LIVER DISEASES remain a major health concern worldwide. The term “liver disease” includes >100 etiologies of hepatic and biliary diseases, all of which can ultimately lead to liver failure. Liver and biliary cancer combined represent the fifth leading cause of death in men and the ninth leading cause of death in women in the United States (69). Unfortunately, mortality from liver and intra- and extrahepatic bile duct cancer continues to rise and is projected to be the third leading cause of cancer-related deaths in the United States by 2030 (59). Current treatment strategies merely aim to prevent disease progression; therefore, more targets are needed to develop curative therapies. Many studies are underway to examine the mechanisms of liver and biliary tumorigenesis in an effort to identify novel treatment modalities.

MicroRNAs (miRNAs) are small noncoding RNAs that influence gene expression independent of any other regulation, typically by direct binding. These small regulatory RNAs have recently garnered a great deal of attention from the scientific community as potential targets for treatment of human disease. Several miRNAs have been shown to be altered during the progression of liver diseases (5). For example, lethal-7 (let-7), one of the first miRNAs discovered, is altered in several forms of liver disease (27). Additionally, let-7 is a major regulator of cellular differentiation in the developing organism.

A main regulator of let-7 is an RNA-binding protein and stem cell marker, Lin28 (48). Lin28 and let-7: roles and regulation in liver diseases. Am J Physiol Gastrointest Liver Physiol 310: G757–G765, 2016. First published March 24, 2016; doi:10.1152/ajpgi.00080.2016.—The diagnosis and treatment of liver disease remain a major health concern worldwide because of the diverse etiologies of this disease. For this reason, new therapeutic targets are greatly needed to halt the progression of this damaging disease. Upon initiation of liver injury by viral infection, autoimmune disease or toxin, and/or hepatitis, chronic disease may develop, which can progress to cirrhosis, hepatocellular carcinoma (HCC), cholangiocarcinoma, liver failure, or death. The Lin28/lethal-7 (let-7) molecular switch has emerged as a central regulator of multiorgan injuries and cancer development. Lin28 is a stem cell marker vital to initiation or maintenance of a stem cell phenotype. Lin28 has not been extensively studied in the liver, despite its ability to induce tissue regeneration via reprogramming of oxidative enzymes in other tissues and its involvement with numerous upstream regulators and downstream targets in liver disease. Theoretically, overexpression of Lin28 in certain forms of liver disease could be a potential treatment that aids in liver regeneration. Alternatively, Lin28 has been implicated numerous times in the progression of diverse cancer types and is associated with increased severity of disease. In this case, Lin28 could be a potential inhibitory target to prevent malignant transformation in the liver. This review seeks to characterize the role of Lin28 in liver disease.

lethal-7; liver disease; hepatic disorders; hepatocellular carcinoma; primary biliary cholangitis; primary sclerosing cholangitis; liver repair
Lin28

Lin28 is a small RNA-binding protein that contains three RNA-binding domains (41). In humans and other mammals, Lin28 has two forms: Lin28A and Lin28B. In humans, these proteins are coded by two loci: Lin28A, located on chromosome 1, and Lin28B, located on chromosome 6 (51). Lin28A is a 209-amino acid monomeric protein with a mass of 22,743 Da; Lin28B is a 250-amino acid with a mass of 27,084 Da (41).

Lin28A is a translational enhancer, capable of promoting protein synthesis by chaperoning its target mRNAs to polyribosomes (29). Lin28A is predominantly a cytoplasmic protein, but it is found in the nucleus 10–15% of the time because of its ability to shuttle between the cytoplasm and the nucleus. Typically, Lin28A tends to localize to cytoplasmic processing bodies and stress granules (55). Although Lin28A is primarily a cytoplasmic regulator, methylation of Lin28 increases its nuclear retention and protein stability. A methylated nuclear form of Lin28A is able to sequester pri-let-7 and block its processing. This nuclear form of Lin28A is able to regulate transcriptional changes in the V-myc avian myelocytomatosis viral oncogene homolog pathway, which leads to maintenance of the stem cell phenotype and inhibition of differentiation (32). Also, Lin28 is able to directly interact with eukaryotic translation initiation factor 3 subunit 2, a component of the eukaryotic translation initiation factor 3 complex, which is required for several steps to initiate protein synthesis (42). Additionally, Lin28A directly interacts with nucleolin, which is the main nuclear protein of growing cells (53). In humans, Lin28 is typically found in embryonic stem cells, placenta, and testes. During development, Lin28 is highly expressed in fetal liver; however, this high level of expression typically decreases as cells become further differentiated.

