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Article type : Review

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Keywords: cholangiocarcinoma, liver cancer, long non coding RNA, biomarker, signaling pathway

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List of abbreviations

CCA, cholangiocarcinoma; ICC, intrahepatic cholangiocarcinoma; HCC, hepatocellular carcinoma; ncRNAs, non coding RNAs; RNA, ribonucleic acid; miRNAs, microRNAs; lncRNAs, long non coding RNAs; circRNAs, circular RNAs; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; HULC, highly upregulated in liver cancer; H19, H19 imprinted maternally expressed transcript; MALAT1, metastasis associated lung adenocarcinoma transcript 1; ceRNA, competing endogenous RNA; BAP1, BRCA1 associated protein 1; NEAT1, nuclear paraspeckle assembly transcript 1; siRNA , small interfering RNA; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; PRC, polycomb repressive complex; H3K27, lysine 27 on histone 3; CDH1, E-cadherin; H3K27me3, trimethylation of H3K27; SNHG, small nucleolar RNA host gene; CDKN1A, cyclin dependent kinase inhibitor 1A; TLR4, toll like receptor 4; NF-κB, nuclear factor kappa B; SPRY4-IT1, SPRY4 intronic transcript 1; KLF2, kruppel like factor 2; LATS2, large tumor suppressor kinase 2; PVT1, PVT1 oncogene; DANCR, differentiation antagonizing non-protein coding RNA; TWIST1, twist family bHLH transcription factor 1; EMT, epithelial to mesenchymal transition; MEG3, maternally expressed 3; BMI1, BMI1 proto-oncogene, polycomb ring finger; RNF2, ring finger protein 2; HOTAIR, HOX transcript antisense RNA; PANDAR, promoter of CDKN1A antisense DNA damage activated RNA; CCAT, colon cancer associated transcript; PCAT1, prostate cancer associated transcript 1; TP73-AS1, TP73 antisense RNA 1; LINC, long intergenic non-protein coding RNA; TUG1, taurine up-regulated 1; BCL-2, BCL2 apoptosis regulator; BAX, BCL2 associated X, apoptosis regulator; MAP, mitogen-activated protein; AFAP1-AS1, AFAP1 antisense RNA 1

MMP, matrix metalloproteinase ; CCND1, cyclin D1 ; MYC, MYC proto-oncogene, bHLH transcription factor; MIAT, MIAT, myocardial infarction associated transcript; UCA1, urothelial cancer associated 1; GSK, glycogen synthase kinase; SOX2-OT, SOX2 overlapping transcript; EPIC1, epigenetically-induced lncRNA1; bHLH, basic helix-loop-helix; LOXL1-AS1, LOXL1 antisense RNA 1; ATP, adenosine triphosphate; ABCA1, ATP-binding cassette transporter; CRNDE, colorectal neoplasia differentially expressed; NNT-AS1, NNT antisense RNA 1; ZEB1-AS1, ZEB1 antisense RNA 1; ZEB1, zinc finger E-box binding homeobox 1; HEIH, hepatocellular carcinoma up-regulated EZH2-associated long non-coding RNA; HECT, homologous to the E6-AP carboxyl terminus; HCCA, hilar cholangiocarcinoma; CXCR, C-X-C motif chemokine receptors; RNAseq, RNA sequencing; TGFβ, transforming growth factor beta; TLINC, TGFβ-induced long noncoding RNA; LFAR1, liver fibrosis-associated lncRNA1; LMCD1-AS1, LMCD1 antisense RNA 1; ANRIL, CDKN2B antisense RNA 1; COL6A3, collagen VI-alpha3 chain; NNT-AS1, NNT antisense RNA 1; ERK, extracellular-signal-regulated kinase; ECC, extrahepatic cholangiocarcinoma; TNM, tumor node metastasis; MIR22HG, MIR22 host gene; ASAP1-IT1, ASAP1 intronic transcript 1; SMO, smoothened, frizzled class receptor; GLI1, GLI family zinc finger 1; SNPs, single nucleotide polymorphisms; GAPLINC, gastric adenocarcinoma predictive long intergenic non-coding; CPS1-IT1, carbamoyl-phosphate synthetase 1 intronic transcript 1; KCNQ1OT1, KCNQ1 opposite strand/antisense transcript 1; FLVCR1-AS1, FLVCR1 antisense RNA 1; FOXO3, forkhead box O protein 3; FU,

fluorouracil; Cdr1as, cerebellar degeneration-related protein 1 antisense RNA; Oct-2, octamer transcription factor-2.

Financial Support: This work was supported by Inserm, Université de Rennes 1, and ITMO Cancer AVIESAN (Alliance Nationale pour les Sciences de la Vie et de la Santé) dans le cadre du Plan cancer (Non-coding RNA in cancerology: fundamental to translational; [Grant C18007NS to CC]). CC is a member of the European Network for the Study of Cholangiocarcinoma (ENSCCA) and participates in the initiative COST Action EURO-CHOLANGIO-NET granted by the COST Association (CA18122).

Conflicts of Interest: The authors declare no conflict of interest.

Abstract: Cholangiocarcinoma (CCA) is the second primary hepatic malignancy after hepatocellular carcinoma. CCA is characterized by its aggressiveness and poor prognosis associated with limited therapeutic options. Thus, a better understanding of molecular pathogenesis of CCA together with the identification of robust biomarkers and therapeutic targets is still necessary to improve the management of patients with CCA. In this review, we discuss the role of long non coding RNAs (lncRNAs) in CCA onset and progression. LncRNAs represent a heterogeneous class of RNA molecules with various genomic location and organization acting at both transcriptional and post-transcriptional levels. Notably, we provide an overview of the functions of lncRNAs in epigenetic modulation and regulation of signaling pathways and cell behaviors leading to tumor progression (e.g. apoptosis, survival, epithelial to mesenchymal transition, migration, invasion, angiogenesis). Moreover, we depict the clinical relevance of lncRNAs as biomarkers and emerging therapeutic targets in CCA.

Introduction

Cholangiocarcinomas (CCA) include malignant tumors originating from the bile ducts and exhibiting cholangiocyte differentiation features. CCA are classified into three types according to the anatomical location: intrahepatic, perihilar and distal CCA (Figure 1) (1, 2). Intrahepatic CCA (ICC) represent the second primary hepatic malignancy after hepatocellular carcinoma (HCC). CCA are aggressive and poor prognosis neoplasms with a median survival of 24 months after the initial diagnosis. Despite the recent identification of molecular alterations involved in CCA pathogenesis, including in the stromal compartment, there is no established molecular targeted therapy that significantly increases patient survival yet (1-5). Surgical resection remains the only potential curative treatment when feasible and performed at early stage of the disease (1, 6). Thus, a better understanding of molecular pathogenesis of CCA together with the identification of robust biomarkers and therapeutic targets is still necessary to improve the management of patients with CCA.

