Therapeutics in Renal Disease: The Road Ahead for Antiproliferative Targets

Peter J. Nelson^a Stuart J. Shankland^b

^aDivision of Nephrology, New York University School of Medicine, New York, N.Y., and

^bDivision of Nephrology, University of Washington Medical Center, Seattle, Wash., USA

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New treatments, redundant · Renal function preservation · Proliferative renal disease · Small-molecule drugs

Abstract

Discovery into the molecular basis of renal disease is occurring at an unprecedented rate. With the advent of the NIH Roadmap, there is a greater expectation of translating this knowledge into new treatments. Here, we review the therapeutic strategy to preserve renal function in proliferative renal diseases by directly inhibiting the mitogenic pathways within renal parenchymal cells that promote G₀ to G₁/S cell-cycle phase progression. Reductionist methodologies have identified several antiproliferative molecular targets, and promising preclinical testing of leading small-molecule drugs to modulate these targets has now led to landmark clinical trials. Yet, this advancement into targeted therapy highlights important differences between the therapeutic goals of molecular nephrology versus molecular oncology and, by extension, the poorly understood role of alternative target activity in drug efficacy. Systems research to clarify these issues should accelerate the development of this promising therapeutic strategy.

Introduction

Starting from the earliest clinicopathologic correlates and on into the current genomic era of experimental molecular nephrology, the fundamental causal relationship between abnormally increased proliferation of renal parenchyma (i.e., renal glomerular and tubular cells) and loss of nephron function has been validated [1]. Over the last decade, the therapeutic implications of directly targeting this pathogenic phenotype have been increasingly explored, culminating recently in clinical trials for mesangial proliferative glomerulonephritis^{1,2}. This progress into targeted therapy for proliferative renal diseases overlaps the commencement of the United States National Institutes of Health (NIH) Roadmap [2]. This significant, ongoing initiative by the NIH is designed to encourage investigators engaged in biomedical research within the public sector to create the research reagents, informatics

¹ Seliciclib in IgA Nephropathy Trial by Cyclacel, Limited (www.cyclacel.com). This was a multi-institutional phase II clinical trial to test a small-molecule cyclin-dependent kinase inhibitor in patients with IgA nephropathy. The trial was halted due to the development of adverse events related to drug administration in some patients.

² Gleevec in IgA Nephropathy Trial by Novartis (www.novartis.com). This was designed as a multi-institutional phase II clinical trial to test a small-molecule platelet-derived growth factor receptor tyrosine kinase inhibitor in patients with IgA nephropathy. The trial was halted due to concerns raised by long-term preclinical toxicity studies in animals.

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platforms and consortiums to facilitate therapeutic translational research (www.nihroadmap.nih.gov). In the light of these advances, we review here the therapeutic strategy to preserve renal function in proliferative renal diseases by directly inhibiting the mitogenic pathways within renal parenchymal cells that promote G_0 to G_1/S cell-cycle phase progression. For examples of additional therapeutic strategies for some proliferative renal diseases, the reader is referred to recent reviews on the treatment of glomerulonephritis by Coppo and Amore [3] and Javai and Quigg [4].

Target Identification

The identification of therapeutic molecular targets for renal diseases has derived largely from studies to determine either the etiologic or the phenotypic basis for the loss of nephron function. Each of these two categories of targets carries inherent therapeutic risks that are unrelated to any potential toxicity of drug treatment. Molecular targets against specific etiologies (e.g., metabolic in diabetic nephropathy, infectious in collapsing glomerulopathy, immune in membranous nephropathy, genetic in polycystic kidney disease, etc.) risk the presence of ‘downstream’ pathogenic mechanisms within the diseased renal parenchyma that have become independent of the etiology and, therefore, are not readily halted or reversed if the etiology is eliminated. Examples of this include the activation of signaling cascades and the increased production of cytokines and growth factors at sites of parenchymal injury that persist despite withdrawal or treatment of the inciting etiology. On the other hand, molecular targets against specific pathogenic phenotypes (e.g., apoptotic, fibrotic, etc.) risk reinjury by the etiology and a lack of specificity and efficacy due to a minor contribution of the targeted phenotype to the loss of nephron function.

