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Marquez RT, Wendlandt E, Galle CS, Keck K, McCaffrey AP. MicroRNA-21 is upregulated during the proliferative phase of liver regeneration, targets *Pellino-1*, and inhibits NK-κB signaling. *Am J Physiol Gastrointest Liver Physiol* 298: G535–G541, 2010. First published February 18, 2010; doi:10.1152/ajpgi.00338.2009.—During liver regeneration, normally quiescent liver cells reenter the cell cycle, nonparenchymal and parenchymal cells divide, and proper liver architecture is restored. The gene expression programs regulating these transitions are not completely understood. MicroRNAs are a newly discovered class of small regulatory RNAs that silence messenger RNAs by binding to their 3′-untranslated regions (UTRs). A number of microRNAs, including miR-21, have been shown to be involved in regulation of cell proliferation. We performed partial hepatectomies on mice and allowed the liver to regenerate for 1, 6, 12, 24, and 48 h and 4 and 7 days. We compared the expression of miR-21 in the posthepatectomy liver to the prehepatectomy liver by Northern blot and found that miR-21 was upregulated during the early stages of liver regeneration. NF-κB signaling is also activated very early during liver regeneration. It has been previously reported that NF-κB upregulates the miR-21 precursor transcript. The predicted miR-21 target, Pellino (*Peli1*), is a ubiquitin ligase involved in activating NF-κB signaling. We observed an inverse correlation between miR-21 and *Peli1* mRNA levels during liver regeneration. miR-21 overexpression in cultured cells inhibited a *Peli1* 3′-UTR luciferase reporter. Using NF-κB reporter assays, we determined that miR-21 overexpression inhibits NF-κB signaling. In conclusion, miR-21 expression was upregulated during early stages of liver regeneration. Targeting of *Peli1* by miR-21 could potentially provide the basis for a negative feedback cycle regulating NF-κB signaling.

Toll-like receptor; interleukin-1

UNLIKE MOST DEVELOPED ORGANS in the body, the liver has a tremendous regenerative potential. Remarkably, one can remove 60% of a mouse's liver (referred to as a partial hepatectomy), and it grows back to its original mass after approximately 1 wk. Rapid biochemical and gene expression changes occur after partial hepatectomy. Significant effort is being devoted to understanding what signals regulate the complex process of liver regeneration (see Refs. 39, 46, 52 for reviews). The highly orchestrated process of liver regeneration after partial hepatectomy can be divided into four interconnected stages. During the priming phase (0–4 h), liver cells are licensed to replicate. In the proliferative phase, hepatocytes (parenchymal cells) proliferate at ~36–40 h, whereas nonparenchymal cells proliferate later, at ~72–96 h. Proper liver architecture is restored during the remodeling phase. Lastly, in the termination phase, cell proliferation is repressed once normal liver mass is restored.

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It is widely accepted that quiescence in the liver is maintained by a balance of proliferative/antiproliferative and apoptotic/antiapoptotic signals. Shortly after partial hepatectomy, this balance shifts toward proliferation. However, once sufficient proliferation has occurred to restore proper liver mass, this balance is reestablished by negative feedback cycles. It has been proposed that interleukin (IL)-1β secretion by nonparenchymal cells (NPCs) of the liver serves as a signal to terminate hepatocyte proliferation after the initial wave of cell division (6). Cell culture studies showed that NPCs isolated from the livers of mice after partial hepatectomy produce IL-1β (6). IL-1β inhibited DNA synthesis in cultured hepatocytes (6, 41) and significantly reduced DNA replication in vivo following partial hepatectomy (6). IL-1β signals through the IL-1 receptor (IL-1R)/Toll-like receptor (TLR) pathway, which leads to NF-κB activation. NF-κB is a regulator of the cell cycle but is also important for preventing apoptosis of hepatocytes (4) Surprisingly, however, NF-κB signaling does not appear to be absolutely essential for liver regeneration after partial hepatectomy (8, 13), possibly attributable to the presence of redundant signaling pathways.

MicroRNAs (miRNAs) are small ~22-nucleotide noncoding RNAs predicted to repress approximately one half of all human genes posttranscriptionally (17) through translational arrest and/or messenger RNA (mRNA) degradation. Humans have >600 miRNAs that regulate the expression of mRNAs by binding to their 3′-untranslated regions (UTRs) (14, 34). miRNAs are transcribed as long primary miRNA precursors (pri-miRNAs), which are processed by Drosha into an intermediate called a pre-miRNA before further processing by Dicer into the mature miRNA (3). There is experimental evidence that miRNAs regulate cell division, differentiation, cell fate decisions, development, oncogenesis, apoptosis, and many other processes (reviewed in Refs. 3 and 49). miRNAs are often dysregulated in cancers, including hepatocellular carcinoma (40), and miRNAs can also act as oncogenes (23).

