MicroRNAs and Their Role in Progressive Kidney Diseases

Mitsuo Kato, Laura Arce, and Rama Natarajan

Gonda Diabetes Center, Beckman Research Institute of City of Hope, Duarte, California

MicroRNAs (miRs) are a family of short non-coding RNAs. These endogenously produced factors have been shown to play important roles in gene regulation. The discovery of miRs has greatly expanded our knowledge of gene regulation at the posttranscriptional level. miRs inhibit target gene expression by blocking protein translation or by inducing mRNA degradation and therefore have the potential to modulate physiologic and pathologic processes. The imperative need to determine their cellular targets and disease relevance has sparked an unprecedented explosion of research in the miR field. Recent findings have revealed critical functions for specific miRs in cellular events such as proliferation, differentiation, development, and immune responses and in the regulation of genes relevant to human diseases. Of particular interest to renal researchers are recent reports that key miRs are highly expressed in the kidney and can act as effectors of TGF-β actions and high glucose in diabetic kidney disease. Moreover, podocyte-specific deletion of Dicer, a key enzyme involved in miR biogenesis, led to proteinuria and severe renal dysfunction in mice. Hence, studies aimed at determining the *in vitro* and *in vivo* functions of miRs in the kidney could determine their value as therapeutic targets for progressive renal glomerular and tubular diseases. Translational approaches could be facilitated by the development of effective inhibitors of specific miRs and methods for optimal delivery of anti-miRs to the kidney. The major goal of this review is to highlight key functions of these miRs and their relationships to human diseases, with special emphasis on diabetic kidney disease.

Clin J Am Soc Nephrol 4: 1255–1266, 2009. doi: 10.2215/CJN.00520109

MicroRNAs (miRs) are a family of short, non-coding RNAs that are approximately 22 nucleotides long. These endogenously produced transcripts have been shown to play important roles in gene regulation (1–12). The discovery of miRs, only approximately 15 yr ago, has greatly expanded our knowledge of gene regulation and provided a new perspective on the mechanisms of gene expression under disease states. In the early 1990s, the first miR, lin-4, was reported as a small, non-coding RNA controlling a specific step in developmental timing in *Caenorhabditis elegans* by downregulating a conventional protein-coding gene (*lin-14*) (13,14). The *lin-14* 3′ untranslated region (3′UTR) harbors multiple sites of imperfect complementarity to the lin-4 small RNA. lin-4 binds to these sites and blocks lin-14 translation. In 2000, the second miR (let-7) and its target, lin-41, were discovered and shown to be conserved across species (1,2,15). These important discoveries provided the foundation for our current understanding of mammalian miR function. Estimates indicate that more than 1000 human miRs target and downregulate at least 60% of human protein-coding genes expressed in the genome (4,8,16–20). It is therefore not surprising that there is an unprecedented explosion in miR research to determine their biologic functions and disease relevance.

Recent findings have revealed critical functions for specific miRs in several cellular and biologic processes, including proliferation, differentiation, and development, and in the regulation of genes relevant to cancer, insulin secretion (5,11,21,22), modulation of immune responses in macrophages (23), and cardiac and muscle differentiation (24–26). Because miRs are important regulators of gene expression, misregulation or mutations of miRs are expected to play key roles in several diseases (5,11,27,28). Cancer was one of the first diseases to be related to abnormal expression or actions of miRs (5,11,27). Several miR genes have been detected at chromosomal breakpoints associated with cancer (29,30). miRs with both tumor suppressor and oncogenic properties have been reported (31–38), and miR research is very active in the cancer field. In addition, miRs are highly expressed in the cardiovascular system and play important roles in cardiovascular development, biology, and pathology (39). Key miRs have been implicated in cardiomyocyte differentiation, growth, and hypertrophy (24,25,40–42). In addition, miR-21 was implicated in vascular smooth muscle cell proliferation and neointimal thickening (43) and myocardial disease (44). Thus, several groups are actively evaluating miRs as potential therapeutic targets for various vascular and cardiac diseases.

Of particular interest to the renal community are reports showing that a cluster of key miRs are highly expressed in the kidney and that there are differences in the miR expression profile in renal cortex *versus* medulla (45,46); however, it was not until recently that specific roles of miRs in renal function were investigated. These studies revealed that key miRs can play roles in TGF-β1 actions and diabetic kidney disease (47,48) and that podocyte-specific deletion of Dicer led to progressive glomerular and tubular damage along with proteinuria and other podocyte defects in mice (49–51). As such, there is in-

Correspondence: Dr. Rama Natarajan, Gonda Diabetes Center Beckman Research Institute of the City of Hope 1500 East Duarte Road, Duarte, CA 91010. Phone: 626-256-4673, ext. 62289; Fax: 626-301-8136; E-mail: rnatarajan@coh.org; or Dr. Mitsuo Kato, Phone: 626-256-4673, ext. 63996; Fax: 626-301-8136; E-mail: mkato@coh.org

ISSN: 1555-9041/407–1255

creasing interest in evaluating the *in vitro* and *in vivo* functions of miRs in the kidney and thereby determine their value as therapeutic targets for renal glomerular and tubular diseases. In this review, we highlight key functions of miRs and their relationships to human diseases, with emphasis on diabetic nephropathy.

Biogenesis and Mechanism of Action of miRs

miR transcripts initially originate as long primary miRs (Figure 1). Primary miRs are processed to a stem-loop (hairpin) structure fragment termed precursor miR in the nucleus by an RNase III enzyme, Drosha, in complex with the double-strand RNA–binding protein DGCR8 (10,12). The approximately 70-nucleotide hairpin structure precursor miRs are then exported to the cytoplasm by Exportin-5 and further cleaved to RNA duplexes by a second RNase III family enzyme, Dicer (10,12). The miR duplexes are then unwound, and one strand, termed "mature miR" guide strand, which contains complementarity to mRNA targets, is loaded into the RNA-induced silencing complex (RISC) (6,10,12,52), which contains Argonaute 2, Dicer, and transactivating response RNA-binding proteins. miRs in the RISC complex then guide the recognition of target genes. If the complementarity is perfect, then RISC usually cleaves the target mRNA (classical RNA interference); however, if the complementarity is not perfect, then RISC induces translational repression of target genes by targeting their 3′UTR (Figure 1) (6,8,10,11). Although complete complementarity is not required for miR-mediated regulation of a target transcript, the "seed sequence," namely seven key nucleotides of the miR 5′ sequence, is critical for target recognition and inhibition (18,20),

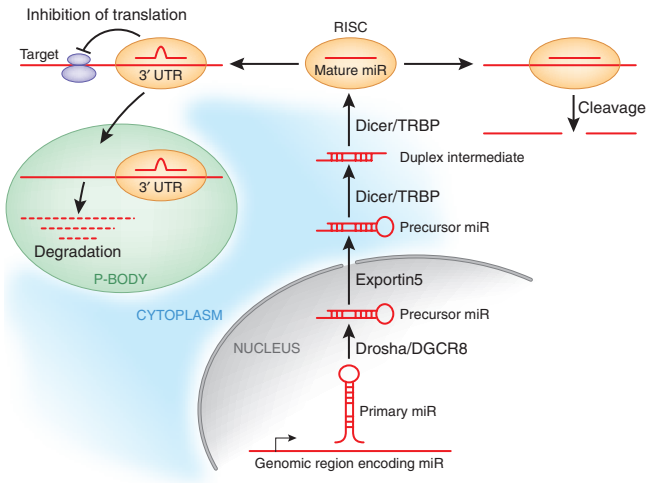

Figure 1. Biogenesis and mechanism of action of microRNAs (miRs). miR transcripts initially originate as primary miRs that are then processed into precursor miRs, which are further cleaved to result in miR–miR duplexes. The miR duplexes are then unwound, and the mature miR guide strand is loaded into the RNA-induced silencing complex (RISC) complex. miRs in the RISC complex then guide the recognition of target RNAs to induce their downregulation depending on the type of complementarity.

whereas other factors, such as a bulge in the central region and reasonable complementarity even at miR 3′ sequence, have also been suggested (53,54). miRs inhibit the initiation and elongation steps of translation to reduce protein expression (55–58). They can also repress gene expression by sequestering targeted mRNAs to cytoplasmic mRNA processing bodies for degradation (59–61). In addition to their role in such posttranscriptional repression, miRs are now implicated in transcriptional gene silencing by targeting the promoter region (62). Thus, miRs can inhibit gene expression *via* translational repression, target mRNA degradation, or transcriptional inhibition.