Non coding RNAs (ncRNAs) belong to a heterogeneous class of RNA molecules involved in numerous biological and pathological processes, including carcinogenesis (7). Based on their size and structure, ncRNAs include microRNAs (miRNAs), long non coding RNAs (lncRNAs) and circular RNAs (circRNAs). In CCA, miRNAs have been largely studied. For example, miR-21 is a well-known oncogenic miRNA overexpressed in CCA and involved in response to chemotherapy by modulating gemcitabine-induced apoptosis (8, 9). Its overexpression in plasma makes it a potential circulating biomarker for CCA diagnosis and prognosis (10). The first lncRNAs implicated in liver carcinogenesis were described in HCC (e.g. HULC, H19, MALAT1) (11). As an example, MALAT1 was identified early on as a prognostic factor in lung cancer and then in liver, breast and colon cancers. Further studies suggested that MALAT1 plays a general role in cell proliferation (12). However, lncRNAs remain poorly investigated in CCA. LncRNAs are defined by a size greater than 200 nucleotides and the lack of a functional open reading frame. They are transcribed by RNA polymerase II and can be polyadenylated. Transcriptome analysis by deep sequencing demonstrated that the number of lncRNAs is greater than the one of mRNAs. Genomic organization of lncRNAs is complex and can be described according

to their relationship to protein-coding genes: intergenic, sense, antisense, intronic and bidirectional (13). LncRNAs have been involved in pluripotency, differentiation or proliferation although the function of most lncRNAs remains largely unknown. They show tissue specific expression and are tightly regulated at multiple levels, involving chromatin marks, independent promoters, regulation by transcription factors and alternative splicing. LncRNAs are located into the nucleus, the cytoplasm and could be also secreted, notably within extracellular vesicles (EVs). Based on their cellular location, lncRNAs have been shown to modulate gene expression, notably by acting as chromatin remodeling factors or by interacting with transcription factors (14). LncRNAs have been also reported to modulate post-transcriptional mRNA processing, protein function or localization, and intercellular signaling through EVs (15). In the cytoplasm, lncRNAs and circRNAs could interact directly with miRNAs acting as competing endogenous RNAs (ceRNAs) (Figure 2) (16, 17). This review highlights lncRNAs according to their role in CCA carcinogenesis (Table 1).

LncRNAs and epigenetic modulation

In the nucleus, lncRNAs could act at epigenetic level, mainly as scaffolding for chromatin or histone modifiers (Figure 3). BAP1, as a chromatin modulator, can involve interactions with several methylation and deacetylase components and result in modulating gene expression. LncRNA NEAT1, a downstream target of BAP1, is upregulated in CCA and HCC as well as in lung, esophageal, and colorectal cancers (18, 19). NEAT1 is involved in responses to therapy as well as in maintenance of proliferative, migratory and invasive capabilities of CCA cells (18). Exogenous silencing of NEAT1 reduces cell proliferation, migration, invasion and colony-forming abilities. EZH2 is the catalytic subunit of polycomb repressive complex (PRC) 2, a protein complex involved in methylating lysine 27 on histone H3 (H3K27). The effect of NEAT1 on modulating EZH2 further supports a role in tumor progression (20). EZH2 recruitment by NEAT1 and subsequent repression of E-cadherin (*CDH1* gene) promote CCA migration and invasion. Depletion of NEAT1 causes a reduction in EZH2 binding to *CDH1* promoter and increases *CDH1* transcription. Chromatin immunoprecipitation using an H3K27me3 antibody found decreased enrichment of H3K27me3 at *CDH1* promoter upon NEAT1 depletion. Taken together, these data suggest that EZH2 is recruited by NEAT1 to the promoter of *CDH1* and that together they repress *CDH1* expression. NEAT1 can also regulate gene expression through its involvement in paraspeckle nuclear body formation (21).

SNHG1 is another example of lncRNA upregulated in liver cancer (22). SNHG1 promotes CCA malignancy by interacting with EZH2 and repressing *CDKN1A* encoding P21 cell cycle inhibitor. Knockdown of SNHG1 lowers the binding of EZH2 as well as H3K27me3 levels at *CDKN1A* promoter, resulting in *CDKN1A* increased transcription and P21 protein levels, thus decreasing CCA growth through cell cycle inhibition (23). SNHG1, as a ceRNA for miR-140, enhances TLR4 expression and activated NF-κB signaling, thereby regulating growth and tumorigenesis in CCA (24).

LncRNA SPRY4-IT1, up-regulated in CCA, exerts oncogenic properties partly through repressing tumor suppressors KLF2 and LATS2 by scaffolding EZH2 (25). SPRY4-IT1 also acts as a molecular sponge for miR-

101-3p, antagonizing its ability to repress EZH2 translation. LncRNAs PVT1 and DANCR promote cell proliferation and migration in CCA cell lines by binding with EZH2 and then by epigenetically repressing the expression of ANGPTL4 and FBP1, respectively (26, 27). In another study, DANCR is suggested to boost CCA cell proliferation, migration, invasion and angiogenesis. Mechanistically, DANCR interacts with miR-345-5p and prevents its binding to TWIST1 mRNA encoding a transcription factor crucial for epithelial to mesenchymal transition (EMT) (28).

In contrast, MEG3 is one of the lncRNAs which exhibit tumor suppressive activities. MEG3 expression is decreased in lung, liver, prostate, and gastric cancer, as well as in multiple myeloma, meningioma, and glioma (29). In CCA, MEG3 exerts its tumor suppressive effects partly by reversing EMT (30). MEG3 represses CCA malignant progression at the epigenetic level by negatively regulating BMI1 and RNF2 expression, two major components of PRC1 complex. MEG3 also exhibits tumor suppressive effects by inhibiting NFkB signaling pathway via miR-361-5p regulation (31).

LncRNAs and cancer-associated signaling pathways

LncRNAs contribute to several hallmarks of cancer in CCA (Figure 4A). The mechanisms of deregulation of different signaling pathways are detailed below and summarized in Figure 4B.

