitriol activates the vitamin D receptor (VDR) in the target cell and is inactivated by CYP24A1 [2].

Co-localisation of VDR, CYP27B1 and CYP24A1 in the human kidney has been shown in PT and to some extent in the distal segments of the nephron [3], but the segmental expression of CYP2R1 and CYP27A1 has previously not been shown [4,5]. Extra-renal expression of CYP27B1, CYP24A1 and occasionally CYP27A1 has been shown in tissues previously thought only to be vitamin D (vD) responsive [34]. This extra-renal metabolism of (vD) appears to be regulated differently, and might be important for the immune system and cell cycle control [6]. This is supported by studies showing $1,25(OH)_2D_3$ mediated inhibition of clonal proliferation in a variety of human malignancies, and calcitriol-promoted differentiation of both normal and malignant cells [2]. During malignant transformation in colon, mammary tissue and prostate, the expression of the 1α-hydroxylase diminishes simultaneously with the loss of VDR [2]. These alterations in the level of CYP27B1 and VDR expression are associated with the histological subtype and grade of the tumour [7].

Renal cell carcinoma (RCC) is a heterogeneous disease and clear cell carcinoma (CCc) is the most frequent (70%) RCC subtype [8]. They are characterised by having multiple chromosomal aberrations, often including a deletion or a mutation on chromosome 3 in the von Hippel–Lindau (VHL) gene. VHL is a tumour suppressor

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Table 1
Primers used for RT-PCR and preparation of ISH probes.

VDR	GGAGAAAACACTTGTAAGTTGCT	TGGTCAGGTTGGTCTCGAACT	208 bp
ISH[a]	AATTAACCCTCACTAAAGGGTTGCTAAACGAGTC	TAATACGACTCACTATAGGGTCTCGAACTCC	182 bp
CYP2R1	AGAGACCCAGAAGTGTTCCAT	GTCTTTCAGCACAGATGAGGTA	259 bp
CYP24A1	CGGACTCTTGACAAGGCAACA	TGAGGCGTATTATCGCTGGCA	171 bp or 369 bp
	GGCCTCTTTCATCACAGAGCT	GCCTATCGCGACTACCGCAA	190 bp
ISH[a]	AATTAACCCTCACTAAAGGGCGACTACCGCAAAG	TAATACGACTCACTATAGGGCATCACAGAGCTCAT	174 bp
CYP27A1	GGCAAGTACCCAGTACGG	AGCAAATAGCTTCCAAGG	292 bp
ISH[a]	AATTAACCCTCACTAAAGGGAGCTATGGAAGGA	TAATACGACTCACTATAGGGTTCCCCGAAGCA	212 bp
CYP27B1	CCTGGCAGAGCTTGAATTGCA	GGGGAAGATGTATACCTTGGT	242 bp
RPS20	AGACTTTGAGAATCACTACAAGA	ATCTGCAATGGTGACTTCCAC	179 bp

[a]The added T3- and T7-promotor sequences, respectively, are underlined. All primers shown in 5′–3′ direction.

gene and impaired function leads to an increase in the production of HIF (hypoxia inducible factor), which promotes tumour formation [9]. The incidence of renal cancer is higher in countries located at higher latitude, and UVB radiation is inversely associated with the development of the cancer, indicating that high circulating vitamin D concentrations may protect against RCC [10–12]. Supportive data showed lower serum concentrations of vD in patients with RCC compared to controls, and patients with T3 and T4 tumours (>7 cm) had lower vD concentrations than patients with T1 and T2 tumours (<7 cm) [13,14]. Previous studies show that CCcs do not express VDR, except for a few cells near the border of the tumour, but the implications for this loss is unknown [15–18]. Alterations in VDR expression or function are only one of several disruptions to vitamin D metabolism, because the cellular response to vD also depends on uptake of substrate and presence of the metabolising enzymes. Therefore, we performed this comprehensive analysis to investigate if the reported loss of VDR expression in CCc is associated with altered expression of CYP2R1, CYP27A1, CYP27B1 and CYP24A1.

2. Materials and methods

2.1. Patients and ethics

Patients were recruited from Rigshospitalet, Denmark, and patient material was obtained in accordance with the Helsinki declaration and initiated after approval from the local ethics committee, journal KF 01 2006-3472. Nine kidneys were obtained after nephrectomy, in eight cases induced by malignancy, while one case was caused by severe hypertension and small kidney size. 7/8 tumours were CCcs, and one was an unclassified carcinoma (uRCC). A pathologist divided the tissue block into normal (subdivided into cortex and medulla) and malignant tissue. One part was frozen at −80 °C, while the remaining was fixed in formalin. The normal tissue was obtained from a small atrophic kidney with no malignant disease and from normal tissue surrounding the tumours. In addition, we purchased normal kidney RNA (Clontech, Takora Bio Europe: named Normal) for RT-PCR analysis.

