Localization of discoidin domain receptors in the normal and fibrotic rat kidney

Rutha Lee

Brain Korea 21 Project for Medical science
The Graduate School, Yonsei University

Localization of discoidin domain receptors in the normal and fibrotic rat kidney

Directed by Professor Dae Suk Han

The Doctoral Dissertation
submitted to the Brain Korea 21
Project for Medical Science
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

Rutha Lee

June, 2002

This certifies that the Doctoral Dissertation
of Rutha Lee is approved.

Thesis Supervisor: Dae Suk Han

Yoav Segal: Thesis Committee Member

Kang Yell Choi: Thesis Committee Member

Hun Joo Ha: Thesis Committee Member

Won Jae Lee: Thesis Committee Member

The Graduate School
Younsei University

June, 2002

ACKNOWLEDGEMENTS

I would like to thank God for his love, mercy and grace for me.

First I acknowledge and thank my advisor, Dr. Dae Suk Han, Professor of Department of Internal Medicine, Division of Nephrology, for his competent guidance and helpful suggestions through all the research that went into this thesis. I would also like to thank Dr. Yoav Segal, Assistant Professor of Department of Medicine, Division of Nephrology and Hypertension, University of Minnesota, who gave me the opportunity to learn and broaden my understanding in basic science and in particular, to these interesting proteins. Without his continued advice and excellent support, this study would not have come to any fruition. Also, special appreciation is acknowledged to Dr. Kang Yell Choi, Hun Joo Ha, Won Jae Lee, for their invaluable contributions as members of the committee.

I wish to express my special appreciation to my parents for their endless love and support throughout my life and to parents- in-law for their special love and support during my stay in the States. I also dedicate this to my husband and to my wonderful children, Hyo Jin and Min Ho, who made this degree achievable.

In the near future, I hope that the successive research on this receptor will shed light on the function of it, especially in the kidney. If, we can expect therapeutic effect through this protein for the patients who suffer from the kidney disease, it would be the most delightful thing to me.

Contents

List of Figures

Abstract

Localization of discoidin domain receptors in the normal and fibrotic rat kidney

Rutha Lee

Brain Korea 21 Project for Medical Science
The Graduate School, Yonsei University

(Directed by Professor Dae Suk Han)

The discoidin domain receptors (DDR's) DDR1 and DDR2 are cardinal members of receptor tyrosine kinase subfamily, activated by collagens. Essential in mammalian development, these receptors are also candidate effectors in tissue fibrosis; DDR2 in particular has been implicated in hepatic fibrosis. We investigated DDR expressions in normal and fibrosing rat kidneys. Immunolocalization studies using multiple antibodies indicated that DDR1 is expressed in endosomal vesicles within proximal tubules, and DDR2 is expressed in apical membranes of select nephron segments, from the loop of Henle to the macula densa. DDR2 was found to exist in a unique high molecular weight (MW), denaturation-resistant form, described herein. The distribution of DDR2 in fibrosing kidneys, obtained from a well established model of progressive renal injury, was similar to that in controls, lacking evidence of novel glomerular, interstitial or vascular expression, or tubular cell redistribution towards expanding collagen matrices. Further, DDR mRNA levels in fibrosing and control kidneys are not significantly different, at 2, 4 and 8 weeks, after induction of injury. This study suggests that DDR localization in the adult kidney is incompatible with predominant roles in cell-matrix interactions. The possibility of alternative roles for this important receptor pair is discussed.

Key Words: discoidin domain receptor, collagen, fibrosis, kidney

Localizition of discoidin domain receptors in the normal and fibrotic rat kidney

Rutha Lee

Brain Korea 21 Project for Medical Science
The Graduate School, Yonsei University

＜Directed by Professor Dae Suk Han＞

I. INTRODUCTION

The receptor tyrosine kinases (RTKs) constitute a vast family of integral membrane receptors, regulating growth, differentiation, survival and behavior. Their overall structure is characterized by the presence of an extracellular ligand-binding domain, a single transmembrane domain, and one cytoplasmic tyrosine kinase domain. Individual RTKs are bound by correspondingly restricted sets of extracytoplasmic ligands, which can include soluble growth and survival factors, cytokines and chemokines, or select integral membrane and extracellular matrix proteins. In response to ligand binding, RTKs undergo oligomerization and phosphorylation, thereupon modulating down-stream signaling pathways.[1]

The recently identified discoidin domain receptors (DDRs) DDR1 and DDR2 represent a unique family of mammalian RTK's, distinguished by an extracellular discoidin domain.[2] Roughly 160 amino acids in length, this putative ligand-binding domain is named after its homology to discoidin, a multifunctional lectin in the slime mold *Dictyostelium discoideum*.[3] Discoidin domains are found in a variety of mammalian membrane and secreted proteins, including coagulation factors V and VIII.[4,5] The major isoforms of DDR1 and DDR2 are 125 kDa and 130 kDa in size, respectively, sharing 78% homology within their discoidin domains. Other DDR1 isoforms are known, and

subject to complex regulation.[2] The DDRs are widely expressed in normal and malignant tissues, and in cell lines.[6-8] DDR1-null mice exhibit defects in embryonic implantation, bone mineralization and mammary development,[9] while DDR2-null mice exhibit defects in chondrocyte proliferation, resulting in dwarfism.[10]

The DDRs were described originally as orphan receptors. Recent identification of collagens as candidate ligands for these receptors has marked them as potential non-integrin extracellular matrix receptors, with corollary roles in cell-matrix interactions.[5,11,12] Several lines of evidence support this idea. First, DDR phosphorylation and second messenger recruitment are induced by collagens I, II, III and V *in vitro*.[11-13] Second, DDR expression influences cellular interactions with matrices, in reconstituted[14] or living systems;[15] indeed, disruptions in cell-matrix interactions have been framed as likely causes for the developmental abnormalities in *Ddr*-targeted mouse lines.[9,10] Third, DDR ligand-binding activity correlates with sites of collagen expression.[9,16,17] Notwithstanding these observations, direct evidence for collagens as the natural ligands of the DDRs is difficult to ascertain, and the possibility of complementary ligands has been raised.[9]

Roles for the DDR's as effectors in matrix turnover and progressive fibrotic diseases have also been postulated, on the basis of their collagen-binding properties, and downstream signaling effects, including regulation of matrix metalloproteinase expression.[11] Of the two, DDR2 has been implicated directly, following observation of its upregulation in a model of hepatic fibrosis.[18] Additional evidence for roles of the DDRs in fibrotic diseases would strengthen the rationale for their therapeutic targeting. The purpose of this study was to investigate DDR expression in normal and fibrosing kidneys, utilizing the rat remnant kidney as a well-established experimental model.

II. MATERIALS AND METHODS

1. Animals and reagents

All studies were carried out using 200～250 gm male Sprague-Dawley rats (Harlan, Madison, WI, USA).

Antibodies directed against the N- or C-terminus of DDR1, or the N- or C-terminus of DDR2

were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The lectin Phaseolus Vulgaris and monoclonal anti-calbindin D antibody were obtained from Sigma (St. Louis, MO, USA). Anti-Tamm-Horsfall protein antibody was obtained from ICN Biomedicals (Aurora, OH, USA). Secondary antibodies included fluorescein isothiocyanate (FITC)-labeled rabbit anti-goat IgG (ICN Biomedicals), Alexa Fluor 594-labeled donkey anti-goat IgG (Molecular Probes, Eugene, OR, USA) and horseradish peroxidase-conjugated donkey anti-goat IgG (Jackson Immunoresearch Laboratories, West Grove, PA, USA). FITC-labeled anionic dextran (average MW 10 kDa) was purchased from Molecular Probes.

2. Tissue sections and immunofluorescence

Rat kidney sections were prepared from fresh frozen tissues, perfusion-fixed tissues or archived paraffin blocks. Fresh frozen tissues were dissected directly, embedded in O.C.T. compound (Sakura Finetek, Torrance, CA, USA) and snap frozen in liquid nitrogen. Cryostat sections (6 m) were placed on poly-L-lysine-coated slides (Sigma), air dried for 30 minutes, fixed in acetone for 10 minutes at room temperature, and rinsed in phosphate-buffered saline (PBS). Kidneys were fixed by perfusion via the abdominal aorta with 2% freshly depolymerized paraformaldehyde in PBS, at 180 mm Hg for 3 minutes, followed by 18% sucrose in PBS, also at 180 mm Hg for 3 minutes. They were then removed, cut into slices, and infused overnight with 18% sucrose in PBS, at 4^{o}C. They were embedded in O.C.T. compound, snap frozen and cryosectioned, as described above. In experiments using FITC-dextran as a marker of fluid-phase endocytosis, anesthetized rats were injected through the right jugular vein with FITC-dextran (1.75 mg/100 g body weight, in 0.5 ml 0.9% NaCl), over a 30-second period. Kidneys were perfusion-fixed 15 minutes thereafter.