Apoptosis and survival

Most of lncRNAs described in CCA so far promotes cell proliferation and reduces cell apoptosis. Thus, knockdown of several lncRNAs upregulated in CCA, including HOTAIR, PANDAR, CCAT2, PCAT1, TP73-AS1 and LINC01061, increases the expression of the pro-apoptotic markers caspase-3 and caspase-9 (32-37). Silencing of PANDAR and TUG1 also restrains the expression of the anti-apoptotic BCL-2 and increase the expression of BAX in CCA cells (33, 38). However, the underlying molecular mechanisms involved remain to be discovered. LncRNA AFAP1-AS1 is upregulated in CCA (39, 40). AFAP1-AS1 silencing decreases cell migration and invasion notably by downregulating MMP2 and MMP9. AFAP1-AS1 silencing also suppresses cell proliferation by arresting cell cycle via decreases of the protein levels of cyclin D1 (CCND1) and c-Myc (MYC). Both tumor volume and tumor weight are significantly decreased in mice xenografted with AFAP1-AS1 knocked down CCA cells (39). MIAT is another example of oncogenic lncRNA that enhances CCA cell proliferation by releasing the negative regulation of miR-551b-3p towards CCND1 mRNA (41). LncRNA UCA1, up-regulated in CCA, affects cell apoptosis and cell cycle by activating AKT/GSK-3β/CCND1 signaling pathway (42). PI3K/AKT pathway is also activated by MALAT1 and SOX2-OT to promote CCA cell proliferation and invasion (43-45).

LncRNAs could alter gene expression by modulating transcription factors. RNA immunoprecipitation experiments demonstrated that EPIC1, a lncRNA upregulated in CCA, directly targets MYC, a key transcription factor regulating the expression of a wide range of genes involved in cell growth, proliferation and survival (46). Accordingly, MYC targets, including cyclin A/D and CDK9, are downregulated following EPIC1 silencing. LncRNA LINC01296 also targets MYCN transcription factor (47). By inhibiting miR-5095, LINC01296

upregulates the expression of MYCN and promotes cell viability, migration and invasion in CCA cells. LncRNA SNHG6 harbors a pro-tumorigenic role in different cancers (48). Its expression is elevated in CCA and related to cell proliferation, migration and angiogenesis. SNHG6 potentiates the expression of E2F8 transcription factor, which is required for normal cell cycle progression from G1 to S phase. Specifically, SNHG6 acts as a ceRNA for miR-101-3p, which targets E2F8 mRNA for degradation. Thus, increased SNHG6 expression inhibits the miR-101-3p-mediated silencing of E2F8 in CCA (49). LncRNA LOXL1-AS1, upregulated in CCA, is associated with unfavorable prognosis. LOXL1-AS1 could facilitate cell proliferation, migration and invasion and attenuate cell apoptosis (50). LOXL1-AS1 acts by sponging miR-324-3p to elevate ATP-binding cassette transporter (ABCA1).

EMT, migration and invasion

Several ncRNAs have been shown to play a key role in migration, invasion and therefore in metastatic process in CCA, notably by regulating EMT. As described above, NEAT1 is one of the lncRNAs involved in the repression of E-cadherin to promote CCA cell migration and invasion (20). *In vitro*, the knockdown of numerous lncRNAs upregulated in CCA (e.g. HOTAIR, CRNDE, PANDAR, TUG1, CCAT1, CCAT2, SPRY4-IT1, UCA1, NNT-AS1) also reduces the expression of mesenchymal markers (Vimentin, N-cadherin) and increases the expression of CDH1 epithelial marker, resulting in EMT reversion (25, 32-34, 38, 42, 51-53). In contrast, MEG3 exhibits tumor suppressive effects and its knockdown increases the expression of mesenchymal markers (30).

The oncogenic effects of lncRNAs through EMT are frequently mediated by their function as ceRNAs (e.g. CCAT1, SPRY4-IT1, TUG1, KCNQ1OT1, UCA1, NNT-AS1) (25, 53-57). As an example, the oncogenic effects of CCAT1, which expression is increased in poor prognosis ICC, are at least partly mediated through miR-152 sponging (53, 58). Indeed, miR-152 inhibitors rescue the cell migration and invasion capability of CCA cells after CCAT1 knockdown. In CCAT1 silenced cells, miR-152 inhibitors increase the expression of N-cadherin and Vimentin, but reduce the expression of E-cadherin. In addition, lncRNA ZEB1-AS1 promotes EMT in CCA cells by sponging miR-200a and subsequently enhancing the expression of its target ZEB1. Interestingly, ZEB1-AS1 reinforces proliferation and metastasis of CCA *in vivo* and its expression correlates with poor prognosis in patients (59). LncRNA HEIH is over-expressed in CCA. HEIH induces cell proliferation, migration and invasion *in vitro* and promotes tumor growth *in vivo*. At the molecular level, HEIH sequesters miR-98-5p away from its target mRNA, including the HECT domain E3 ubiquitin protein ligase 4 (60). LncRNA LINC01410 also promotes CCA cell proliferation and migration. LINC01410 is highly expressed in CCA tissues and cell lines. It up-regulates SMAD5 by sponging miR-124-3p, thereby promoting CCA progression (61).

LncRNA MALAT1 has a key role in multiple cancers and is upregulated in CCA. In hilar cholangiocarcinoma cells (HCCA), MALAT1 knockdown significantly suppresses proliferation, migration and invasion (62). Acting as a ceRNA, MALAT1 exerts its promotive functions by miR-204-dependent CXCR4 regulation. H19 and HULC were up-regulated after both short and long-term oxidative stress, implying their pivotal roles in inflammation promotion and CCA pathogenesis. H19 and HULC regulate cell migration and invasion by increasing pivotal

inflammation cytokine IL-6 and chemokine receptor CXCR4 through sponging let-7a/let-7b and miR-372/miR-373, respectively (63). Xu et al. confirmed in 2017 that H19 promotes cell migration and invasion. Knockdown of H19 decreases proliferation, increases apoptosis and reverses EMT in CCA cells (64).

Transforming Growth Factor beta (TGFβ) pathway is tightly implicated in EMT and CCA progression (65, 66). TLINC is a lncRNA induced by TGFβ and upregulated in ICC. Its long isoform is associated with a migratory phenotype and promotes an inflammatory microenvironment through upregulation of IL8 both *in vitro* and in resected human ICC (67). The liver-enriched lncRNA LFAR1 induces CCA cell proliferation, migration and invasion, by positively regulating components of the TGFβ pathway, such as TGFB1, SMAD2 and SMAD4. These effects are accompanied by a concomitant reduction of E-cadherin and an up-regulation of Vimentin (68). LncRNA LMCD1-AS1, upregulated in CCA, is an E2F1 target. LMCD1-AS1 exerts oncogenic properties in CCA cells by sponging miR-345-5p to upregulate COL6A3 level (69). COL6A3 is a well-known oncogene in several tumors implicated in extracellular matrix remodeling notably through PI3k-AKT and TGFβ/Smad signaling pathways. LncRNA NNT-AS1 upregulated expression is correlated with a poor prognosis in CCA (70). Knockdown of NNT-AS1 impairs CCA cell proliferation, migration and invasion, and tumor growth *in vivo*. NNT-AS1 acts as a ceRNA against several miRNAs: miR-142-5p targeting HMGA2, miR-485 targeting BCL9 implicated in Wnt/β-catenin pathway and miR-203 targeting ZEB1 and IGF1R implicated in EMT, PI3K/AKT and ERK1/2 pathways (51, 54, 70).