2.2. RT-PCR and preparation of probes for in situ hybridisation

Total RNA was extracted from a representative sample of the frozen specimens and isolated with NucleoSpin RNA II purification kit as described by the manufacturer (Macherey-Nagel, Düren, Germany). cDNA was synthesized using a dT20 primer and random hexamers. RT-PCR was performed using specific primers targeting each mRNA and spanning intron–exon boundaries, including RPS20 (ribosomal protein S20) as an internal control (Table 1). Cycle conditions were: one cycle of 5 min at 95 °C; 40 cycles of 30 s at 95 °C, 1 min at 64 °C (CYP27B1, VDR, CYP24A1) or 62 °C (CYP2R1, CYP27A1 and RPS20), 1 min at 72 °C and one cycle of 5 min at 72 °C. Representative bands from each primer combination were excised and sequenced for verification (Eurofins MWG GmbH, Germany).

Probes for ISH were prepared by reamplification of the RT-PCR fragments using specific primers with an added T3- and T7-promotor sequence (Table 1). PCR conditions were: 5 min 95 °C; 5 cycles of 30 s 95 °C, 1 min 45 °C, 1 min 72 °C; 20 cycles of 30 s 95 °C, 1 min 65 °C, 1 min 72 °C and finally 5 min 72 °C. The resulting PCR product was purified on a 1% agarose gel and sequenced (Eurofins MWG). Aliquots of 200 ng were used for *in vitro* transcription labelling, using the MEGAscript-T3 (sense) or MEGAscript-T7 (anti-sense) kits, as described by the manufacturer (Ambion, TX, USA).

2.3. Immunohistochemistry (IHC) and western blot (WB)

Paraffin sections were deparaffinised and rehydrated. Antigen retrieval was accomplished by microwaving the sections for 15 min in TEG buffer (Tris 6.06 g, EGTA 0.95 g in 5 L, pH 9.0). All sections were afterwards incubated with 0.1% bovine serum albumin (BSA) to minimize cross-reactivity. Commercially polyclonal antibodies were purchased from Santa Cruz Biotechnology Inc., Santa Cruz, CA (CYP2R1 (C-15) sc-48985, CYP27A1 (P-17) sc-14835, CYP27B1 (H-90) sc-67261, VDR (H-81): sc-9164, CYP24 (H-87): sc-66851). CYP2R1 and CYP27A1 were goat polyclonal antibodies and CYP27B1, VDR and CYP24 were rabbit polyclonal antibodies. The optimal dilution of the primary antibody was: CYP2R1 1:50, CYP27A1 1:100, CYP27B1 1:100, VDR 1:100 and CYP24 1:200. After 16 h of incubation, the sections were incubated with AP-conjugated goat anti rabbit IgG-AP (sc 3838) or donkey anti-goat IgG-AP (sc 3852) (both diluted 1:250) for 30 min. NBT BCIP (nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate) was used for visualization and levamisol was used to block endogenous alkaline phosphatase (AP). For negative control, the primary antibody was replaced by 0.1% BSA in TBS. Two independent investigators (MBJ and CBA), blinded to patient data, evaluated all slides. Staining was classified according to an arbitrary semi quantitative IHC reference scale ranging from negative to +++ depending on the cytoplasmic/nuclear staining and the proportion of cells stained: +++, strong staining in nearly all cells; ++, moderate staining in most cells; +, weak staining or a lower percentage of cells stained; ±, very weak staining in few cells; neg., no positive cells detected.

Tissue preparation (frozen tissues from clear cell carcinoma and normal tissue surrounding the tumours), SDS-polyacrylamide gel electrophoresis and WB were carried out as previously described [19]. The primary antibodies described in the IHC section were used in 1:200 concentration. A monoclonal mouse anti α-tubulin (1:200) antibody was used as control (Santa Cruz Biotechnology Inc., Santa Cruz, USA, sc-8035). Secondary antibodies described for IHC were diluted 1:1000.