Previously, Hand et al. (22) generated hepatocyte-specific Dicer knockout transgenic mice to examine the role of miRNAs in the liver. Surprisingly, the knockout mice initially developed normally. However, after several months, they exhibited progressive hepatocyte damage and elevated serum aminotransferase levels. Dicer knockout livers were enlarged relative to control livers, suggesting a role for miRNAs in the control of hepatocyte proliferation. Increased apoptosis was also noted, and, in older animals, normal hepatic architecture was lost. Because the knockout in this study was hepatocyte specific, it did not address the role of miRNAs in liver NPCs. Recently, Kren et al. (28) showed that the association of miRNAs with different polysome fractions was altered during liver regeneration in rats. These studies raise the intriguing possibility that miRNAs might regulate liver regeneration.

In our study, we found that miR-21 is upregulated during the proliferative phases of liver regeneration (see Ref. 29 for a recent comprehensive review on miR-21). NF-κB activation is an early event during liver regeneration; in other settings, NF-κB upregulates miR-21 (48). The upregulation of miR-21 during the proliferative phase of liver regeneration is intriguing because miR-21 is upregulated in many cancers including hepatocellular carcinoma (9, 19, 24, 25, 30, 31). Furthermore, miR-21 has been shown to target tumor suppressor genes, such as *Pdcd4* (1, 16), *Pten* (38), *Maspin* (51), and *Tpm1* (50) as well as matrix metalloproteinase inhibitors, such as *Timp3* (43) and *Reck* (20). In a cancer xenograft model, miR-21 inhibition suppressed cell proliferation and increased apoptosis (44), further defining miR-21 as an oncogenic miRNA. Overexpression of miR-21 in cultured human hepatocellular carcinoma cells led to increased tumor cell proliferation and migration, whereas inhibition of miR-21 increased the sensitivity of tumor cells to chemotherapy (5, 37, 44).

Here, we show that miR-21 was upregulated during the proliferative phases of liver regeneration. We observed an inverse correlation between the expression levels of miR-21 and *Peli1*, a putative miR-21 target gene required for IL-1R/TLR-activated NF-κB signaling (27). Upregulation of miR-21 also coincided with a decrease in the NF-κB-responsive gene, *IL-6*, during liver regeneration. We demonstrated that miR-21 directly inhibited a *Peli1* 3′-UTR luciferase reporter. Furthermore, we demonstrated that miR-21 overexpression inhibited NF-κB signaling, thus indicating a possible negative feedback loop.

MATERIALS AND METHODS

Liver regeneration studies and RNA preparation. A partial hepatectomy was performed on C57/BL6 mice whereby the median and left lobes of the liver were removed and homogenized in TRIzol (Invitrogen). The liver was allowed to regenerate and was harvested in TRIzol at seven different time points: 1, 6, 12, 24, and 48 h and 4 and 7 days. All animal studies were approved by the University of Iowa Institutional Animal Care and Use Committee.

Small-transcript Northern blots. Small-transcript Northern blots were performed as described (42). Briefly, 3 μg of total RNA was resolved on a 15% polyacrylamide, 7 M urea and 20 mM MOPS/NaOH gel and transferred to Hybond Nx nylon membrane (Amersham/Pharmacia). RNA was cross linked in 0.16 M *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride in 127 mM 1-methylimidazole (pH 8). An oligonucleotide antisense to miR-21 (5′- TCAACATCAGTCTGATAAGCTA -3′) was 5′-end labeled with [γ-^{32}P] adenosine triphosphate, and membranes were hybridized at 35°C for 16 h in Church's buffer (3× SSC, 5 mmol/l Tris·HCl, pH 7.4, 0.5% sodium dodecyl sulfate, 0.05% Ficol 400, 0.05% polyvinylpyrrolidone, and 0.05% bovine serum album) and washed three times at 45°C in 0.1× SSC. Bands were quantitated using a phosphorimager (Molecular Dynamics). A probe for the U6 spliceosomal RNA was used for normalization. miRNA expression was compared between the liver removed during the partial hepatectomy (prehepatectomy liver) and the regenerated (post-hepatectomy) liver of the same mouse.