PCAT1 is an oncogenic lncRNA upregulated in multiple cancers (71). In extrahepatic cholangiocarcinoma (ECC), PCAT1 regulates tumor progression via the Wnt/β-catenin signaling pathway (37). PCAT1 acts as a ceRNA against miR-122, a well-known tumor suppressor miRNA in HCC (72). In ECC cells, PCAT1 depletion decreases β-catenin levels and increases GSK3β levels. In ECC cells co-transfected with miR-122 inhibitors and PCAT1 siRNAs, protein levels of β-catenin were restored and colony formation increased (37). In contrast, lncRNA MIR22HG acts as a tumor suppressor via Wnt/β-catenin signaling pathway (73). Its down-regulation is related to high TNM stage and poor prognosis in CCA patients. MIR22HG inhibits cell proliferation, migration and invasion in CCA by negatively regulating the expression of proteins involved in the Wnt/β-catenin signaling pathway (e.g. β-catenin, CCND1 and MYC). The effect of MIR22HG overexpression on CCA progression could be partly rescued by activating the Wnt/β-catenin signaling pathway (73). LncRNA ASAP1-IT1 is upregulated in CCA and involved in CCA progression. Knockdown of ASAP1-IT1 impedes cell proliferation, cell migration and EMT. ASAP1-IT1 activates hedgehog pathway through upregulating SMO and GLI1, two hedgehog-related proteins (74).

Angiogenesis

As previously mentioned, several lncRNAs have been associated with angiogenesis in CCA, including PVT1, DANCR and SNHG6. PVT1 localizes downstream of the *MYC* gene. It is upregulated in CCA and promotes cell proliferation and migration (27). Interestingly, gene ontology analysis following PVT1 knockdown indicates that the most markedly overrepresented biological processes were pathways involved in cell proliferation, apoptosis

and angiogenesis. However, the role of PVT1 in angiogenesis remains to be formally confirmed by functional tests in CCA, as it was demonstrated in gastric cancer (75). DANCR was reported to promote angiogenesis in several cancers. In ovarian cancer, DANCR promotes angiogenesis through regulation of miR-145/VEGF axis (76). In CCA, DANCR was shown to affect numerous cellular processes, including cell proliferation, migration, invasion, EMT and angiogenesis (28). However, the underlying molecular mechanisms linking DANCR to angiogenesis remain to be characterized. Lastly, it was proposed that SNHG6 promotes angiogenesis in CCA by sponging miR-101-3p and subsequent activation of E2F8 (49).

LncRNAs and genetic variation

Genetic variations and/or alterations such as single nucleotide polymorphisms (SNPs), mutations and gene fusions referring to coding regions are common events in human carcinogenesis. LncRNAs which are less conserved across species are even more prone to genetic variations. Fujimoto et al. provided a comprehensive whole-genome mutational analysis of 300 liver tumors, including CCA, combined CCA-HCC and a majority of HCC (77). The analysis revealed *NEAT1* and *MALAT1* to be prominently mutated among non-coding genes. The lncRNASNP is a database of SNPs in lncRNAs in human and mouse (78). HULC and MALAT1 SNPs were found to be implicated in HCC carcinogenesis (79). However, no lncRNA SNP has been reported so far in CCA carcinogenesis.

Clinical relevance of lncRNA expression

LncRNAs as biomarkers

Multiple studies focused on evaluating miRNA abundance in tissue, serum and bile as diagnostic biomarkers in CCA (80, 81). Some of them were also identified as promising prognostic biomarkers in serum (80-82) (Figure 5). To date, most of the lncRNAs are overexpressed in CCA cells and tissues and were identified as regulators of carcinogenesis (e.g. epigenetic deregulation, EMT, apoptosis). This overexpression is also frequently associated with a poor prognosis (H19, SOX2OT, CRNDE, HOTAIR, GAPLINC, ANRIL) (32, 43, 52, 64, 83, 84) and poor histologic features (CCAT1, UCA1, AFAP1-AS1, PANDAR, TUG1, SPRY4-IT1, TP73-AS1, LINC01296, CPS1-IT1, KCNQ1OT1, LOXL1-AS1, lnc-PKD2-2-3, NNT-AS1, SNHG3) (25, 33, 35, 39, 42, 47, 50, 54, 56, 85-89). Conversely, MEG3, LINC01714 and MIR22HG are downregulated in CCA tissues (30, 73, 90). MEG3 and LINC01714 decreased expression is correlated with poor survival. Some lncRNAs are not yet relevant for prognosis and only serve as potential diagnostic biomarkers (e.g. NEAT1, SNHG1, PVT1, PCAT1, EPIC1, TLINC, MALAT1, LMCD1-AS1, LINC01061, FLVCR1-AS1) (20, 23, 27, 36, 37, 46, 62, 67, 69, 91). However, one can assume that the ideal biomarkers are those obtained with non-invasive tests from body fluids, such as serum or plasma (Figure 5). In a recent meta-analysis, lncRNAs were found to be of high diagnostic value for HCC and their expression could potentially be used as auxiliary biomarkers in confirming HCC diagnosis (e.g. UCA1, MALAT1, SPRY4-IT1) (57). A recent study found circulating PCAT1, MALAT1 and CPS1-IT1 significantly increased in plasma samples of HCCA patients (92).

LncRNAs as therapeutic targets

The capability of lncRNAs to regulate key oncogenic processes makes them relevant targets for therapeutic research. Although, actual drugs are unable to directly target lncRNAs, promising strategies are in development to achieve transcriptional silencing or functional inhibition of oncogenic lncRNAs (14). *In vivo,* transcriptional silencing of lncRNAs can be achieved by antisense oligonucleotides (ASOs), siRNA-mediated knock-down and CRISPR technology (93). Another way to target oncogenic lncRNAs is by compromising their functional properties, through interfering with lncRNA-protein interactions. Designing RNA/DNA aptamers or small molecules aims to target RNA recognition motifs of RNA-binding proteins or to disorganize the secondary or tertiary structure of lncRNAs (94). The ability of lncRNAs to sponge miRNAs could be also exploited to sequester oncogenic miRNAs. This mechanism has been used experimentally with an artificial lncRNA to overcome sorafenib resistance in HCC (95). In the context of cancer, lncRNA-targeting approaches are still in the pre-clinical phase. Several pre-clinical models, such as genetically engineered mice, patient-derived xenografts, xenografted human cells, patient-derived tumor organoids and zebrafish models are currently used to manipulate the expression of lncRNAs in cancer. To date, there are not complete clinical trials involving therapeutic targeting of lncRNAs. However, two clinical trials involving miRNAs with promising preliminary results and one that makes use of lncRNA H19 promoter to specifically target bladder cancer are the pioneer trials for ncRNA therapeutics (96).