2.4. In situ hybridisation

ISH was performed essentially as described previously [20]. The ISH procedure in brief: deparafinised sections (4 μm) were re-fixed in 4% parformadehyde (PFA), treated with proteinase K

Table 2
Description of specimens and assessment of the IHC expression of VDR, CYP2R1, CYP27A1, CYP27B1, and CYP24A1.

Tissue	VDR	CYP2R1	CYP27A1	CYP27B1	CYP24A1
Normal kidney ($N=8$)	Glom. Bow + PT ++ DT + CD + Int. ±	Glom. negative PT + DT +++ CD+ to ++ Int. negative	Glom. negative PT + DT +++ CD ++ Int. negative	Glom. Bow ± PT +++ DT + CD neg to ± Int. +	Glo.mes, Bow+ PT+ to ++ DT + CD neg to ± Int.+
Atrophic kidney	Atro.tubuli +	Atro.tubuli +++	Atro.tubuli +++	Atro.tubuli +	Atro. tubuli +
CCc 1	Negative	Negative	Negative, few +	Negative	Negative, few ++
CCc 2	Negative, few +	± to +	±	Negative, few+	Negative, few ±
CCc 3	Negative	Negative	± to +	Negative, few ±	Negative
CCc 4	Negative	Negative	Negative	Negative	Negative
CCc 5	Negative	Negative	±	Negative, few ±	Negative, few ±
CCc 6	Negative, few +	Negative	Negative, few ++	Negative, few +	Negative, few +
CCc 7	Negative, few +	Negative, few ±	Negative, few ++	Negative, few +++	Negative, few +++
Unclassified RCC	Negative, few ±	Negative	Negative, few +	Negative, few +	Negative, few ±
Subcellular location	Cytoplasmic/nucl	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic

Neg.: not stained; ±: barely detectable; +: weak staining; ++: moderate staining; +++: strong staining; few: less than 5%; endo: endothelium; Atro.: atrophic; Glom: glomeruli; PT: proximal tubules; DT: distal tubules; CD: collecting ducts; Int.: interstitial cells; Mes: mesangial cells; Bow: Bowman's capsule; nucl: nuclear.

(Sigma–Aldrich, St Louis, MO, USA) (1.0, 2.5, 5.0 or 7.5 mg/mL), post-fixed in PFA, pre-hybridised 1 h at 49 °C, and hybridised overnight at 49 °C with biotinylated or digoxigenin anti-sense or sense control probes. Excess probe was removed with 0.1× standard saline citrate (58 °C) 3 × 30 min. Visualization was performed using anti digoxigenin-AP 1:1000 (Cat. No. 1093274; Roche Diagnostics GmbH, Germany) or streptavidin conjugated with alkaline phosphatase (1:1000) (Cat. No. 1093266; Roche Diagnostics GmbH,