With these potential therapeutic constraints in mind, it is important to appreciate that the therapeutic strategy to preserve renal function by directly inhibiting mitogenic signaling within glomerular and tubulointerstitial cell types derives from the phenotypic category of targets. This therapeutic strategy is based on the knowledge that physiologic structure-function relationships along the mature nephron require the presence of cell-cycle quiescent, functionally and morphologically differentiated renal parenchyma [1]. Unlike the high physiologic rate of proliferation due to normal cell losses in some organ systems, such as in the gastrointestinal, hematopoietic, or skin, there is very little cell proliferation in normal adult

glomeruli and a very low rate of proliferation in tubular cells in a healthy kidney [5, 6]. Proliferation of renal glomerular and tubular cells is, therefore, considered to be an abnormal phenotype. Thus, following injury, the ability to halt abnormal proliferation of specific renal cell types with antiproliferative therapies should be possible without theoretically harming bystander disease-free nephrons or nephron segments, as these typically have little or no proliferation if nondiseased.

In developing this therapeutic strategy over the last decade (fig. 1), the identification of nearly all ‘druggable’ antiproliferative molecular targets within the renal parenchyma, ranging from cell surface receptors to nuclear cell-cycle regulatory proteins (table 1), has resulted from applying research methodologies designed to detect critical molecules controlling proliferation. These ‘reductionist methodologies’ were largely developed and refined in molecular oncology, where one central theory for the cause of proliferative disease is its reducibility to the pathogenic loss- or gain-of-function of specific molecules within the neoplastic cell [7]. The wide success of these reductionist methodologies in molecular nephrology is evidenced by the fact that several target/drug pairs currently under investigation (table 1) were first characterized through studies in molecular oncology.

Caveats clearly exist, however, in stating a general applicability of molecular oncology targets or target/drug pairs to proliferative renal diseases, as follows. First, the ultimate desired therapeutic goal of targeted therapy in molecular oncology is to eliminate neoplastic, malignant growth (cytotoxic growth arrest irrespective of cell cycle phase) within otherwise normal tissue domains [8]. In contrast, the desired therapeutic goal in molecular nephrology is to promote cell-cycle quiescence, cell differentiation, and tissue remodeling at sites of proliferative injury within the kidney. These therapeutic responses are most likely to occur during cytostatic growth arrest in the G_0/G_1 phase of the renal cell-cycle, the physiologic state of normal glomerular and tubular renal cells. If this is indeed correct, it may render targets that induce nonphysiologic growth arrest beyond the G_1/S boundary, such as mitotic spindle disruption, problematic in the kidney (fig. 2). Second, targets or target/drug pairs in the mitogenic signaling cascade from G_0 to the G_1/S boundary may be characterized for their ability to induce cytotoxicity over other phenotypic responses, thereby minimizing important knowledge of additional target activities of therapeutic relevance [8]. Lastly, the prolonged and continuous courses of therapy (and the potential related toxicities) that may be required to treat some indolent pro-

Fig. 1. Flow chart in the development of a therapeutic strategy. The first step is to identify a potential drug target that is predicted to disrupt a critical step in the pathogenesis of disease. The chosen target may harbor alternative target activity that together increases overall efficacy. This alternative target activity may be due to the ability of a chosen target to control multiple cellular processes involved in the pathogenesis of the disease, a therapeutic activity termed ‘target pleiotropy’, and/or to the existence of unintended or unknown off-targets to the chosen target that are also involved in the pathogenesis of the disease, a therapeutic activity termed ‘target paralogy’. However, target pleiotropy and target paralogy can confuse the therapeutic role of the chosen target if the contribution of the former to efficacy is poorly understood. Drug entities are typically screened and selected for their ability to modulate the chosen target in vitro; it may be discovered after a drug is developed that the drug itself harbors therapeutically relevant alternative target activity, raising questions about the specificity-of-action of the drug. Once a drug entity is identified, it must exhibit favorable absorption, distribution, metabolism, excretion, and toxicity (ADMET) in order to at least undertake proof-of-concept studies of the therapeutic strategy in vivo to proceed to clinical trials. At this stage, the best measurements of efficacy include biomarkers (see table 2) that either directly detect or accurately predict the specificity of drug action in preclinical models and in humans.

