Micro-RNA in Cholangiocarcinoma

Subjects: Oncology

Contributor: Pietro Carotenuto, Luca Reggiani Bonetti, Brunella Franco

Bile-duct cancers (BDC) are a group of solid tumors arising from the biliary tree. Despite their classification as rare cancers, the incidence of BDC is increasing worldwide. Poor prognosis is a common feature of this type of cancer and is mainly determined by the following factors: late diagnosis, lack of effective therapeutic approaches, and resistance to conventional treatments. In the past few years, next-generation sequencing technologies has allowed us to study the genome, exome, and transcriptome of BDC deeper, revealing a previously underestimated class of RNA: the noncoding RNA (ncRNA). MicroRNAs (miRNAs) are small ncRNAs that play an important regulatory role in gene expression. The aberrant expression of miRNAs and their pivotal role as oncogenes or tumor suppressors in biliary carcinogenesis has been widely described in BDC. Due to their ability to regulate multiple gene networks, miRNAs are involved in all cancer hallmarks, including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing/accessing vasculature, activating invasion and metastasis, reprogramming cellular metabolism, and avoiding immune destruction. Their use as diagnostic, prognostic, and predictive biomarkers has been widely explored in several human cancers, including BDC. Furthermore, miRNA-based therapeutic strategies are currently the subject of numerous clinical trials that are providing evidence of their efficacy as potent anticancer agents.

Keywords: non-coding RNA ; microRNA ; bile duct cancer ; precision medicine

1. Introduction

Bile-duct cancers (BDC) are a group of rare solid tumors originating from the biliary system. A commonly used anatomical classification subdivides BDC into intra-hepatic (iBDC) and extra-hepatic (eBDC), originating respectively from the biliary tree within and outside the liver parenchyma. The eBDC is further subdivided into distal and perihilar BDC (dBDC, pBDC). Each subtype is characterized by specific molecular and epidemiological features [1][2]. Epidemiologic studies have suggested an increasing incidence of iBDC in most parts of the world. Globally, the incidence and mortality rates of BDC show substantial geographical variations, which reflect, at least partially, differences in geographical, environmental, and genetic risk factors [2][3].

BDCs are mainly characterized by a poor prognosis and a survival limited to a few months. The determinants of the terribly poor prognosis are essentially late diagnoses, the absence of efficient therapies, and drug-resistance. International practice guidelines recommend surgical resection followed by adjuvant therapy as the standard curative approach. Although surgery is the preferred treatment option for all BDC subtypes, only a minority of patients (approximately 30%) are suitable for this treatment. However, even in these cases, only a small percentage (20–40%) benefit from the treatment in terms of overall survival. Moreover, a large part of BDC patients are diagnosed at an advanced stage and are not suitable for surgical treatment. Currently, the combination of gemcitabine and cisplatin is the first-line chemotherapy for BDC patients with advanced-stage BDC who cannot be subjected to surgical resection [3]. However, the response rate is frequently characterized by a progressive disease associated with a poor clinical outcome.

In recent years, the scientific community has put a great deal of effort into the discovery of new therapeutic approaches for BDC, and into the identification of novel diagnostic, prognostic, and predictive biomarkers. The successful applications of genomic technology to BDC molecular pathology led to the discovery of targeted therapy as a promising therapeutic approach [4]. In fact, next-generation sequencing platforms allowed us to identify actionable drivers in BDC including genetic alterations in IDH (*Isocitrate Dehydrogenase*), FGFR2 (*Fibroblast Growth Factor Receptor* 2), and RAF genes. Pemigatinib was the first therapeutic agent targeting the FGFR2 to be approved in BDC, while other drugs targeting IDH and RAF are under investigation in clinical trials [4][5][6][7][8][9]. Immunotherapy, with immune checkpoint inhibitors, and tumor vaccines are emerging therapeutic strategies currently undergoing clinical trials [10].

In the past few years, genomic analysis—extensively investigating the genome, exome, and transcriptome—unveiled a novel class of RNA: the noncoding RNAs (ncRNA).

MicroRNAs (miRNAs) are small ncRNAs that play a pivotal role in regulating gene expression. It has been demonstrated that miRNAs are aberrantly expressed in BDC and promote biliary carcinogenesis [11]. Dysregulated miRNA expression in cancer derives from alterations in various molecular mechanisms, including deletion or amplification of miRNA-genes, altered transcriptional control of miRNAs, irregular epigenetic changes, and defects in the biogenesis machinery [12].