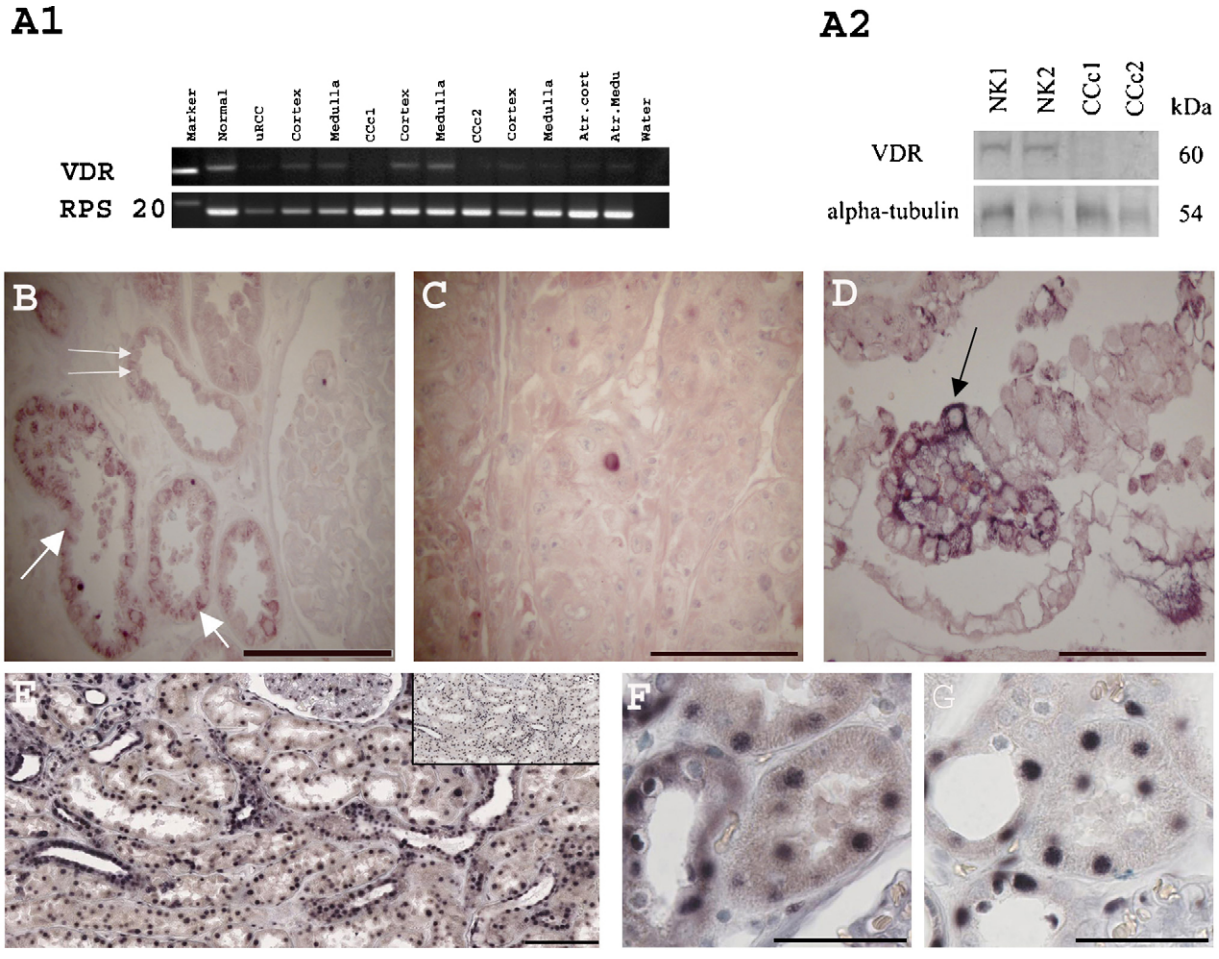

Fig. 1. Expression of VDR in normal and malignant tissue. (A1) RT-PCR analysis of mRNA expression. From left: normal RNA (healthy donor); uRCC, unclassified RCC; followed by cortex and medulla from the same patient; CCc 1, clear cell carcinoma from patient 1, followed by cortex and medulla from the same patient; CCc 2, clear cell carcinoma from patient 2, followed by cortex and medulla from the same patient; Atr. cortex and Atr. medulla, cortex and medulla from patient with atrophic kidney. (A2) Western blot of VDR expression in normal and malignant kidney tissue; alpha-tubulin was included as a control. (B) IHC analysis of VDR in normal kidney; (C) IHC of VDR in CCc. (D) IHC detection of VDR in a CCc with more differentiated cells. (E–G) ISH analysis of the expression of VDR mRNA in normal kidney; (E) overview of VDR mRNA expression with the corresponding sense probe inserted; (F) and (G) high resolution of ISH with VDR anti-sense and sense probes, respectively. White arrows, proximal tubules (PT); double arrows, distal tubules (DT); black arrow indicates more differentiated cells from a CCc. Bar corresponds to 100 μm.