Fig. 2. Depiction of the cell-cycle domain of antiproliferative targets in proliferative renal diseases. Molecular oncology targets favor cytotoxic growth arrest of malignant cells and, therefore, have been identified in all phases of the cell-cycle, i.e., G₁, S, G₂, and M (box A). In contrast, ideal targets in molecular nephrology largely fall within the cell-cycle phase from G₀ to the G₁/S cell cycle checkpoint (box B), the phase where physiologic growth arrest can be achieved. Targets beyond the G₁/S cell-cycle checkpoint induce an undesirable, nonphysiologic state of growth arrest and are often cytotoxic to cells.

Table 1. Antiproliferative molecular targets studied in models of proliferative renal disease

Molecular target	Drug entity ^a	Renal cell model	Animal model (human disease)	Proposed mechanism ^c	References
Cyclin-dependent kinases (CDK)	flavopiridol, roscovitine, TNP-470	mouse podocyte, rat mesangial	mouse Tg26 (collapsing glomerulopathy), rat anti-Thy1.1 (mesangial proliferative glomerulonephritis), mouse anti-glomerular protein (collapsing glomerulopathy)	inhibit G ₁ /S cell-cycle phase progression by preventing CDK activity	27–32
Epidermal growth factor receptor tyrosine kinase (EGFR TK)	EKB-569, EKI-785	rodent kidney explants	mouse BPK (polycystic kidney disease), rat Han:SPRD (polycystic kidney disease)	inhibits EGFR TK activity by preventing EGFR autophosphorylation	33–36
3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA)	lovastatin ^b , pravastatin ^b , simvastatin ^b	human mesangial, rat epithelium, rat mesangial	rat anti-Thy1.1 (mesangial proliferative glomerulonephritis), rat Han:SPRD (polycystic kidney disease)	inhibits prenylation of GTPases by preventing synthesis of isoprenoid	37–47
Mammalian target of rapamycin (mTOR)	rapamycin ^b	human epithelium, mouse epithelium	rat Han:SPRD (polycystic kidney disease)	inhibits translation of cell-cycle control genes	48–50
Peroxisome-proliferator-activated receptor gamma (PPAR γ)	ciglitazone ^b , L-805645, pioglitazone ^b , troglitazone ^b , 15d-PGJ ₂	human fibroblast, mouse mesangial, rat mesangial	rat anti-glomerular basement membrane (crescentic glomerulonephritis)	inhibits serum-responsive genes by activation of PPAR γ -responsive elements	51–54
Phosphodiesterases	cilostamide, cilostazol ^b , lixazinone, pentoxifylline ^b , rolipram	human fibroblast, rat mesangial	rat folate-induced, rat anti-Thy1.1 (mesangial proliferative glomerulonephritis)	inhibit raf signaling by activating PKA through raising cAMP levels	55–59
Platelet-derived growth factor receptor (PDGFR)	trapidil ^b	rat mesangial	rat anti-Thy1.1 (mesangial proliferative glomerulonephritis)	inhibits PDGFR activation by preventing PDGF binding to PDGFR	60, 61
Platelet-derived growth factor receptor tyrosine kinase (PDGFR TK)	imatinib mesylate ^b , Ki6783	rat mesangial	rat anti-Thy1.1 (mesangial proliferative glomerulonephritis)	inhibits PDGFR TK activity by preventing PDGFR autophosphorylation	62, 63
Prostacyclin receptor	beraprost sodium	rat mesangial	mouse MZB/W (lupus glomerulonephritis), rat anti-glomerular basement membrane (crescentic glomerulonephritis)	inhibits ERK signaling by inducing MAPK phosphatase	64–68
Ras	<i>S-trans, trans</i> -farnesylthiosalicylic acid	rat mesangial	rat anti-Thy1.1 (mesangial proliferative glomerulonephritis)	inhibits ras activity by preventing ras membrane localization	69
Retinoic acid receptor (RAR/RXR)	AGN 194204, AGN 195183, all- <i>trans</i> -retinoate ^b , BMS-453, isotretinoin ^b , n-(4-hydroxyphenyl) retinamide, RO-13740, RO-257386	human epithelium, canine epithelium, rat epithelium, rat mesangial	rat anti-Thy1.1 (mesangial proliferative glomerulonephritis), mouse anti-glomerular protein (collapsing glomerulopathy)	inhibits expression of fos, jun, and GATA-2 by activation of RAR/RXR-responsive elements	70–76
Vasopressin V ₂ receptor (VPV2R)	OPC-31260, OPC-41061	human epithelium	mouse pcy (nephronophthisis), mouse Pkd2 ^{-tm1S^{om}} (polycystic kidney disease), rat PCK (polycystic kidney disease)	inhibits paradoxical, Ca ²⁺ -dependent raf activation by PKA through lowering cAMP levels	77–82