Similar to other cancers, miRNAs are implicated in all steps of biliary carcinogenesis by functioning as oncogenes (onco-miRNAs) or onco-suppressor (oncosuppressor-miRNA) genes (**Figure 1**). Their pro- and anti-tumorigenic molecular function greatly influences all the cancer hallmarks, including supporting proliferative signaling, evading cell death, and activating invasion and metastasis. Several clinical investigations proposed miRNAs as potential biomarkers in cancer and highlighted their diagnostic and prognostic power [12]. Furthermore, miRNA-based therapeutic strategies have been recently developed and currently several clinical trials are assessing their efficacy and safety [12].

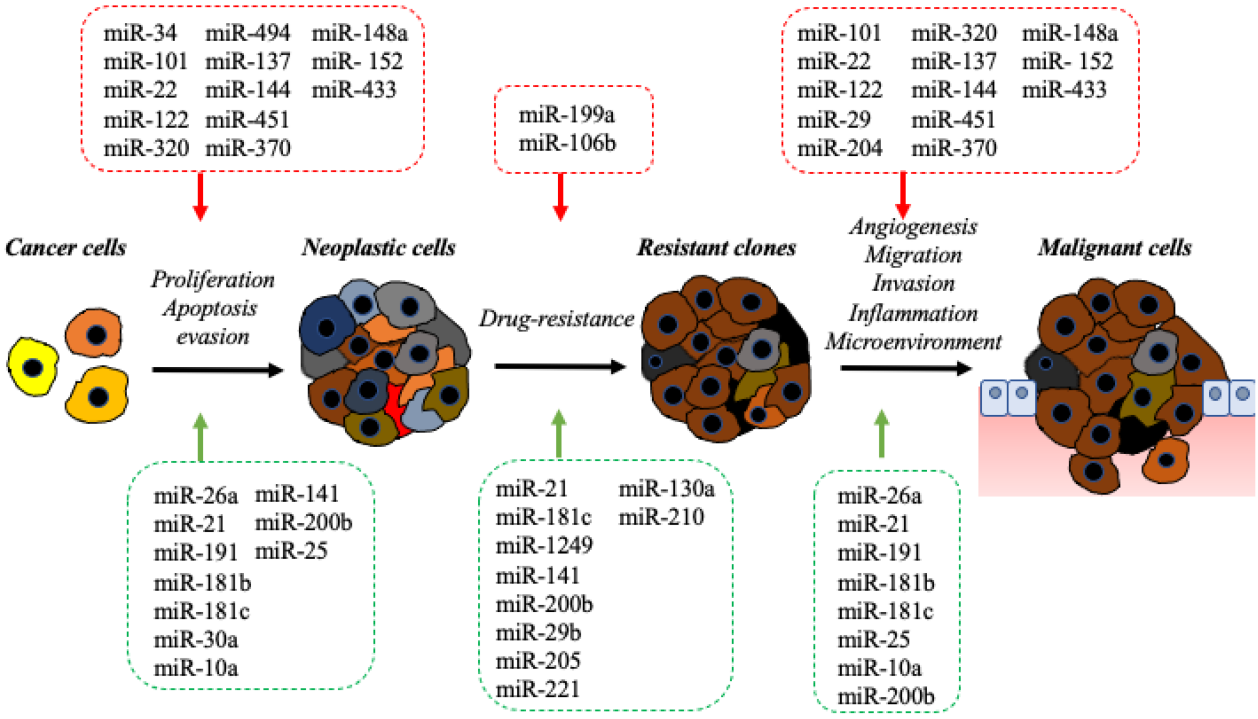

Figure 1. The impact of the miRNA in BDC carcinogenesis.

2. The Impact of the microRNA in Molecular Pathology of BDC

In recent years, the development of powerful technological platforms, capable of performing high-throughput RNA sequencing, allowed us to greatly expand the knowledge of the transcriptome and to reveal its complexity. In particular, the complexity of the eukaryotic transcriptome was made evident by the identification of a new class of RNAs with a structure and function clearly different from those previously known. Based on current knowledge, the eukaryotic transcriptome can be divided into messenger (mRNA) or coding RNAs and a novel class of non-coding RNAs (ncRNAs) [12][13].

The ncRNA represents a class of RNA that is transcribed but not translated into proteins. According to their length, they can be further divided into two subtypes: (a) small ncRNAs ranging from 18 to 200 nucleotides and (b) long ncRNA with length greater than 200 nucleotides [12][13]. NcRNAs influences several physiological and disease processes by playing a pivotal role in the regulation of gene expression.