Remnant kidneys from subtotally nephrectomized rats and control kidneys from sham-operated rats were sectioned from archived paraffin blocks. Generation of experimental groups was similar to that described previously.[19,20] For antibody staining, 6 m sections were deparaffinized in xylene,

and rehydrated using graded ethanol steps (100%, for 5 minutes, twice; 90%, for 5 minutes, twice; and 70%, for 5 minutes, once), followed by immersion in deionized water, for 1 minute.

For DDR1 staining, we used sections from fresh frozen and perfusion-fixed tissues, the latter pre-treated with 0.2% sodium dodecyl sulfate (SDS) in PBS, for 5 minutes at room temperature. We could not detect DDR1 in paraffin sections, despite attempting antigen unmasking by several methods. For DDR2 staining, we used untreated sections from fresh frozen or perfusion-fixed tissues, and Antigen Unmasking Solution (Vector Laboratories, Burlingame, CA, USA)-treated paraffin sections. Sections were blocked with 1.5% normal donkey (Sigma) or rabbit (Vector Laboratories) serum, corresponding to the secondary antibody, in PBS for 1 hour, followed by a 5-minute PBS wash. Primary antibody diluted 1 : 20 in PBS was applied for 1 hour at room temperature, in a moist chamber. Sections were rinsed twice in PBS for 5 minutes, then incubated for 30 minutes with the appropriate secondary antibody, diluted 1 : 1,000 in PBS. Sections were rinsed twice in PBS for 5 minutes, then dehydrated and mounted on cover slips with the Prolong Antifade Kit (Molecular Probes), for examination by fluorescence microscopy.

3. Immunoblotting of membrane fractions

Membrane fractionation was carried out using previously described techniques, with minor modifications.[21] Tissues were isolated from rat, minced, and homogenized in isolation solution (250 mM sucrose, 10 mM triethanolamine, pH 7.6) containing protease inhibitors (1 g/ml leupeptin, 0.1mg/ml phenylmethysulfonyl fluoride). Homogenates were centrifuged at 1,000 g for 10 minutes, and the resulting supernatants centrifuged at 17,000 g, for 20 minutes. Pellets were resuspended in isolation solution, and protein concentrations determined using the Protein Assay Kit (Bio-Rad, Hercules, CA, USA).

For immunoblotting, 30 to 100-㎍ samples of 17,000 g membrane extracts were separated by denaturing SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes (Bio-Rad). Samples were loaded in 1x buffer containing 62.5 mM Tris-HCl, pH 6.8, 10% glycerol;

2% SDS, 2.5% β-mercaptoethanol, and 0.04% bromophenol blue. Membranes were blocked with 3～4% bovine serum albumin and 0.1% Tween 20 in PBS for 60 minutes, then incubated overnight with anti-DDR1 or DDR2 antibody (1 : 1,000 dilution in PBS), at 4°C with gentle agitation. After four washes in PBS, the secondary horseradish peroxidase-conjugated donkey anti goat IgG (1 : 100,000 dilution in PBS) was applied, at room temperature for one hour. After additional washing, antibody binding was visualized using the ECL kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). In control experiments, the primary antibody was pre-incubated with excess blocking peptide (Santa Cruz Biotechnology), according to the manufacturers instructions.

4. Cloning of rat DDR2 cDNA

The rat DDR2 coding region was amplified by polymerase chain reaction (PCR) amplification from a Marathon rat kidney cDNA library (Clontech, Palo Alto, CA, USA). The forward primer (FP) 5'-CATATGCCGCTATCCTCTGGGC-3' was degenerate, designed on the basis of human (nt 437-458, GenBank accession XM_016335) and mouse (nt 568-589, GenBank accession NM_ 022563) sequences. The reverse primer 5'-GCTGGAGTGGCTTAGATGTGGGTG-3' was derived from the partial 3' sequence of rat DDR2 (nt 604-627, GenBank accession NM_031764). The resulting 2.2 kb product was cloned in the TA cloning vector (Invitrogen, Carlsbad, CA, USA), and sequenced. The 5' end of the DDR2 coding region, including the FP annealing site, was verified by PCR amplification, using the Marathon Adaptor Primer 1 forward primer 5'-CCATCCT-AATACGACTCACTAT AGGGC-3', and the DDR2-specific reverse primer 5'-CTCAGGGCACC-AAGCTCCATCC-3', and sequencing. Potential DDR2 splice variants DDR2b and DDR2c (see Results) were identified by BLAST search of the GenBank nr and EST databases.

5. Ribonuclease protection assays (RPAs)

Multiprobe RPAs were used to assess DDR2 splice variability, and to compare patterns of DDR1, DDR2, transforming growth factor (TGF)-β_1 and β-actin mRNA expression, between remnant and control kidneys. Kidneys archived previously at -80°C were used in the latter studies. Total

RNA was prepared using ULTRASPEC RNA (Biotecx Laboratories, Houston, TX, USA).

Antisense riboprobes were transcribed from linearized templates using T7 RNA polymerase (Ambion, Austin, TX, USA) in the presence of [α-32P]UTP, and freed from unincorporated nucleotides using Sephadex G-50 columns (Roche Molecular Biochemicals, Indianapolis, IN, USA). Assays were carried out on 20-μg RNA samples, using the Ambion RPAII kit. In assessing potential splice variant DDR2b (Fig. 8), probes were directed against regions corresponding to DDR2, 3 untranslated region (UTR; nt 2126-3006 GenBank accession NM_031764); DDR2, juxtamembrane domain (nt 1367-1753, GenBank accession); and β-actin (nt 86-196, GenBank accession AI_713427). In assessing potential splice variant DDR2c (Fig. 8), probes were directed against regions corresponding to DDR2, 3'UTR; DDR2, tyrosine kinase (nt 2052-2440, GenBank accession); and β- actin. In comparisons between remnant and control kidneys, probes were directed against DDR1 (nt 1-192, GenBank accession AI_072242); DDR2, 3'UTR; TGF-β_1 (nt 941-1111, GenBank accession NM_021578); and β-actin. Hybridizations were carried out at 60°C overnight, and then digested with RNase T1 for 90 minutes at 37°C. Products were separated on 6% denaturing polyacrylamide gels, and detected by autoradiography or phosphorimagery (Molecular Dynamics, Sunnyvale, CA, USA). Densitometry was carried out using ImageQuant software (Molecular Dynamics).

6. Statistics

Effects of subtotal nephrectomy and experimental time points on DDR1, DDR2, TGFβ_1, β-actin mRNA levels were investigated by two way ANOVA. Post-hoc determination of differences between experimental groups was carried out by Bonferroni method.

III. RESULTS

1. DDR1 is expressed in endosomes of the proximal tubule

The distribution of DDR1 in rat kidney was investigated by indirect immunofluorescence, using antibodies directed against the N- or C-terminus (Fig. 1A and B). With each antibody, a punctate