^a Small molecules are listed; other types of drugs have been studied for some targets.^b FDA-approved.^c Proposed in renal systems; mechanism may differ in other systems.

Table 2. Biomarkers in proliferative renal diseases [adapted from ref. 10]

Type I

- Designed to directly detect modulation of drug target activity
- Can be problematic, if unforeseen drug targets are also captured by the biomarker
- Examples:
 - Protein substrates of kinase targets
 - Metabolic products of enzymatic targets
 - Genes under the control of transcription factor targets

Type II

- Commonly referred to as surrogate markers
- Do not directly capture the specificity of drug action
- Examples:
 - Quantitative immunohistopathology for PCNA or Ki-67 expression
 - Urinary or serologic indices of renal function
 - Whole-kidney size or weight

liferative renal diseases, such as polycystic kidney disease and IgA nephropathy, may halt investigation of some targets or target/drug pairs at proof-of-concept of the therapeutic strategy, necessitating the identification of additional targets or new drug entities (fig. 1). For example, the marked improvement in renal function with little adverse effect from targeting cyclin-dependent kinases in renal parenchyma with the small molecule roscovitine (CYC202, seliciclib) has been validated across a range of preclinical models (table 1 and [ref. 83]). However, the adverse events secondary to continuous drug dosing that occurred in some patients in the Seliciclib in IgA Nephropathy Trial were not anticipated by preclinical studies nor by intermittent dosing schedules defined in antecedent phase I oncology studies.

Alternative Target Activity

The therapeutic response to a drug *in vivo* (i.e., efficacy) is ascribed to several factors and is essentially the sum of the specific intended action against known targets and the actions against unintended or unknown targets. The latter is called target paralogy [9]. This complex relationship between a drug's efficacy and its specificity of action is amplified by the potential involvement of any one target in multiple cellular processes. This is known as target pleiotropy [9]. Thus, as a drug's alternative target activity (i.e., target paralogy and target pleiotropy) increases, so too does the probability for ambiguity between

perceived efficacy and specificity of drug action captured by surrogate/type II biomarkers and type I biomarkers (table 2), respectively [10]. We will use empiric antiviral therapy for the major proliferative renal disease seen in HIV-infected patients, collapsing glomerulopathy, as an example to illustrate this ambiguity. Some groups are investigating the possibility that highly active antiretroviral therapy (HAART) may inhibit the life cycle of HIV within infected renal epithelium, thereby disrupting what is hypothesized as a central pathogenic step in HIV-induced collapsing glomerulopathy. If this is true, the plasma HIV-1 RNA levels may serve as a good correlate for the specificity of HAART activity on HIV-encoded drug targets within renal parenchymal cells – i.e., plasma HIV-1 RNA levels are a true-positive surrogate marker of the antiviral activity of HAART within the renal epithelium [11]. Alternatively, inhibition of the life cycle of HIV by HAART in cell types outside of the renal epithelium (e.g., in infected CD4+ lymphocytes, macrophages, or dendritic cells) or direct modulation by HAART of targets derived from the host, not of targets encoded by HIV, might confer efficacy. If these latter possibilities are true, plasma HIV-1 RNA levels would be a false-positive and false-negative surrogate marker, respectively, for any antiviral activity of HAART within the renal epithelium [10]. Indeed, these latter therapeutic mechanisms of HAART have already been shown to be important in other nonrenal HIV-associated proliferative diseases [12].