Lin28B is primarily an inhibitor of miRNA biogenesis in the nucleus. It inhibits the precursors of let-7 and, possibly, miR-107, miR-143, and miR-200c by binding to the pri-miRNA in the nucleus and sequestering the pri-miRNA from the micro-processing complex. In this way, the miRNA is prevented from maturing and performing its function (55). Lin28B is highly expressed in the placenta and is expressed to a lesser extent in the testes and fetal liver (21). Two isoforms of Lin28B are formed by alternative splicing: the 250-amino acid isoform 1 and the 180-amino acid isoform 2. Lin28B isoform 1 is highly upregulated in the placenta and in poorly differentiated forms of hepatocellular carcinoma (HCC). Lin28B isoform 2 is expressed in the fetal liver and benign liver tissues, as well as in well-differentiated tumor tissues. Lin28B isoform 2 is upregulated in triple-negative breast cancers and in liver, ovarian, and thyroid carcinomas (55). In vitro, Lin28B exhibits an oncogenic expression pattern, where overexpression upregulates stemness markers [octamer-binding transcription factor 4, Nanog, and SRY (sex-determining region Y)-box 2] and enhances tumor sphere formation. Inhibition of Lin28B does the opposite (11).

Mechanisms and Actions of Lin28

Lin28 was discovered in Caenorhabditis elegans as a developmental regulator. In this model, Lin28 expression decreased as cells differentiated (4). Its downstream target is typically the miRNA let-7, but it has been shown to bypass let-7 and affect cell cycle regulators and a myriad of other mRNAs independently. Lin28 is regulated by miR-125 (lin-4 in lower animals) and let-7 (46). The mechanisms of Lin28, its targets, and its regulators are discussed below.

miR-125-Lin28-let-7 pathway. Lin28 typically acts through a universal pathway that involves direct regulation of let-7 and regulation by miR-125. Lin28 is able to directly bind to insulin-like growth factor 2 (IGF2) mRNA, myogenic differentiation 1 mRNA, acidic ribosomal phosphoprotein P0 [acidic ribosomal phosphoprotein P0 (ARBP/36B4)] mRNA, and its own mRNA (67).

The main miRNA target of Lin28, the developmental timing regulator miRNA let-7, was one of the first miRNAs discovered and was characterized as an important developmental regulator in C. elegans. In this organism, deletion of let-7 inhibited maturation, whereas overexpression of let-7 caused early maturation (62). This ability to regulate cellular differentiation points to the possibility that let-7 has the ability to promote cellular transformation in a repairing liver. let-7 has been expanded into a family of miRNAs known as the let-7 family, which regulates cell development and differentiation in vertebrates.

miRNAs, including let-7, typically regulate genes by binding to the 3′-untranslated region of the target miRNA, which destabilizes the mRNA and prevents translation (7). RNA polymerase transcribes primary miRNAs from DNA in the nucleus. These primary miRNAs are cleaved by the Drosha-DGCR8 complex into pre-miRNAs that are exported from the nucleus. In the cytoplasm, pre-miRNAs are cleaved once again by the Dicer-TRBP complex into its mature duplex length. The miRNA then binds to the RNA-induced silencing complex, which guides the mature miRNA to its target (77) (Fig. 1).

