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Expression of Transforming Growth Factor β 1 Promotes Cholangiocarcinoma Development and Progression

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Abstract

Background & Aims—Transforming growth factor beta 1 (TGF β 1) role in cholangiocarcinoma (CCA) initiation and growth requires further definition.

Methods—We employed pharmacological and genetic approaches to inhibit or enhance TGF β 1 signaling, respectively and determine the cellular mechanisms involved.

Results—It was observed that inhibiting TGF β 1 activity with short hairpin RNA (shRNA) or pharmaceutical agents suppressed CCA development and growth, whereas overexpression of TGF β 1 enhanced CCA tumor size and promoted intrahepatic metastasis in a rat model. Suppression of TGF β 1 activity inhibits downstream target gene expression mediated by miR-34a that includes cyclin D1, CDK6, and c-Met. In addition, “knockdown” of TGF β 1 expression revealed a miR-34a positive feedback mechanism for enhanced p21 expression in CCAs. A miR-34a inhibitor reversed the effects of “knocking down” TGF β 1 on cell growth, migration, cyclin D1, CDK6 and c-Met expression, suggesting that TGF β 1 mediated effects occurs in part, through this miR-34a signaling pathway. Overexpression of TGF β 1 was associated with CCA tumor progression.

Conclusions—This study suggests that TGF β 1 is involved in CCA tumor progression and participates through miR-34a mediated downstream cascades, and is a target to inhibit CCA development and growth.

Keywords

TGF β 1 inhibitor; Liver fibrosis; bile duct tumor; miRNA-34a

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1. Introduction

Cholangiocarcinoma, a tumor of bile ducts has a poor prognosis with only a 2 % 5-year survival rate if the disease has spread outside the liver. This tumor accounts for 15 % of all primary liver tumors in the United States (American Cancer Society) and about 10–25 % of primary hepatic malignancies worldwide [1, 2]. Recently, there is evidence for a significant increase in the incidence of CCAs in the United States [3]. Several risk factors have been linked to the initiation, development and progression of this disease such as age over 65, presence of biliary stones, chronic infection with liver flukes, hepatitis B and C viruses, inflammatory bowel disease, cirrhosis, and primary sclerosing cholangitis [4]. Although epidemiologic studies suggest that such risk factors may provide a link to CCA initiation, it is not clear how those factors lead to CCA development and progression.

Previous studies suggest that parasitic infections and inflammatory biliary-tract disorders are important for CCA development. Furthermore, severe hepatic fibrosis has also been recognized as a possible risk factor as well [5]. In this context, meta-analysis supports cirrhosis as one of the cardinal risk factors associated with CCA development but the causes of cirrhosis were not specified [6] [7].

The TGF β 1 expression has been recognized as important in the pathogenesis of liver fibrosis/cirrhosis [8–10] and several preclinical animal models have been employed to suppress hepatic fibrosis progression through targeting the TGF β 1 mediated signaling cascade [11–14]. Such approaches have demonstrated some success in suppressing fibrogenesis associated with different diseases models [15–17]. However, the role of TGF β 1 in tumor progression is controversial [18–20]. Indeed, TGF β 1 inhibits cell growth at early stages of development but promotes metastasis during progression. In this regard, recent studies have demonstrated anti-tumor effects of targeting TGF β 1 in breast, hepatocellular (HCC), and other carcinomas [21–23]. Based on the previous observations that cirrhosis is one of the major risk factors for HCC development and progression and that inhibiting TGF β 1 reveals improvement by reducing tumor growth [24, 25], we hypothesized that TGF β 1 signaling may contribute to CCA progression. Therefore, we evaluated its role in CCA development and growth in an attempt to identify potential new therapeutic targets for this disease.

2. Materials and Methods

2.1 Cell lines, animals, and reagents

Cholangiocarcinoma cell lines, H1, RBE, and SSP25 were kindly provided by Dr. Enjoji of Kyushu University in Japan. These cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS). Rat bile duct epithelial cells which stably expresses HER2/Neu oncogene (BDE-Neu) was a gift from Dr. Alphonse E. Sirica at Virginia Commonwealth University. The BDE-Neu CL24 cell line was a subclone of the parental cell line previously established in our laboratory [26]. For production of the rat intrahepatic cholangiocarcinoma model, BDE-Neu cells were inoculated intrahepatic into Fisher-344 male rats (Harlan lab, Indianapolis, IN) weighing 150–200 g. The surgical approach and

method of BDE-Neu cells inoculation were performed as previously described [27]. All animal procedures were previously approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital. The plasmids, pLKO.1-shRNA-luciferase and pLKO.1-shRNA-TGF β 1 were purchased from GE Healthcare (Lafayette, CO). Antibodies for p21, p27, CDK4, CDK6, cyclin D1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) provided by Santa Cruz Biotech Inc. (Dallas, TX). Antibodies for c-Met and β -catenin were purchased from Cell Signaling Technology (Danvers, MA). All primers were prepared by Sigma-Aldrich (St. Louis, MO).