miR-21 luciferase reporter construct and cloning of 3′-UTRs. The miR-21 luciferase sensor (Fig. 3*A*) was designed with a bulge in the target sequence to prevent reporter degradation through RNA interference (RNAi). Sense and antisense oligonucleotides containing the imperfect miR-21 target sequence and 5′-*Xho*I and 3′-*Not*I overhangs for cloning were synthesized (Integrated DNA Technologies). The primer sequences are as follows: sense, 5′-tcgagTCAACATCAGGACATAAGCTAgc -3′; antisense, 5′-ggccgcTAGCTTATGTCCTGATGTTGAc -3′. The oligonucleotides were annealed to each other, kinased, and cloned downstream of the *Renilla* luciferase in the psiCHECK-2 vector (Promega). The full-length *Peli1* 3′-UTR (accession no. NM_023324) was cloned from mouse liver cDNA (Clontech) by PCR with *Peli1*-F1629: 5′-TGGCTGGTGAACAAGGCTAT-3′ and *Peli1*-R3427: 5′-GCTACAATATAGAGCCTCAACAGAA-3′. The 3′-UTR was cloned downstream of the *Renilla* luciferase in the *Xho*I/*Not*I restriction enzyme sites of the psiCHECK-2 vector.

Quantitative PCR. Total RNA was reverse transcribed using the high-capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real-time PCR was performed using 2× Sybr Green Master Mix (Applied Biosystems). The expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase. Three to four biological replicates were performed for each time point. The following primers were used: *Peli1* (accession no. NM_023324): sense - 5′ CTTCAGTCTGCGTGAAACCA -3′, antisense - 5′ AAGGTTGCACCACAAAGGTC 3′; *IL-6* (accession no. NM_ 031168): sense - 5′ AGAACGATAGTCAATTCCAGAAACCGC 3′, antisense - 5′ AAGGCCGTGGTTGTCACCAGC 3′.

Cell culture, transfections, and luciferase assays. For luciferase reporter assays, human embryonic kidney (HEK)-293 cells were cultured in DMEM supplemented with 10% fetal bovine serum at 37°C and 5% CO_2. Cells were plated in a 24-well plate. Luciferase reporter plasmids (50 ng) were cotransfected with pre-miR-21 (0.5, 5, and 50 nM) or Cy3-labeled negative control pre-miRs (50 nM) (Ambion) using Lipofectamine 2000 (Invitrogen). Cells were harvested 24 h later and assayed using the Dual-Luciferase reporter assay system (Promega) using a MicroLumatPlus luminometer (Berthold Technologies). *Renilla* luciferase values were normalized to firefly luciferase values expressed from the same psiCHECK-2 vector. NF-κB reporter assays were performed as follows: HEK-293 cells stably expressing TLR-4 (provided by Theresa Gioannini, University of Iowa) were cotransfected with 100 ng pNF-κB-luc (Clontech), 2 ng phRL-SV40 *Renilla* (Promega), and 50 nM pre-miR-21 (or negative control pre-miRs) for 24 h. Cells were stimulated with 400 pM lipooligosaccharide (LOS):MD2 prepared as described (21) (provided by Theresa Gioannini, University of Iowa) for 4 h to induce NF-κB signaling through TLR-4, then harvested, and assayed using the Dual-Luciferase reporter assay system. Firefly luciferase values were normalized to the *Renilla* luciferase values. Experiments were performed in triplicate.

Statistics. A paired Student's *t*-test was used to determine statistical significance.

RESULTS

miR-21 is upregulated during liver regeneration. During liver regeneration, gene expression is greatly altered when normally quiescent hepatocytes rapidly reenter the cell cycle. We hypothesized that miRNAs are involved in regulating this rapid change in gene expression during liver regeneration. Because miR-21 has been shown to be a proliferative miRNA whose expression is dysregulated in hepatocellular carcinoma (40), we chose to examine its levels during liver regeneration. We measured the expression level of miR-21 during liver regeneration by small-transcript Northern blot (Fig. 1*A*). miR-21 was upregulated at the 1-, 6-, 12-, 24- and 48-h time points, suggesting possible involvement in regulation of gene expression during the proliferative phases of liver regeneration (Fig. 1*B*). miR-21 upregulation was greater than twofold and peaked at the 12- and 24-h time points. Recently, Si et al. (44) showed that a twofold decrease in miR-21 levels in cultured MCF-7 cells produced a statistically significant reduction in prolifera-