The most widely studied class of ncRNAs is certainly the microRNA (miRNA). MiRNAs are small non-coding RNA (18 to 24 nucleotides) transcribed by the RNA pol II (RNA polymerase II) and further processed in a small, functionally mature RNA. The mature miRNA regulates target genes by binding to specific miRNA-responsive regions in the 3′UTR (untranslated region). The binding between miRNA and its pair target gene causes target-mRNA degradation and/or translational inhibition [14]. Several protein complexes take part in miRNA-mediated gene expression regulation, including via the RISC (RNA-induced silencing complex) which participates in miRNA-binding and further mRNA degradation [14]. The imperfect base pairing between miRNA and the miRNA-binding site makes miRNA an extremely complex and versatile regulatory agent, capable of fine-regulating hundreds of target genes [13][14][15].

The Croce group identified two miRNA genes at the chromosome 13q14 region in B-cell chronic lymphocytic leukemia cells, providing the earliest evidence of miRNA involvement in human cancer [16]. The identification of two miRNA genes, miR-15a and miR-16-1 initiated a series of further studies that revealed the key role of these miRNAs in tumor suppression [16].

In the following years, advanced technologies to profile miRNAs facilitated the accumulation of evidence regarding their aberrant expression in cancer. The miRNA expression profile has also been proposed as potential diagnostic and prognostic biomarker [15].

Regarding their link to BDC, miRNAs have been shown to be implicated in almost all steps of biliarycarcinogenesis by acting either as oncogenes (onco-miRNAs) or tumor suppressors (oncosuppressor-miRNAs) (**Figure 1**). An extensive report of the miRNAs involved in BDC, which is comprehensive of their mechanism of action and their targets, is presented in **Table 1** and **Table 2**.

Table 1. MiRNAs acting as onco-miRNAs in BDC: their targets and molecular function.

miRNA	Target Gene	Mechanism	References
miR-26a	GSK-3β; KRT19	Proliferation, migration, and invasion	[17][18][19]
miR-21	PTEN; PDCD4; TIMP3; PTPN14; 15-PGDH/HPGD	Proliferation, apoptosis, EMT, inflammation	[20][21][22]
miR-191	TET	Proliferation, invasion, and migration	[23]
miR-181b-5p	PARK2	Proliferation, migration, and invasion	[24][25][26]
miR-181c	NDRG2	Proliferation, drug-resistance, and metastasis	[27]
MiR-30a-5p	SOCS3	Proliferation	[28]
miR-25	DR4	Proliferation, invasion, and apoptosis	[29][30]
miR-10a-5p	PTEN	Proliferation	[31]

Table 2. MiRNAs acting as oncosuppressor-miRNAs in BDC: their targets and molecular function.

miRNA	Target Gene	Mechanism	References
miR-34	MYC, MET, CDK4/6, BCL2, CD44, NOTCH1, NOTCH2, JAGGED1	Proliferation, apoptosis	[32][33]
miR-101	EZH2, COX-2, APP, MCL-1, VEGF	Proliferation, apoptosis; angiogenesis, inflammation; transcriptional repression	[34][35][36][37]
miR-22	SIRT1, CDK6, SP1, HDAC6	Proliferation, senescence, invasion, metastasis; ciliogenesis, histone modifications	[38][39]

miRNA	Target Gene	Mechanism	References
miR-122	ALDOA, CLIC1	Proliferation and invasion	[40][41][42]
miR-29-3p	ITGA6, ITGB1	Cell migration and invasion	[43]
miR-204	SLUG	Cell migration, invasion, EMT	[44][45]
miR-320	VEGF, NRP-1	Proliferation, invasion, EMT, tumor migration, and metastasis	[46][47]
miR-494	CCNB1, CDK2, CDK4, CDK6, CCND1, CCNE2, HDAC1, RB1, PLK1, PTTG1, TOP2A	Proliferation, cell cycle	[48]
miR-137	WNT2B	Proliferation, migration, and invasion	[49]
miR-144-5p/miR-451	ST8SIA4	Proliferation, migration, and invasion	[50]
miR-370	MAP3K8	Proliferation, inflammation, tumor microenvironment	[51][52]
miR-148a	RASSF1	Proliferation, inflammation, tumor microenvironment	[53][54]
miR-152	CDKN2A	Proliferation, inflammation, tumor microenvironment	[53][54]

3. The Impact of miRNAs in Precision Medicine of BDC

In the last few years, the recent advances in the genomic knowledge of BDC have sped up the idea of ‘precision medicine’. A precision medicine-based approach aims to select the most appropriate treatment dependent on the specific molecular alterations displayed by a single patient. Due to the heterogeneity of BDC, the absence of diagnostic predictive tools, and the inefficacy and toxicity of current treatment, a precision medicine approach could greatly benefit the clinical management of BDC. Moreover, the specificity and sensitivity of available biomarkers determined in serum and biopsy samples are not sufficient to assist in the clinical management of BDC. The availability of predictive biomarkers, which could aid patient stratification and treatment response identification, still represents an unmet clinical need. Extensive research is being carried out to identify novel biomarkers that could contribute to a better understanding of the molecular pathogenesis of BDC, as well as to provide new diagnostic, prognostic, and predictive tools [5][26][55][56][57].