Lin28 regulates let-7 expression by three separate methods: 1) inhibition of pri-let-7 processing by binding to Drosha (73, 74), 2) inhibition of pre-let-7 cleavage by Dicer via direct binding to pre-let-7 and subsequent unwinding at the Dicer binding region (38, 64), and 3) recruitment of RNA uridylyltransferase 4 to the Lin28-pre-let-7 complex, which leads to uridylation and degradation of pre-let-7 (22, 23, 36).

miR-125 is a regulatory miRNA that controls the expression of several downstream signals involved in cellular proliferation, metastasis, immunity, apoptosis, and differentiation, including Lin28. miR-125 directly targets and inhibits Lin28 mRNA, which stops translation of the Lin28 protein, effectively blocking its expression (78). Interestingly, let-7 has also been shown to be an inhibitor of Lin28 in a feedback-loop mechanism (64). Higher levels of let-7 expression are associated with increased organism differentiation and decreased Lin28 expression. let-7 is able to accomplish this inhibition of Lin28 by activation of tripartite motif containing 71, which targets Lin28 for ubiquitin-mediated proteasomal degradation (35) (Fig. 1).

Alternate Lin28 signaling pathways. Outside the miR-125-Lin28-let-7 pathway, there are alternative downstream targets of Lin28. For example, IGF2, a protein secreted by the liver, is a known target of Lin28, and this interaction is primarily independent of let-7 (6, 56). IGF2 is mostly active during development and displays a strong antiapoptotic effect on cells (54). Although Lin28 can bind IGF2 directly, it has also been shown that transcription factor RelB, a member of the nuclear factor-κ light chain enhancer of activated B cells (NF-κB) family, is able to form a multiprotein complex with Lin28 and...
IGF2 mRNA-binding protein (IMP3) to enhance the translation efficiency of IGF2 (50, 70).

Several miRNAs share the same tetranucleotide sequence motif (GGAG) as let-7 in the terminal loop and are regulated by Lin28 in the same way as let-7 (23): miR-107, miR-143, and miR-200c. miR-107 has been shown to promote tumor growth and cancer progression. Interestingly, miR-107 achieves this ability to promote tumor progression by negative regulation of let-7 via direct binding (9). miR-143 is typically portrayed as a tumor suppressor gene and is important in the progression of cancer in numerous studies (28, 31, 85).

Lin28 in cellular transformation. Cellular transformation, transition from an epithelial phenotype to a mesenchymal or stem cell-like phenotype, is vital to liver repair and maintenance. Lin28 has been shown to be integral to cellular transformation, when it was shown to be a key factor in the creation of induced pluripotent stem cells (83). Thus, cellular transformation is a main function of Lin28 and is most likely important in cellular transformation required for liver repair. Recent studies have shown that miR-200c, another miRNA that is regulated by Lin28, may be important in the epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET). Overexpression of miR-200c promotes MET, which is important in termination of cancer progression (24, 25). It has been theorized that the EMT-MET balance dictates the ability to either recover from injury or progress to a cirrhotic condition. More EMT promotes liver fibrosis, whereas more MET promotes liver healing and regeneration (12). In addition, this morphological transition is vital to induce the final repair of the liver and prevent subsequent tumor formation (80). These earlier studies have suggested that cellular transformation and the EMT-MET balance, which is regulated by miR-200c via Lin28, may be important in liver pathology and therapy, although further studies are required to clarify the link between cellular transformation and liver conditions.

The liver is a plastic organ that is able to respond to external or internal damage and maintain its own homeostasis. A controversial theory is that, in order for the liver to regenerate itself, mature hepatocytes and/or cholangiocytes must transform into a more stem-like state to produce replacements for injured areas. Unfortunately, the same transformation responsible for repair can also be dysregulated, leaving the cells in a mesenchymal phenotype. These cells undergo malignant transformation and proliferate, forming tumors and replacing healthy tissue. For transformation from an epithelial to a mesenchymal phenotype, cells dedifferentiate to move to new locations. Typically, when the cell relocates, it undergoes a redifferentiation to reintegrate into the tissue. Because Lin28 expression promotes a stem cell-like phenotype, it is vital to cellular transformation.