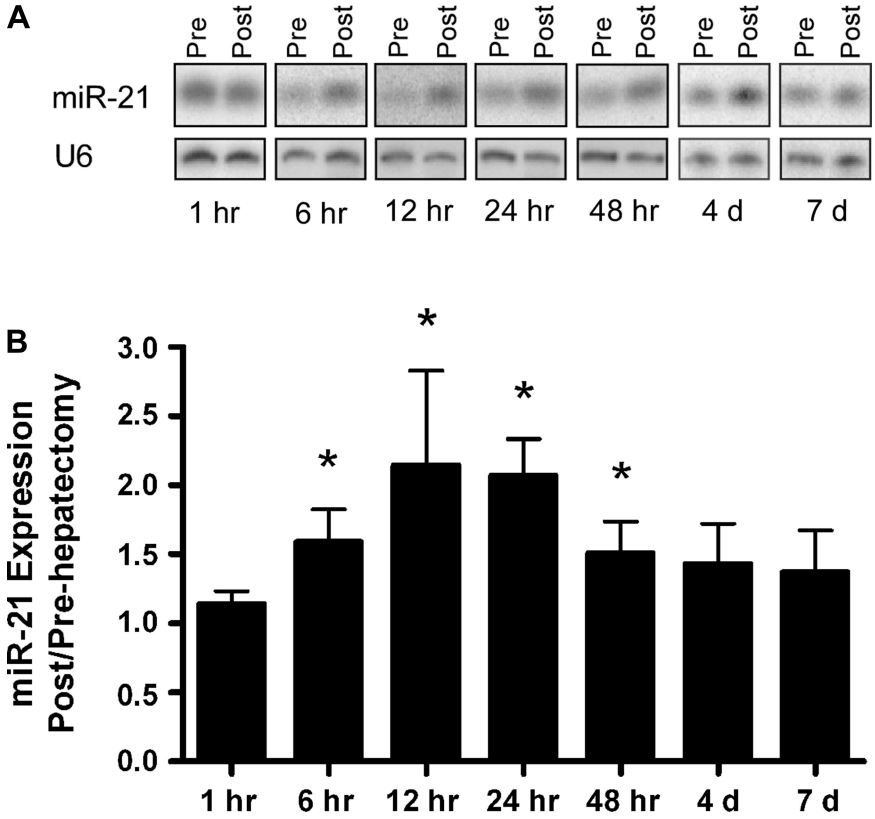

Fig. 1. miR-21 is upregulated during the proliferative phases of liver regeneration. *A*: miR-21 expression was determined by small-transcript Northern blot. Representative blots for each time point are depicted. Pre, prehepatectomy livers; Post, posthepatectomy livers from the same mouse. miR-21 expression levels were normalized to U6 expression. *B*: data are means ± SE; $n = 5$; $^*P < 0.05$.

tion. Importantly, these results show that some miRNAs are differentially expressed during liver regeneration.

Prediction of putative miR-21 target genes. To determine how miR-21 could potentially regulate liver regeneration, we sought to identify novel miR-21 target genes. miRNAs primarily recognize their target mRNAs through hybridization of the miRNA "seed sequence" (nucleotides 2–8) with "seed matches" in the 3′-UTRs of the mRNA. Algorithms, such as TargetScan, use the seed match, conservation among species, and additional context features to identify putative miRNA/mRNA interactions (32, 33). Using TargetScan, we identified *Peli1* as a putative miR-21 target. *Peli1* functions as an adapter in the IL-1R/TLR signaling cascade. *Peli1* forms a complex with IL-1R-associated kinase (IRAK) 1, IRAK4, and TNF receptor-associated factor 6 (TRAF6), which mediates NF-κB activation in response to IL-1R/TLR signaling (27) (Fig. 2*A*).

Expression levels of the miR-21 target gene, Peli1, and the NF-κB-responsive gene, IL-6, inversely correlate with miR-21 during the proliferative phase of liver regeneration. In addition to silencing gene expression by translational repression, miRNAs can also decrease the stability of target mRNAs (reviewed in Ref. 35). We utilized quantitative RT-PCR to measure the levels of *Peli1* mRNA during liver regeneration. At 12 h, as miR-21 levels peak, we observed a statistically significant reduction in *Peli1* mRNA levels (Fig. 2*B*). To determine whether the decrease in *Peli1* mRNA levels affected