Mouse models of Dicer, Dgcr8 or Argonaute 2 knockout, displayed embryonic lethality (63–66). Tissue-specific conditional Dicer knockout mice showed defects in various organs, suggesting that miRs are essential for specific tissue and organ development (3,24,67–69). Cardiac development defects were observed in miR-1– to -2–deficient mice (24) and cardiac hypertrophy in miR-208–deficient mice (25), whereas miR-133 was shown to control cardiac hypertrophy (67). Myeloid-specific miR-223 could regulate progenitor cell proliferation and granulocyte function by targeting Mef2c (68). Thus, miR function seems essential not only for embryonic development but also for adult tissue/organ development; therefore, miRs can be expected to play important roles in disease.

miRs in Diabetes and Metabolism

Whereas several studies have evaluated the role of miRs in cancer, much less is known in the field of diabetes. Overexpression of miR-375, an islet-specific miR, could suppress glucose-induced insulin secretion and, conversely, inhibition of endogenous miR-375–enhanced insulin secretion (21). Myotrophin, a protein that inhibits insulin secretion, was a predicted target of miR-375. Recent reports showed that miR-375 also targets phosphoinositide-dependent protein kinase-1 and regulates glucose-induced biologic response in pancreatic β-cells (70), whereas miR-9 could control insulin secretion (71). A correlation between miR-204/211 expression and insulinoma also suggests that these miRs may be related to insulin secretion (72). miR-122, a liver-expressed miR, was implicated in cholesterol and lipid metabolism and hence a potential target for the treatment of hypercholesterolemia (73,74). miR-124a was shown to play a role in glucose homeostasis (28,75) by targeting Rab27 and FOXA2 and MTPN. FOXA2 may regulate several targets that are relevant to diabetes, including insulin and potassium channel subunits, and also affect glucose homeostasis and pancreas development (28,75). It is interesting that 27 miR genes were reported to be located in nine of 19 insulin-dependent diabetes (IDDM) susceptibility loci (76). Their potential targets were autoimmune- and β cell–related genes. miR-192 and miR-375 were located in IDDM4 and IDDM13, respectively. A single-nucleotide polymorphism (SNP) identified in the miR-192 precursor sequence might alter its secondary structure to play a role in diabetes (77). These results suggest that specific miRs may serve as novel targets for the treatment of diabetes.

Diabetic Nephropathy and TGF-β Actions

Diabetic nephropathy (DN) is a progressive kidney disease and a major debilitating complication of both type 1 and type 2 diabetes that can lead to ESRD and related cardiovascular disorders. The cellular mechanisms underlying diabetes-induced dysfunction of key renal cells have been studied extensively, and several therapies for DN are available; however, patients with diabetes are still reaching ESRD at alarming proportions, and it therefore is imperative to evaluate newer molecular mechanisms and therapeutic targets.

Histologically, DN is characterized by glomerular basement membrane thickening, mesangial expansion, and extracellular matrix (ECM) accumulation. This ECM accumulation is due to coordinate alterations in ECM proteins such as types I and IV collagen, laminin, and fibronectin (78–80); ECM regulatory enzymes such as matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases (81,82); and growth factors such as PDGF, TGF-β, and angiotensin II (83–86). Podocyte effacement and albuminuria are also major features of DN (87,88). Factors that are relevant to the pathogenesis of DN can increase TGF-β expression in glomerular mesangial cells (MCs) *in vitro* and *in vivo* (84,89–93). TGF-β is a profibrotic agent with several effects in renal cells, including the production of ECM proteins such as type I and II collagens, laminin, fibronectin, and plasminogen activator inhibitor-1 (84,90,91,94–96). Thus, TGF-β has been studied as a major target for DN treatment (97).

Smad transcription factors have been studied extensively as the major effectors of TGF-β signaling (98,99). Evidence shows that ECM genes including collagen type I-α1 and 2 (Col1a1/a2) are regulated by TGF-β *via* Smads in MCs (96,100). Interaction of TGF-β with its receptors induces the phosphorylation and nuclear translocation of the receptor-regulated Smad2/3 transcription factors (98,99,101) to regulate gene expression (95,96,100,102). TGF-β also activates the phosphoinositide-3-kinase/Akt kinase pathway in MCs (103–110), and this has been implicated in its fibrotic responses, such as expression of collagen and fibronectin (105,106). Activated Akt kinase phosphorylates several downstream proteins, including GSK3-β and Forkhead (FoxO) transcription factors, to control cell growth, survival, and protein synthesis (111,112). Akt activated by TGF-β phosphorylates and inactivates FoxO3a and thereby downregulates key FoxO3a targets, such as the proapoptotic Bim and antioxidant manganese superoxide dismutase genes (103,109). The combination of these events in response to TGF-β can result in enhanced MC survival, oxidative stress, and hypertrophy and thereby accelerate kidney diseases such as DN.

Although several studies showed that TGF-β signaling events are crucial in regulating its fibrotic effects in MCs and other renal cells, the subtle molecular mechanisms are not fully clear. Using cDNA microarray profiling of genes regulated by TGF-β in mouse MCs (47), we discovered that TGF-β decreases the expression of Zeb1, also known as δEF1 (Figure 2). ZEB1 is a repressor of E-cadherin (113), osteocalcin (114), and E2 box (115). Notably, it is also a repressor of collagen type I and type II genes in various cells (116–118). E-box elements are located in the far-upstream enhancer region of the collagen gene (116,118). It is interesting that TGF-β also decreased the expression of another E-box repressor belonging to the Zeb1 family, namely Zeb2 (also called Smad-interacting protein-1) (119). These results suggested that TGF-β can increase Col1a2 gene expression in MCs by downregulating the E-box repressors ZEB1 and ZEB2 (Figure 2) (47).

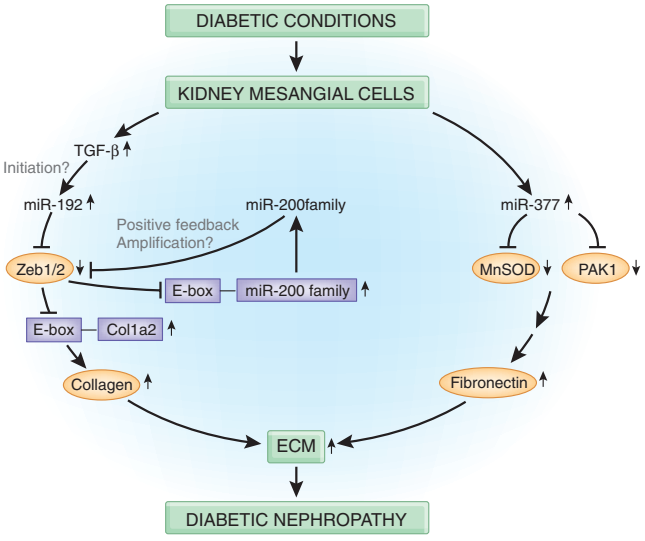

Figure 2. miR-dependent mechanisms for TGF-β–induced expression of extracellular matrix (ECM) genes related to the pathogenesis of diabetic nephropathy (DN). The expression of miR-192 is increased in diabetic kidney glomeruli in mouse models along with that of collagen type I-α 2 (Col1a2) and TGF-β expression (47). Col1a2 expression is increased by TGF-β or diabetic conditions *via* decrease in ZEB2 targeted by miR-192 upregulated by TGF-β in mesangial cells (MCs) (47). Because E-boxes are also present in the upstream genomic regions of the miR-200 family, miR-200 family members might themselves be regulated by ZEB1 and ZEB2 (136,138). miR-192 may initiate signaling from TGF-β also to upregulate the miR-200 family, and then the miR-200 family might amplify or accelerate the signaling by further upregulating themselves *via* downregulation of E-box repressors (Zeb1/Zeb2). The miR-192–regulated circuit may amplify TGF-β signaling under diabetic conditions. miR-377 has also been shown to induce fibronectin (ECM protein) expression *via* downregulation of manganese superoxide dismutase (MnSOD) and p21-activated kinase (PAK1) in MCs (48).

