Expression of G protein coupled receptor 87 (GPR87) in end stage renal disease and associated tumours

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Abstract

End stage renal disease (ESRD) and acquired cystic renal disease (ACRD) are characterized by structural remodelling through chronic inflammation and by frequent development of cancers. So-called eosinophilic-vacuolated and chromophobe-like renal cell carcinoma develop exclusively in ACRD kidney. Previous Affymetrix array analysis detected GPR87 as one of the highly expressed genes in ESRD/ACRD kidneys. Experimental and clinical studies suggested a correlation between activation of GPR87 signalling and proliferation and migration of tumour cells. In this study we have analysed normal and ESRD/ACRD kidneys and related tumours for GPR87 expression by PCR, RT-PCR, and immunohistochemistry. Immunohistochemistry revealed a strong GPR87 expression in proliferating epithelial cells in ESRD/ACRD kidneys and as well as in cells of eosinophilic- vacuolated and chromophobe-like renal cell carcinoma. Our study suggests that GPR87 signalling play an important role in structural remodelling of ESRD/ACRD kidney and development of ACRD-associated tumours with unique histology.

Introduction

End stage renal disease (ESRD) is the final stage of chronic kidney disease leading to loss of renal function. ESRD kidney is characterized by chronic interstitial inflammation and considerable stromal fibrosis replacing the normal kidney parenchyma [1]. After long-term intermittent maintenance haemodialysis ESRD kidneys undergo diffuse cystic changes resulting in acquired cystic renal disease (ACRD). The long-lasting inflammatory microenvironment (IME) may lead to development of renal cell carcinoma (RCC) with unusual histology and genetic alteration [2, 3]. The histology and genetics of conventional and papillary RCC that develops in ESRD/ACRD kidneys correspond to those arising in general population. However, eosinophil-vacuolated RCC (evRCC), and chromophobe-like RCC (chlRCC), and their precursor lesions occur exclusively in ARCD kidneys [2, 3].

The molecular mechanism behind the development of these two unique types of cancer is not yet known. The IME in ESRD/ACRD kidney consists of high number of naive activated fibroblasts (NAF) and immune cells producing cytokines and growth factors. Expression of IL6 and TGFβ in ESRD/ACRD kidneys trigger the generation of reactive oxygen and nitrogen species (RONS). Elevated levels of RONS impair the function of important proteins, interfere with metabolic pathway. One of the common mediators of carcinogenesis is the oxidative stress induced by inflammation.

A gene expression analysis of ESRD/ACRD kidneys using Affymetrix array revealed characteristic fingerprint of functionally associated genes such as cytokines, growth factors, laminins, and keratins among others [4]. The G protein-coupled receptor 87 (GPR87) was the third most prominently expressed gene in ESRD/ACRD kidneys. The GPR87 RNA and encoded protein has been shown to be overexpressed in pancreatic, urothelial, and hepatic carcinoma cell lines in vitro and associated with increased cell proliferation [5–9]. To confirm the result of previous Affymetrix analyses we searched for expression of GPR87 in ESRD kidneys and associated tumours by applying RT-PCR and immunohistochemistry.

Material And Methods

RNA isolation and reverse transcription Shock frozen normal foetal and adult kidneys, ESRD/ACRD kidneys and tumours of the general populations were homogenized in TRIzol (Invitrogen, Germany) as described previously [5]. RNA was isolated according to the manufacturer's instructions. The concentration of total RNA was measured by spectrophotometry at 260 nm. The quality of RNA was checked by electrophoresis on 1% agarose gel. Reverse transcription of 2 µg RNA was carried out in 25 µl reaction volume with 200 U Superscript II reverse transcriptase (Invitrogen, Germany), 4 µM oligo dT primer, 0.8 mM dNTP, and 40 U RNase inhibitor (Invitrogen, Germany). The reaction was carried out for 60 min at 42°C, followed by 30 min at 50°C.