In CCA, few lncRNAs were found to influence current therapeutics. NEAT1 is a downstream target of BAP1, a frequently mutated chromatin modulator, and contributes to sensitivity to gemcitabine in CCA cells (18). Inhibition of NEAT1 restores cell response to gemcitabine and could be a potential sensitizer. Conversely, the sensitivity of cells to gemcitabine could be enhanced by LINC01714, a downregulated tumor suppressive lncRNA in CCA (90). LncRNA lnc-PKD2-2-3, upregulated in CCA tissues and associated to poor prognosis, increases cancer stem cell marker expression in CCA cell lines (87). Lnc-PKD2-2-3 also increases drug resistance to 5-FU in CCA cell lines and enhances sphere formation efficiency indicating that it may promote the acquisition of CCA stemness features. Accordingly, lnc-PKD2-2-3 expression is correlated with cancer stem cells markers in CCA suggesting an application to target cancer stem cells and to reverse drug resistance.

New players in the field: circRNAs

In a previous study, we highlighted circular isoforms of TLINC, a TGFβ induced lncRNA, in CCA tissues (67). CircRNAs are particular ncRNAs mostly greater than 200 nucleotides characterized by covalently closed loop structures with neither 5′ to 3′ polarity nor polyadenylated tail (97). This structure is formed by a back-splicing that ligates a downstream splice donor site reversely with an upstream splice acceptor site (98). This circular structure renders circRNAs particularly resistant to the action of RNAses. Thus, circRNAs may represent interesting stable biomarkers in tissues or circulating in EVs such as exosomes detectable in serum (Figure 5) (98, 99).

Some circRNAs were already identified as potential molecular biomarkers in CCA e.g. circRNA Cdr1as, Hsa_circ_0001649 and Circ2174 (100-102). Hsa_circ_0001649 is aberrantly downregulated in CCA tissues and cells, and its downregulation is associated with tumor size and differentiation grade in CCA (102). Overexpressed circRNA Cdr1as may serve as a potential molecular biomarker to predict the aggressive tumor progression and worse prognosis for CCA patients (100). Circ2174, an intergenic circRNA increased in ICC, can act as a sponge to regulate the expression of miR-149 and thereby modulating Oct-2 and interleukin-16 signaling pathways in CCA (101).

Conclusions

CCA are aggressive neoplasms resulting from a complex multifactorial carcinogenesis process. LncRNAs are implicated in a large variety of functions in cancer development. Their potential interactions with DNA, RNA and protein result in regulation of gene expression at epigenetic, transcriptional and post-transcriptional levels. They act as regulators of key processes in cancer, including EMT, apoptosis, and proliferation. Thus, lncRNAs represent a promising class of biomarkers for CCA diagnosis and prognosis, as well as promising therapeutic targets.

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Author names in bold designate shared co-first authorship.

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Figure legends

Figure 1: Classification of biliary tract malignancies according to their anatomical location. Cholangiocarcinoma (CCA) are broadly classified into three main entities based on their anatomical location: intrahepatic CCA (iCCA) that develop within the liver from the second-order bile ducts (BD) and proximal intrahepatic BD. Extrahepatic CCA include perihilar CCA (pCCA) arising from both left and right hepatic ducts, and distal CCA (dCCA) found further down the BD, closer to Vater's ampulla. Adapted from (2).

Figure 2: Molecular functions of nuclear (left panels) and cytoplasmic (right panels) lncRNAs.

Figure 3: LncRNAs involved in epigenetic regulation. **A:** Oncogenic lncRNAs (e.g. NEAT1) recruit EZH2 (one component of PRC2) involved in H3K27 trimethylation (H3K27me3). Subsequently, increased H3K27me3 levels at the promoter of genes result in decreased gene transcription. **B:** Tumor suppressor lncRNA MEG3 negatively regulates PRC1 components (BMI1 and RNF2). Subsequently, ubiquitination of H2A could be decreased and transcription of tumor suppressor gene could be restored. PRC: polycomb repressive complex. TF: transcription factor. RNA pol II: RNA polymerase II. H3K27: histone H3 lysine 27. H2A: histone 2A. ub: ubiquitination.

