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Mechanisms of Cholangiocarcinoma, Molecular Subtypes, and Emerging Therapeutic Strategies

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Abstract

Cholangiocarcinoma (CCA) is a highly heterogeneous malignancy arising from the biliary epithelium and is characterized by an insidious onset, aggressive biological behavior, and poor overall prognosis. With the widespread application of high-throughput sequencing and multi-omics technologies, the molecular pathogenesis and classification of CCA have been progressively elucidated. Accumulating evidence suggests that genetic alterations, epigenetic reprogramming, a chronic inflammation-driven tumor microenvironment, and dysregulated immune surveillance collectively contribute to CCA initiation and progression. Moreover, CCAs originating from distinct anatomical sites and histological subtypes exhibit substantial differences in molecular features, clinical behavior, and therapeutic responsiveness. This review systematically summarizes current knowledge on the molecular mechanisms underlying CCA, delineates major molecular subtypes, and highlights recent advances in clinical management, with particular emphasis on targeted therapies and immunotherapies. In addition, molecular biomarkers associated with prognosis are discussed, aiming to provide a theoretical framework for molecular stratification, prognostic assessment, and biomarker-guided individualized treatment strategies in CCA.

Keywords

Cholangiocarcinoma, Molecular Pathogenesis, Molecular Subtypes, Targeted Therapy, Immunotherapy

1. Introduction

Cholangiocarcinoma is a malignant tumor originating from biliary epithelial cells. Based on anatomical location along the biliary tract, CCA is commonly classified

into three major subtypes: intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), and distal cholangiocarcinoma (dCCA) [1]. Among these entities, iCCA represents the most prevalent subtype, and epidemiological data indicate a continuous increase in its incidence worldwide [2]. The clinical burden of CCA is closely related to its complex clinicopathological features and heterogeneous molecular background. Clinical manifestations are often nonspecific; early symptoms such as fatigue, jaundice, anorexia, and weight loss frequently overlap with other hepatobiliary disorders. As a result, many patients are diagnosed at advanced stages, which substantially contributes to the poor prognosis associated with CCA [3]. Current therapeutic options remain limited, particularly for advanced disease, and conventional chemotherapy and radiotherapy provide only modest survival benefit, with generally low 5-year overall survival (OS) rates.

Advances in molecular biology have substantially improved our understanding of CCA pathogenesis. Increasing evidence indicates that CCA development and progression are driven by diverse molecular alterations, including somatic mutations, epigenetic dysregulation, and dynamic interactions within the tumor microenvironment. Clinically, iCCA is frequently associated with underlying liver diseases such as cirrhosis, chronic viral hepatitis, and non-alcoholic fatty liver disease (NAFLD), whereas pCCA is more commonly linked to chronic inflammatory biliary disorders, particularly primary sclerosing cholangitis (PSC) [4].

At present, surgical resection remains the mainstay of potentially curative treatment; however, most patients present with unresectable disease at diagnosis. Even among patients undergoing curative-intent surgery, recurrence rates remain high [3]. Nevertheless, the rapid development of molecularly targeted therapies and immunotherapeutic approaches has begun to improve outcomes for selected patient subsets. Therefore, a deeper understanding of the molecular mechanisms underlying CCA—particularly subtype-specific molecular features and differential therapeutic responses—appears essential for improving early detection, refining risk stratification, and optimizing treatment selection. With continued progress in molecular diagnostics and immuno-oncology, CCA management is expected to become increasingly precise, potentially translating into improved patient outcomes.

2. Epidemiology and Risk Factors

Cholangiocarcinoma is generally considered a relatively rare malignancy; however, its incidence and mortality have increased globally over recent decades, with pronounced geographic variation. Epidemiological analyses estimate that CCA accounts for approximately 3% of all gastrointestinal malignancies worldwide [3] [5].

2.1. Global Incidence and Mortality

The epidemiological patterns of CCA vary substantially across regions. In Asia—particularly Southeast Asia—CCA incidence is notably high, reaching up to 85

cases per 100,000 population in certain endemic areas. This phenomenon is largely attributed to liver fluke infection and environmental exposures. For example, Thailand, South Korea, and selected regions of China report relatively high age-standardized incidence rates [6]. In these settings, chronic infection with liver flukes such as *Opisthorchis viverrini* is considered a major etiological factor, as sustained biliary inflammation promotes carcinogenesis. In contrast, CCA incidence in Western countries remains lower (approximately 0.5 - 3.4 per 100,000 population), although a gradual upward trend has also been observed. In the United States and several European countries, accumulating data indicate a rise in iCCA-related incidence and mortality, whereas pCCA and dCCA mortality rates have remained relatively stable [3].