PCR and quantitative PCR The reaction was performed with 6µl of 1:16 diluted cDNA, 0.133 µM of each forward and reverse primers, 0.2 mM dNTP, 1.5 mM MgCl2, and 0.75 U Taq polymerase (Invitrogen, Germany) over 32 cycles; with denaturation for 30 sec at 94°C, annealing for 30 sec at 61°C, elongation for 45 sec at 72°C and an additional 5 min elongation at 72°C. The PCR product was separated by electrophoresis on 1.5% agarose gel and stained with ethidium bromide. Quantitative PCR was performed using a Real Time PCR Machine (Opticon, MJ Research Inc.). 6 µl of 1:16 diluted cDNA was amplified with 0.133 µM of each forward and reverse primer and 7.5 µl of the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, Germany). Cycling conditions were: 2 min at 50°C and 3 min at 95°C followed by 40 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec 61°C and elongation for 45 sec at 72°C, and an additional 5 min elongation at 72°C. Samples were parallel amplified with gene specific and ß-actin primers. Standard curves were generated from a normal and ESKD kidney cDNA dilution series for both gene specific and ß-actin reactions. The concentration of target genes was calculated by comparing the cycle numbers of the log-linear phase of the samples with cycle numbers of the standards. Data were expressed as the ratio between the amounts of each transcript of interest versus amount of the ß-actin. Melting curves were analysed to determine the specificity of PCRs. Forward and reverse primers used for PCR and real-time PCR analysis were: GPR87 (5'-GGG TTC AAC TTG ACG CTT GCA AAA T-3' and 5'-GGG TTC AAC TTG ACG CTT GCA AAA T-3'). ß-actin (5'-ATG GAT GAT GAT ATC GCC GCG-3' and 5'-GTC CAT CAC GAT GCC AGT GGT AC-3'),

Immunohistochemistry Twelve representative slides were selected from six ESRD and six ACRD kidneys removed due to cancer and analysed by immunohistochemistry. The diagnosis of evRCC was established as proposed by Tyckoo et al. [3]. After removing the paraffin and rehydration the 4 µm sections were subjected to heat-induced epitope retrieval in citrate buffer, pH 6.0 in 2100-Retriever (Pick-Cell Laboratories, Amsterdam, The Netherlands). Endogenous peroxidase activity was blocked for 10 min at room temperature. Endogenous peroxidase activity was blocked by incubating the slides with Envision FLEX Peroxydase Blocking Reagent (DAKO) for 10 min. The slides were then incubated for one hour with polyclonal rabbit anti-GPR87 antibody (ab13945, abcam, Cambridge, UK) at the dilution of 1:100 at room temperature. EnVision FLEX horse-radish-peroxidase conjugated secondary antibody (DAKO) was applied for 30 min at room temperature and colour was developed with 3-amino-9 ethylcarbazole (AEC) substrate (DAKO). Tissue sections were counterstained with Mayer's haematoxylin (Lillie's modification, DAKO) and

after 10 seconds bluing mounted with Glycergel (DAKO). Photographs were taken by a Leitz DMRBE microscope, equipped with HC PLAN APO 20x0.70 microscope objective, and a ProgRes C14 camera.

Results

Expression of GPCR87 RNA in ESRD/ACRD kidney The PCR product was separated by electrophoresis on 1.5% agarose gel and stained with ethidium bromide. Results of PCR analysis of GPR87 RNA in normal fetal and adult kidneys, ESRD/ACRD kidneys, and in conventional, papillary and chromophobe RCCs, and renal oncocytoma that developed in ESRD/ACRD kidneys is shown in Fig. 1A. GPR87 is expressed exclusively in ESRD/ACRD kidneys. The RT-PCR showed similar results albeit at slightly different copy number of PCR products (Fig. 1B). The data were calculated as the ratio between the amounts of each transcript of GPR87 versus amount of the ß-actin. Therefore, expression of GPR87 in foetal kidney from 15 weeks of gestation might be explained by a low expression of ß-actin in relation to GPR87.

GPR87 immunohistochemistry of normal, ESRD kidneys and associated tumours The GPR87 protein was detected exclusively in the collecting ducts of normal adult kidneys (Fig. 2A). In ESRD kidneys the proliferating tubular-epithelial cells showed strong GPR87 cytoplasmic expression, whereas atrophic tubuli or those mimicring thyroid tissues were negative (Fig. 2B). No GPR87 expression was seen in renal oncocytoma, papillary and conventional RCC arised in ESRD/ACRD. However, the two types of renal cancers occurring exclusively in ESRD/ACRD kidneys, ev-RCC, chlRCC displayed GPR87 protein expression. A strong immune reaction with GPR87 antibody was seen in the cytoplasm of evRCC and their precursor lesions (Fig. 2C). The chlRCC, which display a nearly empty cytoplasm when staining with hematoxylin and eosin, showed a weak reticular staining with the GPR87 antibody (Fig. 2D).