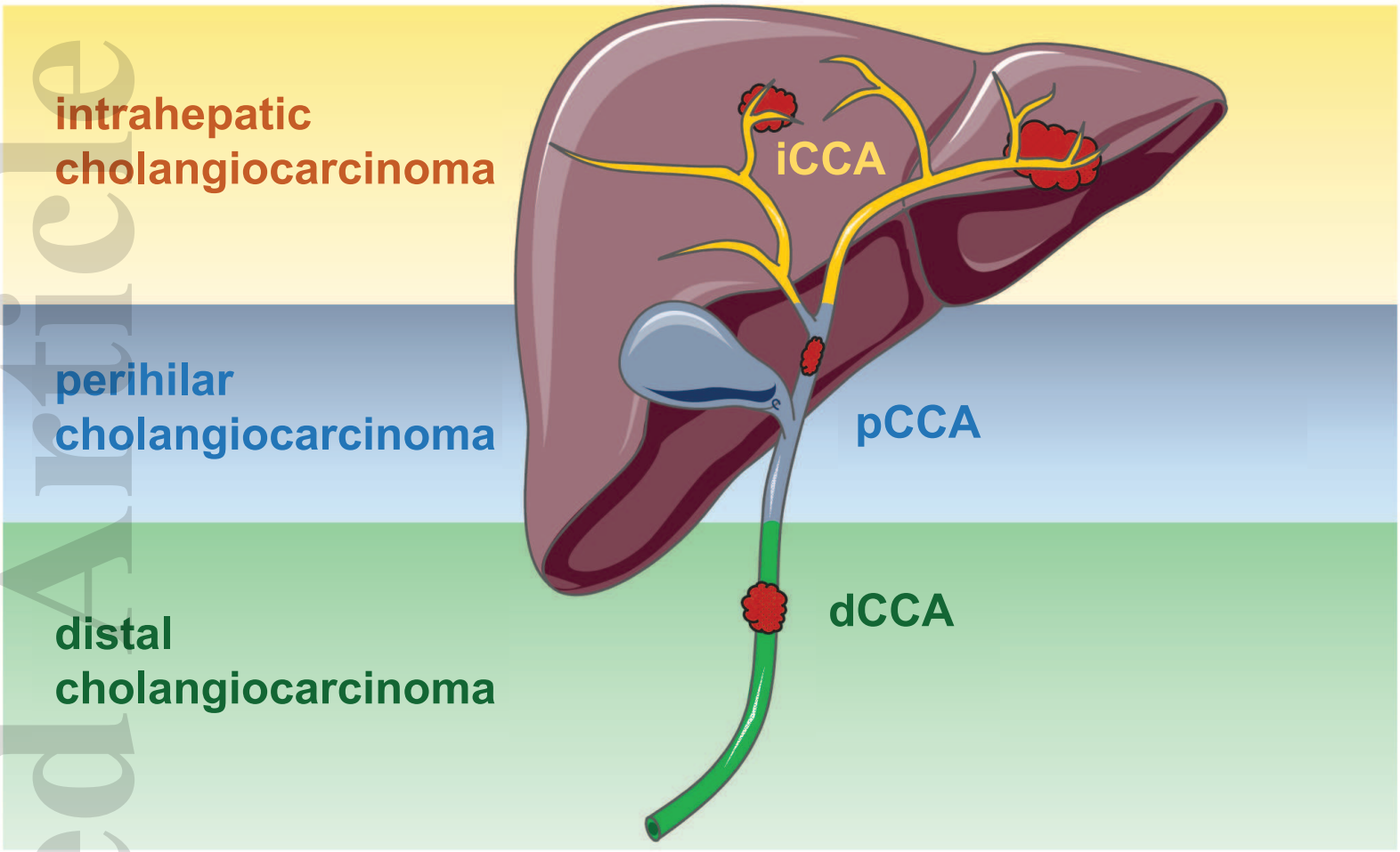
Figure 4: LncRNAs regulate CCA progression through multiple ways. **A:** Examples of lncRNAs affecting the hallmarks of cancer, during CCA development. **B:** Signaling pathways such as NF-κB, TGFβ, Ras/MEK/ERK, PI3K/Akt, Wnt and Hedgehog play crucial roles in CCA and they regulate or they are modulated by lncRNA. LncRNAs induced or repressed in CCA are indicated with red and green color, respectively.

Figure 5: Non coding RNAs as innovative biomarkers in CCA. Biological material is obtained either by minimally invasive (blood, bile) or by invasive techniques (biopsies or tumor tissue from surgical hepatic resection). Non coding RNA expression (miRNA, lncRNA or circRNA) could be relevant for diagnosis and/or prognosis in the management of patients with CCA.

Table 1: Role of lncRNAs in CCA carcinogenesis.

lncRNA	up/dn	mechanism of action	biological functions	related pathways	targets	biomarker	ref.
NEAT1	up	epigenetic, mutation	proliferation, migration, invasion, EMT, resistance to therapeutic		EZH2, E-cadherin	diagnostic	18-21
SNHG1	up	epigenetic, ceRNA	proliferation, invasion, cell cycle	TLR4 NF-kB	EZH2, CDKN1A, miR-140	diagnostic	22-24
SPRY4-IT1	up	epigenetic, ceRNA	apoptosis, proliferation, migration, invasion, EMT		EZH2, KLF2, LATS2, miR-101-3p	diagnostic	25
PVT1	up	epigenetic	apoptosis, proliferation, migration		ANGPTL4	diagnostic	27
DANCR	up	epigenetic, ceRNA	proliferation, migration, invasion, angiogenesis, EMT		FBP1, EZH2, miR-345-5p, TWIST1	diagnostic	26,28
MEG3	down	ceRNA, epigenetic	proliferation, migration, invasion, EMT	NFkB	miR-361-5p, TRAF3, PRC1	diagnostic, prognostic	29-31
HOTAIR	up		apoptosis, migration, invasion, EMT			diagnostic, prognostic	32
PANDAR	up		apoptosis, proliferation, migration, invasion, EMT			diagnostic, prognostic	33
CCAT2	up		apoptosis, migration, invasion, EMT			diagnostic	34
PCAT1	up	ceRNA	apoptosis, proliferation, migration, invasion	Wnt/ β-catenin	miR-122	diagnostic	37,71
TP73-AS1	up		apoptosis, migration, invasion, EMT			diagnostic	35
LINC01061	up	ceRNA	apoptosis, proliferation, migration		miR-612, SEMA4D	diagnostic	36
TUG1	up	ceRNA	apoptosis, proliferation, migration, invasion, EMT		miR-145,Sirt3, GDH	diagnostic, prognostic	38,89
AFAP1-AS1	up		proliferation, migration, invasion, cell cyle	MAPK		diagnostic, prognostic	39,40
MIAT	up	ceRNA	apoptosis, proliferation, cell cycle		miR-551b-3p, CCND1	diagnostic	41
UCA1	up	ceRNA	apoptosis, cell cycle, proliferation, migration, invasion, EMT	AKT GSK-3β CCND1	miR-122	diagnostic, prognostic	42.55
MALAT1	up	mutation, ceRNA	proliferation, migration, invasion	CXCR4, PI3K/AKT	miR-204	diagnostic	44,62
SOX2-OT	up		proliferation, invasion	PI3K/AKT		diagnostic, prognostic	43,45
EPIC1	up	TF	apoptosis, proliferation		MYC, cyclin A/D, CDK9	diagnostic	46
LINC01296	up	ceRNA	cell viability, migration, invasion		MYC, miR-5095	diagnostic, prognostic	47
SNHG6	up	TF, ceRNA	proliferation, migration, angiogenesis		miR-101-3p, E2F8	diagnostic	48,49
LOXL1-	up	ceRNA	proliferation, migration,		miR-324-3p,	diagnostic,	50