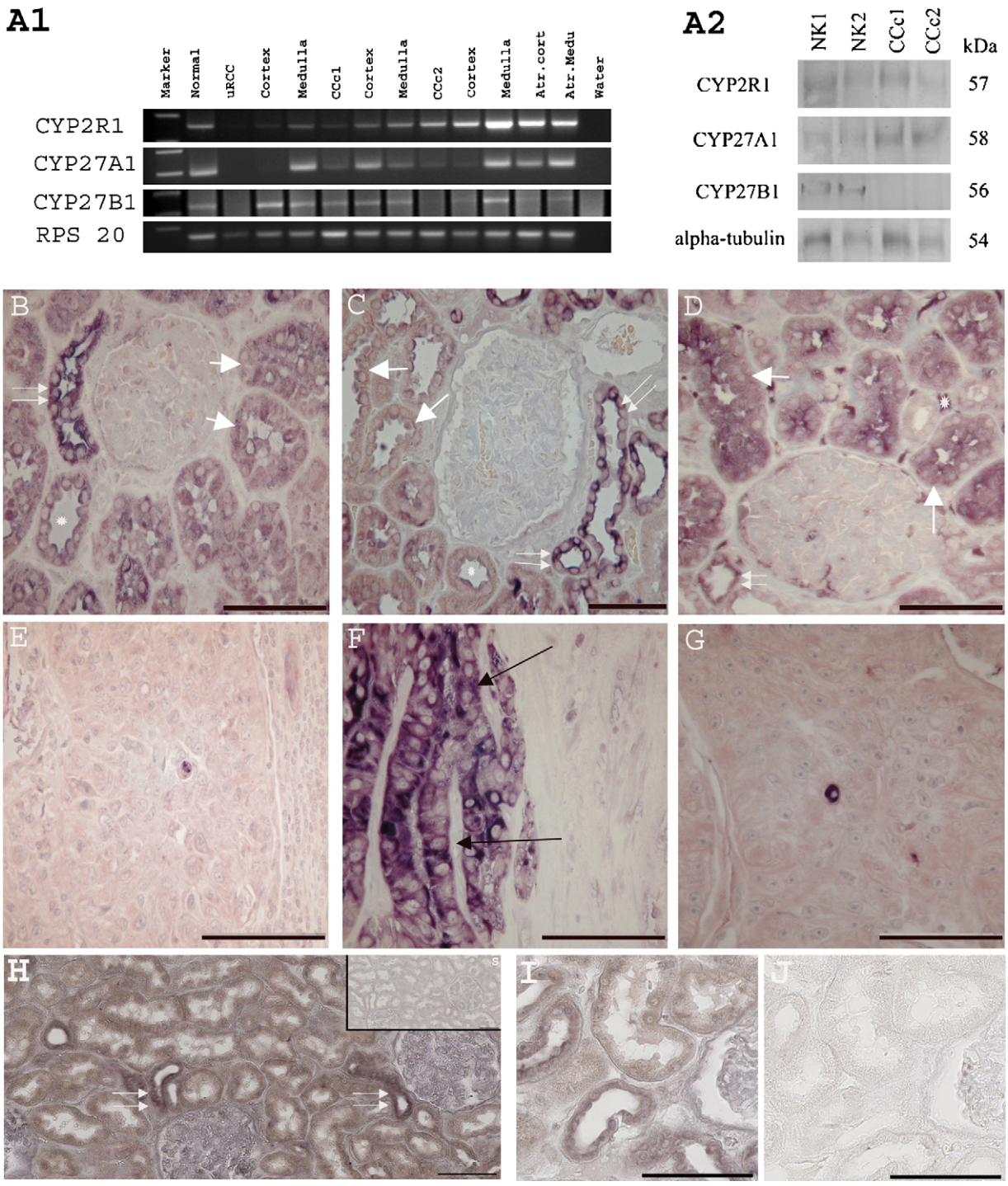

Fig. 2. Expression of the activating enzymes CYP2R1, CYP27A1, and CYP27B1 in normal and malignant tissue. (A1) RT-PCR with all the activating enzymes. From left: normal RNA (healthy donor); uRCC, unclassified RCC; followed by cortex and medulla from the same patient; CCc 1, clear cell carcinoma from patient 1, followed by cortex and medulla from the same patient; CCc 2, clear cell carcinoma from patient 2, followed by cortex and medulla from the same patient; Atr. cortex and Atr. medulla, cortex and medulla from patient with atrophic kidney. (A2) Western blot of CYP2R1, CYP27A1 and CYP27B1 expression in normal and malignant kidneys. (B–D) IHC detection of CYP2R1 (B); CYP27A (C); and CYP27B1 (D) in normal kidney. (E–G) IHC analysis of clear cell carcinoma: CYP2R1 (E); CYP27B1 (F); CYP27B1 (G). (H–J) ISH analysis of the expression of CYP27A1 mRNA in normal kidney; (H) overview of mRNA expression with hybridisation with the corresponding sense probe inserted; (I) and (J) high resolution of ISH with anti-sense and sense probes, respectively. White arrows: PT; double arrows: DT; black arrows: differentiated cells in CCc. The center of a CD is indicated by a white *. Bar corresponds to 100 μm.

Germany) followed by development with NBT BCIP (262.5 μg/mL *p*-nitro-blue tetrazolium chloride; 225 μg/mL 5-bromo-4-chloro-3-indolyl phosphate dipotassium salt dissolved in 100% and 70% dimethyl formamide, respectively) with levamisol (1.25×10^{-6} M). The individual probe sets were tested on four different kidney samples, including PFA and formalin fixed tissues with at least two different Proteinase K treatments in each experiment. We used both biotin and digoxigenin labelled probes, but we recommend the use of DIG-labelled probes because of higher background staining with the biotin-labelled probes. ISH with probes for CYP27B1 and CYP2R1 gave inconsistent results, with either no staining or identical staining with sense and anti-sense probes.