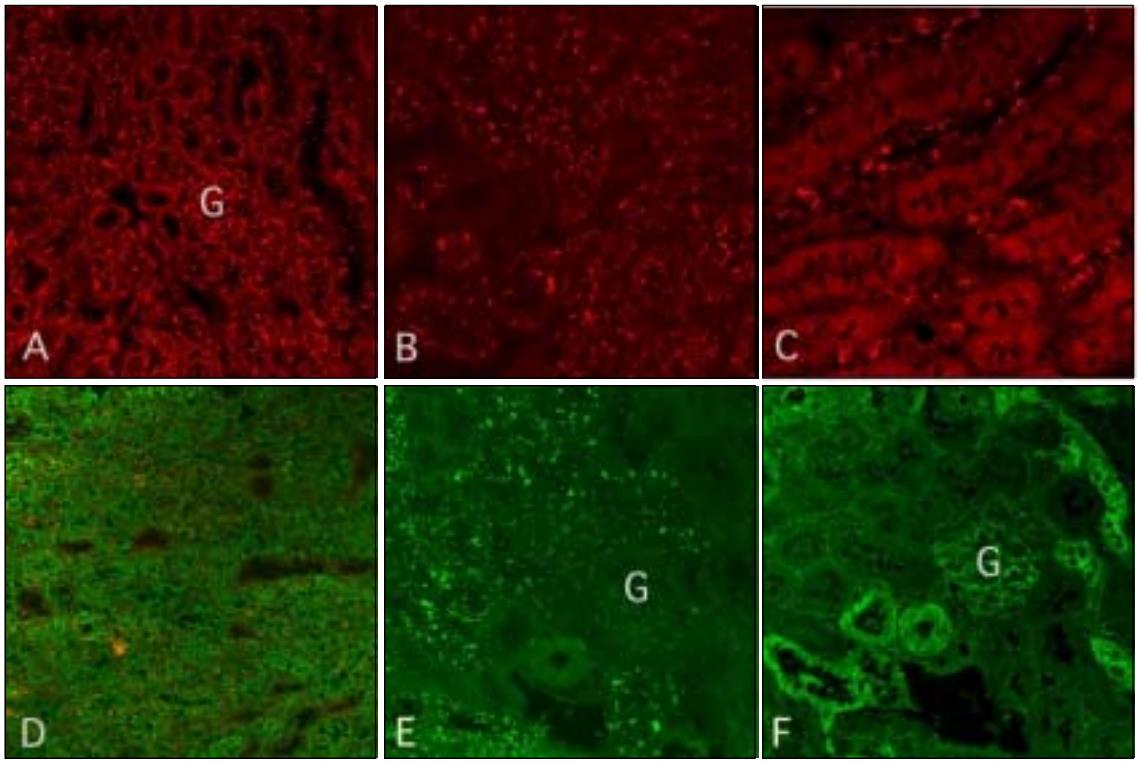

Fig. 1. Kidney distribution of DDR1. DDR1 staining (red) demonstrates a punctate distribution in cortex (A, B) and medulla (C). Dual staining with anti-DDR1 antibody (red) and lectin *Phaseolus vulgaris* (green), a marker for the proximal tubule (D) suggests a partial overlap in the tubular distribution patterns. Serial sections stained for DDR1 (green) (E) and Tamm-Horsfall protein (green) (F), within the ascending loop of Henle, indicates no apparent overlap in distribution. Interestingly, the cellular distribution of DDR1 appears incompatible with extracellular matrix interactions. G: glomerulus. Frozen sections.

staining pattern was observed, predominantly within renal cortical tubules. In some sections, we also observed same staining pattern in medullary tubules (Fig. 1C). There was no glomerular, interstitial or vascular staining. Staining was abolished by pre-incubation of the primary antibodies with corresponding blocking peptides (not shown). Positively staining cortical tubules were identified as proximal tubules, by their dual staining with the marker lectin Phaseolus vulgaris (Fig. 1D), and their lack of staining with antibody against Tamm-Horsfall protein (Fig. 1E and F).

To further define DDR1-containing structures, we carried out dual staining with an antibody directed against EEA1, a marker of early endosomes (Fig. 2A and B).[22] Consistent with previous

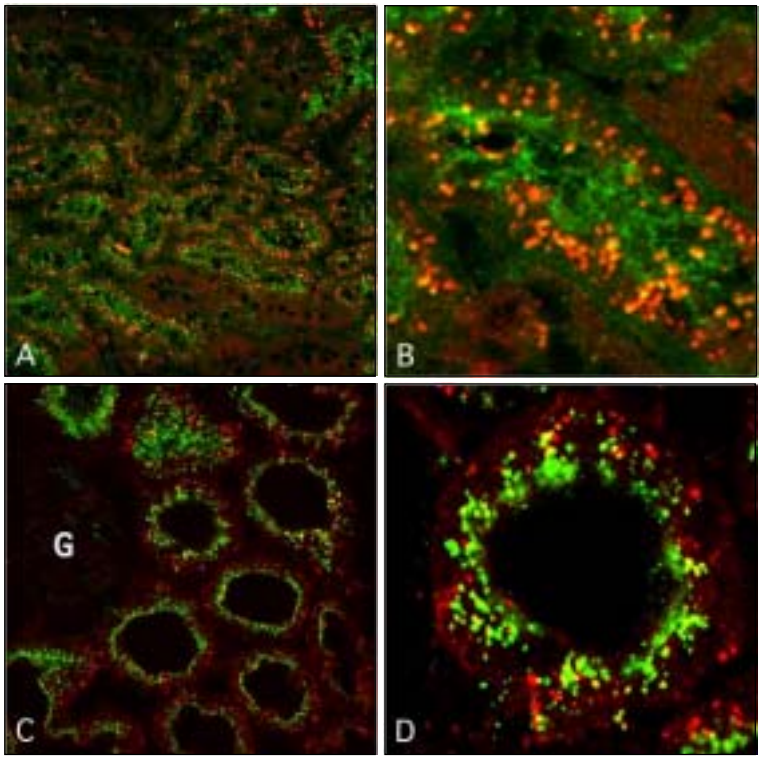

Fig. 2. Co-localization of DDR1 with endosomal markers in proximal tubule. Dual staining of DDR1 (red) and EEA1 (green), an apical membrane and early endosome marker, revealed significant overlap in their distribution (yellow; A and B). FITC-dextran was used as a marker of fluid-phase endocytosis in proximal tubules (C and D). Sections from kidneys harvested 15 minutes after bolus infusion exhibited significant overlap (yellow) of DDR1 staining (red) and FITC-dextran (green) G: glomerulus. Fresh frozen sections.

reports,[23] and knowledge of extensive apical membrane endocytosis in proximal tubules,[24] EEA1 immunoreactivity was detected in apical and subapical regions of proximal tubular cells. DDR1 and EEA1 staining patterns overlapped partially, indicating that DDR1 is present in early endosomes, and other structures as well, possibly including late endosomes, lysozomes, post-Golgi vesicles and/or basolateral structures. At least a fraction of these structures are derived from the apical membrane, as we observed overlap between DDR1 staining, and infused FITC-dextran, filtered across the glomerular capillary wall, and taken up by fluid-phase endocytosis (Fig. 2C

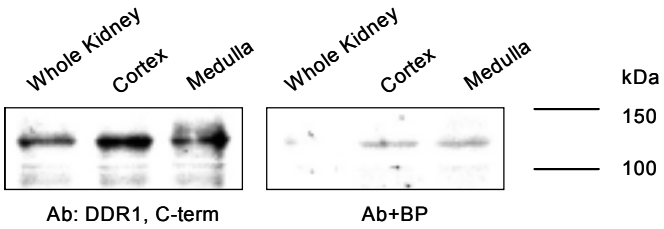

Fig. 3. Immunoblotting of DDR1 in kidney membrane fractions. Western blot analysis using anti-DDR1 C-terminal antibody detected a 125 kDa band in whole kidney, cortical, or medullary 17,000 g membrane fractions. DDR1 abundance was enriched in the cortical fraction. Immunoreactivity of the antibody is largely abolished by preincubation with a blocking peptide. Ab, antibody; BP, blocking peptide.

and D).

A predominant band of 125 kDa, corresponding to the reported MW of DDR1 was identified by immunoblotting of subcellular membrane fractions with N- or C-terminal antibody (Fig. 3). Lower MW bands were also identified, suggesting the possibility of alternative forms of DDR1,[5,6] or partial degradation products. Bands were enriched in the renal cortex, consistent with the findings on immunofluorescence (Fig. 3). Immunoreactivity was abolished by pre-incubation of the primary antibody with blocking peptide.

2. DDR2 is expressed in apical membranes of distinct nephron segments

Indirect immunofluorescent staining of rat kidney sections with antibodies directed against the N- or C-terminus of DDR2 revealed apical membrane expression in select renal tubules in the cortex and outer medulla, especially the macula densa (Fig. 4A and B). No glomerular, interstitial or vascular staining was observed. Staining was abolished by pre-incubation of the primary antibodies with corresponding blocking peptides (not shown). In dual labeling experiments, we could identify co-localization of DDR2 antibody staining not with that of Phaseolus vulgaris Ligand (Fig. 4C) or calbindin D antibody (Fig. 4D), a marker of principal cells within distal and collecting tubules, but with that of Tamm-Horsfall protein antibody (Fig. 4E and F). These findings were