2.2 Lentivirus production and infection of cholangiocarcinoma cells (supplemental information)

2.3 Immunoblotting (supplemental information)

2.4 RNA preparation and quantitative RT-PCR (supplemental information)

2.5 Cell migration assay (supplemental information)

2.6 Cell growth assay (supplemental information)

2.7 MTT assay (supplemental information)

2.8 Colony formation in soft agar assay (supplemental information)

2.9 Statistical analysis (supplemental information)

3. Results

3.1 Inhibition of TGF β 1 reduces the malignant phenotype in CCA cell lines

Colony formation in soft agar is one of the important criteria or tests for malignant transformation. In order to determine whether TGF β 1 promotes this process in biliary epithelial cells, we overexpressed TGF β 1 in the rat bile duct epithelial cell line which stably overexpresses HER2/Neu oncogene (BDE-Neu) via the lentiviral system and measured subsequent colony number and size in vitro. Since the lentiviral vector construct simultaneously express both the target gene and green fluorescence protein (GFP), the GFP generated signal was monitored to determine the virus transfection efficacy. Results reveal that more than 95% of the transfected cells were GFP positive. Furthermore, there was an increase of TGF β 1 messenger RNA (mRNA) expression of approximately 130 fold upon lentiviral transduction (Fig. 1A). There was also a substantial increase in TGF β 1 protein levels in culture supernatants derived from CCA cell lines by ELISA assay (Supplemental Fig. 1). It was observed that TGF β 1 expression significantly enhanced, by 2.5-fold, colony formation in BDE-Neu cells (Fig. 1B). To determine if the endogenous TGF β 1 effects CCA malignant potential, TGF β 1 and epithelial-mesenchymal transition (EMT) associated proteins were also examined in 5 CCA cell lines. Among 5 CCA cell lines, H1, RBE, and SSP25 were found to have active TGF β 1 mediated signal transduction (Supplemental Fig. 2). In this context, these three cell lines were used to evaluate the effects of endogenous TGF β 1 expression on CCA colony formation which included cell growth and migration measurements as well. Inhibition of TGF β 1 with pharmacological compounds, such as, LY2157299 and SB431542 were found to substantially suppress colony formation in human H1 CCA cells (Fig. 1C and D). Since pharmacological compounds may have an impact on other unknown downstream targets, we inhibited TGF β 1 expression specifically with a

shRNA to determine its role on colony formation in soft agar. The results reveal that shRNA-TGF β 1 significantly inhibits TGF β 1 mRNA expression and subsequent colony formation in soft agar (Fig. 1E and F), suggesting that targeting TGF β 1 signaling may have anti-tumor effects on CCA development and growth.

3.2 Altering TGF β 1 signaling modulates cell growth in CCA

Previously, TGF β 1 has been shown to inhibit cell growth upon short term exposure with recombinant protein [18]. In addition, several studies have subsequently demonstrated that targeting TGF β 1 inhibits tumor progression [18, 19, 23]. To clarify the effects of TGF β 1 protein on CCA cell growth, we either overexpressed or “knocked down” TGF β 1 in CCA cell lines. It is noteworthy that both manipulations of TGF β 1 inhibited cell growth. The “knockdown” of TGF β 1 was effective and suppressed CCA cell growth by up to 90% (Fig. 2A and B). Targeting TGF β 1 signaling with pharmacological compounds, such as LY2157299 and SB431542, had a consistent suppressive effect on the growth of RBE and SSP25 cell lines (Fig. 2C and D).