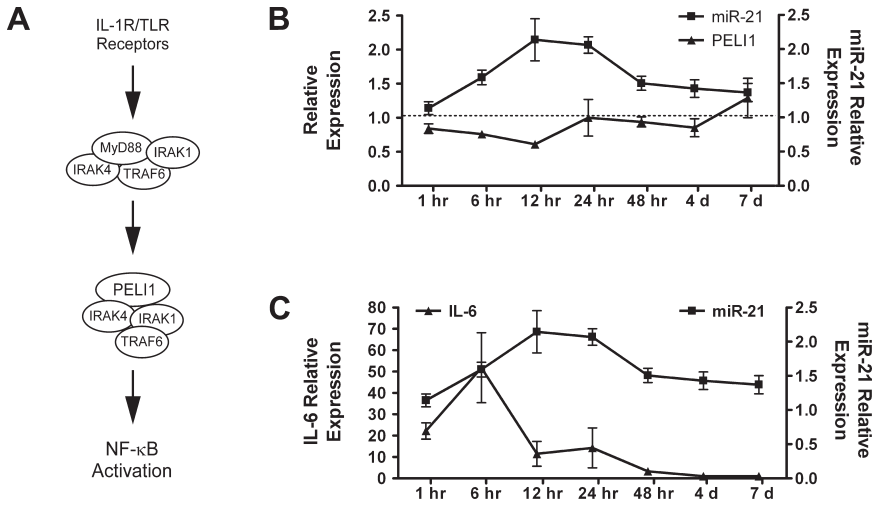

Fig. 2. miR-21 expression inversely correlates with expression of target, *Pellino-1* (*Peli1*), and NF-κB-responsive gene, IL-6, during liver regeneration. *A*: schematic showing the role of PELI1 as an adapter in IL-1 receptor/Toll-like receptor (IL-1R/TLR) signaling. NF-κB activation leads to transcription of IL-6. *A* and *B*: mRNA expression of *Peli1* and *IL-6* during liver regeneration was measured by quantitative PCR and normalized to GAPDH. *B*: *Peli1* expression was significantly downregulated at 12 h during liver regeneration, coinciding with upregulation of miR-21. *C*: *IL-6* expression is downregulated at the 12- and 24-h time points correlating with *Peli1* downregulation and inversely correlating with miR-21 expression. Data are means ± SE; $n = 3–4$. IRAK1, IL-1R-associated kinase 1; TRAF6, TNF receptor-associated factor 6.