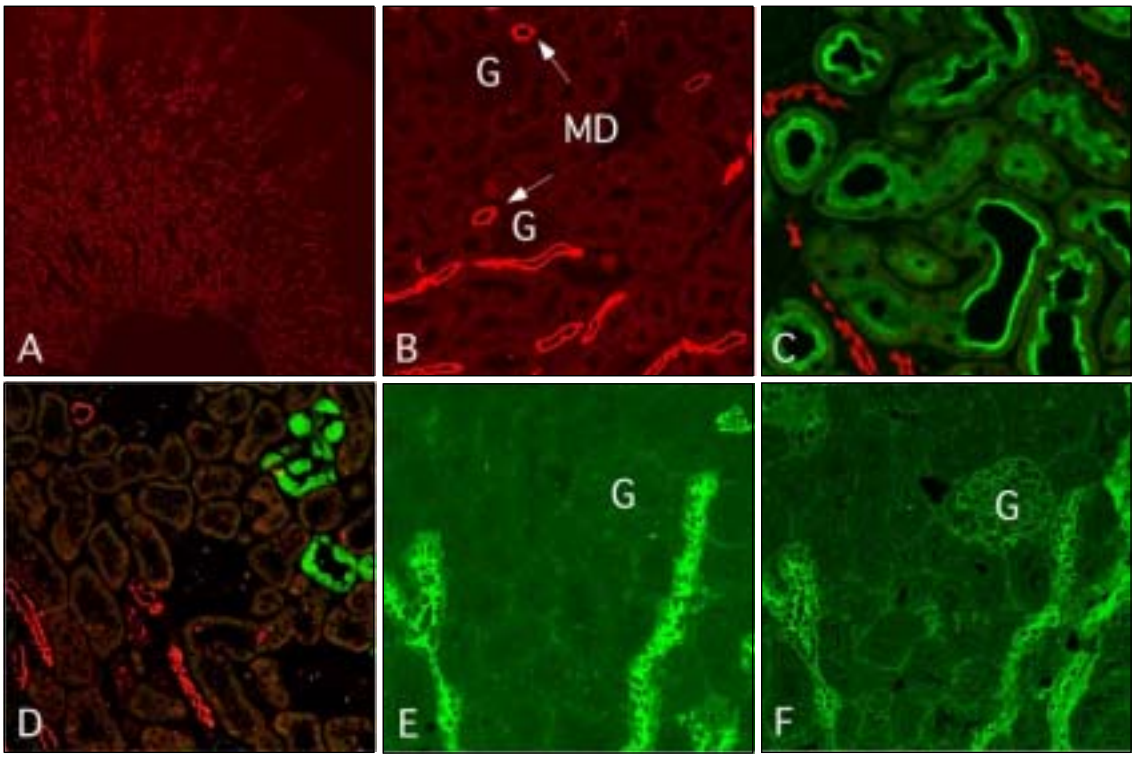

Fig. 4. Kidney distribution of DDR2. Select tubules of the renal cortex and medulla exhibit apical DDR2 staining (A). Staining is prominent in the macula densa (B). Dual staining with anti-DDR2 antibody (red) and lectin *Phaseolus vulgaris* (green) (C), or anti-calbindin antibody (green) (D), reveals no overlap in their respective tubular distributions. Serial sections stained for DDR2 (E) and Tamm-Horsfall protein (F) indicates that DDR2 is present within the loop of Henle. G, glomerulus; MD, macula densa. Paraffin sections.

considered as evidence of continuous expression, from the ascending loop of Henle to the macula densa. Thus, DDR2, like DDR1, is inappropriately situated for major roles in cell-matrix interactions.

DDR2 immunoreactivity was detected in plasma membrane fractions from kidney, enriched in the medulla, using both C- and N-terminal antibodies (Fig. 5 and data not shown). Predominant bands migrated at an apparent MW between 150 and 250 kDa, compared to the expected MW of 130 kDa. Bands of the expected MW were detected in membrane fractions prepared from other tissues, including heart, lung and brain (Fig. 6), suggesting fidelity of the antibody reagents. DDR2

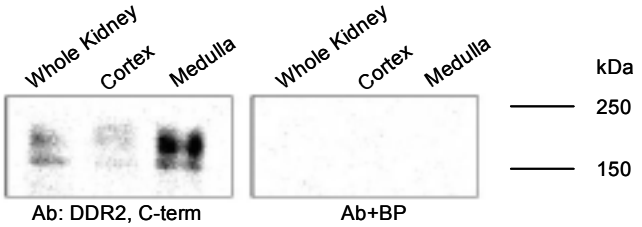

Fig. 5. Immunoblotting of DDR2 in kidney membrane fractions. A predominant band of apparent molecular weight 200 kDa is detected in 17,000 g fractions from whole kidney, cortex and medulla, enriched in the medulla. Immunoreactivity of the C-terminal antibody is abolished by pre-incubation with a blocking peptide.

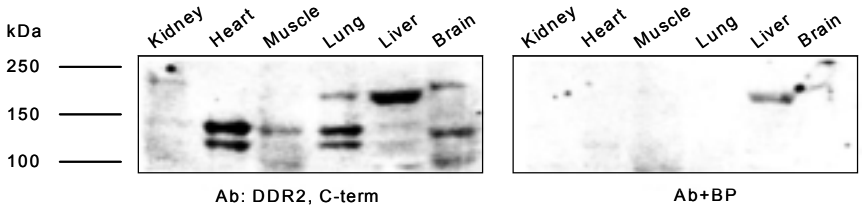

Fig. 6. Immunoblotting of DDR2 in tissue membrane fractions. Membrane fractions from the indicated tissues were blotting with an antibody directed against the C-terminus of DDR2. Multiple bands were detected, with the majority abolished by pre-incubation of the antibody with blocking peptide. Major bands of 130 kDa, corresponding to the expected molecular weight of DDR2, were present in several tissues. Higher molecular weight bands were present in kidney, lung, liver and brain. Lower molecular weight forms of uncertain origin were also identified in several tissues.

migration was not affected by varying heat pre-treatment or urea concentration of the samples, maneuvers influencing the electrophoretic behavior of membrane proteins subject to heat- and SDS-induce aggregation.[25]

3. A high MW, SDS-resistant form of DDR2 is predominant in kidney

On the basis of our findings, it's possible that DDR2 exists in a unique high MW form in kidney. In principle, this could arise as a tissue-specific isoform, product of post-translational processing, e.g. glycoform, and/or persistence of a denaturation-resistant homo/hetero-oligomer, exhibiting true or anomalous migration by SDS-PAGE. Structural features of the kidney-specific form of DDR2

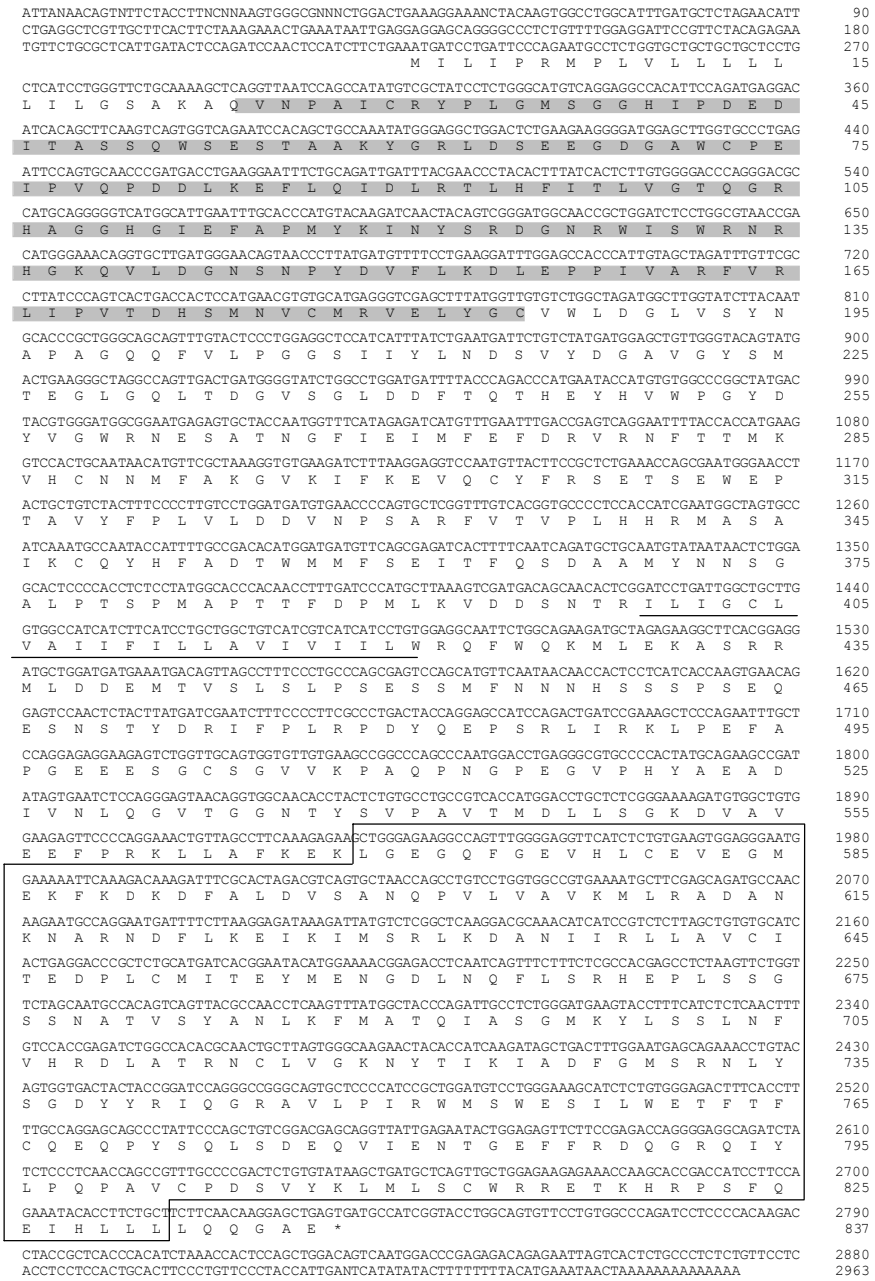

Fig. 7. Nucleotide and deduced amino acid sequences of rat DDR2. The discoidin domain (shaded), transmembrane domain (underlined), tyrosine kinase domain (boxed) are indicated.

might be expected to influence its biological properties, including membrane targeting, accounting for the specialized apical membrane distribution.