An alternate theory of liver repair is that hepatocytes or cholangiocytes simply undergo mitosis to replace the damaged area of the liver, which would not involve Lin28. In all likelihood, liver repair is a combination of several different repair mechanisms, which may involve the contribution of mitosis of uninjured cells, cellular transformation, and/or liver stem cell differentiation.

Regulation of cellular transformation during development. Lin28 was initially shown in C. elegans to regulate the fate of cells both in early development and terminal differentiation (3, 4, 46). In more recent years, Lin28 has been seen as a regulator of stem cell pluripotency in all organisms and is downregulated as stem cells differentiate into their terminal cell types (17, 47, 63, 81).

In the development of the liver, the expression levels of the transcription factors hepatocyte nuclear factor (HNF) 4α and HNF6 are inversely correlated with those of let-7. Inhibition of let-7b was shown to increase the levels of HNF4α, HNF6, and miR-122 and to cause an accumulation of mesenchymal stem cells, which triggered initiation of hepatic commitment (2).
Upregulation of Lin28 and downregulation of let-7 are essential to the maintenance of a stem cell phenotype. In an immature organism, the Lin28-to-let-7 ratio will lean toward Lin28; however, as the organism matures, the ratio changes to favor let-7. In other words, cell differentiation occurs when Lin28 decreases and let-7 increases, whereas cells become less differentiated when Lin28 expression increases and levels of let-7 decrease. This see-saw-type mechanism is essential not only to the development of an organism, but also to cellular transformation, as these are the mechanisms critical for liver repair.

**Cell cycle regulation.** Cell cycle regulation is important in the maintenance of normal growth, as well as stability of the organism, regulation of repair, and cellular proliferation. The Lin28-let-7 pathway has been shown to be vital for maintenance of the cell cycle in several instances. For example, overexpression of let-7 has been shown to lead to the loss of cell growth and subsequent induction of apoptosis, which is vital to the developing organism’s ability to form organs and limbs.

The mechanisms by which Lin28 and let-7 regulate the cell cycle are varied. For example, let-7c has been shown to directly target the 3’-untranslated region of the M-phase inducer cell division cycle 25A (CDC25A), leading to its inhibition and causing the cells to remain in the G1 phase and making them unable to divide. Inhibition of CDC25A, in turn, causes decreases in other cell cycle regulators such as cyclin D1, cyclin-dependent kinase 6, retinoblastoma protein, and E2F transcription factor 2 (87) (Fig. 2). Overexpression of let-7g or let-7i has been shown to suppress DNA replication, which leads to inhibition of proliferation and promotion of apoptosis by inhibition of the antiapoptotic protein Bcl-xL (79). The ability of Lin28 to regulate the cell cycle points to an important role for the Lin28-let-7 pathway not only during development, but also during liver repair and progression to liver cancer.

**Lin28 in Liver Disease**

**Hepatitis.** Inflammation of the liver, hepatitis, is induced by viral infection, autoimmune disorders, toxin exposure, or other etiologies and is characterized by heat, edema, and an influx of inflammatory cells. Theoretically, Lin28 should be actively involved in repair mechanisms following acute liver injury; however, most studies performed in hepatitis characterize transformation to HCC via Lin28 expression. For example, when the subatomic genome of the hepatitis C virus was expressed in cultured cells, expression of cancer stem cell markers, including Lin28, was increased (1). Similarly, it has been shown that the hepatitis B virus decreases let-7 expression by modification of Lin28 expression (79).

Other targets of Lin28 have also been studied in hepatitis. For example, reduced expression of miR-107 was found in chronically infected hepatitis C patients (65). In addition, miR-200c is upregulated in hepatitis C patients with fibrotic lesions. miR-200c reduces Fas-associated phosphatase 1 expression, which subsequently increases expression of the proto-oncogene tyrosine-protein kinase Src (Src) kinase and liver fibrosis (60).