AS1			invasion and apoptosis		ABCA1	prognostic	
CRNDE	up		proliferation, migration, invasion, EMT			diagnostic, prognostic	52
CCAT1	up	ceRNA	migration, invasion, EMT		miR-152	diagnostic, prognostic	53,58,85
NNT-AS1	up	ceRNA	proliferation, migration, invasion, EMT	Wnt/ β-catenin, PI3K/AKT, ERK1/2	miR-485, BCL9, miR-142-5p, HMG2, miR-203	diagnostic, prognostic	51,54,70
KCNQ1OT1	up	ceRNA	proliferation, migration, invasion, apoptosis, EMT		miR-140-5p, SOX4	diagnostic, prognostic	56
ZEB1-AS1	up	ceRNA	proliferation, EMT		miR-200a, ZEB1	diagnostic, prognostic	59
HEIH	up	ceRNA	proliferation, migration, invasion		miR-98-5p, HECTD4	diagnostic	60
LINC01410	up	ceRNA	proliferation, migration		miR-124-3p, SMAD5	diagnostic	
H19	up	ceRNA	proliferation, migration, invasion,EMT, apoptosis	CXCR4 IL6	let-7a, miR-372	diagnostic, prognostic	63,64
HULC	up	ceRNA	migration, invasion	CXCR4 IL6	let-7b, miR-373	diagnostic	63,79
TLINC /CASC15	up		migration	TGFβ		diagnostic	67
LFAR1	up		proliferation, migration, invasion, EMT	TGFβ	TGFβ1, SMAD2, SMAD4	diagnostic	68
LMCD1-AS1	up	ceRNA	proliferation, invasion, apoptosis		miR-345-5p, COL6A3	diagnostic	69
MIR22HG	down		proliferation, migration and invasion	Wnt/β-catenin		diagnostic, prognostic	73
ASAP1-IT1	up		proliferation, migation, EMT	Hedgehog	SMO, GLI1	diagnostic, prognostic	74
GAPLINC	up		proliferation, migration, invasion			diagnostic, prognostic	84
ANRIL	up					prognostic	83
CPS1-IT1	up		proliferation, apoptosis			diagnostic, prognostic	86
lnc-PDK2-2-3	up		sphere formation, resistance to therapeutic			diagnostic, prognostic	87
SNHG3	up					diagnostic, prognostic	88
LINC01714	down		proliferation, migration, invasion, resistance to therapeutic		FOXO3	diagnostic, prognostic	90
FLVCR1-AS1	up	ceRNA	proliferation, migration, invasion			diagnostic	91



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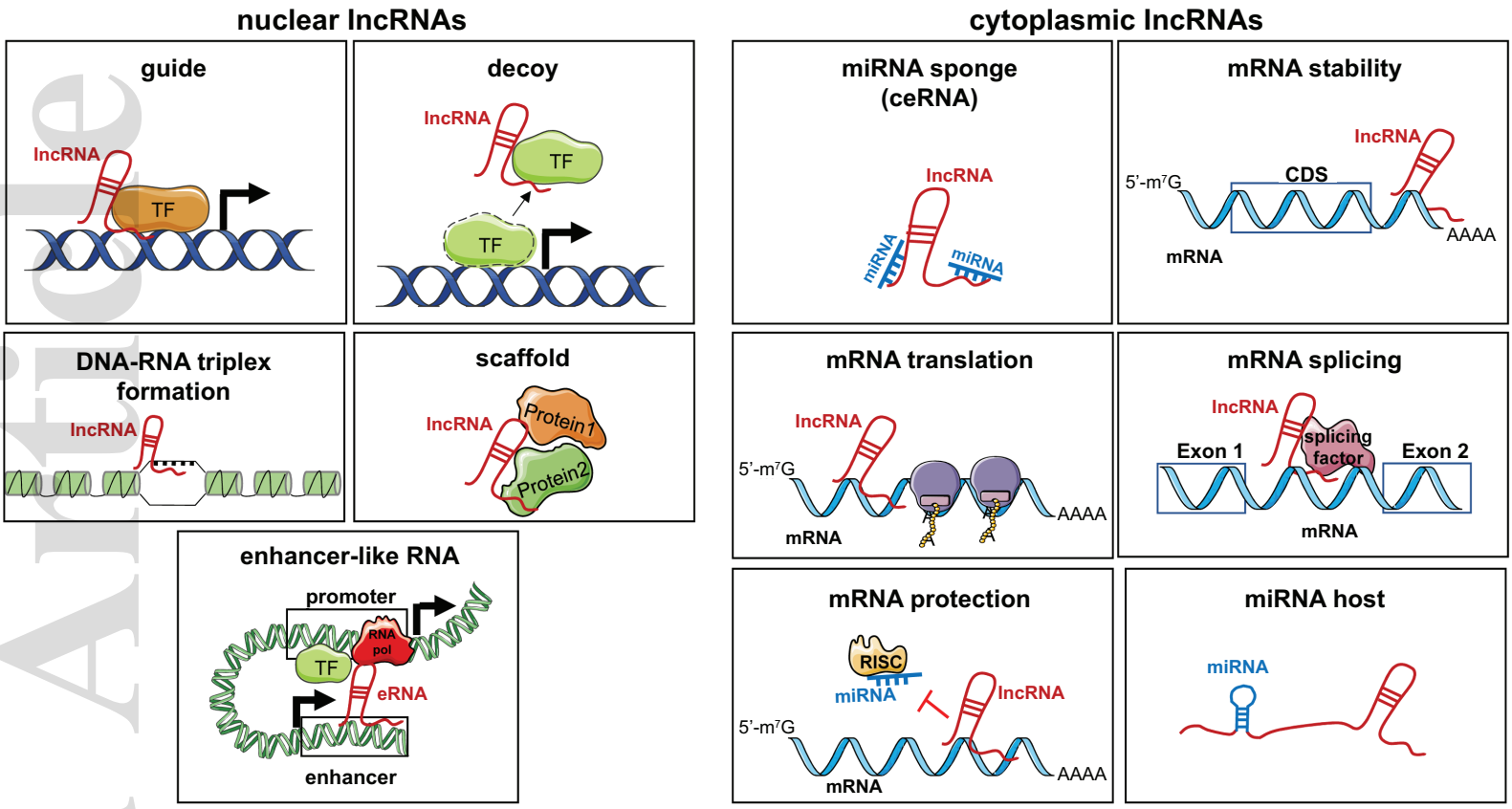

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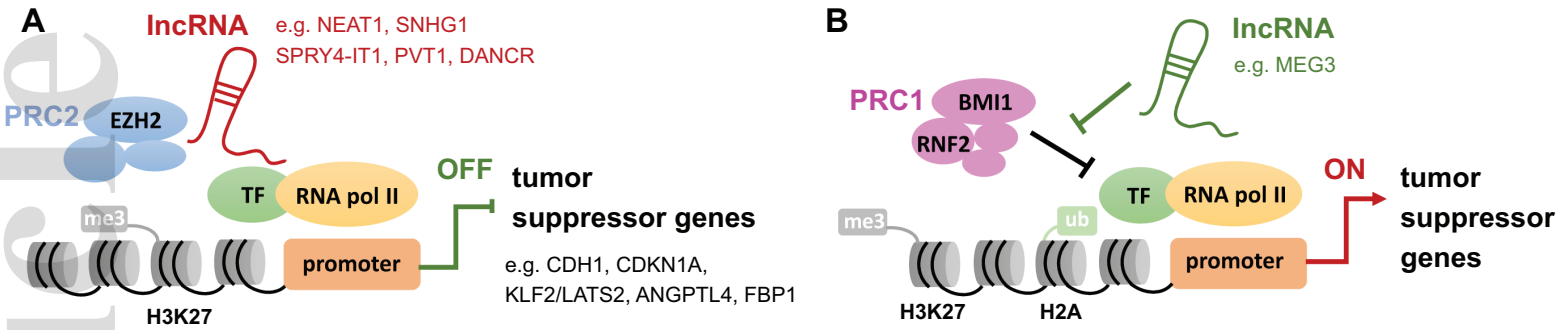