DDR2 coding region was cloned from a rat kidney cDNA library. The sequence predicts an open reading frame of 2562 bp, corresponding to 854 aa, with an estimated MW of 94 kDa (Fig. 7). The predicted amino acid sequence was 96.6 and 98.9% identical to the human and mouse sequences, respectively, without insertions to account for high MW species. Splice variants of DDR2 predicting larger gene products have not been reported. Through searching the EST database, two potential splice variants were identified, these are termed DDR2b and DDR2c, from human sources (GenBank accessions AV707701 and AV705759, respectively), each predicted to truncate the cytoplasmic domain internally, by 18 aa (Fig. 8). As splicing variability exerts potential influence over RTK oligomerization/activation state[26-28] and targeting.[29] Rat orthologues of DDR2b and DDR2c were postulated, and investigated the relative expression of these splice variants (Fig. 8). Consistent with previous findings,[30] DDR2 mRNA is widely expressed. The

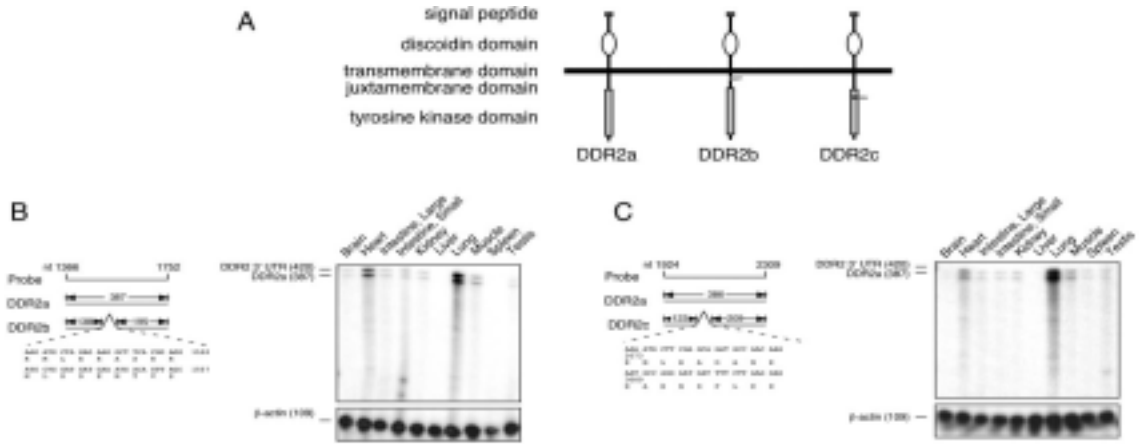

Fig. 8. mRNA expression of DDR2 and its potential splice variants. Tissue-specific splicing variability is a potential basis for specialized targeting of the DDR's in kidney. Potential splice variants DDR2b and DDR2c, identified in dbEST, are compared to the known form of DDR2 (DDR2a) in (A). RNase protection assays for DDR2b and DDR2c are shown in (B) and (C), respectively. Riboprobes targeted against regions of potential splicing variability are shown schematically, indicating the sizes of protected bands, for the expected mRNA species. Protected bands for DDR2b and DDR2c were not identified.

known form of DDR2 (DDR2a) accounted for its expression in all examined tissues, including kidney, without detectable contributions from potential splice variants DDR2b and DDR2c. Thus, the kidney form of DDR2 is unlikely to arise from splice variants encoding tissue-specific isoforms.

It looked possible that the high MW form of DDR2 is a unique glycoform, exhibiting anomalous migration by SDS-PAGE. Deglycosylation of kidney membrane fractions with N-glycanase, for removal of N-linked oligosaccharides, did not alter the electrophoretic behavior of high MW DDR2 (not shown).

Together, these findings suggest a unique oligomeric form of DDR2, arising in kidney by one of several possible mechanisms (see Discussion).

4. DDR mRNA levels are not altered in experimental renal fibrosis

It was an unexpected finding that DDR expression was removed from major sites of the extracellular matrix in kidney. In the tubulointerstitium, in which the DDR's are expressed, the major extracellular matrix networks are tubular basement membrane, composed largely of type IV collagen, and the interstitial matrices, containing fibrillar collagens I, III and V.[31-33] We proceeded to investigate DDR expression in fibrosing remnant kidneys from subtotally nephrectomized rats, in which fibrillar collagens I and III are present as major components of the expanding extracellular matrices, within the glomeruli and tubulointerstitium.[33,34] We report results for DDR2 alone, as it was not possible to detect DDR1 in paraffin sections. DDR2, in particular, has already been implicated in experimental models of progressive fibrosis.[18,35]

In remnant kidneys obtained 2 and 4 weeks after nephrectomy, characteristic histologic changes of glomerular hypertrophy, interstitial expansion, and mesangial and peritubular fibrosis were observed (not shown). Cortical and medullary DDR2 expression remained associated exclusively with apical membranes, without evidence of redistribution towards the expanding matrices (Fig. 9A and B). There was no evidence of glomerular or interstitial expression, occurring *de novo*. By contrast, we observed intense expression of vimentin, well described as a marker of activated glomerular, tubular and interstitial cells, in this model (Fig. 10D, E and F).[33]

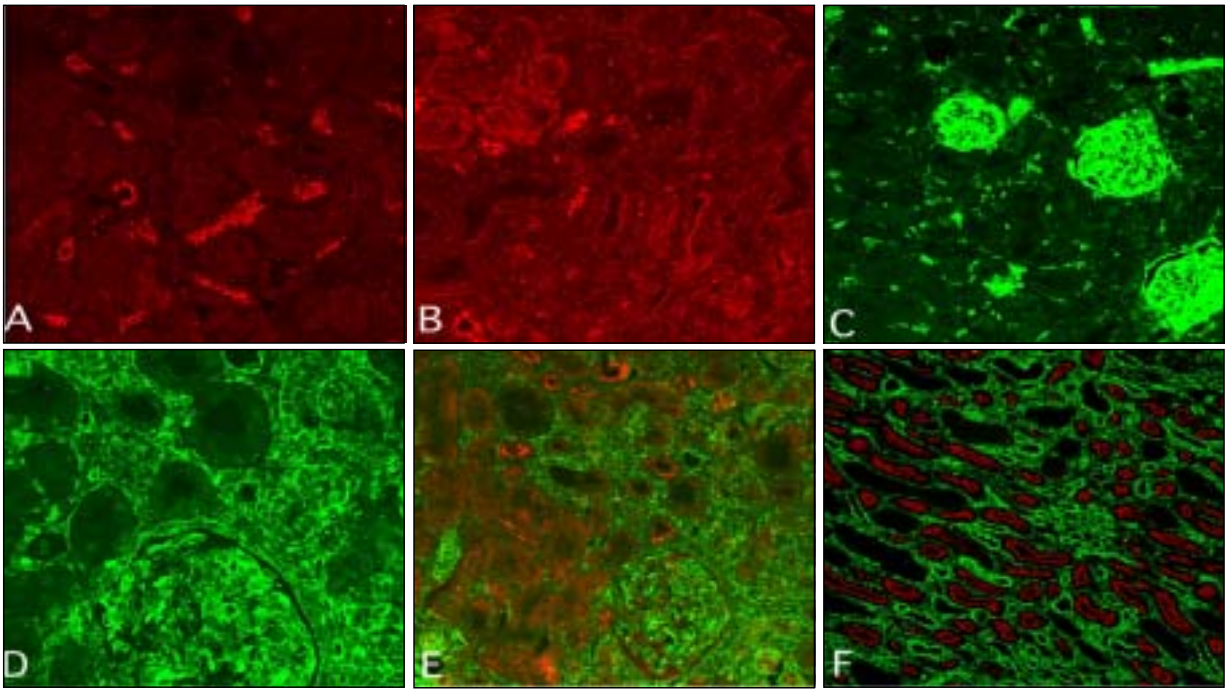

Fig. 9. DDR2 localization in 5/6 remnant rat kidneys. DDR2 staining remains comfined to the apical surface of select renal tubules, at 2 (A) and 4 (B) weeks. Vimentin (green) is a marker of glomerular podocytes and interstitial cells in normal rat kidney (C). Its upregulation in remnant kidneys is evidenced within an enlarging population of interstitial cells, as well as tubular epithelial cells (D). Dual staining for DDR2 (red) and vimentin (green) in cortex (E) and medulla (F) reveals no evidence of overlapping expression.