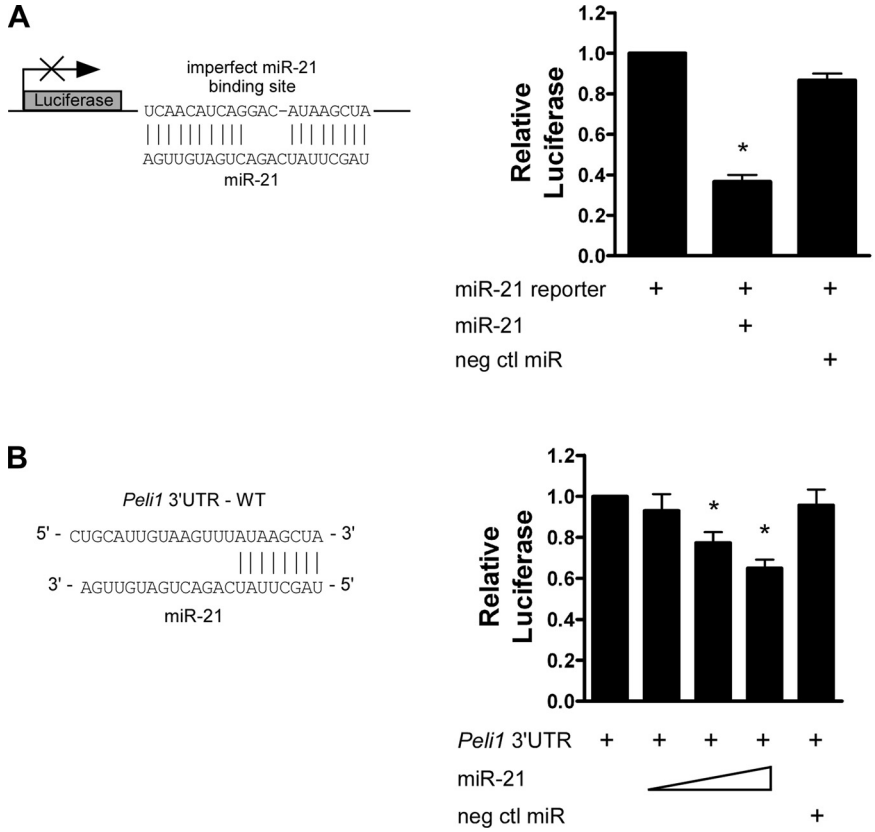

Fig. 3. miR-21 targets the 3′-untranslated region (UTR) of *Peli1*. *A*: predicted base pairing between miR-21 and the 3′-UTR of the miR-21 sensor is shown. Human embryonic kidney (HEK)-293 cells were cotransfected with a miR-21 luciferase sensor construct containing an imperfect miR-21 binding site and pre-miR-21 (50 nM) or negative control pre-miR (50 nM). Luciferase activity was inhibited ~60% in pre-miR-21-transfected cells. *B*: TargetScan's predicted interaction between miR-21 and the putative *Peli1* miR-21 binding site. HEK-293 cells cotransfected with luciferase reporter construct containing the 3′-UTR of *Peli1* and increasing concentrations (0.5, 5, and 50 nM) of miR-21 or negative control pre-miR (50 nM). Pre-miR-21 inhibition of luciferase production is dose dependent. Relative luciferase is *Renilla*/firefly luciferase. Data are means ± SE; $n = 3$; $^*P < 0.05$. Representative data from three independent experiments are shown. WT, wild-type.

NF-κB signaling, we measured expression levels of *IL-6*, an NF-κB-responsive gene. *IL-6* mRNA expression is upregulated immediately following partial hepatectomy with maximum induction at 6 h. Consistent with *Peli1* expression, we see an inverse correlation between *IL-6* and miR-21 expression levels between 12 and 24 h (Fig. 2*C*). These results are consistent with miR-21-mediated targeting of *Peli1* and subsequent inhibition of NF-κB signaling during liver regeneration; however, further studies, such as in vivo miR-21 mouse knockout studies, will be required to establish a direct role for miR-21 in regulating liver regeneration.

A functional assay for miR-21 overexpression. miR-21 levels can be increased in cells by transfection with miRNA mimics called pre-miRs (Ambion). To establish conditions for efficient pre-miR transfection, we constructed a miR-21 sensor. The miR-21 reporter contained an imperfect miR-21 binding site downstream of the *Renilla* luciferase gene in psiCHECK-2. This binding site confers miR-21-mediated silencing (Fig. 3*A*). A bulge was introduced to prevent degradation of our sensor construct via RNAi. We conducted experiments in HEK-293 cells, which express very low endogenous levels of miR-21 (data not shown). Thus studies in HEK-293 cells allow us to examine 3′-UTR responsiveness in the presence or absence of miR-21. Cotransfection of the miR-21 sensor with miR-21 into HEK-293 cells resulted in a 60% reduction in luciferase production, thus demonstrating that we can successfully manipulate miR-21 levels in cultured cells (Fig. 3*A*).

miR-21 directly targets Peli1. To investigate whether *Peli1* was a miR-21 target, we cloned the full-length *Peli1* 3′-UTR downstream of the *Renilla* luciferase gene of the psiCHECK-2

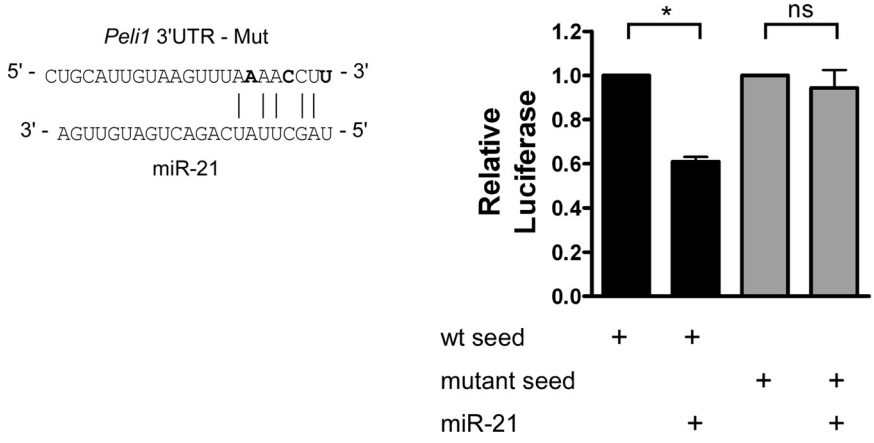

Fig. 4. miR-21 does not inhibit mutated (Mut) 3′-UTR of *Peli1*. Three nucleotides in the miR-21 binding seed sequence in *Peli1* 3′-UTR (indicated in bold) were mutated to determine specificity of miR-21-mediated inhibition of *Peli1*. HEK-293 cells were cotransfected with wild-type or mutant luciferase reporter constructs and no pre-miR or pre-miR-21 (5 nM). The mutant luciferase reporter demonstrated derepression of miR-21 inhibition. Data are means ± SE; $n = 3$; $^*P < 0.0001$. ns, not statistically significant. Relative luciferase is *Renilla*/firefly luciferase. Representative data of three independent experiments are shown.