Discussion

ESRD/ACRD kidney displays a remodelling of kidney structure by strong proliferation of blood vessels, naive activated fibroblasts (NAF) and immune cells embedded in fibrillary extracellular matrix (ECM). The microenvironment with persistence of NAF, continous expression of cytokines, growth factors, oxydative and metabolic stress, genetic instability, and remodeling of ECM may explain the frequent findings of cancer in ESRD/ACRD kidneys. However, the molecular pathway of evRCC and chlRCC development in ACRD is not yet cleared. The evRCC and its precursors show strong cytoplasmic and nuclear staining of TXNIP and only weak expression of TXN suggesting that impaired redox homeostasis might be associated with development of evRCC (not yet published).

We showed in this study, that GPR87 is upregulated in proliferating epithelial cells in ESRD/ACRD kidneys. Interestingly, GPR87 is expressed in evRCC and chlRCC but none of the conventional or papillary RCCs, which occurring not only in ESRD/ACRD kidneys but also in general population. These data suggests that the development of evRCC and chlRCC is associated with upregulation of GPR87. The strong inflammation in ESRD/ACRD kidneys leads to oxidative stress, production of genotoxic agents which contribute to mitochondrial and genomic DNA damage and in this way to cancer initiation [10]. It

was shown that expression of GPR87 is necessary for p53-dependent cell survival in response to genotoxic agents and that GPR87 is upregulated by DNA damage in a p53-dependent manner [5]. This finding may explain the high expression of GPR87 in ESRD/ACRD kidneys and evRCC and chlRCC.

The lysophosphatidic acid (LPA), the ligand of GPR87 mediates diverse biological activities such as cell proliferation, cell motility by binding to its receptor GPR87. GPR87 expression promotes cells growth and metastasis through upregulation of CD133 positive cancer stem cell like cells in hepatic cancer [6]. GPR87 overexpression activates Akt, which suppress p53, thereby preventing apoptosis. This finding points towards GPR87 being an anti-apoptotic gene that induces cell proliferation [7]. Overexpression of GPR87 promotes migratory and invasive properties in vitro and increased tumour initiation in vivo [6]. GPR87 enhances pancreatic cancer aggressiveness by activating NF-kB signalling pathway [8]. Clinical studies also suggest that overexpression of GPR87 promotes tumour initiation and progression. Expression of GPR87 significantly correlated with clinicopathological parameters and patients with high GPR expression in pancreatic cancers had a shorter overall survival compared to the patients with lowed GPR87 levels [8]. Patients with GPR87 negative urinary bladder carcinomas have a longer intravesical recurrence-free survival [9]. Both experimental and clinical studies confirmed the correlation between activation of the LPA/GPR87 signalling and proliferation and migration of tumour cells. Our study suggests that GPR87 signalling play an important role in remodelling of ESRD/ACRD kidney and development of ACRD-associated tumours with unique histology.

Declarations

Ethics approval: The kidney tissues were collected from different countries during 1995 and 1998 (see Acknowledgements). For the retrospective study an informed consent waiver was obtained from the Ethics Commission I of the Medical Faculty of University of Heidelberg. The use of tissue samples for this study was approved by the Ethics Committee of the University Pecs, Hungary (No. 8466.PTE 2020). All procedures were in accordance with the ethical standards of Institutional Research Committee and with the 1964 Helsinki Declaration.

Consent for publication: not applicable

Data availability: The data generated during the current study are available from the corresponding author upon reasonable request.

Competing interest: Authors have no competing of interests to declare.

Author contributions: A.N. performed the RNS analysis, B.H. made the immunohistochemistry, T.B. conceived the project and wrote the draft, K.B. and K.Zs reviewed the manuscript. All authors have read and agreed to the final version of the manuscript.