DDR mRNA levels were determined by RNase protection assays, in remnant and control kidneys (Fig. 10). There were no significant differences between the groups in DDR and β-actin mRNA levels, at 2, 4 or 8 weeks. By contrast, mRNA levels for the major fibrogenic cytokine TGF-β_1 were significantly higher in remnant kidneys, as reported for this model.[36]

IV. DISCUSSION

The DDR's are known to be essential in mammalian development. Identified originally as orphan receptor tyrosine kinases, they have attracted considerable attention as potential non-integrin

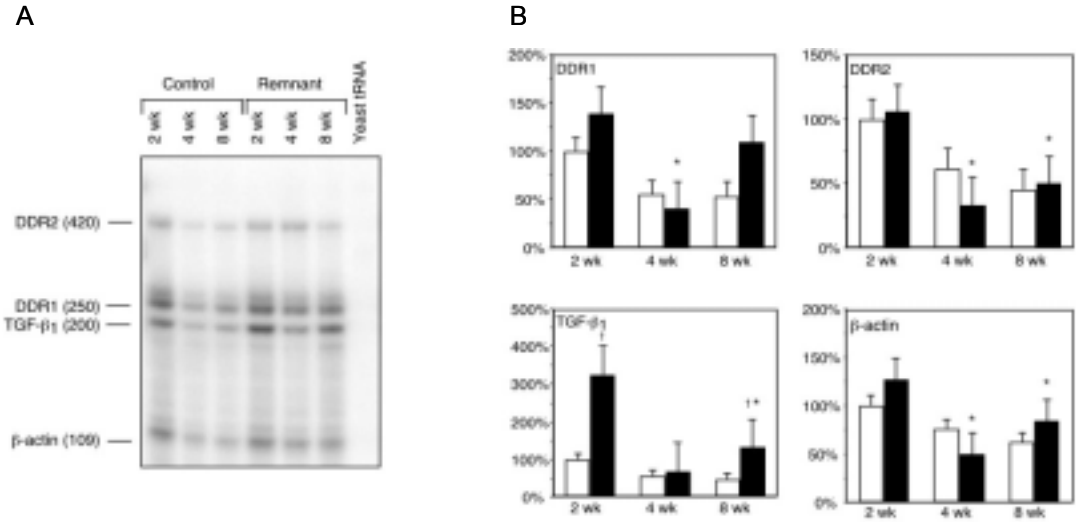

Fig. 10. Comparison of DDR mRNA levels in remnant and control kidneys. Representative RNase protection assays for DDR1, DDR2, TGF-β1 and β-actin are shown in (A). Molecular sizes (in bases) of protected bands are indicated. Samples were obtained from remnant or control kidneys, 2, 4 and 8 weeks after nephrectomy. Results of densitomertric analysis are summarized in (B). For each mRNA, data represent mean±SEM (n=6), and are normalized to the mean value for 2-week control kidneys. White bars: control. Black bars: remnant. Statistically significant differences are indicated. *: vs. 2-weeks ($P<0.05$). † : control vs. remnant ($P<0.05$).

collagen-binding receptors. Indeed, the observed phenotypes of *Ddr*-targeted mice are fully compatible with this view, inasmuch as cell-matrix interactions are central in the development of affected organs, including the bony skeleton and breast.[37,38] Notwithstanding, proof of DDR activation by collagens *in vivo* represents an important experimental challenge.

In this study, DDR distribution was investigated in normal and fibrosing adult rat kidneys, postulating effector pathways in progressive renal injury, originating from interactions with the expanding interstitial matrices. Surprisingly, the DDR's are removed from potential sites of interaction with renal extracellular matrices, a finding also applying to DDR2 in fibrosing kidneys, despite its prior implication of DDR2 in experimental fibrosis.[18,35] Here, limitations on current findings, and then the implications of those corroborated by use of multiple antibodies are discussed.

1. Limitations on the experimental findings

This study has been focused on DDR expression in progressive renal injury, engendered in a well-established experimental model. This study does not fortify evidence for the roles of the DDR's in mediating cell-matrix interactions, attendant to their regulating cellular behaviors. However, several restrictions on our conclusions, as they relate to DDR localization in the kidney, warrant consideration.

First, it was not possible to localize DDR1 in remnant kidneys, archived in paraffin blocks. Pending direct investigation, it is possible that DDR1 is activated in resident and/or invading cell types of the fibrosing kidney, or that DDR1 protein is redistributed towards the basolateral membrane of renal tubular cells. Indeed, DDR1 is located predominantly in the renal cortex, preferentially involved in pathways of glomerular hypertrophy and sclerosis, tubular trans-differentiation, and interstitial fibrosis, which are defining histological features of this model, but from our study, DDR1 mRNA levels are not significantly different between remnant and control kidneys. However, this finding is uninformative with respect to potential forms of post-transcriptional regulation, which might be the case in these settings.

Second, this study does not exclude a role for DDR2 in mediating matrix interactions for rare cell types, particularly fibroblasts. It has been postulated that the DDR's exert their effects on matrix turnover through stromal fibroblasts, rather than differentiated organotypic cells.[15,18,35,39] Central in fibrogenesis, renal fibroblasts have variable ontogeny, are phenotypically diverse, and undergo population-based shifts, in response to fibrogenic stimuli.[40,41] In reviewing sections from control and remnant kidneys, we detected DDR2 expression in tubular epithelial cells only, and vimentin in the interstitium, populated by fibroblasts. However, it is possible that DDR2 is present at undetectable levels in renal fibroblasts, or is expressed uniquely in an uncommon subset of these cells.

Third, this study does not exclude roles for the DDR's in mediating cell-matrix interactions

within the kidney, in other developmental or pathophysiologic states. For example, it is possible that the DDR's can be localized to sites of the extracellular matrix in the developing kidney, in which cell-matrix interactions serve critical and multifaceted roles.[42] The DDR's might also be postulated to serve in recovery from acute tubular necrosis, during which tubular epithelial cells rely on cell-matrix interactions, in repopulatation of the nephron.[43] The DDR's may be more easily implicated in other forms of progressive renal fibrosis, including other experimental models, differing from the remnant kidney model in their pathophysiological mechanisms.

2. Kidney distribution of the DDR's

Major findings of this study relate to the kidney distribution of the DDR's. DDR1 is localized predominantly to subcellular vesicle populations within the proximal tubule, and DDR2 to apical membranes from the ascending loop of Henle, to the macula densa. For DDR2, this study couldn't show novel sites of expression in well-established fibrosing remnant kidney models.

Despite advancing knowledge of DDR biology, there remains relatively little information regarding their tissue localization. DDR1 immunoreactivity is present within several stratified epithelial, stromal and endothelial cell types of the cornea,[44] arterial adventitial fibroblasts following experimental injury,[15] and in cortical neurons of the cerebellum.[16] DDR2 is co-expressed with DDR1 in the cornea.[44] DDR1 is present in the polarized epithelium of the mammary gland,[9] but its cellular distribution is unclear. Recently, basolateral localization of DDR1 in bronchial epithelium has been reported.[17]

The distribution of DDR1 in select renal tubules implies major abundance in a recyclable pool, trafficking, at least partially, through the apical membrane. It was not determined whether DDR1 can traffic through the basolateral membrane. It is instructive to compare the distribution of DDR1 to those of integrin basement membrane receptors in the proximal tubule, confined to basolateral membranes. If DDR1 does indeed transduce the state of basement membrane or interstitial matrices, then its ligand specificities, dynamic range, and signal response characteristics are likely

to be wholly distinct from those of the integrins. The cellular synthesis, sorting, turnover and sites of activity of DDR1 are overlapping issues still to be addressed.

The existence of a unique ~200 KDa form of DDR2 in kidney may provide important clues regarding its apical membrane localization. While subject to complex interplay among targeting determinants, membrane proteins of polarized epithelia appear to be under major influences of primary peptide sequences, post-translational modification, and oligomerization state.[45-47] It was not possible to identify splice isoforms of DDR2, predicted to retain immunoreactivity, or N-linked glycosylated forms, susceptible to deglycosylation with PNGase F. Denaturation-resistant homo- or heterooligomeric forms of DDR2 in kidney remain a major possible explanation for our findings. Several RTK's are now known to exist in oligomeric forms, which precede ligand-induced activation, or are themselves subject to ligand-independent activation.[48] Some RTK's also appear to undergo irreversible covalent dimerization.[49]

3. Considering novel functions for the DDR

DDR localization in the kidney raises the possibility of alternative roles for this receptor pair. In contrast to other RTK's, DDR activation by collagen follows slow kinetics and can take up to 18 hr to reach maximal kinase activity.[11] Indeed, the possibility of alternative ligands has been considered previously.[45]

The extracellular domains of the DDR's are each dominated by the presence of a discoidin domain, named after its homology to the discoidin I protein in the slime mold, *Dictyostelium discoideum*. In slime mold, this protein is upregulated during starvation, accounting for as much as 1% of total protein, as single cells aggregate adaptively, to produce multicellular forms of the organism. Despite extensive study, the functions and natural ligands of discoidin I remain obscure. Divergent roles in mediating cell-cell[51] and cell-matrix[52] interactions have been described, but more recent evidence suggests that these are cytoplasmic proteins regulating cytoskeletal reorganization during chemotaxis.[53] Indeed, the weight of evidence suggests multiplicity of functions for this protein.