3.3 Targeting TGF β 1 signaling reduces CCA migration

Expression of TGF β 1 may be one of the major contributors to tumor metastatic spread¹¹. We determined whether TGF β 1 was potentially involved in CCA metastases and employed pharmacological compounds and specific shRNAs to target TGF β 1 signaling with respect to effects on CCA migration. Consistent with previous observations [5, 9], “knockdown” of TGF β 1 substantially suppressed cell migration of the human CCA cell lines, RBE and SSP25 (Fig. 3A and B). Consistent with shRNA results, the two pharmacological TGF β 1 inhibitors reduced CCA cell migration as well but to a lesser degree (Fig. 3C and D).

3.4 Inhibition of TGF β 1 restricts CCA tumor cell growth through miR-34a-associated signaling pathways

Expression of TGF β 1 has been linked to regulation of non-coding RNA, such as micro RNA (miRNA) which may modulate a number of biological responses through targeting downstream gene expression. For example, treating human renal epithelial cells with recombinant human TGF β 1 protein revealed that miR-34a was subsequently down-regulated [28]. In addition, it has been proposed that TGF β 1 transcriptionally suppresses miR-34a gene expression in hepatocellular carcinoma cells [29], whereas inhibition of TGF β 1 signaling transcriptionally promotes miR-34a expression. It is of interest that miR-34a appears to be the most well-characterized of the tumor suppressor miRNAs [30]. These findings raise the possibility that targeting TGF β 1 may restrict CCA progression through modulating miR-34a-associated downstream signaling pathways. Indeed, expression of TGF β 1 in CCAs negatively correlated with miR-34a expression (Supplemental Fig. 3). In this regard, the CDK6 and CCND1 genes have been identified as potential miR-34a downstream targets and are highly involved in cell proliferation (Fig. 4A). To determine whether “knockdown” of TGF β 1 would inhibit expression and function of these two miR-34a target genes, expression levels were measured in both RBE and SSP25 CCA cells. As suspected, shTGF β 1 significantly inhibited CDK6 and CCND1 mRNA expression in RBE and SSP25 CCA cells (Fig. 4A). In addition, shTGF β 1 also suppresses CDK6 and cyclin D1 protein expression without influencing a non-miR-34a regulated target, such as

CDK4 in RBE and SSP25 CCA cells (Fig. 4B). In addition, miR-34a has been previously shown to have positive feedback effects on p21 expression through down-regulation of MDM4 and subsequently suppress tumor progression [31]. To determine if this positive feedback loop involving growth exists in CCA cells, MDM4 mRNA expression was measured in RBE and SSP25 CCA cells after shRNA-TGF β 1 treatment (Fig. 4C). Indeed, shTGF β 1 was found to inhibit MDM4 mRNA expression which results in enhanced p21 protein but not p27 expression in both RBE and SSP25 CCA cells (Fig. 4D). These results suggest that shTGF β 1 modulates miR-34a to produce its anti-tumor properties. In order to further clarify this proposed mechanism, an anti-miR-34a was employed to treat RBE and SSP25 CCA cells with or without shTGF β 1 exposure. In this context, the anti-miR-34a reversed the shTGF β 1-mediated protein expression of CDK6, and cyclin D1 (Fig. 4E). In addition, anti-miR-34a significantly antagonized the effect of shTGF β 1 on growth of CCA cell lines (Fig. 4F), indicating that miR-34a was an important component in shTGF β 1-mediated signaling in CCA.

3.5 Inhibition of TGF β 1 signaling inhibits CCA cell migration through miR-34a

The c-Met gene has been identified to be one of the downstream targets of miR-34a involved in generation of a metastatic tumor phenotype (Fig. 5A). To determine whether c-Met may be a gene involved in TGF β 1-mediated cell migration, the mRNA expression levels of c-Met in RBE and SSP25 CCA were explored. Results suggest that shTGF β 1 inhibits c-Met mRNA protein expression in these two CCA cell lines and protein expression was reduced as well following transfection with a shTGF β 1 expression construct (Fig. 5B and C). In addition, it has been previously demonstrated that TGF β 1 promotes tumor metastasis, in part, through an EMT mechanism[32]. Therefore, EMT associated pathways were examined in shTGF β 1 treated CCAs cell lines. One of the EMT markers, such as β -catenin, was reduced in two CCA cell lines transfected with shTGF β 1 [33] (Fig. 5C), suggesting that TGF β 1 may promote CCA tumor cell migration through both an EMT and miR-34a-associated c-Met signal transduction pathway. To further clarify this hypothesis, we expressed anti-miR-34a in shTGF β 1 treated CCA cells and measured c-Met expression. It was observed that, expression of anti-miR-34a reversed c-Met over expression as well as cell migration in shTGF β 1 treated CCA cells (Fig. 5D, E and F), suggesting that TGF β 1 promotes cell migration through a miR-34a-mediated and c-Met dependent signaling cascade.