4. The Impact of miRNA-Based Therapeutic in BDC

MiRNA-based therapy is emerging as a promising novel strategy to treat cancers. miRNA-based therapeutic approaches operate by silencing overexpressed onco-miRNAs or replacing downregulated oncosuppressor-miRNAs [15]. Several miRNA-based therapeutics are under investigation in clinical trials according to www.clinicaltrials.gov (accessed on 22 January 2022) [58].

From the methodological point of view, the following strategies were adopted to efficiently perform onco-miRNA silencing both in vitro and in vivo: anti-miRNA oligonucleotides (AMOs), anti-miRNA locked nucleic acid (LNA), anti-miRNA sponges, and genetic knockouts based on the CRISPR/Cas9 genome-editing technologies [15]. On the other hand, miRNA replacement therapy has generally achieved restoration of onco-suppressor-miRNA by introducing synthetically modified oligonucleotides (miRNA mimics) or viral vectors [15].

However, while promising, miRNA-based therapeutics has raised the following issues to be considered: delivery, selectivity to specific target cells, degradation, and toxicity. Chemical modification of nucleotides or of the RNA backbone through methylation or LNAs, together with the development of vehicles to encapsulate the RNAs, are the main strategies put in place to protect miRNA from degradation [15]. Moreover, toxicity and side effects represent perhaps the biggest obstacle so far encountered in miRNA-based therapeutics [15]. Viral and non-viral vectors have been developed to improve delivery efficiencies to target cells, but the risk of adverse immunogenicity has restricted their use. Lipid-based and polymer-based nanoparticles (NPs) resulted as promising technical approaches as they guarantee efficient delivery and a good safety profile [15][59].

MRX34, a formulation based on miR-34 mimics in liposomal particles, was the first miRNA restoration strategy performed, subsequently entering a clinical trial recruiting patients with solid tumors, including hepatocellular carcinoma [60]. Preliminary results emerging from clinical investigations reported a good safety profile and significant anticancer activity for MRX34 treatment [60]. Recent findings showing the inhibition of BDC cell growth by the miR-34 mimic strongly suggested that miRNA-34-based therapy may be a potential efficient and safe therapeutic approach in BDC [32].

Li et al. first found that stroma-derived extracellular vesicles (EVs) containing miR-195 inhibit the proliferation, migration, and metastasis of BDC cells [59]. Furthermore, they observed that the systemic injection of miR-195-loaded EVs inhibited BDC tumor growth and prolonged survival in animal models [59].

Xie et al. explored a new therapeutic approach based on the use of nanoparticles loaded with a cholesterol-modified polymeric antagonist of CXCR4 (*C-X-C receptor type 4*) and anti-miR-210 [61]. They showed that CXCR4 antagonist- and anti-miR-210-loaded nanoparticles cooperate synergistically in inducing apoptosis and sensitizing BDC cells to Gem/Cis treatment. They presented results showing that the novel nano-therapeutic approach combining the silencing of both CXCR4 and miR-210 suppress tumor growth in BDC cell lines and animal models [61].

Another miRNA-based therapeutic approach is represented by the combination of chemotherapy and miRNA to establish a synergistic antitumor effect. Recently, Zhang et al. assembled NPs loaded with gemcitabine—oleic acid prodrugs (GOA) and miR-122 to form GOA/miRNAs NPs [18]. They demonstrated that GOA/miR-122-loaded NPs were efficiently delivered to the tumor area and inhibited hepatocellular carcinoma tumor growth in vivo, without significantly affecting the biosafety profile [18].

To date, miRNA-based therapeutics have been investigated by a huge number of preclinical studies, but only a small number have moved up to clinical trials. Major obstacles are still represented by the delivering of miRNA-based drugs, their stability, and safety profile. However, promising novel strategies are emerging, combining novel delivery platforms and low-toxicity profiles, providing a basis for innovative miRNA-based therapeutic approaches.