miRs in the Kidney: Role in TGF-β Actions, ECM Production, and the Diabetic Kidney

Sun *et al.* (45) identified five miRs (192, 194, 204, 215, and 216) that were highly expressed in human and mouse kidney using miR microarray. Tian *et al.* (46) combined miR microarray and proteomics to identify a differential profile of miR expression in rat renal cortex *versus* medulla, as well as several miR-target protein pairs. Recent reports using new proteomic approaches to profile and identify miR targets demonstrated that miRs repress their targets at both the mRNA and translational levels and that the effects are mostly relatively mild (120–122). Such complementary methods are expected to identify key miRs

more accurately as well as their targets under normal *versus* disease states.

Reports have implicated miRs in TGF-β signaling and actions in various systems. Zebrafish miR-430 was found to affect the expression of the Nodal (TGF-β) agonist and antagonist (123). *Xenopus laevis* miR-15 and miR-16 could alter development processes by targeting the Nodal type II receptor (124). miR-24 was implicated in the inhibition of skeletal muscle differentiation by TGF-β (26). Certain gastric cancer cells were resistant to TGF-β because the miR-106b-25 cluster was upregulated and inhibited the expression of p21 (cell-cycle arrest gene) and Bim (apoptotic gene) that are downstream of TGF-β (125). It is interesting that the processing of miR-21 was reported to be enhanced by Smad proteins phosphorylated by TGF-β (126).

We demonstrated that the expression miR-192, one of the highly expressed miRs in human and mouse kidney (45) and rat kidney cortex (46), is increased in renal glomeruli obtained from mouse models of type 1 diabetes (streptozotocin injected) and type 2 diabetes (db/db mice) relative to the corresponding control mice (47). It is interesting that the expression of miR-192 was increased by TGF-β in mouse MCs, whereas, conversely, the expression of its target, Zeb2, was decreased (47). This also paralleled the increased *Col1a2* and TGF-β expression (47). These results suggested that the increase in TGF-β *in vivo* in diabetic glomeruli and *in vitro* in MCs can induce miR-192 expression, which can target and downregulate Zeb2 thereby to increase Col1a2. This is supported by the report showing that miR-192 is upregulated in human MCs treated with high glucose (48). Recent computational analysis of miRs and mRNA in colon cancer cells also revealed ZEB2 as a target of miR-192 (127). The molecular mechanisms by which miR-192 is induced by TGF-β needs further investigation. It is interesting that the human miR-192 promoter is regulated by hepatocyte nuclear factor 1α (128), a factor whose mutations have been associated with maturity-onset diabetes of the young (129).

TGF-β induced downregulation of Zeb2 (*via* miR-192), and Zeb1 (*via* potentially another miR) can cooperate to enhance *Col1a2* expression *via* de-repression at E-box elements (47) (Figure 2). A recent report also showed that bone morphogenic protein 6–induced miR-192 decreases the expression of ZEB1 in breast cancer cells (130). Enhanced expression of miR-192 and collagen genes were reported in nasopharyngeal carcinomas (131). Thus, miR-192 might regulate collagen expression by targeting E-box repressors not only in kidney disease but also in various cancers.

Several articles have shown that the miR-200 family targets ZEB1 and ZEB2 (132–138). We also observed that Zeb1 is a target of certain miR-200 family members that are also upregulated by TGF-β in MCs (M.K. and R.N., unpublished observations). Thus, TGF-β–induced increase in the expression of key miRs (miR-192 and miR-200 family members) might coordinately downregulate E-box repressors Zeb1 and Zeb2 to increase Col1a2 expression in MCs related to the pathogenesis of DN. The proximal promoter of the *Col1a2* gene responds to TGF-β *via* Smads and SP1 (100). Conversely, the downregulation of ZEB1 and ZEB2 by TGF-β *via* miR-200 family and miR-192 can affect upstream E-box regions (47). Because E-boxes are also present in the upstream genomic regions of the miR-200 family, miR-200 family members may themselves be regulated by ZEB1 and ZEB2 (136,138). It is possible that the miR-200 family upregulated by TGF-β or in diabetic glomeruli under early stages of the disease can also regulate collagen expression related to diabetic kidney disease by targeting and downregulating E-box repressors. miR-192 might initiate signaling from TGF-β to upregulate miR-200 family members, which subsequently could amplify the signaling by further regulating themselves through downregulation of E-box repressors (Figure 2). Such events could lead to progressive renal dysfunction under pathologic conditions such as diabetes, in which TGF-β levels are enhanced. Conversely, there are several reports that miR-200 family members and miR-192 can be downregulated by TGF-β, and this promotes epithelial-to-mesenchymal transition (EMT) in cancer and other kidney-derived epithelial cell lines *via* subsequent upregulation of targets ZEB1 and ZEB2 to repress E-cadherin (135–139). Thus, the effects of renal miRs may be cell type specific, and the miR signaling networks that mediate the effects of TGF-β on MCs and epithelial cells and on metastatic and fibrotic EMT may not be identical.

These miR circuits may also operate in cancer, because cyclin-dependent kinase inhibitor p21-mediated cell-cycle arrest but not apoptosis of cancer cells by miR-192 *via* the p53 pathway was recently reported (140–142). miR-192 activated the promoter of the antiapoptotic Survivin gene (143); therefore, miR-192 likely induces only cell-cycle arrest but not apoptosis. Given that the p53 and Survivin promoters have E-boxes (144,145), these genes may be regulated by ZEB1 and ZEB2 targeted by miR-192 and miR-200 family. Because p21-mediated cell-cycle arrest plays a role in MC hypertrophy (146), miR-192 might also promote glomerular hypertrophy by activating the p21 and p53 pathways.

Wang *et al.* (48) demonstrated that in cultured human MCs exposed to high glucose or TGF-β, as well as in mouse DN models *in vivo*, there was a significant upregulation of miR-377 that could downregulate p21-activated kinase and manganese superoxide dismutase and thereby enhance fibronectin production. Thus, miR-377 might also be a key regulator of DN. Recent studies showed that Akt kinase is activated *via* downregulation of Pten (phosphatase and tensin homolog) targeted by miR-216a and miR-217 that are upregulated by TGF-β in mouse MCs (109). Furthermore, these miRs could promote MC hypertrophy and survival similar to TGF-β (109). It is anticipated that other miRs that are expressed not only in MCs but also in podocytes and tubular and other renal cells and that regulate renal functions under diabetic conditions will be reported in the near future.

Other Kidney Diseases

Studies showed that key miRs are also involved in nondiabetic kidney diseases. miR-15a was reported to modulate the expression of the cell-cycle regulator Cdc25A and affect hepatic cystogenesis in a rat model of polycystic kidney disease (147). A microarray-based study in rats revealed that 30 miRs were differentially expressed in polycystic kidney disease, and two

of these, miR-31 and miR-217, were not previously identified in the kidney (148). A comprehensive study of human lupus nephritis identified 66 miRs that were differentially expressed between patients with lupus nephritis and healthy control subjects (149). miR-17-92 cluster targets Pten and Bim and, interestingly, transgenic mice of miR-17-92 cluster had enlarged kidney glomeruli, hypercellularity, mesangial expansion, and proteinuria, features similar to DN (Table 1) (32).