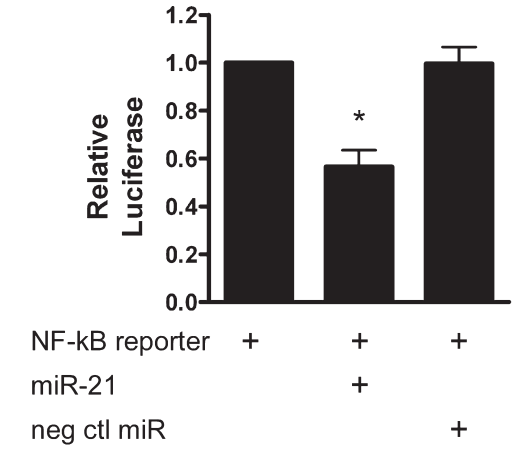

Fig. 5. Overexpression of miR-21 inhibits NF-κB signaling. HEK-293 cells stably expressing TLR-4 were cotransfected with an NF-κB-responsive luciferase reporter and pre-miR-21 (50 nM) or negative control pre-miR (50 nM) to measure the effect of miR-21 on NF-κB signaling. After 24 h, cells were treated with lipooligosaccharide:MD2 for 4 h to activate IL-1R/TLR signaling. Cells transfected with pre-miR-21 inhibited luciferase production by ~40%. Cells were cotransfected with a *Renilla* luciferase plasmid for normalization. Relative luciferase is firefly/*Renilla* luciferase. Data are means ± SE; $n = 3$; *$P < 0.05$.

vector. We cotransfected into HEK-293 cells the *Peli1* 3′-UTR reporter with miR-21 or negative control. We observed a significant dose-dependent miR-21-mediated decrease ($P < 0.05$) in luciferase activity (Fig. 3*B*). The ~35% decrease in luciferase expression is consistent with large-scale proteomics studies that showed that most miRNA/mRNA interactions result in silencing of ~30% or less (2).

If miR-21 does indeed target *Peli1*, then mutating the seed match in the *Peli1* 3′-UTR luciferase reporter should alleviate miR-21 repression. Indeed, mutant *Peli1* reporter levels in cultured cells did not decrease upon pre-miR-21 cotransfections (Fig. 4). Importantly, these results establish that the interaction between miR-21 and *Peli1* is direct and further confirm that *Peli1* is an authentic miR-21 target. Furthermore, we established a novel role for miR-21 in regulating NF-κB signaling.

miR-21 overexpression reduces NF-κB reporter signaling. We have identified *Peli1* as a novel miR-21 target. *Peli1* forms a complex with IRAK, IRAK4, and TRAF6 to activate NF-κB signaling (Fig. 2*A*). We hypothesized that, if miR-21 inhibits *Peli1*, then we would expect overexpression of exogenous miR-21 to inhibit NF-κB signaling. We used the known TLR-4 ligand, LOS:MD2 (21), to induce NF-κB signaling in HEK-293 cells stably expressing TLR-4 and transfected them with miR-21 (or negative control miR). We found that, upon stimulation, cells overexpressing miR-21 (but not cells overexpressing a control miRNA) had ~40% less NF-κB activity (Fig. 5). This response may be due to inhibition of *Peli1* or additional miR-21 targets involved in NF-κB signaling.

DISCUSSION

Liver regeneration triggers many gene expression changes to induce proliferation of parenchymal and nonparenchymal cells. We found that miR-21 was upregulated during the proliferative phase of liver regeneration (Figs. 1 and 2). This is intriguing because miR-21 is upregulated in many cancers (9, 19, 24, 25, 30, 31) and has been shown to target tumor suppressor genes (1, 16, 20, 38, 43, 50, 51).

Upregulation of miR-21 during liver regeneration may be induced by multiple transcription factors. The miR-21 promoter is regulated by transcription factors such as NF-κB (48), activation protein-1, NFIB, and STAT3 (18, 36). Many of these transcription factors are activated during the early stages of liver regeneration (47).