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Figures

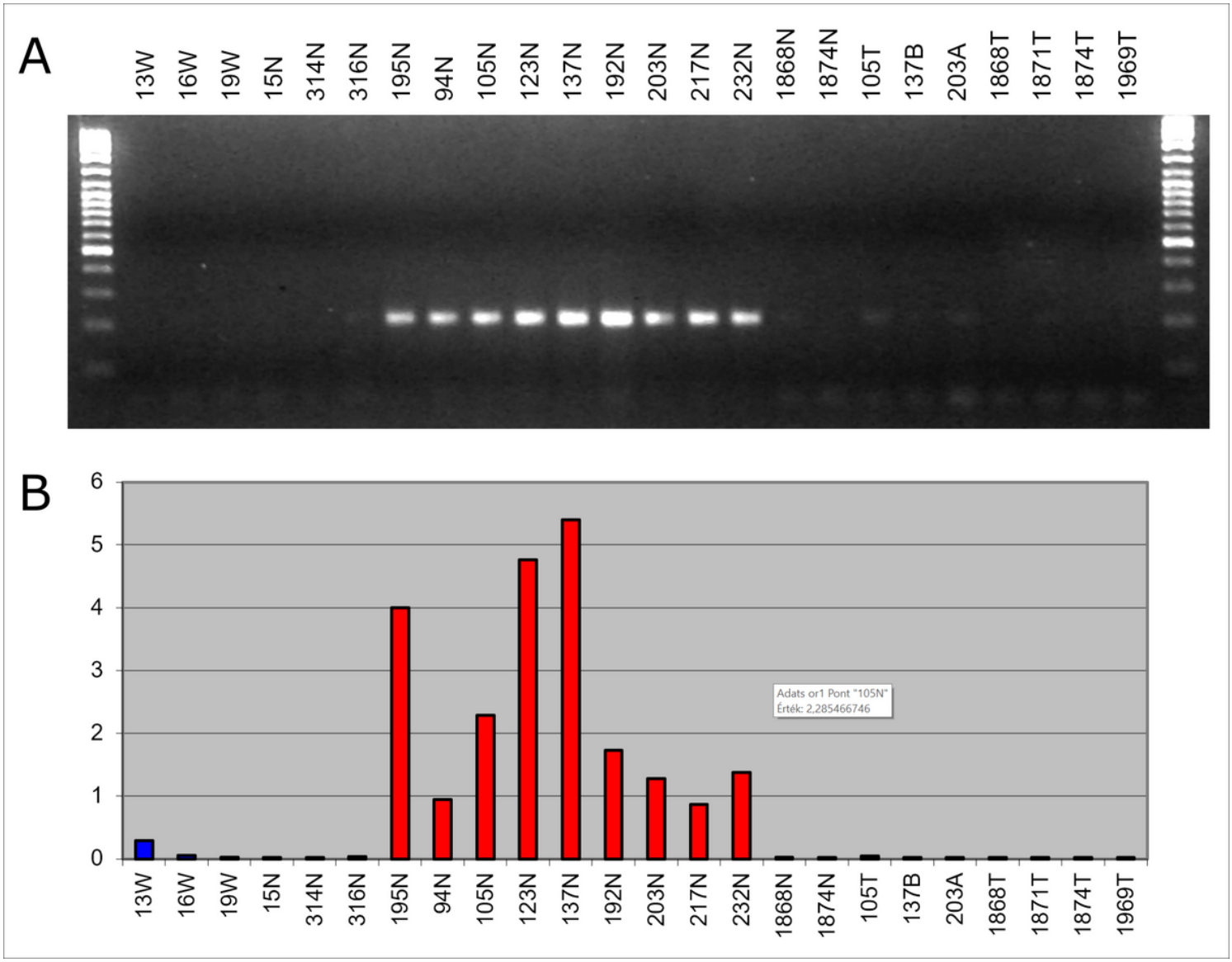

Figure 1

Expression of GPR87 RNA in fetal kidneys (13-19 W), adult kidneys (15N-316N and 1868N-1874N), ESRD/ACRD kidneys 195N-232N), in renal oncocytoma (105T), conventional (137B, 203A) and papillary RCC (1868T-1969T). **A**PCR product loaded onto agarose gel show expression of GPCR87 exclusively in ESRD/ACRD kidneys. **B.** RT-PCR shows the expression of GPR87 only in ESRD-ACRD kidneys, albeit at different levels. The images of agarose gel and of RT-PCR analysis are original uncropped images