Recently, a series of reports by investigators who used mice with podocyte-specific deletion of Dicer suggested that miRs play critical roles in podocyte biology and pathology (49–51). It is interesting that all three reports showed major renal abnormalities in these mice. The major phenotypes were proteinuria, podocyte foot process effacement, changes in podocyte genes, and glomerular basement membrane abnormalities. There was a rapid progression of renal disease, glomerulosclerosis and tubulointerstitial fibrosis, and renal failure and death by 6 to 8 wk. The investigators also identified changes in specific miRs (especially miR-30 family) under these conditions and their potential involvement in glomerular disease (Table 1) (49–51). It is interesting that the miR-30 family is reported to target connective tissue growth factor (150), a profibrotic factor that is also downstream of TGF-β. Thus, the targets of these miRs may regulate critical glomerular and podocyte functions. These exciting studies highlight the essential roles of Dicer and miRs in renal physiology and pathology.

Clinical Applications, Therapeutic Strategies, and Perspectives

Overwhelming evidence implicating miRs in the pathology of key human diseases has sparked tremendous interest in development of modalities to block specific miRs and their function *in vitro* and *in vivo*. Small non-coding RNAs and miRs, such as miR-192 and miR-377, may be novel targets for DN and other diabetic complications. Vector-based expression of small interfering RNA is a powerful tool to inhibit the expression of targets *in vitro* and *in vivo* (151) that has shown promise in the treatment of certain diseases (152,153). Expression of tandem repeats of miR-binding sites (Decoy or Sponge) is an efficient method to inhibit miR action (67,154), as well as chemically modified oligonucleotide (oligo) inhibitors (74,155). Cholesterol-tagged small interfering RNA against 12/15-lipoxygenase, whose expression is enhanced under diabetic conditions (156), ameliorated key features of DN in mice with type 1 diabetes (157). Such cholesterol-tagged antagomirs targeting miR-122 were also very effective *in vivo* (74). Thus, chemically modified inhibitors (antagomirs, or locked nucleic acid–modified anti-miRs) (44,74,155) targeting key miRs seem to be efficient inhibitors of miR actions *in vivo* and may be developed as therapies for the prevention and treatment of human DN. Inhibition of miR-122 by 2′-O-methoxyethyl phosphorothioate antisense oligos, cholesterol-tagged 2′-O-Me antisense oligo, or antisense locked nucleic acid (LNA–anti-miR) modified oligos improved hypercholesterolemia in mouse models (73,74,158). LNA–anti-miR-122 also reduced plasma cholesterol levels in a nonhuman primate model without any toxicity, thereby illustrating the potential of such modified oligos to be developed as a new class of therapeutics for disease-associated miRs (155). miR inhibitors, such as LNA–anti-miRs or antagomiRs, injected into animal models of kidney diseases could be evaluated for potential use in similar human kidney disorders (Figure 3).

It is now clear that key miRs are expressed in specific renal cells; modulate the actions of TGF-β and diabetic conditions; alter MC and podocyte functions; and lead to ECM accumulation, podocyte dysfunction, albuminuria, and EMT and thereby affect renal physiology. This picture is just emerging, and we will undoubtedly see increasing numbers of reports of other miRs and their targets with multiple functions in the kidney. Future studies with overexpression or deletion of individual miRs in a cell-specific manner would provide very useful data

Table 1. miRs with known functions in the kidney[a]

miR	Validated Targets	Expression and Relation to Kidney Disease	References
miR-15a	Cdc25A	Rat model of PKD	(147)
miR-17-92 cluster	PTEN and Bim	Enlarged kidney glomeruli, hypercellularity, mesangial expansion, proteinuria	(32)
miR-30 family	CTGF	High expression in kidney glomeruli (and loss in podocyte-specific Dicer KO)	(49-51,150)
miR-192	ZEB1 and ZEB2	Enhanced expression in diabetic mouse kidney and by TGF-β in mouse MC; enhanced expression by high glucose in human MCs	(47,48,109,127,130)
miR-200 family	ZEB1 and ZEB2	EMT in cultured kidney and cancer cells	(132-138)
miR-216a, miR-217 cluster	PTEN	Enhanced expression in diabetic mouse kidney and by TGF-β in mouse MCs	(109)
miR-377	PAK1 and MnSOD	Enhanced expression by high glucose in human MC	(48)

[a]CTGF, connective tissue growth factor; EMT, epithelial to mesenchymal transition; KO, knockout; MC, mesangial cell; miR, microRNA; MnSOD, manganese superoxide dismutase; PAK1, p21-activated kinase; PKD, polycystic kidney disease; PTEN, phosphatase and tensin homolog.

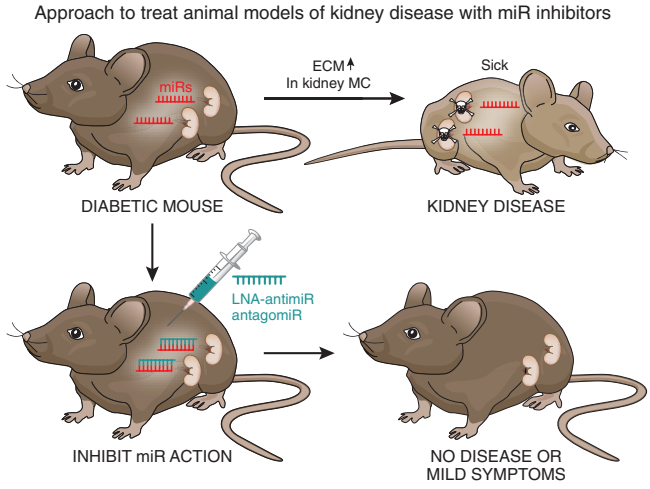

Figure 3. Treating renal disorders in mouse models by targeting specific miRs *in vivo* with oligonucleotide (oligo) inhibitors such as locked nucleic acid (LNA)-modified anti-miRs or other chemically modified antagomiRs. Cholesterol-tagged anti-miR oligos (antagomiRs) or LNA-modified oligo anti-miRs could be developed as efficient inhibitors of key disease-related miRs. The chemistry of these oligos can be engineered for optimal renal cell targeting, accumulation, and miR inhibition *in vivo*. Both type 1 and type 2 diabetic mice could be tested by injecting them with specific miR inhibitors or control oligos and examining whether they can prevent or delay key features of DN. Such studies will determine whether the increased rates of DN in the diabetic mice can be attributed at least in part to the aberrant expression of the specific miR being targeted.

on their role in renal biology and pathobiology. Reports of single miR knockouts in a whole-animal or tissue-specific manner have shown dramatic phenotype (24,25,67,68), confirming that miRs can indeed play critical roles in mammalian organ physiology or pathology. Studies in the kidney are still in their infancy but represent an exciting avenue for new therapies for debilitating renal diseases. These translational approaches can be further facilitated by examining the expression of miRs and their targets in renal biopsies obtained from patients with kidney disease. Recently, SNPs that could affect response to miRs were noted in miR target site sequences (159,160). Correlations between miR polymorphisms and SNPs in miR target sites with risk of bladder and colorectal cancers were reported (161–163). Hence, examination of SNPs in miR-binding sites may also provide key insights into various human diseases. Given the unprecedented progress in miR research, we anticipate several discoveries to be reported in the upcoming years.

Glossary of Terms

Apoptosis/apoptotic: Programmed cell death

DGCR8: DiGeorge syndrome critical region gene 8, an essential co-factor for Drosha

Dicer: A key enzyme involved in miR biogenesis in cytoplasm

Drosha: Another key enzyme involved in miR biogenesis in the nucleus

E-box: DNA consensus sequence CANNTG (where N is any nucleotide) that typically lies upstream of a genomic promoter sequence and recruits basic helix-loop-helix transcription factors to regulate the transcription of the downstream gene

ECM: The extracellular tissue/material secreted by cells and can provide mechanical support for the cells in addition to performing various other important functions.