2.2. Risk Factors for Cholangiocarcinoma

CCA development is influenced by multiple intrinsic and extrinsic factors, including hepatobiliary disorders, chronic inflammation, metabolic diseases, and environmental exposures.

Biliary tract disorders: Chronic biliary diseases are among the most important risk factors for CCA. PSC, a chronic immune-mediated cholangiopathy, is strongly associated with pCCA; patients with PSC have an approximately 400-fold increased risk of developing CCA compared with the general population [7]. Congenital biliary anomalies, such as choledochal cysts, and hepatolithiasis are also recognized risk factors, particularly in Asian populations [8].

Chronic liver disease and cirrhosis: Cirrhosis represents a major risk factor for iCCA. Persistent liver injury, inflammation, and fibrogenesis create a permissive microenvironment for malignant transformation. Epidemiological studies suggest that the relative risk of iCCA in patients with cirrhosis is markedly increased [4]. In addition, NAFLD and its progressive form, non-alcoholic steatohepatitis (NASH), have been increasingly implicated in iCCA development, particularly in individuals with obesity and type 2 diabetes.

Viral hepatitis: Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) is well established as a major risk factor for hepatocellular carcinoma, and growing evidence indicates an association with iCCA as well [9] [10]. HBV- and HCV-infected individuals appear to have an increased risk of developing iCCA compared with uninfected populations.

Metabolic disorders: The rising prevalence of metabolic syndrome may contribute to the increasing incidence of CCA. Type 2 diabetes mellitus has been associated with elevated iCCA risk, and epidemiological studies suggest a modest but significant risk increase [11]. Obesity and fatty liver disease have also been implicated as important risk factors [12].

Environmental exposures: Alcohol consumption has been associated with increased iCCA risk, particularly in individuals with underlying liver disease. Smoking has likewise been identified as a contributory exposure factor [10].

Parasitic infections: In several Asian countries, liver flukes and Clonorchis

sinensis are major etiological agents of CCA. Chronic biliary infection induces sustained inflammation and epithelial injury, thereby facilitating carcinogenesis. Epidemiological data suggest that approximately 10% of individuals with long-standing liver fluke infection may eventually develop CCA [13].

3. Molecular Mechanisms of Cholangiocarcinoma

CCA development represents a multistep process driven by diverse molecular events, with pronounced heterogeneity at genomic, epigenomic, and microenvironmental levels. Accumulating evidence indicates that genetic alterations, epigenetic reprogramming, chronic inflammation-associated tumor microenvironments, and immune dysregulation collectively promote CCA initiation and progression. Importantly, CCAs arising from distinct anatomical sites and histological subtypes display substantial molecular differences, which likely underlie variability in clinical behavior and therapeutic responsiveness.

3.1. Key Driver Genes and Dysregulated Signaling Pathways

Large-scale genomic sequencing studies have identified multiple driver mutations and pathway alterations in CCA, involving the RAS-RAF-MEK-ERK, PI3K-AKT-mTOR, TP53/cell-cycle regulation, TGF-β/SMAD, and chromatin remodeling pathways [14]. The distribution of these alterations varies significantly across CCA subtypes. In iCCA, particularly the small-duct subtype, IDH1/2 mutations and FGFR2 fusions are frequently observed, often accompanied by alterations in chromatin remodeling genes such as BAP1, ARID1A, and PBRM1 [15] [16]. In contrast, large-duct iCCA as well as pCCA and dCCA more commonly harbor KRAS, TP53, and SMAD4 alterations, resulting in molecular profiles that resemble pancreatic ductal adenocarcinoma-like phenotypes [17]. These distinct mutational spectra support the concept that CCA comprises a group of biologically diverse tumor entities rather than a single disease.

3.2. Epigenetic Alterations and Dysregulated Transcriptional Programs

Beyond somatic mutations, epigenetic abnormalities play important roles in CCA initiation and progression. Aberrant DNA methylation [18], altered histone modifications [19], and chromatin remodeling changes are frequently observed and may lead to silencing of tumor suppressor genes or activation of oncogenic transcriptional programs. IDH1/2 mutations represent a critical link between metabolic dysregulation and epigenetic reprogramming [20]. Mutant IDH enzymes produce 2-hydroxyglutarate (2-HG), which inhibits α-ketoglutarate-dependent dioxygenases and results in widespread DNA and histone hypermethylation, thereby disrupting normal biliary epithelial differentiation and facilitating tumorigenesis. In addition, dysregulated non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have been implicated in CCA proliferation, invasion, and therapeutic resistance [21] [22].