References

1. Rizvi, S.; Khan, S.A.; Hallemeier, C.L.; Kelley, R.K.; Gores, G.J. Cholangiocarcinoma—Evolving concepts and therapeutic strate-gies. Nat. Rev. Clin. Oncol. 2018, 15, 95–111.
2. Khan, S.A.; Tavolari, S.; Brandi, G. Cholangiocarcinoma: Epidemiology and risk factors. Liver Int. 2019, 39, 19–31.
3. Bertuccio, P.; Malvezzi, M.; Carioli, G.; Hashim, D.; Boffetta, P.; El-Serag, H.B.; Negri, E. Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma. J. Hepatol. 2019, 71, 104–114.
4. Kelley, R.K.; Bridgewater, J.; Gores, G.J.; Zhu, A.X. Systemic therapies for intrahepatic cholangiocarcinoma. J. Hepatol. 2020, 72, 353–363.
5. Braconi, C.; Roessler, S.; Kruk, B.; Lammert, F.; Krawczyk, M.; Andersen, J.B. Molecular perturbations in cholangiocarcinoma: Is it time for precision medicine? Liver Int. 2019, 39, 32–42.
6. Adeva, J.; Sangro, B.; Salati, M.; Edeline, J.; La Casta, A.; Bittoni, A.; Berardi, R.; Bruix, J.; Valle, J.W. Medical treatment for cholangiocarcinoma. Liver Int. 2019, 39, 123–142.
7. De la Fouchardiere, C. Towards greater clarity in the treatment of cholangiocarcinoma. Lancet Oncol. 2020, 21, 738–739.
8. Salati, M.; Caputo, F.; Baldessari, C.; Carotenuto, P.; Messina, M.; Caramaschi, S.; Dominici, M.; Bonetti, L.R. The Evolving Role of FGFR2 Inhibitors in Intrahepatic Cholangiocarcinoma: From Molecular Biology to Clinical Targeting. Cancer Manag. Res. 2021, 13, 7747–7757.