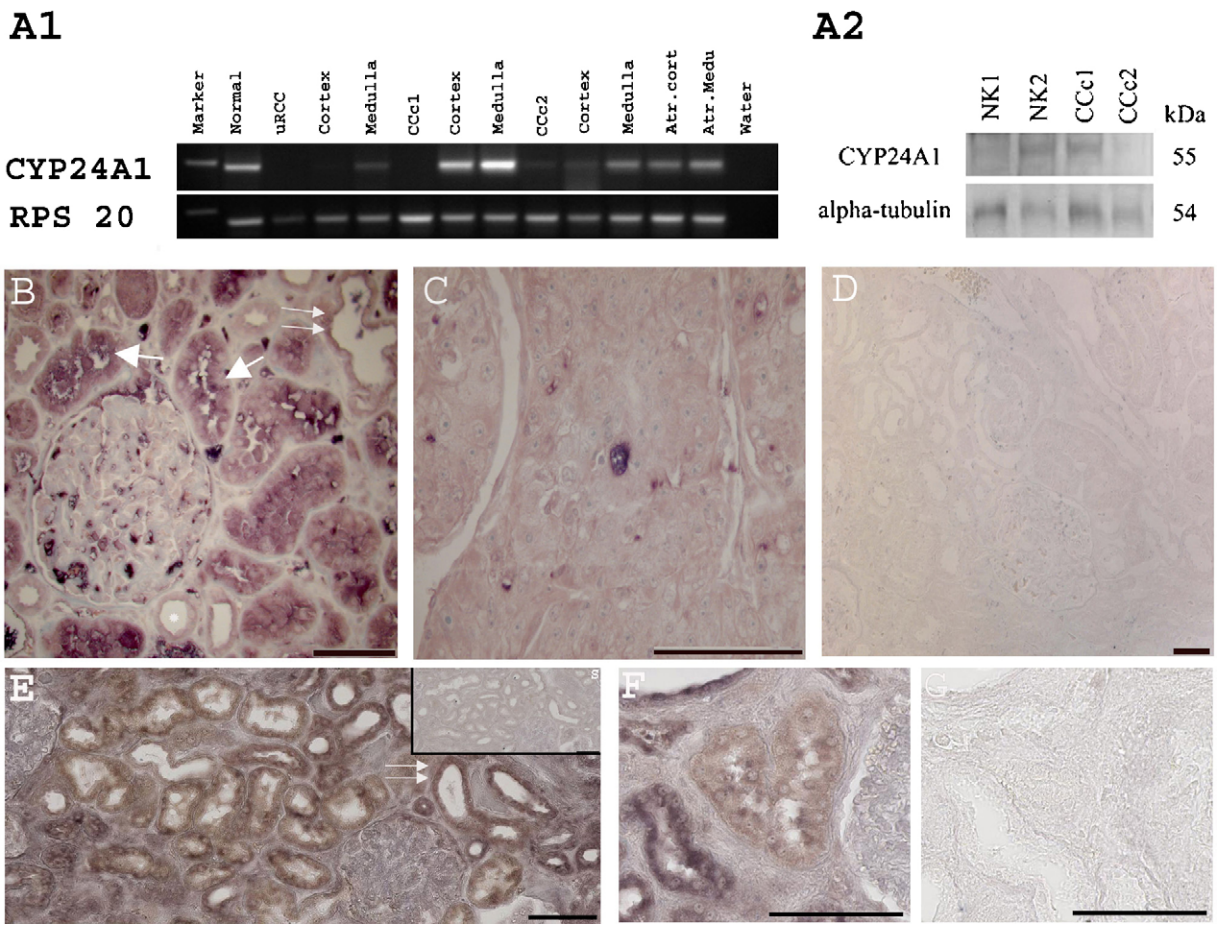

Fig. 3. Expression of the inactivating enzyme CYP24A1 in normal and malignant tissue. (A1) RT-PCR analysis of CYP24A1. From left: normal RNA (healthy donor); uRCC, unclassified RCC; followed by cortex and medulla from the same patient; CCc 1, clear cell carcinoma from patient 1, followed by cortex and medulla from the same patient; CCc 2, clear cell carcinoma from patient 2, followed by cortex and medulla from the same patient; Atr. cortex and Atr. medulla, cortex and medulla from patient with atrophic kidney. (A2) WB of normal and malignant kidney samples. (B) IHC detection of CYP24A1 normal kidney; (C) IHC detection of CYP24A1 in clear cell carcinoma; (D) IHC control with no primary antibody. (E–G) ISH analysis of the expression of CYP24A1 mRNA in normal kidney; (E) overview of mRNA expression with hybridisation with the corresponding sense probe inserted; (F) and (G) high resolution of ISH with anti-sense and sense probes, respectively. White arrows indicate PT; double arrows DT. Bar corresponds to 100 μm.