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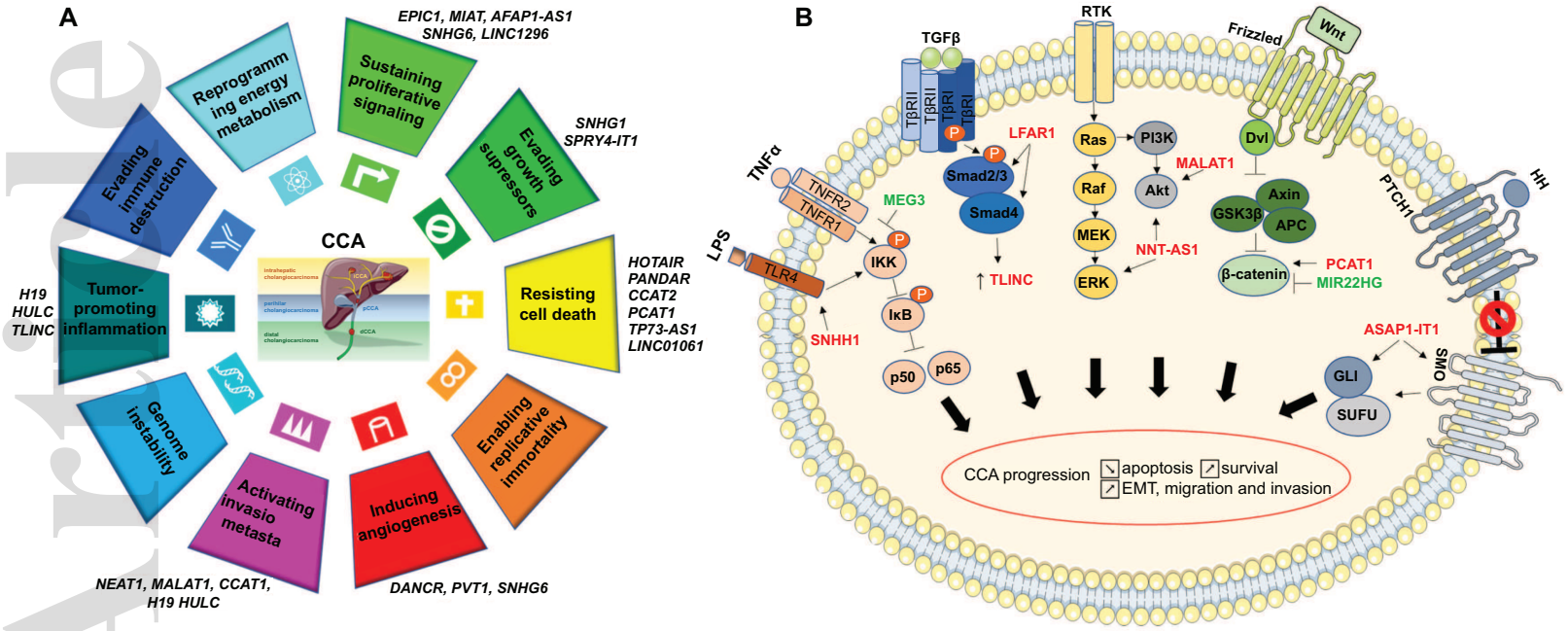

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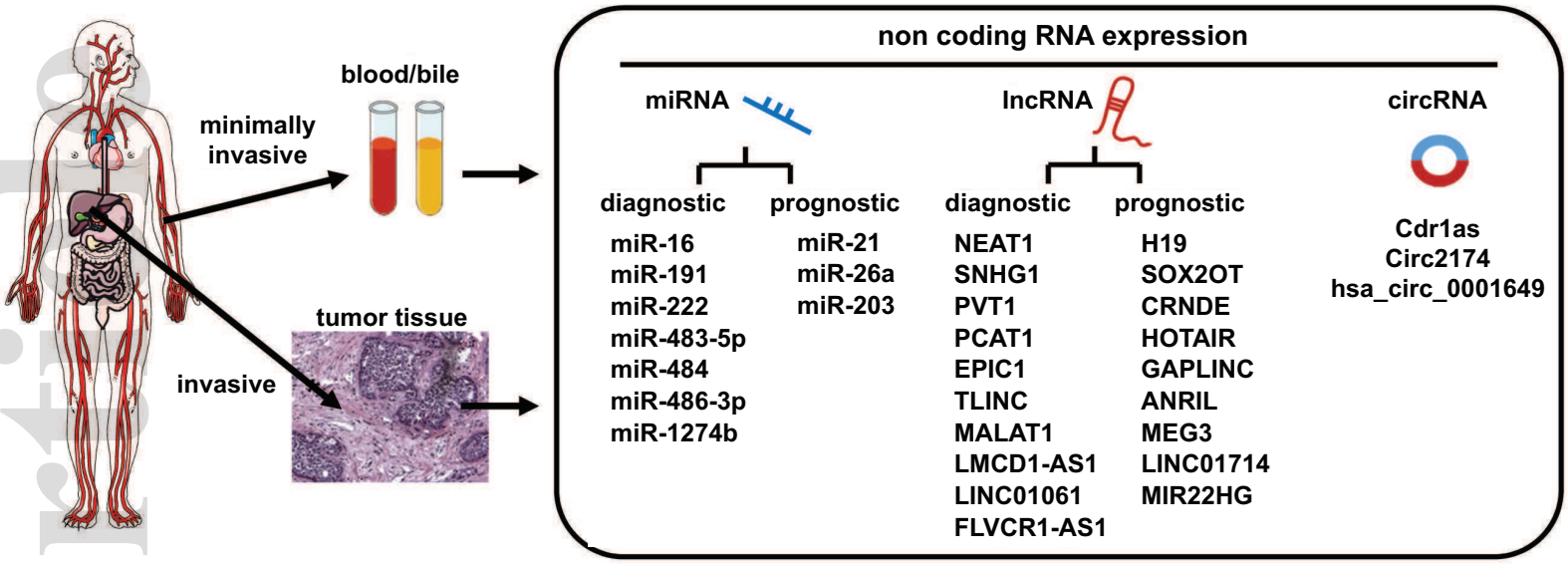

hep_31534_f5.eps