9. Franco, B.; Clarke, P.; Carotenuto, P. Pemigatinib. Fibroblast growth factor receptor inhibitor, Treatment of cholangiocarci-noma. Drugs Future 2019, 44, 923.
10. Guo, X.; Shen, W. Latest evidence on immunotherapy for cholangiocarcinoma (Review). Oncol. Lett. 2020, 20, 381.
11. Carotenuto, P.; Hedayat, S.; Fassan, M.; Cardinale, V.; Lampis, A.; Guzzardo, V.; Braconi, C. Modulation of Biliary Cancer Chemo-Resistance Through MicroRNA-Mediated Rewiring of the Expansion of CD133+ Cells. Hepatology 2020, 72, 982–996.
12. Salati, M.; Braconi, C. Noncoding RNA in Cholangiocarcinoma. Semin. Liver Dis. 2018, 39, 013–025.
13. Anastasiadou, E.; Jacob, L.S.; Slack, F.J. Non-coding RNA networks in cancer. Nat. Rev. Cancer 2018, 18, 5–18.
14. Gebert, L.F.R.; Macrae, I.J. Regulation of microRNA function in animals. Nat. Rev. Mol. Cell Biol. 2019, 20, 21–37.
15. Rupaimoole, R.; Slack, F.J. MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. Nat. Rev. Drug Discov. 2017, 16, 203–222.
16. Adrian, C.G.; Dan, D.C.; Shimizu, M.; Bichi, R.; Zupo, S.; Noch, E.; Croce, C.M. Frequent Deletions and Down-Regulation of Micro-RNA Genes miR15 and miR16 at 13q14 in Chronic Lymphocytic Leukemia . Available online: https://www.pnas.org/doi/abs/10.1073/pnas.242606799 (accessed on 22 January 2022).
17. Wang, P.; Lv, L. miR-26a induced the suppression of tumor growth of cholangiocarcinoma via KRT19 approach. Oncotarget 2016, 7, 81367–81376.
18. Zhang, H.-T.; Sun, J.; Yan, Y.; Cui, S.-H.; Wang, H.; Wang, C.-H.; Zhang, Q. Encapsulated microRNA by gemcitabine prodrug for cancer treatment. J. Control. Release 2019, 316, 317–330.
19. Zhang, J.; Han, C.; Wu, T. MicroRNA-26a Promotes Cholangiocarcinoma Growth by Activating β-catenin. Gastroenterology 2012, 143, 246–256.e8.
20. Wang, L.-J.; He, C.-C.; Sui, X.; Cai, M.-J.; Zhou, C.-Y.; Ma, J.-L.; Zhu, Q. MiR-21 Promotes Intrahepatic Cholangiocarcinoma Proliferation and Growth In Vitro and In Vivo by Targeting PTPN14 and PTEN . Volume 6. Available online: www.impactjournals.com/oncotarget (accessed on 22 January 2022).
21. Liu, Z.; Jin, Z.-Y.; Liu, C.-H.; Xie, F.; Lin, X.-S.; Huang, Q. MicroRNA-21 regulates biological behavior by inducing EMT in human cholangiocarcinoma. Int. J. Clin. Exp. Pathol. 2015, 8, 4684–4694.
22. Meng, F.; Henson, R.; Lang, M.; Wehbe, H.; Maheshwari, S.; Mendell, J.T.; Jiang, J.; Schmittgen, T.D.; Patel, T. Involvement of Human Micro-RNA in Growth and Response to Chemotherapy in Human Cholangiocarcinoma Cell Lines. Gastroenterology 2006, 130, 2113–2129.
23. Li, H.; Zhou, Z.-Q.; Yang, Z.-R.; Tong, D.-N.; Guan, J.; Shi, B.-J.; Nie, J.; Ding, X.-T.; Li, B.; Zhou, G.-W.; et al. MicroRNA-191 acts as a tumor promoter by modulating the TET1-p53 pathway in intrahepatic cholangiocarcinoma. Hepatology 2017, 66, 136–151.
24. Indrieri, A.; Carrella, S.; Carotenuto, P.; Banfi, S.; Franco, B. The Pervasive Role of the miR-181 Family in Development, Neurodegeneration, and Cancer. Int. J. Mol. Sci. 2020, 21, 2092.
25. Jiang, Z.L.; Zhang, F.X.; Zhan, H.L.; Yang, H.J.; Zhang, S.Y.; Liu, Z.H.; Ke, R.S. miR-181b-5p Promotes the Progression of Cholangiocarcinoma by Targeting PARK2 via PTEN/PI3K/AKT Signaling Pathway. Biochem. Genet. 2022, 60, 223–240.
26. Sun, C.; Zhu, J.; Wu, B.; Chen, J.; Zhu, Z.; Cai, P.; Guo, W.; Gu, Z.; Wang, J.; Huang, S. Diagnostic and prognostic value of microRNAs in cholangiocarcinoma: A systematic review and meta-analysis. Cancer Manag. Res. 2018, 10, 2125–2139.
27. Wang, J.; Xie, C.; Pan, S.; Liang, Y.; Han, J.; Lan, Y.; Liu, L. N-myc downstream-regulated gene 2 inhibits human cholangiocarci-noma progression and is regulated by leukemia inhibitory factor/MicroRNA-181c negative feedback pathway. Hepatology 2016, 64, 1606–1622.
28. Zhang, J.W.; Wang, X.; Li, G.C.; Wang, D.; Han, S.; Zhang, Y.D.; Luo, C.H.; Wang, H.W.; Jiang, W.J.; Li, C.X.; et al. MiR-30a-5p promotes cholangiocarcinoma cell proliferation through targeting SOCS3. J. Cancer 2020, 11, 3604–3614.
29. Liu, H.; Ma, L.; Wang, J. Overexpression of miR-25 is associated with progression and poor prognosis of cholangiocarcinoma. Exp. Ther. Med. 2019, 18, 2687–2694.
30. Razumilava, N.; Bronk, S.F.; Smoot, R.L.; Fingas, C.D.; Werneburg, N.W.; Roberts, L.; Mott, J.L. miR-25 targets TNF-related apoptosis inducing ligand (TRAIL) death receptor-4 and promotes apoptosis resistance in cholangiocarcinoma. Hepatology 2011, 55, 465–475.
31. Gao, L.; Yang, X.; Zhang, H.; Yu, M.; Long, J.; Yang, T. Inhibition of miR-10a-5p suppresses cholangiocarcinoma cell growth through downregulation of Akt pathway. OncoTargets Ther. 2018, 11, 6981–6994.

32. Han, Y.; Meng, F.; Venter, J.; Wu, N.; Wan, Y.; Standeford, H.; Alpini, G. miR-34a-dependent overexpression of Per1 decreases chol-angiocarcinoma growth. J. Hepatol. 2016, 64, 1295–2130.

33. Slabáková, E.; Culig, Z.; Remšík, J.; Souček, K. Alternative mechanisms of miR-34a regulation in cancer. Cell Death Dis. 2017, 8, e3100.