Taken together, we suggest that because there is likely a multifactorial basis for most proliferative renal diseases, all current targets or target/drug pairs under investigation (table 1) are similarly challenged to define specificity of action. In contrast to the desire to eliminate malignant cell growth in the oncology field, where modulation of drug targets in nonmalignant tissue is often trivialized (unless toxic) [8], alternative target activity may contribute significantly to drug efficacy in proliferative renal diseases. One known pleiotropic effect from modulating antiproliferative targets is the concomitant effect on other undesirable pathways, such as inflammatory, fibrogenic, and secretory pathways [13, 14], that have harnessed the same target for activity. These additional pathways are referred to as 'target-sharing' pathways which have been aberrantly coactivated and likely also contribute to tissue injury and loss of nephron function. However, the primary intent of promoting cell-cycle quiescence in the renal parenchyma by inhibiting proliferation and any secondary pleiotropic benefit quickly lose definition, if alternative and therapeutically relevant targets exist either within or outside the kidney.

The recent renal clinical trial target/drug entrant, platelet-derived growth factor receptor/imitanib mesylate (Gleevec/Glivec), illustrates this fact. Imatinib mesylate, an FDA-approved small-molecule drug of the family of the pyrido[2,3-*d*]pyrimidines [15], was considered for clinical trials on patients with IgA nephropathy to inhibit platelet-derived growth factor receptor tyrosine kinase activity within the renal parenchyma. Imatinib mesylate and its derivatives also inhibit tyrosine kinases that are highly similar to the platelet-derived growth factor receptor such as fibroblast growth factor receptor, c-kit, and Bcr-Abl [16]. By baiting with immobilized drug, Wissing et al. [16] recently discovered that this drug class binds and more potently inhibits the serine/threonine kinases RICK and p38 α based on a shared specificity determining threonine residue across seemingly disparate kinase domains. This activity conferred previously unknown potent anti-inflammatory properties to this drug class that is independent of its antiproliferative activity [16]. Moreover, imatinib mesylate was also recently discovered to modulate adaptive immune responses by inhibiting T lymphocyte proliferation and dendritic cell differentiation, reportedly through its known tyrosine kinase targets [17–19]. Since targeting the inflammatory, immunologic component of IgA nephropathy may be efficacious [20], these new therapeutic mechanisms for imatinib mesylate may have misdirected subsequent steps, such as the selection of newer drug entities and appropriate biomarkers, in the development of this particular therapy. While any decision to investigate a potential target or target/drug pair is made with incomplete knowledge, this example highlights the growing need for systems research to delineate the contribution of alternative target activity to drug efficacy in proliferative renal diseases.

Systems Biology

In part due to the rapidly expanding repertoire of potential targets in proliferative renal diseases, there is a growing appreciation for identifying targets that may carry therapeutic activity in addition to inhibiting proliferation of the renal parenchyma. In practice, this requires an integrative approach to proliferative renal disease biology – often called a ‘systems biology’ approach – to identify these exceptional targets. For example, unlike reductionist methodologies applied to one disease phenotype, systems biology approaches to target identification are currently being designed to select molecular targets that emerge from integrating a broad range of disease biology,

with the goal that these targets may impart the greatest therapeutic impact [21]. These systems-biology-derived targets have been coined ‘nodes’, because they are predicted to function simultaneously in several pathogenic pathways that together contribute to the development of disease [21]. Thus, modulation of these ‘nodes’ with drugs may be very effective at reconfiguring the entire disease state back towards normal [21].