Polymorphisms in IGF2, a downstream target of Lin28, are associated with decreased clearance of the hepatitis B virus and increased risk of HCC (33). Additionally, IGF2 has been found to be hypomethylated in patients with cirrhotic hepatitis C that progressed to HCC. Therefore, this hypomethylation could be predictive of HCC progression (14).

Although not in the liver, Lin28 has been shown to interact with other inflammatory molecules, which are often found in hepatitis. For example, in an acute spinal contusion model in rats, Lin28 was able to act via NF-κB to participate in lipopolysaccharide (LPS)-induced inflammatory responses in astrocytes (84). Lin28 and interleukin (IL)-6 interact in a unique way. It has been shown that Src, a proto-oncogene, is able to activate NF-κB, which directly activates Lin28. Activation of Lin28 leads to repression of let-7. Since let-7 typically represses IL-6, activation of Src causes an increase in IL-6, which promotes inflammation and activates signal transducer and activator of transcription 3. This pathway upregulates NF-κB, promoting a positive-feedback loop (26). IL-1β is another inflammatory marker in hepatitis. In non-small cell lung cancer cells, IL-1β acts through cyclooxygenase 2-hypoxia-inducible factor 1α to repress miR-101, a miRNA in-

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**Fig. 2.** Upregulation of let-7 is able to regulate the cell cycle by inhibiting the mitotic checkpoint regulator cell division cycle 25A (CDC25A). Inhibition of CDC25A causes downregulation of the G1 regulators cyclin D1, cyclin-dependent kinase 6 (CDK6), retinoblastoma protein (pRb), and E2F transcription factor 2 (E2F2). This combined effect leads to cells being halted in the G0 phase and unable to divide.
volved in tumor suppression, which subsequently increased Lin28B and allowed for cellular transformation to a cancer cell phenotype (75) (Fig. 3).

**Alcoholic and nonalcoholic fatty liver disease.** Alcoholic liver disease (ALD) occurs as a result of excess alcohol consumption over time and is characterized by steatosis (fatty liver) and the sequelae of injury and inflammation leading to fibrosis (scarring) and, eventually, cirrhosis. Patients with alcoholic liver injury are also more prone to other hepatic insults, such as nonalcoholic fatty liver disease (NAFLD) and chronic viral hepatitis (19). Although the role of Lin28 has not been studied in ALD directly, it can be speculated that Lin28 is active during repair mechanisms induced by alcoholic liver injury. In addition, Lin28 should be upregulated as ALD progresses to cirrhosis and cells take on a malignant phenotype.

This can be observed indirectly through Lin28 targets. In the serum of ethanol (EtOH)-fed mice, let-7a, let-7b, and let-7g are decreased, indicating an upregulation of Lin28 (44). In addition, miR-107 is elevated in rats that were fed EtOH over 5 wk (15). Because Lin28 typically blocks the action of miR-107, this indicates that EtOH has the ability to suppress Lin28 expression, which leads to upregulation of miR-107. This result could be indicative of EtOH repressing the liver’s ability to repair.

NAFLD is characterized by steatosis of the liver typically correlated with obesity but can be a result of certain medications. The incidence is increasing in the United States because of excessive consumption of fat- and sugar-laden foods. In addition to NAFLD, sugary foods can lead to type 2 diabetes. The combination of NAFLD and diabetes often leads to nonalcoholic steatohepatitis (NASH), which is characterized by progressive hepatitis. Left untreated, NASH can progress to cirrhosis and ultimately, liver failure.