3. Results

3.1. Expression of VDR

The VDR mRNA was detected in all normal kidney samples (Fig. 1A1); the mRNA was also present in most tumours, but at a lower level and undetectable in both CCcs (Fig. 1A). In accordance, WB revealed that VDR was present in the two normal kidney samples, but essentially undetectable in the tumour samples (Fig. 1A2). In the normal kidney the VDR was primarily present in the cytoplasm of tubular cells in proximal (PT) and distal tubules (DT) (Fig. 1 and Table 2). In contrast, the VDR was only expressed in a few isolated clear cells in 3/7 CCcs and the uRCC, while it was undetectable in the remaining tumours. However, VDR was present in tumours with more differentiated structures, where it localised to structures resembling primitive tubules (Fig. 1D). The expression of VDR was confirmed by ISH, which showed cytoplasmic staining in proximal and distal tubules (Fig. 1E–G), with the strongest staining in distal tubules and no staining in the glomerulus. Note that both the sense and anti-sense probes also show a distinct nuclear staining in all cells, including the cells in the glomerulus; this is probably caused by the presence of large AS-S transcripts that are unrelated to the expression of the VDR mRNA [21].

3.2. Expression of the activating enzymes (CYP2R1, CYP27A1, and CYP27B1)

mRNAs encoding the 25-hydroxylases CYP2R1 and CYP27A1 were expressed in all normal samples and in CCcs, however, CYP27A1 was absent in uRCC, which expressed CYP2R1 (Fig. 2A1). The 1α-hydroxylase (CYP27B1) was present in all normal samples, although the expression level varied, being low in the atrophic kidney. The expression of CYP27A1 in the tumours varied; it was undetectable in the uRCC and in one of the CCcs, whereas it was detectable in tumours with more differentiated structures, which was corroborated by the WB (Fig. 2A2). The IHC expression of CYP2R1 and CYP27A1 was localised to DT, collecting ducts (CD), and a minor expression in PT, which was supported by ISH, where the CYP27A1 mRNA had a marked expression in DT and was present in PT. (Fig. 2 and Table 2). CYP27B1 was predominantly expressed in PT (Fig. 2 and Table 2). The expression of the activating enzymes was except for CYP27B1 higher in the regenerating tubules compared to the normal samples. In CCc, the activating enzymes were generally not expressed, however in 3/7 CCcs a few isolated cells, which resembled clear cells, expressed the activating enzymes and VDR (Table 2). These cells were not exclusively found at the border of the tumour, but appeared randomly distributed in the malignant tissue. In tumours with differentiated structures the enzymes

were expressed at a high level in the differentiated tubule-like structures.

3.3. Expression of the inactivating enzyme (CYP24A1)

CYP24A1 mRNA was detectable in normal kidney tissue from all patients (Fig. 3A1). In tumours, the CYP24A1 mRNA was barely detectable, and its presence seemed to be dependent on the presence or absence of differentiated structures. There was no difference in the expression of the two splice-variants [22] of CYP24A1 (not shown). As observed for the mRNA, the CYP24A1 protein was present in both normal kidney and tumours by WB, however the level varied between samples (Fig. 3A2). CYP24A1 was expressed in DT and PT, and at a low level also in the CD; in contrast to the activating enzymes, CYP24A1 was also present in the mesangial cells in glomerulus (Fig. 3B and Table 2). The clear cells in the tumours were generally negative for CYP24A1; however the differentiated parts of the tumours and a few randomly distributed clear cells expressed CYP24A1 (Fig. 3 and Table 2). The protein localisation was confirmed by ISH that showed cytoplasmic staining in PT and DT, with the strongest staining in the distal segments of the nephron. There was no staining of the cells in the glomerulus, which suggest that the mesangial staining might be unspecific (Fig. 3E–G).