3.3. Chronic Inflammation and the Tumor Microenvironment

Chronic inflammation is widely considered a key driver of CCA development, particularly in clinical contexts such as PSC, liver fluke infection, and prolonged cholestasis [23]. Persistent biliary injury and regeneration increase oxidative stress and DNA damage, accompanied by sustained activation of pro-inflammatory signaling pathways, including interleukin-6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa B (NF-κB), thereby creating a permissive environment for malignant transformation [24]. CCA is also characterized by a prominent desmoplastic tumor microenvironment. Tumor-associated fibroblasts, immunosuppressive macrophages, and abundant extracellular matrix components collectively promote tumor growth and dissemination and contribute to resistance to chemotherapy and targeted therapies [25].

These components not only support tumor cell proliferation but also play critical roles in tumor dissemination and metastasis. Cancer-associated fibroblasts (CAFs) facilitate tumor cell migration and invasion through the secretion of various cytokines, chemokines, and extracellular matrix components. Tumor-associated macrophages (TAMs), in turn, suppress antitumor immune responses by releasing immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), thereby further promoting tumor growth and metastatic progression.

Immune cell populations within the tumor microenvironment exert profound effects on cancer development, particularly through the regulation of T-cell subsets and macrophage phenotypes. In cholangiocarcinoma, T cells—especially regulatory T cells (Tregs)—play a pivotal role in immune evasion. Tregs facilitate tumor immune escape by suppressing the activity of other immune effector cells, including cytotoxic T lymphocytes. Meanwhile, TAMs predominantly exhibit a pro-tumorigenic phenotype, contributing to tumor growth and metastasis through multiple mechanisms and exerting a substantial impact on resistance to chemotherapy and targeted therapies. A more comprehensive understanding of these specific immune cell populations will therefore provide important insights into the immune evasion mechanisms of cholangiocarcinoma and may identify novel targets for immunotherapeutic intervention.

Overall, chronic inflammation and the tumor microenvironment interact in a self-reinforcing vicious cycle that drives cholangiocarcinoma initiation and progression. Consequently, elucidating the roles of these microenvironmental factors and their underlying mechanisms is of considerable clinical significance for the development of novel therapeutic strategies, particularly for achieving breakthroughs in immunotherapy.

3.4. Dysregulated Developmental Pathways and Phenotypic Plasticity

Multiple studies indicate that CCA progression is accompanied by aberrant activation of developmental signaling pathways, including Notch, Wnt/β-catenin, Hedgehog, and Hippo-Yes-associated protein (YAP)/transcriptional coactivator

with PDZ-binding motif (TAZ) pathways [26]-[28]. These pathways are essential for bile duct development and epithelial differentiation, and their dysregulation may promote malignant transformation, epithelial-mesenchymal transition (EMT), and maintenance of tumor stemness, thereby enhancing invasive and metastatic potential [29]. Importantly, activation of these developmental pathways appears to be subtype- and site-specific rather than uniformly distributed across all CCAs, providing a biological basis for molecular classification.

4. Molecular Subtypes

CCA exhibits marked heterogeneity at both anatomical and molecular levels. According to the site of origin and molecular features, CCA is generally categorized into iCCA, pCCA, and dCCA. In addition, iCCA can be further divided into large-duct and small-duct types based on molecular and histological characteristics [30]. These subtypes differ systematically in driver alterations, transcriptional programs, and tumor microenvironmental composition, which substantially influence clinical behavior and therapeutic responsiveness.

4.1. Intrahepatic Cholangiocarcinoma

iCCA is the most common CCA subtype, with a sustained increase in incidence worldwide, particularly in Western countries. iCCA arises within the intrahepatic bile duct system and is frequently associated with chronic liver disease backgrounds (e.g., cirrhosis and NAFLD). At the molecular level, large-duct iCCA is more common in patients with cirrhosis and is characterized by alterations in TP53, KRAS, and SMAD4, with an overall molecular profile resembling pancreatic ductal adenocarcinoma-like phenotypes [31]. Clinically, this subtype often demonstrates aggressive behavior and relative tolerance to conventional chemotherapy. In contrast, small-duct iCCA is enriched for IDH1/2 mutations and FGFR2 fusions [32], frequently accompanied by chromatin remodeling gene alterations. These features provide actionable therapeutic entry points, enabling a subset of patients to benefit from FGFR or IDH inhibitors.

4.2. Perihilar Cholangiocarcinoma

pCCA occurs at the hepatic hilum and is often presented with symptoms related to biliary obstruction. Its molecular landscape differs from iCCA, with common driver events including KRAS mutations, TP53 inactivation, and CDKN2A (p16) loss [31]. Alterations in HRAS and SMAD4 are also observed at relatively high frequencies. Epidemiologically, pCCA in Asia has been closely linked to liver fluke infection (e.g., Opisthorchis viverrini and Clonorchis sinensis). Overall, pCCA tends to exhibit aggressive biological behavior, lower respectability, poorer prognosis, and considerable heterogeneity in response to systemic therapies.