NAFLD causes excessive inflammation as a result of increased steatosis. NF-κB-mediated inflammation has been linked to a strong increase in Lin28, which quickly decreases let-7 and subsequently induces cellular transformation (26). Therefore, it is quite likely that Lin28 increases as NAFLD progresses to the more severe NASH. In addition, comparison of steatosis with steatohepatitis has shown a contrast in let-7 levels: let-7b expression is increased in steatohepatitis, but let-7d is decreased in steatosis (30). In addition, the downstream Lin28 target miR-200c was upregulated in a rat model of steatohepatitis (18). The reduced levels of let-7 in steatosis indicate an increased level of Lin28 in this acute disease, indicating an active role of liver repair. In contrast, the increased levels of the downstream targets of Lin28, miR-200c and let-7, in steatohepatitis indicate that liver repair is reduced or nonexistent as the liver progresses to cirrhosis.

**HCC.** HCC is an aggressive malignancy that arises from hepatocytes, typically in the setting of chronic hepatitis or cirrhosis. Typically, HCC is a result of the liver’s efforts to repair cirrhosis by unrestricted cellular proliferation. HCC is frequently locally advanced or metastatic at the time of diagnosis, and most patients succumb to the disease within 3–6 mo as a result of late diagnosis and/or inability to resect the tumor (69). Stem-like cells play a role in HCC development, as Lin28 and let-7 have been implicated multiple times in this disease both in basic science and clinical studies.

Multiple cell lines have been derived from HCC and are easily testable for Lin28 and let-7 levels, as well as manipulation of these markers. For example, members of the let-7 family have been shown to be decreased in HCC cell lines: HepG2, Hep3B, and Huh7 (34). Additionally, the Hep3B cell line has been shown to express high levels of Lin28 (71). The ability of let-7 to inhibit the HCC phenotype was examined by let-7 overexpression was shown to inhibit the viability and mobility of HCC cells. In this model, let-7 appeared to remain in the cytoplasm to induce changes in organelles, including autophagy (39). Additionally, HCC proliferation was inhibited.
after transfection of let-7g mimics, while let-7g inhibitors showed the opposite effect (34).

Targeting of Lin28 in HCC cell lines also shows promise. For instance, RNAi knockdown of Lin28B in the HCC cell line HCC36 decreased cellular proliferation in vitro and reduced tumor growth in the xenograft model. On the other hand, overexpression of Lin28B in the HCC cell line HA22T resulted in heightened tumorigenicity and induced EMT, leading to tumor invasion (76). This is expected, as increased Lin28 should induce more of a stem-like phenotype. Additionally, in this study, knockdown of Lin28B by RNAi in the HCC cell line HCC36 suppressed proliferation in vitro and reduced in vivo tumor growth in nonobese diabetic/severe combined immunodeficiency mice. In contrast, overexpression of Lin28B in the HCC cell line HA22T enhanced tumorigenicity (76). This indicates that Lin28 is a promising potential therapeutic target in HCC.

In vivo, Lin28B has been implicated as the prime generator of hepatoblastoma and HCC in mouse models. Overexpression of Lin28B alone was sufficient to initiate tumor formation, whereas liver-specific knockout of both Lin28A and Lin28B reduced tumor burden and extended survival. In the same study, systemic inhibition of Lin28B with siRNA reduced tumor burden and extended survival as well (49). In a separate study in a mouse xenograft model using HCC cells, cholesterol-conjugated let-7 overexpression inhibited tumor growth and invasion, as well as metastasis (39). In the same model but in a separate study, tumor growth was suppressed in the mouse xenograft model using let-7c-overexpressed HepG2 cells (87).

In human patients, higher levels of Lin28B have been found in the circulating peripheral blood nucleated cells of HCC patients than non-HCC patients. In addition, increased Lin28B expression was also associated with a larger tumor size, higher tumor grade, high American Joint Committee on Cancer stage, and high Barcelona Clinic Liver Cancer stage (11). Upon tumor resection, Lin28 was shown to be higher in HCC than non-HCC tumor tissues, and high levels of Lin28 were also correlated with larger HCC tumors (58). When tested for let-7, these same HCC tissues had significantly lower levels of let-7c than adjacent normal tissue, and a correlation was seen between low let-7c levels and poor tissue differentiation (88). Along the same lines, high-grade HCC tumors with high α-fetoprotein levels have also been shown to express Lin28B more abundantly than normal tissue (76). These patient studies underline the importance of Lin28 in the development and maintenance of the cancer phenotype and point out that Lin28 could be a strong potential therapeutic target in the fight against HCC.