34. Au, S.L.-K.; Wong, C.C.L.; Lee, J.M.-F.; Fan, D.N.-Y.; Tsang, F.H.; Ng, I.O.-L.; Wong, C.-M. Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. Hepatology 2012, 56, 622–631.

35. Strillacci, A.; Griffoni, C.; Sansone, P.; Paterini, P.; Piazzi, G.; Lazzarini, G.; Tomasi, V. MiR-101 downregulation is involved in cycloox-ygenase-2 overexpression in human colon cancer cells. Exp. Cell Res. 2009, 315, 1439–1447.

36. Su, H.; Yang, J.-R.; Xu, T.; Huang, J.; Xu, L.; Yuan, Y.; Zhuang, S.M. MicroRNA-101, down-regulated in hepatocellular carcinoma, pro-motes apoptosis and suppresses tumorigenicity. Cancer Res. 2009, 69, 1135–1142.

37. Zhang, J.; Han, C.; Zhu, H.; Song, K.; Wu, T. miR-101 Inhibits Cholangiocarcinoma Angiogenesis through Targeting Vascular Endothelial Growth Factor (VEGF). Am. J. Pathol. 2013, 182, 1629–1639.

38. Wang, J.; Li, Y.; Ding, M.; Zhang, H.; Xu, X.-M.; Tang, J. Molecular mechanisms and clinical applications of miR-22 in regulating malignant progression in human cancer (Review). Int. J. Oncol. 2016, 50, 345–355.

39. Mansini, A.P.; Pisarello, M.J.L.; Thelen, K.M.; Cruz-Reyes, M.; Peixoto, E.; Jin, S.; Howard, B.N.; Trussoni, C.E.; Gajdos, G.B.; LaRusso, N.F.; et al. MicroRNA (miR)-433 and miR-22 dysregulations induce histone-deacetylase-6 overexpression and ciliary loss in cholangiocarcinoma. Hepatology 2018, 68, 561–573.

40. Fu, X.; Calin, G.A. miR-122 and hepatocellular carcinoma: From molecular biology to therapeutics. EBioMedicine 2018, 37, 17–18.

41. Xu, Z.; Liu, G.; Zhang, M.; Zhang, Z.; Jia, Y.; Peng, L.; Zhu, Y.; Hu, J.; Huang, R.; Sun, X. miR-122-5p Inhibits the Proliferation, Invasion and Growth of Bile Duct Carcinoma Cells by Targeting ALDOA. Cell. Physiol. Biochem. 2018, 48, 2596–2606.

42. Kong, L.; Wu, Q.; Zhao, L.; Ye, J.; Li, N.; Yang, H. Upregulated lncRNA-UCA1 contributes to metastasis of bile duct carcinoma through regulation of miR-122/CLIC1 and activation of the ERK/MAPK signaling pathway. Cell Cycle 2019, 18, 1212–1228.

43. Hozaka, Y.; Seki, N.; Tanaka, T.; Asai, S.; Moriya, S.; Idichi, T.; Wada, M.; Tanoue, K.; Kawasaki, Y.; Mataki, Y.; et al. Molecular Pathogenesis and Regulation of the miR-29-3p-Family: Involvement of ITGA6 and ITGB1 in Intra-Hepatic Cholangiocarcinoma. Cancers 2021, 13, 2804.

44. Zhang, L.; Wang, X.; Chen, P. MiR-204 down regulates SIRT1 and reverts SIRT1-induced epithelial-mesenchymal transition, anoikis resistance and invasion in gastric cancer cells. BMC Cancer 2013, 13, 290–299.

45. Chung, T.; Lau, T.; Cheung, T.; Yim, S.; Lo, K.; Siu, N.; Chan, L.; Yu, M.; Kwong, J.; Doran, G.; et al. Dysregulation of microRNA-204 mediates migration and invasion of endometrial cancer by regulating FOXC1. Int. J. Cancer 2011, 130, 1036–1045.

46. Liang, Y.; Li, S.; Tang, L. MicroRNA 320, an Anti-Oncogene Target miRNA for Cancer Therapy. Biomedicines 2021, 9, 591.

47. Zhu, H.; Jiang, X.; Zhou, X.; Dong, X.; Xie, K.; Yang, C.; Jiang, H.; Sun, X.; Lu, J. Neuropilin-1 regulated by miR-320 contributes to the growth and metastasis of cholangiocarcinoma cells. Liver Int. 2017, 38, 125–135.