3.4. Co-localisation of all vitamin D metabolising enzymes and VDR

Concomitant expression of VDR and the metabolising enzymes was evaluated in serial sections and all enzymes and VDR co-localised in both PT and DT, although the abundance of the expression varied along the nephron (Table 2). VDR followed to some extend the IHC pattern of the 1α-hydroxylase and CYP24A1, with the strongest expression located in PT. Minor expression was found in DT and CD, where the 25-hydroxylases had a marked expression. VDR, CYP27B1 and CYP24A1 were, in addition to the intratubular expression, detected with some inconsistency in Bowman's capsule, scattered endothelial cells, and the thick limb of Henle, which also had a marked expression of both 25-hydroxylases. In general the CCc did not express VDR or any of the metabolising enzymes except for the co-localised expression in isolated tumour cells.

4. Discussion

To our knowledge this is the first investigation describing the segmental expression of the 25-hydroxylases in the human kidney. The marked expression in the distal segments could be influenced by the expression of megalin and cubilin in PT, while the distal segments rely on intracellular hydroxylation [23]. In CD expression of CYP27B1 and CYP24A1 diminished, while expression of the remaining enzymes and VDR seemed comparable with the expression pattern found in DT. Our results are in concert with earlier reports, however, with a few discrepancies: the 1α-hydroxylase was not consistently positive in CD in our study, and some of the stromal cells were positive for CYP24A1, which is in contrast to the data from ISH and the results described by Kumar et al. [24]. These minor differences could be due to differences in antibody properties, use of different fixatives or method selection.

Microarray data from CCc relate the cancer cells to the proximal nephron epithelium, which overexpress megalin, cubilin, genes involved in angiogenesis, and genes related to VHL/HIF1A dysregulation, known to promote tumour formation [25]. Earlier studies investigating VDR in CCc have consistently found a low proportion of tumour cells expressing VDR [16–18]. We show here that the absence of VDR is concomitant with an abolished intracellular activation of $25(OH)D_3$. Our findings are consistent with the hypothesis that CCc arise from PT epithelium, although we only detected expression of the 1α-hydroxylase, VDR, and CYP24A1 in very few tumour cells. The simultaneous loss of VDR, CYP27B1, CYP24, CYP2R1 and to some extend CYP27A1 could be caused by endocrine, paracrine or autocrine regulators of either VDR or the metabolising enzymes. However, none of the known endocrine regulators (FGF23, Klotho, PTH, PTHrP, calcitonin) are able to down regulate VDR, CYP27B1 and CYP24A1 simultaneously.

The diminished expression of VDR and the metabolising enzymes could be caused by the malignant transformation, alternatively CCc may arise from progenitor cells instead of differentiated proximal tubules cells. The latter is reinforced by the primitive tubules we often observe in the heterogeneous tumours that could originate from either atrophic DT or from differentiating tumour cells. Unlike the surrounding tumour cells that do not resemble renal epithelial cells, these differentiated cells express both VDR and the metabolising enzymes. Recent publications have shown tumour initiating stem cells in human renal carcinomas [26,27]. These cells express stem cell markers, are able to differentiate into epithelial or endothelial cell types (bipotent), and can generate *in vivo* serially transplantable tumours [28,29]. The RCC do not express VDR, but $1,25(OH)_2D_3$ induce expression of VDR in various cell lines, and activated VDR may thus be able to modulate the behaviour of these progenitor cells. HIF is essential for renal cancer formation and progression and calcitriol has *in vitro* been shown to decrease the expression of HIF and inhibit angiogenesis through the HIF-dependent upregulation of VEGF [30]. The anti-neoplastic effect is also found in *in vivo* studies, where induced tumours were larger and had higher levels of HIF in VDR knockout mice compared to controls [31]. Furthermore, $1,25(OH)_2D_3$ treatment of mice with RCC increased survival, and inhibited tumour growth, angiogenesis and metastasis [32,33].

In conclusion we show a segmental expression of the 25-hydroxylases, 1α-hydroxylase, VDR and 24- hydroxylases in the normal kidney. The abolishment of VDR in renal clear cell carcinoma seems to be associated with diminished expression of the 1α-hydroxylase, the 24-hydroxylase and to some extent the 25-hydroxylases. Vitamin D may have a positive impact on prognosis in RCC patients, but further studies are needed to elucidate whether activated vitamin D may have a therapeutic effect.

Note added in proof

Supportive to the data presented here we recently found that VDR and the metabolising enzymes were either absent or co-expressed in the same cells in other mesenchymal-derived organs, including the testis, epididymis, and prostate [34].

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