**Biliary diseases.** In primary biliary cholangitis, also known as primary biliary cirrhosis (PBC), the intrahepatic small- to medium-sized bile ducts are destroyed by an autoimmune mechanism, which leads to cholestasis (40). PBC is typically found in a 10:1 ratio of women to men. Patients are typically 35–70 yr of age (8). Primary sclerosing cholangitis (PSC) is a biliary disease that leads to cholestasis via inflammation and obstructive biliary fibrosis. PSC is commonly associated with inflammatory bowel disease and occurs in men 70% of the time; patients are, on average, 41 yr of age (16). PSC will progress to cirrhosis and liver failure without hepatic transplantation and can be a mitigating factor in the development of HCC or cholangiocarcinoma. Although Lin28 expression has not been studied extensively in cholangiopathies, numerous studies examining members of the let-7 family have been published, allowing an extrapolation of the results to speculate on the activities of Lin28 in biliary diseases. As in most liver diseases, there are two points where cellular transformation and, thus, Lin28 would be active: 1) repair after injury and 2) progression to cholangiocarcinoma.

Because inflammation is a critical element in the etiology of diverse cholangiopathies, it is important to examine its effects on cholangiocytes. LPS release by intestinal bacteria upon liver injury continues to be an important mediator of bile duct inflammation due to the link between the gut and the liver. In vitro it has been shown that let-7i has the ability to regulate expression of the LPS receptor Toll-like receptor in cholangiocytes, indicating the ability of let-7 to regulate immune responses due to specific bacterial infections (10). The ability of let-7i to regulate this inflammation indicates that Lin28 may be overexpressed during inflammatory processes in the bile ducts. During cancer transformation, let-7 would typically be downregulated and Lin28 would be upregulated in a cholangiocarcinoma tumor. Interestingly, using cholangiocarcinoma cells, we have shown that overexpression of let-7a increases cell survival following chemotherapy (45).

In vivo, we showed that inhibition of let-7a or miR-125b in bile duct-ligated mice increased intrahepatic bile duct mass and increased expression of nerve growth factor (20). This is expected, because without let-7 or miR-125 to inhibit Lin28, cellular transformation, especially EMT, should be upregulated and there should be a proliferation of cholangiocytes. Diethylnitrosamine-treated mice that were subjected to bile duct ligation surgery and subsequently formed cholangiocarcinoma showed repression of let-7 and upregulation of Lin28B (82). This is expected and also is consistent with the HCC data, where let-7 is decreased and Lin28 is upregulated in tumors.

In humans, most of the work on let-7 has been done in PBC patients. For instance, levels of let-7b were lower in the peripheral blood cells of patients with PBC than healthy controls. Additionally, let-7b levels decreased in parallel to the increases in disease severity (57). In a separate study of end-stage PBC patients, let-7d was downregulated in PBC liver tissue compared with normal tissue (52). This is expected in these diseased samples, because Lin28 should be upregulated due to the liver’s attempts to repair itself in the early stages of disease or in end-stage disease, the progression to HCC or cholangiocarcinoma. Although miRNAs have been examined in human PSC samples, let-7 family members were either not selected as miRNAs of interest or the results were unclear (37, 66).