3.6 TGF β 1 promotes intrahepatic CCA tumor growth and metastasis

We further evaluated the effects of TGF β 1 on CCA progression in *in vivo* using a rat intrahepatic model. Overexpression of TGF β 1 significantly increased the CCA tumor volume and number of metastatic foci (Fig. 6A and B). To determine whether TGF β 1 also promotes CCA progression through miR-34a associated cascades, expression levels of miR-34a target genes, CCND1, CDK6, and c-Met were also evaluated. In line with *in vitro* finding that shTGF β 1 inhibits miR-34a target gene expression, we observed that mRNA expression levels of CCND1, CDK6 and c-Met were also significantly increased in tumor tissue upon overexpression of TGF β 1 (Fig. 6C and D). The protein expression levels of cyclin D1, CDK6, and c-Met were enhanced by overexpressing TGF β 1 as well. Finally,

TGFβ1 significantly promotes the expression of the EMT marker, (β-catenin) in this rat intrahepatic model system (Fig. 6E and F).

4. Discussion

Parasitic infections and biliary-tract inflammatory disorders have been established as risk factors for CCA development. Recent meta-analysis suggests that cirrhosis is a major risk factor for this disease as well [5]. In this context, expression of TGFβ1 is highly associated with the development of progressive fibrosis leading to cirrhosis [16]. However, it has been controversial regarding the role of TGFβ1 expression in tumor development and growth. One hypothesis presented is that TGFβ1 inhibits cell cycle progression and proliferation at early stages of tumor growth and promotes metastasis through EMT progression at later stages of the disease [18]. Recently, several novel findings have suggested that TGFβ1 expression promotes cancer stem cell expansion and resistance to chemotherapy. Thus, targeting TGFβ1 was predicted to enhance the effectiveness of chemotherapy in breast cancer, for example [22]. In support of this concept, TGFβ1-FOXO signaling may be required for maintaining leukemia initiating cells in chronic myeloid leukaemia [34]. Several targeting approaches directed against TGFβ1 have demonstrated some anti-tumor activity. A specific inhibitor of the TGFβ1 receptor, SB-431542 displayed therapeutic potential against several human tumors [23]. Another TGFβ1 receptor inhibitor, LY-2157299 has completed phase II and has entered phase III clinical trials for several types of tumors [35] and suggest that TGFβ1 may be a viable oncogenic target.

From previous studies, cirrhosis has been demonstrated to be a major risk factor for CCA development and since TGFβ1 is highly related to pathogenesis of this disease process, we raised the possibility that TGFβ1 may be important for CCA development and growth and a potential therapeutic target for this disease. However, there is controversy regarding the role of TGFβ1 on CCA growth and progression [36, 37]. Most studies on TGFβ1 signaling in CCA are related to short-term exposure with recombinant TGFβ1 protein and it is not apparent if these effects continue to be active under long-term *in vivo* conditions associated with tumor progression. In this study, targeting TGFβ1 both with specific shRNA and pharmacological compounds substantially inhibited CCA growth; the underlying mechanisms for TGFβ1 effects were explored. Previous findings indicate that TGFβ1 promotes p21 but suppresses cyclin D1 expression[36, 38], likewise, we also observed that inhibition of TGFβ1 promotes p21 but inhibits cyclin D1 expression. In addition, miR-34a was found to be important in shTGFβ1-mediated CCA tumor progression. Recent investigations suggests that TGFβ1 may transcriptionally suppress miR-34a expression in a dose dependent manner in HCC and such a phenomenon has been associated with HCC progression[29]. What are the possible explanations for these divergent results? For example, TGFβ1 has been shown to be involved in cellular differentiation. Thus, two of the critical events that modulate cellular differentiation are cell cycle arrest and apoptosis and TGFβ1 has been implicated as being involved in both these two processes[39]. However, our findings with shTGFβ1 are compatible with another investigation that suggests TGFβ1 may transcriptionally regulate the expression of miR-34a which has a tumor suppressor function which implies that shTGFβ1 may promote miR-34a expression through transcriptional regulation and thus inhibit tumor progression by that proposed mechanism. Indeed, our

findings obtained both *in vitro* and *in vivo* support the concept that TGF β 1 may promote CCA progression. In addition, targeting or inhibiting TGF β 1 mediated signaling may suppress this phenotype and alter tumor progression through miR-34a expression (Fig. 7).