Discoidin domains have now been identified widely in mammalian proteins, and their putative functions have been reviewed.[4,5] Prominent examples include the C2 domains of coagulation factors V and VIII, which have afforded a structural basis for interactions with phospholipids of the activated endothelium.[54,55] Likewise, the discoidin domain of lactadherin, first described as a milk protein, may serve its primary function by mediating interactions with exosomes, phospholipid vesicles discharged by dendritic and other cell types, for the purposes of transmitting cargo to nearby cells.[56] The case of the neuropilins may be particularly instructive in considering alternative functions for the DDR's. Neuropilins-1 and 2 are single-transmembrane domain receptors, each containing two extracellular discoidin domains, binding class 3 semaphorins, as plexin co-receptors,[57-60] and vascular endothelial growth factor (VEGF) isoforms, as VEGF co-receptors.[61] Additionally, the neuropilins can serve as receptors for other as yet unidentified cell adhesion ligands.[62] Together, the properties of these and other discoidin domain-containing proteins are illustrative of several principles: first, that these proteins are multifunctional; second, that discoidin domains can interact with unconventional as well as conventional ligands, including phospholipids; and third, that full reconstitution of biological activity requires co-receptors or other signaling molecules.

In kidney, roles of the DDR's remain to be established. Both DDR1 and DDR2 must be considered potential receptors and/or co-receptors for ligands at the apical surface, chiefly constituents of the glomerular ultrafiltrate. DDR1 may also function in subcellular membrane compartments, while DDR2 may function as a constitutive homo- or hetero-oligomer. Interestingly, triple-helical collagen degradation products are filtered into the urinary space,[63] where they could interact with DDR's, but unlikely at a concentration sufficient to effect receptor activation. This suggests the possibility of alternative roles for the DDR's.

4. Summary and future directions

Identification of collagen as ligands for the DDR's was a major step forward in their

understanding.[11,12] It is worthwhile considering the possibility that these receptors have complementary ligands, and/or functions independent of collagen-binding. Tissue systems, represented here, should prove invaluable in defining important biochemical properties of this receptor pair *in vivo*, enhancing knowledge of their signaling pathways, in a variety of biological settings.

V. CONCLUSION

In conclusion, this study showed DDR expression in normal and 5/6 nephrectomy rat kidney. DDR localization in the adult rat kidney is incompatible with predominant role in cell-matrix interaction.

REFERENCES

1. van der Geer P, Hunter T, Lindberg RA. Receptor protein-tyrosine kinases and their signal transduction pathways. Annu Rev Cell Biol 1994;10:251-337.
2. Alves F, Vogel W, Nossie K, Milauer B, Höfler H, Ulrich A, et al. Distinct structural characteristics of discoidin I subfamily receptor tyrosine kinases and complementary expression in human cancer. Oncogene 1995;10:609-18.
3. Poole S, Firtel RA, Lamar E, Rowekamp W. Sequence and expression of the discoidin I gene family in Dictyostelium discoideum. J Mol Biol 1981;153:273-89.
4. Baumgartner S, Hofmann K, Chiquet-Ehrismann R, Bucher P. The discoidin domain family revisted: new members from prokaryotes and homology-based fold prediction. Protein Sci 1998;7:1626-31.
5. Vogel W. Discoidin domain receptors: structural relations and functional implication. FASEB J 1999;13:S77-82.
6. Alves F, Saupe S, Ledwon M, Schaub F, Hiddemann W, Vogel W. Identification of two novel, kinase-deficient variants of discoidin domain receptor 1: differential expression in human colon cancer cell lines. FASEB J 2001;15:1321-3.
7. Lai C, Lemke G. Structure and expression of the tyro10 receptor tyrosine kinase. Oncogene 1994;9:877-83.
8. Zerlin M, Julius MA, Goldfarb M. NEP: A novel receptor-like tyrosine kinase expressed in proliferative neuroepithelia. Oncogene 1993;8:2731-9.
9. Vogel WF, Aszdi A, Alves F, Pawson T. Discoidin domain receptor 1 tyrosine kinase has

an essential role in mammary gland development. Mol Cell Biol 2001;21:2906-17.
10. Labrador JP, Azcoitia V, Tuckermann J, Lin C, Olaso E, Mañes S, et al. The collagen receptor DDR2 regulates proliferation and its elimination leads to dwarfism. EMBO Rep 2001; 2:446-52.
11. Shrivastava A, Radziejewski C, Campbell E, Kovak L, McGlynn M, Ryan TE, et al. An orphan receptor tyrosine kinase family whose members serve as nonintegrin collagen receptors. Mol Cell 1997;1:25-34.
12. Vogel W, Gish GD, Alves F, Pawson T. The discoidin domain receptor tyrosine kinases are activated by collagen. Mol Cell 1997;1:13-23.
13. Curat CA, Eck M, Dervillez X, Vogel WF. Mapping of epitopes in discoidin domain receptor 1 critical for collagen-binding. J Biol Chem 2001;7:45952-8.
14. Kamohara H, Yamashiro S, Galligan C, Yoshimura T. Discoidin domain receptor 1 isoform-a (DDR1alpha) promotes migration of leukocytes in three-dimensional collagen lattices. FASEB J 2001;15:2724-6.
15. Hou G, Vogel W, Bendeck MP. The discoidin domain receptor tyrosine kinase DDR1 in arterial would repair. J Clin Invest 2001;107:727-35.
16. Bhatt RS, Tomoda T, Fang Y, Hatten ME. Discoidin domain receptor 1 functions in axon extension of cerebellar granule neurons. Genes Dev 2000;14:2216-28.
17. Sakamoto O, Suga M, Suda T, Ando M. Expression of discoidin domain receptor 1 tyrosine kinase on the human bronchial epithelium. Eur Respir J 2001;17:969-74.
18. Ankoma-Sey V, Matli M, Chang KB, Lalazar A, Donner DB, Wong L, et al. Coordinated induction of VEGF receptors in mesenchymal cell types during rat hepatic wound healing. Oncogene 1998;17:115-21.
19. Anderson S, Rennke HG, Brenner BM. Therapeutic advantages of converting enzyme inhibitiors in arresting progressive renal disease associated with systemic hypertension in the rat. J Clin Invest 1986;77:1993-2000.
20. Lafayette RA, Mayer G, Park SK, Meyer TW. Angiotensin II receptor blockade limits glomerular injury in rats with reduce renal mass. J Clin Invest 1992;90:766-71.
21. Ecelbarger CA, Terris J, Frindt G, Echevarria M, Marples D, Nielsen S, et al. Aquaporin-3 water channel localization and regulation in rat kidney. Am J Physiol 1995;269:F663-F72.
22. Mu FT, Callaghan JM, Steele-Mortimer O, Stenmark H, Parton RG, Campbell PL, et al. EEA1, an early endosome-associated protein: EEA1 is a conserved alpha-helical peripheral membrane protein flanked by cysteine “fingers” and contains a calmodulin-binding IQ motif. J Biol Chem 1995;270:13503-11.
23. Keusch I, Traebert M, Ltscher M, Kaissling B, Murer H, Biber J. Parathyroid hormone and dietary phosphate provoke a lysosomal routing of the proximal tubular Na/Pi-cotransporter type II. Kidney Int 1998;54:1224-32.