**Polycystic liver disease.** Polycystic liver diseases (PCLD) encompass a spectrum of autosomal-dominant and -recessive disorders that may occur in association with polycystic kidney diseases. Multiple gene mutations have been associated with disease progression that often leads to multiple hepatic cysts and, ultimately, hepatic fibrosis. The mutated genes encode proteins primarily expressed in cilia, which leads to ciliary malformation and malfunction. Isolated liver disease includes autosomal-dominant PCLD, which occurs in ~1 in 100,000 people (13, 43). Recent evidence demonstrates that cholangiocytes involved in PCLD have the ability to undergo EMT, which indicates a probable role of Lin28 in this disease (43). Recent microarray analysis confirmed that 68 miRNAs are
differentially expressed in cystic cholangiocytes of polycystic kidney rats. The majority of these miRNAs had decreased expression, which included many members of the let-7 family (let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, and let-7i) (43). Lin28 has not been studied in PCLD; however, the let-7 data suggest that Lin28 would be upregulated, promoting cellular transformation and cholangiocyte proliferation. More research is needed to confirm the roles of Lin28 and the let-7 family in hepatic cirrhosis; however, these current findings suggest that targeting Lin28 may prove to be beneficial in halting cyst growth and subsequent hepatic fibrosis.

**Therapeutic Potentials**

Theoretically, since Lin28 is a progenitor cell regulator, it should be involved in two distinct stages of liver disease: 1) liver repair by cellular transformation and 2) progression to cancer. In the mouse, Lin28A was shown to promote tissue repair of hair follicles, bone, and cartilage by reprogramming oxidative enzymes. In these models, let-7 repression was necessary, but not sufficient, to repair tissue injuries independently of Lin28A (68). Similar mechanisms could be utilized in liver repair as a wound healing-like response to injury.

Since overexpression of Lin28 is able to regulate the regeneration of other mesenchymal tissues, it is conceivable that targeted overexpression of Lin28 in the progression of liver disease will aid in the organ’s regenerative capabilities. Overexpression of Lin28 could progress to cancer if unchecked; therefore, it may be necessary to follow the Lin28 overexpression with an expression vector directed to let-7 and/or miR-125 to silence the Lin28 followed by excess cellular transformation.

In vivo studies have shown that repression of Lin28 in xenograft and other tumorigenic models has the ability to inhibit and/or slow tumor formation. This demonstrates that Lin28 is a good and viable target for potential therapeutics to prevent the progression of chronic liver diseases to cancer. Although it would be best for a targeted liver therapy to repress Lin28 overexpression, because Lin28 is rarely expressed in adult tissues, it would be feasible to repress Lin28 systemically in liver cancer patients.

**Conclusions/Future Perspectives**

Lin28 regulation shows great promise as a potential therapeutic for treatment of liver disease. Recent studies have suggested that Lin28 is integral to the transformation of cancer cells and Lin28 is most likely involved during the regulation of repair in the liver following injury. Lin28 has been shown in this review to be critical in the liver’s ability to undergo cellular transformation and maintain homeostasis.

Unfortunately, Lin28 appears to be a rather potent signal and may potentially have the ability to dedifferentiate cells to an almost stem-like state, as shown by its requirement in induction of pluripotent stem cells. This strong signal to dedifferentiate and undergo cellular transformation has been shown to induce HCC and other types of cancers. In addition, higher expression of Lin28 has been shown to correlate with more malignant tumors, which also correlate with a less differentiated cell.

It is possible that, in many cases, liver injury is not severe enough to activate the Lin28-let-7 pathway. However, when liver injury is severe, the liver may decrease let-7 expression, allowing an increase in Lin28 in an effort to repair the injury by induction of cellular transformation and more active proliferation. As Lin28 levels increase, there may be a checkpoint that does not occur and that could lead to cancer as less differentiated cells overtake the liver. Overall, there seems to be a fine balance between Lin28 and let-7 in liver homeostasis and repair. More Lin28 and let-7 studies need to be performed in the liver to elucidate the influence of these important factors on liver disease and repair.

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The views expressed in this article are those of the authors and do not necessarily represent the views of the Department of Veterans Affairs.

**AUTHOR CONTRIBUTIONS**

K.M., C.H., and K.S. prepared the figures; K.M. and K.S. drafted the manuscript; C.H., K.S., T.L., M.M., S.G., F.M., and G.A. edited and revised the manuscript; T.L., M.M., S.G., F.M., and G.A. approved the final version of the manuscript; G.A. developed the concept and designed the research.

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