However, theoretical, this analytic approach provides a useful framework to investigate the therapeutic role of target pleiotropy and target paralogy that results from applying reductionist methodologies to proliferative renal diseases. By assuming post hoc that a target (intended or unintended) may affect any aspect of proliferative renal disease biology, one can ask whether modulation of that target in renal parenchymal cell types or in other cell types contributes to (and may be paramount in) or is nonparticipatory to perceived efficacy [21]. It may be discovered, for example, that the target directly impacts mitogenic pathways within the renal parenchyma, other renal parenchymal phenotypes that contribute to the loss of nephron function, extrarenal disease phenotypes (an important consideration in multisystem diseases), or the proposed etiology, if known (fig. 3).

Alternative target activity that increases the overall efficacy-to-toxicity ratio (i.e., increases the therapeutic index) would be clearly desirable, whereas exacerbation of disease phenotypes or other adverse outcomes may eliminate or limit the applicability of specific targets or target/drug pairs. For example, modulation of the mammalian target of rapamycin (mTOR) within the renal epithelium may be efficacious for polycystic kidney disease (table 1), but will require a better understanding of how prolonged CD4⁺ T lymphocyte anergy (with its potential pitfalls) may contribute, if at all, to perceived efficacy. In contrast, modulation of this same target within mesangial cells in mesangial proliferative glomerulonephritis appears to significantly worsen mesangial remodeling, reportedly due to suppression of mesangial cell migration, but may also involve a paradoxical proinflammatory lymphoid response [22, 23]. Yet, to date, the quest to answer these types of systems-based questions on intended and unintended target activity across a range of cell types in vivo has been hampered by the lack of type I biomarkers [10]. Indeed, the failure of surrogate/type II biomarkers to predict the specificity of drug action is addressed by NIH Roadmap initiatives to develop molecular probes of small-molecule activity [2], a needed step towards systems research in proliferative renal diseases.

Fig. 3. Interrogation of targets through systems research in proliferative renal diseases. **a** The systems biology approach to target identification integrates the interaction of elements of disease biology (depicted by the interaction of circles 'A' through 'D') to identify emergent, high-impact targets, coined 'nodes', that are predicted to function simultaneously in several pathogenic pathways that together contribute to the development of disease. When modulated with drugs, these nodes will theoretically reconfigure the entire disease state back towards normal. **b** By assuming that any one target (intended or unintended) identified through reductionist methodologies may affect any aspect of proliferative renal disease biology, one can ask if modulation of that one target impacts important parameters of disease, such as parenchymal hyperplasia, other secondary parenchymal disease phenotypes, extrarenal disease phenotypes, or the etiology.

Drug Entities

The current drive to explore FDA-approved or other well-developed drug entities that modulate antiproliferative targets in renal cells affected by specific disease processes is predicated not only on the hope for favorable pharmacology in their off-label application to renal disease [24], but also on the calculation that these drug entities will provide a greater therapeutic index over existing therapies. This is no trivial motivation, as the vast majority of promising new drug entities detected through in

vitro screens subsequently fail in preclinical and clinical development due to problems with absorption, distribution, metabolism, excretion, or toxicity (ADMET) [25]. However, since no drug entities investigated to date for use in proliferative renal diseases were specifically developed for this disease indication (table 1), it must be remembered that important factors, such as renal insufficiency and adjuvant therapy, can profoundly influence drug pharmacokinetics and dynamics (e.g., as was shown for mycophenolic acid, a nontargeted therapy in clinical trials for a number of renal diseases) [26]. In any respect,

NIH Roadmap initiatives to provide interrogational small-molecule libraries and predictive ADMET technology to the public sector are poised to change this trend in the future [2].

Conclusions

The development of a therapeutic strategy from target identification to clinical trials is one of the most challenging and complex research endeavors in both industry and academia. Notwithstanding, research to explore the therapeutic strategy that renal function may be preserved in proliferative renal diseases by directly inhibiting G₀ to G₁/S cell-cycle phase progression in the renal parenchyma has made significant progress over the last decade. The application of reductionist methodologies has created a rapidly expanding repertoire of potential targets, and pre-

clinical testing of well-developed drug entities that modulate many of these targets has validated this therapeutic strategy across a range of models. Yet, because many pathogenic factors cooperate to induce most proliferative renal diseases, the ability to clearly link drug efficacy to modulation of intended targets is often elusive, a challenge that may best be addressed by rigorous systems-based research.

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