24. Marshansky V, Bourgoin S, Londono I, Bendayan M, Maranda B, Vinay P. Receptor-mediated endocytosis in kidney proximal tubules: recent advances and hypothesis. Electrophoresis 1997;18:2661-76.
25. Soulie S, Moller JV, Falson P, le Maire M. Urea reduces the aggregation of membrane proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Anal Biochem 1996;236:363-4.
26. Clary DO, Reichardt LF. An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. Proc Natl Acad Sci USA 1994;91: 11133-7.
27. Collesi C, Santoro MM, Gaudino G, Comoglio PM. A splicing variant of the *RON* transcript induces constitutive tyrosine kinase activity and an invasive phenotype. Mol Cell Biol 1996;16: 5518-26.
28. Kwong KY, Hung MC. A novel splice variant of *HER2* with increased transformation activity. Mol Carcinog 1998;23:62-8.
29. Kryl D, Yacoubian T, Haapasalo A, Castren E, Lo D, Barker PA. Subcellular localization of full-length and truncated Trk receptor isoforms in polarized neurons and epithelial cells. J Neurosci 1999;19:5823-33.
30. Lai C, Lemke G. Structure and expression of the tyro10 receptor tyrosine kinase. Oncogene 1994;9:877-83.
31. Jones CL, Buch S, Post M, McCulloch L, Liu E, Eddy AA. Pathogenesis of interstitial fibrosis in chronic purine aminonucleoside nephrosis. Kidney Int 1991;40:1020-31.
32. Martinez-Hernandez A, Gay S, Miller EJ. Ultrastructural localization of type V collagen in rat kidney. J Cell Biol 1982;92:343-9.
33. Muchaneta-Kubara EC, el Nahas AM. Myofibroblast phenotypes expression in experimental renal scarring. Nephrol Dial Transplant 1997;12:904-15.
34. Floege J, Alpers CE, Burns MW, Pritzl P, Gordon K, Couser WG, et al. Glomerular cells, extracellular matrix accumulation, and the development of glomerulosclerosis in the remnant kidney model. Lab Invest 1992;66:485-97.
35. Norman JT, Fine LG. Progressive renal disease: fibroblasts, extracellular matrix, and integrins. Exp Nephro 1999;l7:167-77.
36. Junaid A, Rosenberg ME, Hostetter TH. Interaction of angiotensin II and TGF-β1 in the rat remnant kidney. J Am Soc Nephrol 1997;8:1732-8.
37. Olsen BR, Reginato AM, Wang W. Bone development. Annu Rev Cell Biol 2000;16:191-220.
38. Silberstein GB. Postnatal mammary gland morphogenesis. Microsc Res Tech 2001;52:155-62.
39. Chin GS, Lee S, Hsu M, Liu W, Kim WJ, Levinson H, et al. Discoidin domain receptors and their ligand, collagen, are temporally regulated in fetal rat fibroblasts in vitro. Plast Reconstr Surg 2001;107:769-76.
40. Okada H, Ban S, Nagao S, Takahashi H, Suzuki H, Neilson EG. Progressive renal fibrosis

in murine polycystic kidney disease: an immunohistochemical observation. Kidney Int 2000;58:587-97.
41. Zeisberg M, Strutz F, Mller GA. Renal fibrosis: an update. Curr Opin Nephrol Hypertens 2001;10:315-20.
42. Pohl M, Stuart RO, Sakurai H, Nigam SK. Branching morphogenesis during kidney development. Annu Rev Physiol 2000;62:595-620.
43. Zuk A, Bonventre JV, Brown D, Matlin KS. Polarity, integrin and extracellular matrix dynamics in the postischemic rat kidney. Am J Physiol 1998;275:C7111-C731.
44. Mohan RR, Mohan RR, Wilson SE. Discoidin domain receptor (DDR) 1 and 2: collagen-activated tyrosine kinase receptors in the cornea. Exp Eye Res 2001;72:87-92.
45. Caplan MJ. Membrane polarity in epithelial cells: protein sorting and establishment of polarized domains. Am J Physiol 1997;272:F425-F9.
46. Keller P, Simons K. Post-Golgi biosynthetic trafficking. J Cell Science 1997;110:3001-9.
47. Reddy PS, Corley RB. Assembly, sorting and exit of oligomeric proteins from the endoplasmic reticulum. Bioessays 1998;20:564-554.
48. Jiang G, Hunter T. Receptor signaling: When dimerization is not enough. Curr Biol 1999;9:R568-R71.
49. van der Vliet A, Hristova M, Cross CE, Eiserich JP, Goldkorn T. Peroxynitrite induces covalent dimerization of epidernal growth factor receptors in A431 epidermoid carcinoma cells. J Biol Chem 1998;273:31860-6.
50. Vogel WF, Aszdi A, Alves F, Pawson T. Discoidin domain receptor 1 tyrosine kinase has an essential role in mammary gland development. Mol Cell Biol 2001;21:2906-17.
51. Gabius H-J, Springer WR, Barondes SH. Receptor for the cell binding site of discoidin I. Cell 1985;42:449-56.
52. Springer WR, Cooper DN, Barondes SH. Discoidin I is implicated in cell-substratum attachment and ordered migration of Dicytostelium discoideum and resembles fibronectin. Cell 1984;39: 557-64.
53. Alexander S, Sydow LM, Wessels D, Soll DR. Discoidin proteins of Dictyostelium are necessary for normal cytoskeletal organization and cellular morphology during aggregation. Differentiation 1992;51:149-61.
54. Macedo-Ribeiro S, Bode W, Huber R, Quinn-Allen MA, Kim SW, Ortel TL, et al. Crystal structures of the membrane-binding C2 domain of human coagulation factor V. Nature 1999;402:434-9.
55. Pratt KP, Shen BW, Takeshima K, Davie EA, Fujikawa K, Stoddard BL. Structure of the C2 domain of human factor VIII at 1.5 Å resolution. Nature 1999;402:439-42.
56. Théry C, Regnault A, Garin J, Wolfers J, Zitvogel L, Ricciardi-Castagnoli P, et al. Molecular

characterization of dendritic cell-derived exosomes: selective accumulation of the heat shock protein hsc73. J Cell Biol 1999;147:599-610.
57. Chen H, He Z, Bagri A, Tessier-Lavigne M. Semaphorin-neuropilin interactions underlying sympathetic axon responses to class III semaphorins. Neuron 1998;21:1283-90.
58. Giger RJ, Rowe Urquhart E, Gillespie SKH, Levengood DV, Ginty DD, Kolodkin AL. Neuropilin 2 is a receptor for semaphorin IV: insight into the structural basis of receptor function and specificity. Neuron 1998;21:1079-92.
59. Takahashi T, Fournier A, Nakamura F, Wang L-H, Murakami Y, Kalb RG, et al. Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. Cell 1999;99:59-69.
60. Tamagnone L, Artigiani S, Chen H, He Z, Ming G, Song H, et al. Plexins are a large family of receptors for transmembrane, secreted and GPI-anchored semaphorins in vertebrates. Cell 1999;99:71-80.
61. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by tumor and endothelial cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998;92:735-45.
62. Shimizu M, Murakami Y, Suto F, Fujisawa H. Determination of cell adhesion sites of neuropilin-1. J Cell Biol 2000;148:1283-93.
63. Banu N, Hara H, Kataoka S, Egusa G, Yamakido M. A novel method for concentrating urinary type IV collagen based on precipitation with polyethylene glycol: application to its measurement by enzyme radioimmunoassay. Ann Clin Biochem 1994;31:485-91.

국문요약

정상 및 섬유화된 백서 신장에서 **Discoidin Domain Receptor**의 분포

배경: Discoidin domain receptor (DDR1과 DDR2)는 콜라겐에 의해 활성화되는 타이로신 키나아제 수용체의 아족이다. 이 수용체들은 포유류 발생에 불가결하며, 조직 섬유화의 후보적인 효과기이다. 특히 DDR2는 간 섬유화에 관련된 것으로 제시되었다. 이에 연자 등은 정상과 섬유화된 백서 신장에서 DDR의 분포를 조사하였다.

방법: 신장에서 DDR1과 DDR2의 분포를 간접 면역 형광법, 면역 탁본법, 리보누클레아제 보존 분석법 등을 이용하여 조사하였다. 아전 신절제술을 시행 받은 섬유화 백서 및 겉보기 수술을 시행 받은 대조 백서의 신장으로부터 손상 유발 후 2, 4, 8주 시점에서 표현 형태를 비교하였다.

결과: 두 종류의 항체를 이용한 면역 위치의 연구에서 DDR1은 근위세뇨관 내의 내포 (endosomal vesicle)에 위치하고 있었고, DDR2는 헨르 고리로부터 치밀반 (macula densa)까지의 신원 분절에 위치하고 있었다. 신장에서의 DDR2는 독특한 고분자량 (high molecular weight), 변성 저항 형태로 존재한다. 섬유화 신장에서 DDR2의 분포는 대조 백서에서와 비슷하였다. 특히 이 질환 모델에서 콜라겐 기질이 팽창하는 쪽으로의 DDR2의 새로운 사구체, 간질 또는 혈관이나 신세관에의 재분포는 관찰되지 않았다. 더욱, 섬유화와 대조 신장에서 어떤 실험 시점에서도 DDR mRNA 수준의 증가는 관찰되지 않았다.

결론: 성인 백서 신장에서 DDR 위치는 세포-기질 상호작용에 우세한 역할을 하기에 적합하지 않다. 이 수용체의 다른 역할의 가능성에 대해 논의하고자 한다.

핵심되는 말: discoidin domain receptor, 콜라겐, 섬유화, 신장