Glomeruli/glomerulus: A capillary tuft surrounded by Bowman's capsule in renal nephrons

Glomerular basement membrane: Filtration structure consisting of the basal laminal portion of the glomerulus

Hypertrophy: An increase in size of an organ/cell or in a select area of the tissue

miR: Short, non-coding RNAs that regulate gene expression

Nephropathy: Damage to or disease of the kidney

Non-coding RNA: RNAs that are not translated to proteins; they serve to regulate gene expression or other cellular processes

Oligonucleotide: A short nucleic acid polymer, usually containing $\leq$20 bases

Phosphorylation: Addition of a phosphate group to a protein/chemical

Podocyte: Cells in the renal visceral epithelium forming part of the glomerular filtration barrier

Progenitor cell: A cell that can differentiate into a specific type of cell

Promoter: Regulatory genomic DNA sequence that regulates transcription, typically located upstream of the gene

Proteinuria: Presence of excess serum proteins in the urine, a hallmark of DN and typically indicative of renal malfunction

Renal cortex: Portion of the kidney between the renal capsule and the renal medulla

Renal medulla: Innermost part of the kidney

Target gene: Gene regulated by a given miR

TRBP: Transactivating response RNA-binding protein: A co-factor for Dicer

Transcription: The process of copying DNA to RNA by an enzyme called RNA polymerase

Translation: The first stage of protein biosynthesis, *via* the production of proteins by decoding mRNA and generating an amino acid polymer.

Tubules: Small renal structures that filter blood and produce urine.

Acknowledgments

The authors are supported by grants from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, and the Juvenile Diabetes Research Foundation.

We are grateful to members of the Natarajan laboratory for helpful discussions and suggestions.

Disclosures

None.

References

1. Ambros V: The evolution of our thinking about microRNAs. *Nat Med* 14: 1036–1040, 2008

2. Ruvkun G: The perfect storm of tiny RNAs. *Nat Med* 14: 1041–1045, 2008
3. Stefani G, Slack FJ: Small non-coding RNAs in animal development. *Nat Rev Mol Cell Biol* 9: 219–230, 2008
4. Sontheimer EJ, Carthew RW: Silence from within: Endogenous siRNAs and miRNAs. *Cell* 122: 9–12, 2005
5. Croce CM, Calin GA: miRNAs, cancer, and stem cell division. *Cell* 122: 6–7, 2005
6. Zamore PD, Haley B: Ribo-gnome: The big world of small RNAs. *Science* 309: 1519–1524, 2005
7. Ambros V: The functions of animal microRNAs. *Nature* 431: 350–355, 2004
8. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116: 281–297, 2004
9. He L, Hannon GJ: MicroRNAs: Small RNAs with a big role in gene regulation. *Nat Rev Genet* 5: 522–531, 2004
10. Filipowicz W, Bhattacharyya SN, Sonenberg N: Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? *Nat Rev Genet* 9: 102–114, 2008
11. Chang TC, Mendell JT: microRNAs in vertebrate physiology and human disease. *Annu Rev Genomics Hum Genet* 8: 215–239, 2007
12. Kim VN: MicroRNA biogenesis: Coordinated cropping and dicing. *Nat Rev Mol Cell Biol* 6: 376–385, 2005
13. Lee RC, Feinbaum RL, Ambros V: The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75: 843–854, 1993
14. Wightman B, Ha I, Ruvkun G: Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell* 75: 855–862, 1993
15. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G: Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 408: 86–89, 2000
16. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M, Tuschl T: A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* 129: 1401–1414, 2007
17. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y, Bentwich Z: Identification of hundreds of conserved and nonconserved human microRNAs. *Nat Genet* 37: 766–770, 2005
18. Lewis BP, Burge CB, Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120: 15–20, 2005
19. Friedman RC, Farh KK, Burge CB, Bartel DP: Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 19: 92–105, 2009
20. Bartel DP: MicroRNAs: Target recognition and regulatory functions. *Cell* 136: 215–233, 2009
21. Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M: A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* 432: 226–230, 2004
22. Chen CZ: MicroRNAs as oncogenes and tumor suppressors. *N Engl J Med* 353: 1768–1771, 2005
23. Taganov KD, Boldin MP, Chang KJ, Baltimore D: NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A* 103: 12481–12486, 2006
24. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D: Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1–2. *Cell* 129: 303–317, 2007
25. van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN: Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 316: 575–579, 2007
26. Sun Q, Zhang Y, Yang G, Chen X, Zhang Y, Cao G, Wang J, Sun Y, Zhang P, Fan M, Shao N, Yang, X: Transforming growth factor-beta-regulated miR-24 promotes skeletal muscle differentiation. *Nucleic Acids Res* 36: 2690–2699, 2008
27. Esquela-Kerscher A, Slack FJ: Oncomirs: MicroRNAs with a role in cancer. *Nat Rev Cancer* 6: 259–269, 2006
28. Hennessy E, O'Driscoll L: Molecular medicine of microRNAs: Structure, function and implications for diabetes. *Expert Rev Mol Med* 10: e24, 2008
29. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM: Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 99: 15524–15529, 2002
30. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM: Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A* 101: 2999–3004, 2004
31. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce, CM: A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A* 103: 2257–2261, 2006
32. Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, Henderson JM, Kutok JL, Rajewsky K: Lymphoproliferative disease and autoimmunity in mice with increased miR-17–92 expression in lymphocytes. *Nat Immunol* 9: 405–414, 2008
33. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM: miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A* 102: 13944–13949, 2005
34. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E: Let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell* 131: 1109–1123, 2007
35. Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, Chin L, Brown D, Slack FJ: The let-7 microRNA

represses cell proliferation pathways in human cells. *Cancer Res* 67: 7713–7722, 2007
36. Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massague J: Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 451: 147–152, 2008
37. Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, Lund E, Dahlberg JE: Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc Natl Acad Sci U S A* 102: 3627–3632, 2005
38. Ota A, Tagawa H, Karnan S, Tsuzuki S, Karpas A, Kira S, Yoshida Y, Seto M: Identification and characterization of a novel gene, C13orf25, as a target for 13q31–q32 amplification in malignant lymphoma. *Cancer Res* 64: 3087–3095, 2004
39. Zhang C: MicroRNAs: Role in cardiovascular biology and disease. *Clin Sci (Lond)* 114: 699–706, 2008
40. Cheng Y, Ji R, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C: MicroRNAs are aberrantly expressed in hypertrophic heart: Do they play a role in cardiac hypertrophy? *Am J Pathol* 170: 1831–1840, 2007
41. Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M: MicroRNAs play an essential role in the development of cardiac hypertrophy. *Circ Res* 100: 416–424, 2007
42. van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, Richardson JA, Olson EN: A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci U S A* 103: 18255–18260, 2006
43. Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C: MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. *Circ Res* 100: 1579–1588, 2007
44. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Koteliansky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J, Engelhardt S: MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* 456: 980–984, 2008
45. Sun Y, Koo S, White N, Peralta E, Esau C, Dean NM, Perera RJ: Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. *Nucleic Acids Res* 32: e188, 2004
46. Tian Z, Greene AS, Pietrusz JL, Matus IR, Liang M: MicroRNA-target pairs in the rat kidney identified by microRNA microarray, proteomic, and bioinformatic analysis. *Genome Res* 18: 404–411, 2008
47. Kato M, Zhang J, Wang M, Lanting L, Yuan H, Rossi JJ, Natarajan R: MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. *Proc Natl Acad Sci U S A* 104: 3432–3437, 2007
48. Wang Q, Wang Y, Minto AW, Wang J, Shi Q, Li X, Quigg RJ: MicroRNA-377 is up-regulated and can lead to increased fibronectin production in diabetic nephropathy. *FASEB J* 22: 4126–4135, 2008
49. Harvey SJ, Jarad G, Cunningham J, Goldberg S, Schermer B, Harfe BD, McManus MT, Benzing T, Miner JH: Podocyte-specific deletion of dicer alters cytoskeletal dynamics and causes glomerular disease. *J Am Soc Nephrol* 19: 2150–2158, 2008
50. Shi S, Yu L, Chiu C, Sun Y, Chen J, Khitrov G, Merkenschlager M, Holzman LB, Zhang W, Mundel P, Bottinger EP: Podocyte-selective deletion of dicer induces proteinuria and glomerulosclerosis. *J Am Soc Nephrol* 19: 2159–2169, 2008
51. Ho J, Ng KH, Rosen S, Dostal A, Gregory RI, Kreidberg JA: Podocyte-specific loss of functional microRNAs leads to rapid glomerular and tubular injury. *J Am Soc Nephrol* 19: 2069–2075, 2008
52. Kim DH, Rossi JJ: Strategies for silencing human disease using RNA interference. *Nat Rev Genet* 8: 173–184, 2007
53. Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP: MicroRNA targeting specificity in mammals: Determinants beyond seed pairing. *Mol Cell* 27: 91–105, 2007
54. Nielsen CB, Shomron N, Sandberg R, Hornstein E, Kitzman J, Burge CB: Determinants of targeting by endogenous and exogenous microRNAs and siRNAs. *RNA* 13: 1894–1910, 2007
55. Kiriakidou M, Tan GS, Lamprinaki S, De Planell-Saguer M, Nelson PT, Mourelatos Z: An mRNA m7G cap binding-like motif within human Ago2 represses translation. *Cell* 129: 1141–1151, 2007
56. Chendrimada TP, Finn KJ, Ji X, Baillat D, Gregory RI, Liebhaber SA, Pasquinelli AE, Shiekhattar R: MicroRNA silencing through RISC recruitment of eIF6. *Nature* 447: 823–828, 2007
57. Petersen CP, Bordeleau ME, Pelletier J, Sharp PA: Short RNAs repress translation after initiation in mammalian cells. *Mol Cell* 21: 533–542, 2006
58. Maroney PA, Yu Y, Fisher J, Nilsen TW: Evidence that microRNAs are associated with translating messenger RNAs in human cells. *Nat Struct Mol Biol* 13: 1102–1107, 2006
59. Rossi JJ: RNAi and the P-body connection. *Nat Cell Biol* 7: 643–644, 2005
60. Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R: MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat Cell Biol* 7: 719–723, 2005
61. Sen GL, Blau HM: Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat Cell Biol* 7: 633–636, 2005
62. Kim DH, Saetrom P, Snove O Jr, Rossi JJ: MicroRNA-directed transcriptional gene silencing in mammalian cells. *Proc Natl Acad Sci U S A* 105: 16230–16235, 2008
63. Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ: Dicer is essential for mouse development. *Nat Genet* 35: 215–217, 2003
64. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K: Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev* 19: 489–501, 2005
65. Wang Y, Medvid R, Melton C, Jaenisch R, Blelloch R: DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat Genet* 39: 380–395, 2007
66. Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L, Hannon GJ: Argo-