48. Olaru, A.V.; Ghiaur, G.; Yamanaka, S.; Luvsanjav, D.; An, F.; Popescu, I.; Selaru, F.M. MicroRNA down-regulated in human cholangio-carcinoma control cell cycle through multiple targets involved in the G1/S checkpoint. Hepatology 2011, 54, 2089–2098.

49. Chen, T.; Lei, S.; Zeng, Z.; Pan, S.; Zhang, J.; Xue, Y.; Sun, Y.; Lan, J.; Xu, S.; Mao, D.; et al. MicroRNA-137 suppresses the proliferation, migration and invasion of cholangiocarcinoma cells by targeting WNT2B. Int. J. Mol. Med. 2020, 45, 886–896.

50. Fu, W.; Yu, G.; Liang, J.; Fan, P.; Dong, K.; Zhang, B.; Chen, X.; Zhu, H.; Chu, L. miR-144-5p and miR-451a Inhibit the Growth of Cholangiocarcinoma Cells Through Decreasing the Expression of ST8SIA4. Front. Oncol. 2021, 10, 3104.

51. Meng, F.; Wehbe-Janek, H.; Henson, R.; Smith, H.; Patel, T. Epigenetic regulation of microRNA-370 by interleukin-6 in malig-nant human cholangiocytes. Oncogene 2008, 27, 378–386.

52. An, F.; Yamanaka, S.; Allen, S.; Roberts, L.R.; Gores, G.J.; Pawlik, T.M.; Xie, Q.; Ishida, M.; Mezey, E.; Ferguson-Smith, A.C.; et al. Silencing of miR-370 in Human Cholangiocarcinoma by Allelic Loss and Interleukin-6 Induced Maternal to Paternal Epigenotype Switch. PLoS ONE 2012, 7, e45606.

53. Omar, H.A.; El-Serafi, A.T.; Hersi, F.; Arafa, E.A.; Zaher, D.M.; Madkour, M.; Arab, H.H.; Tolba, M.F. Immunomodulatory MicroRNAs in cancer: Targeting immune checkpoints and the tumor microenvironment. FEBS J. 2019, 286, 3540–3557.

54. Shi, T.; Morishita, A.; Kobara, H.; Masaki, T. The Role of microRNAs in Cholangiocarcinoma. Int. J. Mol. Sci. 2021, 22, 7627.

55. Hu, J.; Wang, Y.-N.; Song, D.-J.; Tan, J.-P.; Cao, Y.; Fan, J.; Zhou, J. A High-Accuracy Model Based on Plasma miRNAs Diagnoses In-trahepatic Cholangiocarcinoma: A Single Center with 1001 Samples. Diagnostics 2021, 11, 610.

56. Macias, R.I.; Banales, J.; Sangro, B.; Muntané, J.; Avila, M.; Lozano, E.; Perugorria, M.J.; Padillo, F.J.; Bujanda, L.; Marin, J. The search for novel diagnostic and prognostic biomarkers in cholangiocarcinoma. Biochim. Biophys. Acta (BBA)—Mol. Basis Dis. 2018, 1864, 1468–1477.

57. Lawrie, C.H.; Gal, S.; Dunlop, H.M.; Pushkaran, B.; Liggins, A.P.; Pulford, K.; Banham, A.H.; Pezzella, F.; Boultwood, J.; Wainscoat, J.S.; et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br. J. Haematol. 2008, 141, 672–675.

58. Clinical Trial Database. Available online: www.clinicaltrials.gov (accessed on 22 January 2022).

59. Li, L.; Piontek, K.; Ishida, M.; Fausther, M.; Dranoff, J.A.; Fu, R.; Selaru, F.M. Extracellular vesicles carry micro RNA -195 to intrahepatic cholangiocarcinoma and improve survival in a rat model. Hepatology 2017, 65, 501–514.

60. Hong, D.S.; Kang, Y.-K.; Brenner, A.J.; Sachdev, J.C.; Ejadi, S.; Borad, M.J.; Kim, T.-Y.; Lim, H.Y.; Park, K.; Becerra, C.; et al. MRX34, a liposomal miR-34 mimic, in patients with advanced solid tumors: Final dose-escalation results from a first-in-human phase I trial of microRNA therapy. J. Clin. Oncol. 2016, 34, 2508.

61. Xie, Y.; Wang, Y.; Li, J.; Hang, Y.; Jaramillo, L.; Wehrkamp, C.J.; Phillippi, M.A.; Mohr, A.M.; Chen, Y.; Talmon, G.A.; et al. Cholangiocarcinoma therapy with nanoparticles that combine downregulation of MicroRNA-210 with inhibition of cancer cell invasiveness. Theranostics 2018, 8, 4305–4320.