An assessment of the cellular malignant phenotype is often through evidence obtained from colony formation assays in soft agar [40]. Although there is uncertainty whether TGF β 1 promotes CCA oncogenesis, it has been previously demonstrated that inhibiting the kinase activity mediated by TGF β 1 type II receptor (TGF β R2) activation promotes hepatocellular carcinogenesis [41]. In this regard, our *in vitro* assays suggest that TGF β 1 further enhances CCA cellular transformation [42, 43]. More important, the *in vivo* intrahepatic CCA model findings also support the role of TGF β 1 in CCA tumor growth and progression particularly since it promotes intrahepatic metastases (Fig. 6). Although there is one study demonstrating that a stromal specific knockout of TGF β R2 enhances prostatic oncogenesis, the protein expression results indicate a robust increase of nuclear staining of phosphorylated Smad2 in epithelial cells[44]. Given the fact that phosphorylated Smad2 is the TGF β 1 signaling transcriptional initiator, it remains a formal possibility that activation of TGF β 1 signaling in epithelial cells may initiate oncogenesis. Interestingly, similar findings on signaling transductions processes have also been observed in HCC and reveal that TGF β 1 was overexpressed in 40% of HCC tumors[45] but in contrast, TGF β R2 has been found to be decreased in 37–70% of HCC tumors[46, 47]; further studies will be required.

Although TGF β R2 appears to be down-regulated in HCC tumors, TGF β 1 expression is increased. Furthermore, there is little expression of TGF β 1 in normal human bile ducts but about 88% are positive for TGF β 1 signaling in CCA tumor tissue. In contrast, TGF β R2 expression, is detectable in 100% of both normal bile ducts and CCA tissue samples[36]. Considering that there has been considerable evidence to support the oncogenic role of TGF β 1 signaling in CCA development and progression, we are led to believe from the results presented here that upregulation of TGF β 1 signaling is important in the pathogenesis of CCA. Therefore, inhibiting TGF β 1 signaling could suppress tumor progression and serve as a novel therapeutic target in CCA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CCA	cholangiocarcinoma
TGFβ1	transforming growth factor beta1
miR-34a	microRNA-34a

shRNA	short hairpin RNA
FBS	fetal bovine serum
BDE-Neu	HER2/Neu oncogene
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GFP	green fluorescence protein
miRNA	micro RNA

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Highlights

- TGF β 1 promotes malignant transformation of cholangiocarcinoma (CCA).
- Overexpression of TGF β 1 accelerates CCA progression.
- TGF β 1 alters CCA growth through miR-34a targeted genes.

Figure 1. TGFβ1 promotes CCA malignant transformation

(A) Left panel: GFP and phase contrast images are shown in BDE-Neu cells transfected with lentivirus containing empty vector (EV) or a TGFβ1 expressing cDNA. Right panel: TGFβ1 mRNA expression level was determined as indicated. (B) Colony formation in soft-agar was determined in BDE-Neu cells. Colony formation was examined in human CCA H1 cells treated with vehicle or TGFβ1 inhibitors, (C) 10 μM LY2157299 or (D) 5 and 10 μM SB431542. (E) TGFβ1 mRNA expression was determined in H1 cells infected with

lentivirus containing EV (shLuc) or shTGF β 1 expressing cDNA. (F) Colony number was measured in H1 cells as indicated. *, $p<0.05$; **, $P<0.01$; ***, $p<0.001$.

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Figure 2. Inhibition of TGFβ1 expression alters CCA cell growth

(A) TGFβ1 mRNA expression was determined in RBE and SSP25 CCA cells infected with lentivirus containing shRNA-luciferase (shLuc) or shRNA-TGFβ1 (shTGFβ1) expressing cDNA. (B) Relative cell growth was measured in RBE and SSP25 CCA cells as indicated by using the MTT assay. Pharmacological TGFβ1 inhibitors, LY2157299 and SB431542 were used to determine the effects of CCA cell growth in (C) RBE and (D) SSP25 CCA cells. *, $p < 0.05$; **, $P < 0.01$; ***, $p < 0.001$.