naute2 is the catalytic engine of mammalian RNAi. *Science* 305: 1437–1441, 2004
67. Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Hoydal M, Autore C, Russo MA, Dorn GW 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G: MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 13: 613–618, 2007
68. Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, Brummelkamp TR, Fleming MD, Camargo FD: Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature* 451: 1125–1129, 2008
69. Yang WJ, Yang DD, Na S, Sandusky GE, Zhang Q, Zhao G: Dicer is required for embryonic angiogenesis during mouse development. *J Biol Chem* 280: 9330–9335, 2005
70. El Ouaamari A, Baroukh N, Martens GA, Lebrun P, Pipeleers D, van Obberghen E: miR-375 targets 3′-phosphoinositide-dependent protein kinase-1 and regulates glucose-induced biological responses in pancreatic beta-cells. *Diabetes* 57: 2708–2717, 2008
71. Plaisance V, Abderrahmani A, Perret-Menoud V, Jacquemin P, Lemaigre F, Regazzi R: MicroRNA-9 controls the expression of Granuphilin/Slp4 and the secretory response of insulin-producing cells. *J Biol Chem* 281: 26932–26942, 2006
72. Roldo C, Missiaglia E, Hagan JP, Falconi M, Capelli P, Bersani S, Calin GA, Volinia S, Liu CG, Scarpa A, Croce CM: MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. *J Clin Oncol* 24: 4677–4684, 2006
73. Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanot S, Monia BP: miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab* 3: 87–98, 2006
74. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M: Silencing of microRNAs *in vivo* with 'antagomirs.' *Nature* 438: 685–689, 2005
75. Baroukh N, Ravier MA, Loder MK, Hill EV, Bounacer A, Scharfmann R, Rutter GA, Van Obberghen E: MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic beta-cell lines. *J Biol Chem* 282: 19575–19588, 2007
76. Zhou L, He H, Mi JX, Li C, Lee B, Mi QS: MicroRNA genes. *Ann N Y Acad Sci* 1150: 72–75, 2008
77. Yang J, Zhou F, Xu T, Deng H, Ge YY, Zhang C, Li J, Zhuang SM: Analysis of sequence variations in 59 microRNAs in hepatocellular carcinomas. *Mutat Res* 638: 205–209, 2008
78. Ayo SH, Radnik RA, Glass WF 2nd, Garoni JA, Rampt ER, Appling DR, Kreisberg JI: Increased extracellular matrix synthesis and mRNA in mesangial cells grown in high-glucose medium. *Am J Physiol* 260: F185–F191, 1991
79. Pugliese G, Pricci F, Pugliese F, Mene P, Lenti L, Andreani D, Galli G, Casini A, Bianchi S, Rotella CM, *et al.*: Mechanisms of glucose-enhanced extracellular matrix accumulation in rat glomerular mesangial cells. *Diabetes* 43: 478–490, 1994
80. Ihm CG, Lee GS, Nast CC, Artishevsky A, Guillermo R, Levin PS, Glassock RJ, Adler SG: Early increased renal procollagen alpha 1(IV) mRNA levels in streptozotocin induced diabetes. *Kidney Int* 41: 768–777, 1992
81. Kitamura M, Kitamura A, Mitarai T, Maruyama N, Nagasawa R, Kawamura T, Yoshida H, Takahashi T, Sakai O: Gene expression of metalloproteinase and its inhibitor in mesangial cells exposed to high glucose. *Biochem Biophys Res Commun* 185: 1048–1054, 1992
82. Nakamura T, Fukui M, Ebihara I, Osada S, Tomino Y, Koide H: Abnormal gene expression of matrix metalloproteinases and their inhibitor in glomeruli from diabetic rats. *Ren Physiol Biochem* 17: 316–325, 1994
83. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA: Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci U S A* 90: 1814–1818, 1993
84. Sharma K, Ziyadeh FN: Hyperglycemia and diabetic kidney disease: The case for transforming growth factor-beta as a key mediator. *Diabetes* 44: 1139–1146, 1995
85. Anderson PW, Zhang XY, Tian J, Correale JD, Xi XP, Yang D, Graf K, Law RE, Hsueh WA: Insulin and angiotensin II are additive in stimulating TGF-beta 1 and matrix mRNAs in mesangial cells. *Kidney Int* 50: 745–753, 1996
86. Rincon-Choles H, Kasinath BS, Gorin Y, Abboud HE: Angiotensin II and growth factors in the pathogenesis of diabetic nephropathy. *Kidney Int Suppl* 8–11, 2002
87. Wolf G, Chen S, Ziyadeh FN: From the periphery of the glomerular capillary wall toward the center of disease: Podocyte injury comes of age in diabetic nephropathy. *Diabetes* 54: 1626–1634, 2005
88. Susztak K, Raff AC, Schiffer M, Bottinger EP: Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes* 55: 225–233, 2006
89. Hoffman BB, Sharma K, Zhu Y, Ziyadeh FN: Transcriptional activation of transforming growth factor-beta1 in mesangial cell culture by high glucose concentration. *Kidney Int* 54: 1107–1116, 1998
90. Reeves WB, Andreoli TE: Transforming growth factor beta contributes to progressive diabetic nephropathy. *Proc Natl Acad Sci U S A* 97: 7667–7669, 2000
91. Sharma K, Ziyadeh FN, Alzahabi B, McGowan TA, Kapoor S, Kurnik BR, Kurnik PB, Weisberg LS: Increased renal production of transforming growth factor-beta1 in patients with type II diabetes. *Diabetes* 46: 854–859, 1997
92. Kim YS, Xu ZG, Reddy MA, Li SL, Lanting L, Sharma K, Adler SG, Natarajan R: Novel interactions between TGF-beta1 actions and the 12/15- lipoxygenase pathway in mesangial cells. *J Am Soc Nephrol* 16: 352–362, 2005
93. Chen S, Cohen MP, Lautenslager GT, Shearman CW, Ziyadeh FN: Glycated albumin stimulates TGF-beta 1 production and protein kinase C activity in glomerular endothelial cells. *Kidney Int* 59: 673–681, 2001
94. Douthwaite JA, Johnson TS, Haylor JL, Watson P, El Nahas AM: Effects of transforming growth factor-beta1 on renal extracellular matrix components and their regulating proteins. *J Am Soc Nephrol* 10: 2109–2119, 1999
95. Poncelet AC, Schnaper HW: Regulation of human mesangial cell collagen expression by transforming growth factor-beta1. *Am J Physiol* 275: F458–F466, 1998
96. Tsuchida K, Zhu Y, Siva S, Dunn SR, Sharma K: Role of Smad4 on TGF-beta-induced extracellular matrix stimulation in mesangial cells. *Kidney Int* 63: 2000–2009, 2003

97. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, Chen S, McGowan TA, Sharma K: Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice. *Proc Natl Acad Sci U S A* 97: 8015–8020, 2000
98. Roberts AB, McCune BK, Sporn MB: TGF-beta: Regulation of extracellular matrix. *Kidney Int* 41: 557–559, 1992
99. Yang YC, Piek E, Zavadil J, Liang D, Xie D, Heyer J, Pavlidis P, Kucherlapati R, Roberts AB, Bottinger EP: Hierarchical model of gene regulation by transforming growth factor beta. *Proc Natl Acad Sci U S A* 100: 10269–10274, 2003
100. Poncelet AC, Schnaper HW: Sp1 and Smad proteins cooperate to mediate transforming growth factor-beta 1-induced alpha 2(I) collagen expression in human glomerular mesangial cells. *J Biol Chem* 276: 6983–6992, 2001
101. Zhang Y, Feng X, We R, Derynck R: Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. *Nature* 383: 168–172, 1996
102. Schiffer M, Schiffer LE, Gupta A, Shaw AS, Roberts IS, Mundel P, Bottinger EP: Inhibitory smads and TGF-beta signaling in glomerular cells. *J Am Soc Nephrol* 13: 2657–2666, 2002
103. Kato M, Yuan H, Xu ZG, Lanting L, Li SL, Wang M, Hu MC, Reddy MA, Natarajan R: Role of the Akt/FoxO3a pathway in TGF-beta1-mediated mesangial cell dysfunction: A novel mechanism related to diabetic kidney disease. *J Am Soc Nephrol* 17: 3325–3335, 2006
104. Mahimainathan L, Das F, Venkatesan B, Choudhury GG: Mesangial cell hypertrophy by high glucose is mediated by downregulation of the tumor suppressor PTEN. *Diabetes* 55: 2115–2125, 2006
105. Ghosh Choudhury G, Abboud HE: Tyrosine phosphorylation-dependent PI 3 kinase/Akt signal transduction regulates TGFbeta-induced fibronectin expression in mesangial cells. *Cell Signal* 16: 31–41, 2004
106. Runyan CE, Schnaper HW, Poncelet AC: The phosphatidylinositol 3- kinase/Akt pathway enhances Smad3-stimulated mesangial cell collagen I expression in response to transforming growth factor-beta1. *J Biol Chem* 279: 2632–2639, 2004
107. Bakin AV, Tomlinson AK, Bhowmick NA, Moses HL, Arteaga CL: Phosphatidylinositol 3-kinase function is required for transforming growth factor beta-mediated epithelial to mesenchymal transition and cell migration. *J Biol Chem* 275: 36803–36810, 2000
108. Dufour C, Holy X, Marie PJ: Transforming growth factor-beta prevents osteoblast apoptosis induced by skeletal unloading *via* PI3K/Akt, Bcl-2, and phospho-Bad signaling. *Am J Physiol Endocrinol Metab* 294: E794–E801, 2008
109. Kato M, Putta S, Wang M, Yuan H, Lanting L, Nair I, Gunn A, Nakagawa Y, Shimano H, Todorov I, Rossi JJ, Natarajan R: TGF-β activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. *Nat Cell Biol* June 21, 2009 [epub ahead of print]
110. Yi JY, Shin I, Arteaga CL: Type I transforming growth factor beta receptor binds to and activates phosphatidylinositol 3-kinase. *J Biol Chem* 280: 10870–10876, 2005
111. Garcia Z, Kumar A, Marques M, Cortes I, Carrera AC: Phosphoinositide 3-kinase controls early and late events in mammalian cell division. *EMBO J* 25: 655–661, 2006
112. Ghosh Choudhury G, Lenin M, Calhaun C, Zhang JH, Abboud HE: PDGF inactivates forkhead family transcription factor by activation of Akt in glomerular mesangial cells. *Cell Signal* 15: 161–170, 2003
113. Eger A, Aigner K, Sonderegger S, Dampier B, Oehler S, Schreiber M, Berx G, Cano A, Beug H, Foisner R: DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. *Oncogene* 24: 2375–2385, 2005
114. Sooy K, Demay MB: Transcriptional repression of the rat osteocalcin gene by deltaEF1. *Endocrinology* 143: 3370–3375, 2002
115. Sekido R, Murai K, Funahashi J, Kamachi Y, Fujisawa-Sehara A, Nabeshima Y, Kondoh H: The delta-crystallin enhancer-binding protein delta EF1 is a repressor of E2-box-mediated gene activation. *Mol Cell Biol* 14: 5692–5700, 1994
116. Ponticos M, Partridge T, Black CM, Abraham DJ, Bou-Gharios G: Regulation of collagen type I in vascular smooth muscle cells by competition between Nkx2.5 and deltaEF1/ZEB1. *Mol Cell Biol* 24: 6151–6161, 2004
117. Terraz C, Toman D, Delauche M, Ronco P, Rossert J: Delta Ef1 binds to a far upstream sequence of the mouse pro-alpha 1(I) collagen gene and represses its expression in osteoblasts. *J Biol Chem* 276: 37011–37019, 2001
118. Murray D, Precht P, Balakir R, Horton WE Jr: The transcription factor deltaEF1 is inversely expressed with type II collagen mRNA and can repress Col2a1 promoter activity in transfected chondrocytes. *J Biol Chem* 275: 3610–3618, 2000
119. Verschueren K, Remacle JE, Collart C, Kraft H, Baker BS, Tylzanowski P, Nelles L, Wuytens G, Su MT, Bodmer R, Smith JC, Huylebroeck D: SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5′-CACCT sequences in candidate target genes. *J Biol Chem* 274: 20489–20498, 1999
120. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP: The impact of microRNAs on protein output. *Nature* 455: 64–71, 2008
121. Selbach M, Schwanhausser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N: Widespread changes in protein synthesis induced by microRNAs. *Nature* 455: 58–63, 2008
122. Grosshans H, Filipowicz W: Proteomics joins the search for microRNA targets. *Cell* 134: 560–562, 2008
123. Choi WY, Giraldez AJ, Schier AF: Target protectors reveal dampening and balancing of Nodal agonist and antagonist by miR-430. *Science* 318: 271–274, 2007
124. Martello G, Zacchigna L, Inui M, Montagner M, Adorno M, Mamidi A, Morsut L, Soligo S, Tran U, Dupont S, Cordenonsi M, Wessely O, Piccolo S: MicroRNA control of Nodal signalling. *Nature* 449: 183–188, 2007
125. Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, lliopoulos D, Pilozzi E, Liu CG, Negrini M, Cavazzini L, Volinia S, Alder H, Ruco LP, Baldassarre G, Croce CM, Vecchione A: E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer Cell* 13: 272–286, 2008
126. Davis BN, Hilyard AC, Lagna G, Hata A: SMAD proteins control DROSHA-mediated microRNA maturation. *Nature* 454: 56–61, 2008