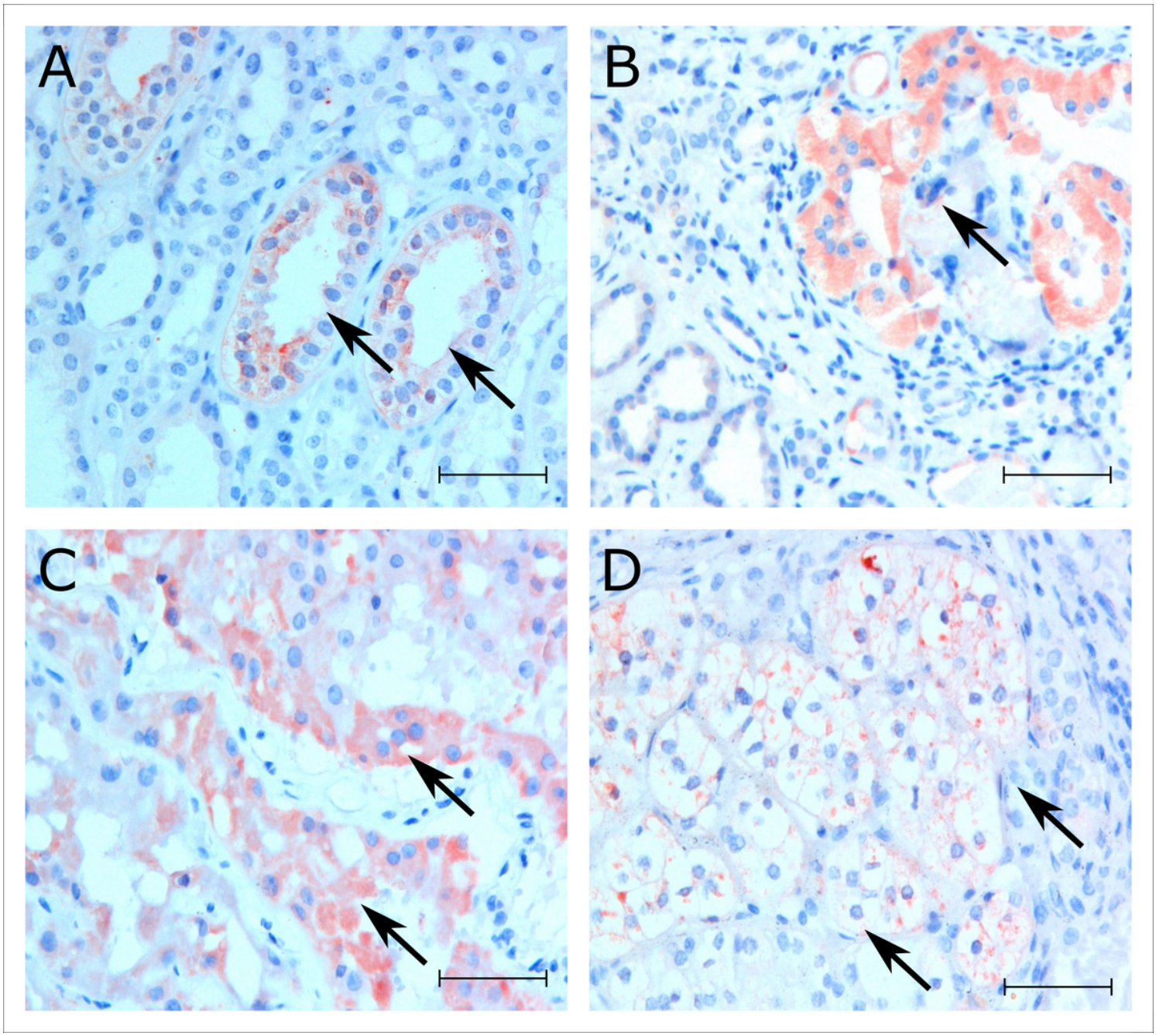

Figure 2

Immunohistochemistry of GPR87. **A** Cells of the collecting duct express GPR87 protein preferentially at the luminal surface of cells (arrows). **B** Irregular growing tubules with large cells show a strong positive cytoplasmic staining with the GPR87 antibody (arrow). Small atrophic tubules are negative. **C** Eosinophilic vacuolated tumour shows intensive cytoplasmic immunostaining with GPR87 antibody (arrows). **D** Chromophobe-like precursor lesion displays weak reticular cytoplasmic positivity with GPR87 antibody (arrows). Scale bar: 40 um