Figure 3. Targeting TGFβ1 restricts CCA motility

Cell migration was determined in (A) RBE and (B) SSP25 CCA cells infected with lentivirus containing shLuc or shTGFβ1 expressing constructs. Graphs shown the number of migrated cells. Representative images are shown below the graphs. The TGFβ1 inhibitors, 10 μM LY2157299 or 10 μM SB431542 were used to determine the effects of suppressing TGFβ1 signaling on CCA cell migration in (C) RBE and (D) SSP25 CCA cells. The representative cell migration images are shown below the graphs. *, $p < 0.05$; **, $P < 0.01$; ***, $p < 0.001$.

Figure 4. Knockdown of TGFβ1 suppresses CCA cell growth through modulating of miR-34a downstream target genes

(A) Upper panel: miR-34a target site resides at 3'-UTR of the CCND1 and CDK6 genes. Lower panel: The mRNA expression levels of CCND1 and CDK6 were determined in RBE and SSP25 treated with shLuc or shTGFβ1. (B) Immunoblotting (IB) results of CDK6, CDK4, cyclin D1, and GAPDH expression are shown in RBE and SSP25 CCA cells treated with shLuc or shTGFβ1 expressing constructs. (C) MDM4 mRNA expression in RBE and SSP25 cells transfected with shLuc or shTGFβ1 expression constructs. (D) IB results of p21 and p27 expression are showed in RBE and SSP25 cells transfected with shLuc or shTGFβ1 expression constructs. (E) IB results of cyclin D1, CDK6, and GAPDH expression were determined in RBE and SSP25 cells transfected with shLuc or shTGFβ1 in the presence of

anti-miR negative control (NT) as a control or anti-miR-34a. (F) Cell growth was determined in RBE and SSP25 cells as indicated. *, $p<0.05$; **, $P<0.01$; ***, $p<0.001$.

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Figure 5. Inhibition of TGFβ1 suppresses CCA migration through a miR-34a-mediated mechanism

(A) The miR-34a target site resides in the 3'-UTR of the c-Met gene. (B) c-Met mRNA expression levels were determined in RBE and SSP25 treated with shLuc or shTGFβ1. (C) IB results of c-Met, β-catenin, and E-Cadherin are shown in RBE and SSP25 cells transfected with shLuc or shTGFβ1 expression construct. (D) IB results of c-Met and GAPDH were determined in RBE and SSP25 treated with shLuc or shTGFβ1 in the presence NC or anti-miR-34a. Migrated cells are displayed in (E) SSP25 and (F) RBE cells as indicated. *, $p < 0.05$; **, $P < 0.01$; ***, $p < 0.001$.

Figure 6. Overexpression of TGFβ1 promotes CCA progression

(A) Macroscopic images of intrahepatic CCA and metastatic tumors in a rat model inoculated with BDE-Neu cells transfected with lentivirus containing empty vector (EV) or TGFβ1 expressing cDNAs. Right panel shows tumor volume as indicated. (B) Metastatic foci were determined as indicated in this rat intrahepatic CCA model. The mRNA expression levels of (C) CCND1, CDK6, and (D) c-Met were measured in tumor tissues as indicated. (E) Immunoblotting results of CCND1, CDK6, β-catenin, and c-Met expression

are shown. Coomassie blue served as loading control. (F) Protein expression levels of cyclin D1, CDK6, β -catenin and c-Met relative to control are shown. *, $p<0.05$; **, $p<0.01$.

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Figure 7. Proposal of how TGFβ1 affects miR-34a downstream targets and consequent tumor progression

Expression of TGFβ1 activates TGFβ1/TGFβR mediated signal transduction and initiates phosphorylation of the Smad signaling cascade. The activated Smad complex may interrupt the function of the transcriptional activator, CREB-binding protein (CBP) and transcriptional factor (TF) to inhibit the transcription of a miR-34a precursor, resulting in down-regulation of miR-34a. Thus, targeting TGFβ1 signaling with shRNA and TGFβ1 inhibitors, such as LY2157299 and SB431542 may promote miR-34a expression to suppress its downstream targets, such as CDK6, cyclin D1 and c-Met and therefore, reduce CCA cell growth and migration. In addition, targeting TGFβ1 may inhibit CCA cell migration through epithelial–mesenchymal transition (EMT) as well. CBP, CREB-binding protein; TFs, transcriptional factors; EMT, epithelial–mesenchymal transition; SB431542, a specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase; LY2157299, a potent TGFβ receptor I inhibitor.