127. Kim S, Choi M, Cho KH: Identifying the target mRNAs of microRNAs in colorectal cancer. *Comput Biol Chem* 33: 94–99, 2009
128. Hino K, Tsuchiya K, Fukao T, Kiga K, Okamoto R, Kanai T, Watanabe M: Inducible expression of microRNA-194 is regulated by HNF-1 alpha during intestinal epithelial cell differentiation. *RNA* 14: 1433–1442, 2008
129. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Bell GI, *et al.*: Mutations in the hepatocyte nuclear factor-1 alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 384: 455–458, 1996
130. Yang S, Du J, Wang Z, Yan J, Yuan W, Zhang J, Zhu T: Dual mechanism of deltaEF1 expression regulated by bone morphogenetic protein-6 in breast cancer. *Int J Biochem Cell Biol* 41: 853–861, 2009
131. Sengupta S, den Boon JA, Chen IH, Newton MA, Stanhope SA, Cheng YJ, Chen CJ, Hildesheim A, Sugden B, Ahlquist P: MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. *Proc Natl Acad Sci U S A* 105: 5874–5878, 2008
132. Christoffersen NR, Silahtaroglu A, Orom UA, Kauppinen S, Lund AH: miR-200b mediates post-transcriptional repression of ZFHX1B. *RNA* 13: 1172–1178, 2007
133. Hurteau GJ, Carlson JA, Spivack SD, Brock GJ: Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. *Cancer Res* 67: 7972–7976, 2007
134. Park SM, Gaur AB, Lengyel E, Peter ME: The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev* 22: 894–907, 2008
135. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ: The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 10: 593–601, 2008
136. Burk U, Schubert J, Wellner U, Schmalhofer O, Vincan E, Spaderna S, Brabletz T: A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. *EMBO Rep* 9: 582–589, 2008
137. Korpal M, Lee ES, Hu G, Kang Y: The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J Biol Chem* 283: 14910–14914, 2008
138. Bracken CP, Gregory PA, Kolesnikoff N, Bert AG, Wang J, Shannon MF, Goodall GJ: A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. *Cancer Res* 68: 7846–7854, 2008
139. Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, Cheng JQ: MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. *Mol Cell Biol* 28: 6773–6784, 2008
140. Braun CJ, Zhang X, Savelyeva I, Wolff S, Moll UM, Schepeler T, Orntoft TF, Andersen CL, Dobbelstein M: p53-Responsive microRNAs 192 and 215 are capable of inducing cell cycle arrest. *Cancer Res* 68: 10094–10104, 2008
141. Song B, Wang Y, Kudo K, Gavin EJ, Xi Y, Ju J: miR-192 regulates dihydrofolate reductase and cellular proliferation through the p53-microRNA circuit. *Clin Cancer Res* 14: 8080–8086, 2008
142. Georges SA, Biery MC, Kim SY, Schelter JM, Guo J, Chang AN, Jackson AL, Carleton MO, Linsley PS, Cleary MA, Chau BN: Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. *Cancer Res* 68: 10105–10112, 2008
143. Gou D, Zhang H, Baviskar PS, Liu L: Primer extension-based method for the generation of a siRNA/miRNA expression vector. *Physiol Genomics* 31: 554–562, 2007
144. Roy B, Beamon J, Balint E, Reisman D: Transactivation of the human p53 tumor suppressor gene by c-Myc/Max contributes to elevated mutant p53 expression in some tumors. *Mol Cell Biol* 14: 7805–7815, 1994
145. Cosgrave N, Hill AD, Young LS: Growth factor-dependent regulation of survivin by c-myc in human breast cancer. *J Mol Endocrinol* 37: 377–390, 2006
146. Wolf G, Ziyadeh FN: Molecular mechanisms of diabetic renal hypertrophy. *Kidney Int* 56: 393–405, 1999
147. Lee SO, Masyuk T, Splinter P, Banales JM, Masyuk A, Stroope A, Larusso N: MicroRNA15a modulates expression of the cell-cycle regulator Cdc25A and affects hepatic cystogenesis in a rat model of polycystic kidney disease. *J Clin Invest* 118: 3714–3724, 2008
148. Pandey P, Brors B, Srivastava PK, Bott A, Boehn SN, Groene HJ, Gretz N: Microarray-based approach identifies microRNAs and their target functional patterns in polycystic kidney disease. *BMC Genomics* 9: 624, 2008
149. Dai Y, Sui W, Lan H, Yan Q, Huang H, Huang Y: Comprehensive analysis of microRNA expression patterns in renal biopsies of lupus nephritis patients. *Rheumatol Int* November 8, 2008 [epub ahead of print]
150. Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, Herias V, van Leeuwen RE, Schellings MW, Barenbrug P, Maessen JG, Heymans S, Pinto YM, Creemers EE: miR-133 and miR-30 regulate connective tissue growth factor: Implications for a role of microRNAs in myocardial matrix remodeling. *Circ Res* 104: 170–178, 2009, 6p following 178
151. Lee NS, Dohjima T, Bauer G, Li H, Li MJ, Ehsani A, Salvaterra P, Rossi J: Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. *Nat Biotechnol* 20: 500–505, 2002
152. Aagaard L, Rossi JJ: RNAi therapeutics: Principles, prospects and challenges. *Adv Drug Deliv Rev* 59: 75–86, 2007
153. Castanotto D, Rossi JJ: The promises and pitfalls of RNA-interference-based therapeutics. *Nature* 457: 426–433, 2009
154. Ebert MS, Neilson JR, Sharp PA: MicroRNA sponges: Competitive inhibitors of small RNAs in mammalian cells. *Nat Methods* 4: 721–726, 2007
155. Elmen J, Lindow M, Schutz S, Lawrence M, Petri A, Obad S, Lindholm M, Hedtjarn M, Hansen HF, Berger U, Gullans S, Kearney P, Sarnow P, Straarup EM, Kauppinen S: LNA-mediated microRNA silencing in non-human primates. *Nature* 452: 896–899, 2008
156. Natarajan R, Gu JL, Rossi J, Gonzales N, Lanting L, Xu L, Nadler J: Elevated glucose and angiotensin II increase 12-lipoxygenase activity and expression in porcine aortic smooth muscle cells. *Proc Natl Acad Sci U S A* 90: 4947–4951, 1993

157. Yuan H, Lanting L, Xu ZG, Li SL, Swiderski P, Putta S, Jonnalagadda M, Kato M, Natarajan R: Effects of cholesterol-tagged small interfering RNAs targeting 12/15-lipoxygenase on parameters of diabetic nephropathy in a mouse model of type 1 diabetes. *Am J Physiol Renal Physiol* 295: F605–F617, 2008
158. Elmen J, Lindow M, Silahtaroglu A, Bak M, Christensen M, Lind-Thomsen A, Hedtjarn M, Hansen JB, Hansen HF, Straarup EM, McCullagh K, Kearney P, Kauppinen S: Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. *Nucleic Acids Res* 36: 1153–1162, 2008
159. Chen K, Rajewsky N: Natural selection on human microRNA binding sites inferred from SNP data. *Nat Genet* 38: 1452–1456, 2006
160. Saunders MA, Liang H, Li WH: Human polymorphism at microRNAs and microRNA target sites. *Proc Natl Acad Sci U S A* 104: 3300–3305, 2007
161. Yu Z, Li Z, Jolicoeur N, Zhang L, Fortin Y, Wang E, Wu M, Shen SH: Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers. *Nucleic Acids Res* 35: 4535–4541, 2007
162. Landi D, Gemignani F, Naccarati A, Pardini B, Vodicka P, Vodickova L, Novotny J, Forsti A, Hemminki K, Canzian F, Landi S: Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. *Carcinogenesis* 29: 579–584, 2008
163. Yang H, Dinney CP, Ye Y, Zhu Y, Grossman HB, Wu X: Evaluation of genetic variants in microRNA-related genes and risk of bladder cancer. *Cancer Res* 68: 2530–2537, 2008