An emerging theme is that miRNAs participate in feedback loops with transcription factors that regulate their transcription. A recent study showed that NF-κB activates the primary miR-21 promoter in hepatocytes treated with unsaturated fatty acids (48). Activation of NF-κB is one of the earliest signaling events following partial hepatectomy (11, 15). Thus NF-κB could possibly be involved in miR-21 upregulation at the early stages of liver regeneration. Our results provide additional evidence for a relationship between miR-21 and IL-1R/TLR-mediated NF-κB signaling. We identified a novel miR-21 target, *Peli1*, which is required for NF-κB activation (27), and we demonstrated an inverse correlation between the expression levels of both *Peli1* and the NF-κB-responsive gene, *IL-6* (Fig. 2, *B* and *C*). This suggests the possibility that miR-21 could inhibit NF-κB signaling by targeting *Peli1* during the proliferative phase of liver regeneration. miR-21 knockout studies in mice could definitively establish this connection. We propose a model by which NF-κB and miR-21 form a negative feedback loop via targeting of *Peli1* (Fig. 6).

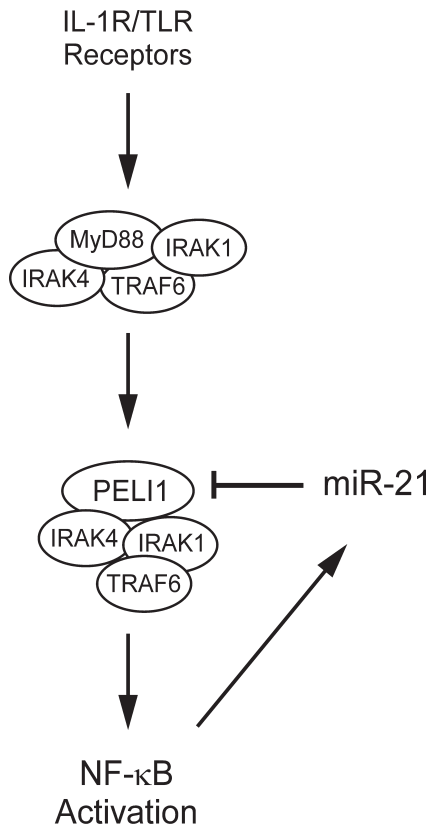

Fig. 6. Negative feedback regulation of NF-κB signaling by miR-21 targeting of *Peli1*. NF-κB upregulates miR-21 expression levels. We show that miR-21 downregulates NF-κB signaling, possibly by targeting *Peli1*, and therefore forms a negative feedback loop.

There is precedence for targeting of the IL-1R/TLR signaling pathway by miRNAs. For example, the Baltimore group (45) showed that miR-146a/b expression was upregulated in THP-1 cells in response to lipopolysaccharide treatment and that it targeted *Irak1* and *Traf6* (45). Thus miR-146a/b induction by lipopolysaccharide may serve to terminate or dampen the inflammatory response to endotoxin. Ceppi et al. (7) also showed that miR-155 targeted human transforming growth factor-β (TGF- β)-activated kinase 1-binding protein 2 (TAB2), which functions downstream of the IRAK1/IRAK4/TRAF6/PELI1 complex (7). They also reported that mouse *Peli1* was targeted by miR-155. Our report implicates miR-21 as an additional regulator of IL-1R/TLR signaling.

Although increased expression of mature miR-21 may be due to activating transcription factors, processing of primary miR-21 may also be affected. Previous studies have shown that TGF-β can upregulate mature miR-21 by stimulating DROSHA-mediated processing of the miR-21 precursor (12). TGF-β is an antiproliferative, apoptogenic factor that is produced in the liver shortly after partial hepatectomy (26). Interestingly, TGF-β signaling upregulates SMAD6, which has been shown to bind and sequester *Peli1*, thus preventing NF-κB signaling. It is therefore intriguing to speculate that TGF-β could induce upregulation of mature miR-21, which would then target *Peli1* to reinforce SMAD6-mediated repression of NF-κB signaling (10).

In conclusion, we have shown that miR-21 is upregulated during the proliferative phase of liver regeneration. We also identified *Peli1*, a mediator of IL-1R/TLR signaling, as a novel miR-21 target. Furthermore, we demonstrated that miR-21 overexpression inhibits NF-κB signaling (Fig. 5). Because NF-κB induces miR-21 expression and miR-21 in turn inhibits NF-κB signaling, we propose the hypothesis that these two molecules form a negative feedback loop to help terminate or fine tune NF-κB signaling during liver regeneration. These studies highlight several exciting new roles for miR-21.

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DISCLOSURES

The authors declare that they do not have anything to disclose regarding funding or conflict of interest.

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