4.3. Distal Cholangiocarcinoma

dCCA primarily arises in the distal bile duct, often involving the common bile

duct or periampullary region. Although its molecular features partially overlap with pCCA and iCCA, dCCA more closely resembles pancreatic ductal adenocarcinoma at the molecular level. MAPK pathway-related alterations such as KRAS and BRAF mutations are commonly detected, and this "pancreatic-like" background has implications for systemic treatment strategies. Transcriptomic studies suggest that a subset of dCCAs may exhibit enrichment of mesenchymal-like or EMT-related expression programs; however, this pattern more likely reflects phenotypic characteristics rather than a specific oncogenic driver mechanism [33].

4.4. Clinical Implications of Molecular Subtypes

Molecular subtyping has important implications for prognostic assessment and treatment selection in CCA. iCCA, particularly small-duct iCCA, is more likely to benefit from FGFR2- or IDH-targeted therapies [34] [35], whereas large-duct iCCA and extrahepatic CCAs tend to demonstrate relative tolerance to conventional chemotherapy [36]. Therefore, molecular subtype-informed precision strategies are essential for optimizing clinical outcomes in CCA.

5. Clinical Treatment

CCA is characterized by molecular and clinical heterogeneity; treatment selection should integrate anatomical subtype, disease stage, molecular features, and patient performance status. Because most patients are diagnosed at advanced stages, conventional modalities offer limited overall benefit, and systemic therapy remains challenging. With advances in molecular classification and precision diagnostics, biomarker-driven decision-making is increasingly reshaping clinical management of CCA.

5.1. Surgical Treatment

According to the latest clinical practice guidelines from the NCCN and EASL, surgical resection remains the only potentially curative treatment for patients with resectable cholangiocarcinoma, particularly those with early-stage and localized disease [37]. For iCCA and pCCA, the primary goal is complete tumor removal with negative margins (R0 resection). Common procedures include hepatectomy, extended liver resection, and bile duct resection [38]. In pCCA, surgery often requires combined hilar bile duct resection and hepatic resection, and in some cases complex biliary reconstruction [39]. However, only a minority of patients are eligible for curative resection due to advanced-stage presentation. For unresectable disease, palliative procedures and medical management aim to relieve biliary obstruction, improve quality of life, and modestly prolong survival [40].

5.2. Chemotherapy and Radiotherapy

For unresectable advanced CCA, chemotherapy and radiotherapy remain major systemic treatment modalities [40]. Gemcitabine plus cisplatin is the most widely used first-line regimen and is broadly applied in advanced or recurrent iCCA and

pCCA [41]. Although this combination provides tumor shrinkage in some patients, overall efficacy is limited and acquired resistance commonly develops [36]. Radiotherapy is primarily used as an adjunct modality, particularly in locally advanced disease or after surgery with positive margins, often combined with chemotherapy to improve local control. Nevertheless, curative effects are limited by tumor biology and proximity to critical organs, and treatment-related toxicities may include hepatic dysfunction and gastrointestinal adverse events.

5.3. Targeted Therapy

With increasing understanding of CCA molecular features, targeted therapy directed against specific driver alterations has become an important component of systemic treatment. Recent studies demonstrate that a subset of patients harbor actionable molecular changes, providing a biological rationale for targeted drug development and clinical use.

FGFR2 inhibitors: FGFR2 fusions are present in approximately 10% - 20% of iCCA cases. FGFR inhibitors such as infigratinib and pemigatinib have shown favorable objective response rates and disease control in clinical studies, indicating that molecularly selected populations can achieve meaningful benefit from targeted therapy [42].

IDH1/2 inhibitors: For patients with IDH1/2 mutations, IDH inhibitors such as ivosidenib can modulate aberrant metabolic pathways and delay disease progression, demonstrating clinical benefit in selected patients [42].

Although FGFR2 fusion- and IDH1/2 mutation-targeted therapies have been extensively investigated and increasingly applied in small-duct intrahepatic cholangiocarcinoma (iCCA), therapeutic strategies targeting other driver mutations that are more prevalent in large-duct iCCA and extrahepatic cholangiocarcinoma (pCCA/dCCA), such as KRAS and TP53, remain largely exploratory and lack robust clinical evidence.

KRAS mutations are widely observed across multiple solid tumor types and are particularly common in extrahepatic cholangiocarcinoma, including pCCA and dCCA, where they are generally associated with increased tumor aggressiveness and poor prognosis. In recent years, targeted therapies directed against KRAS mutations—most notably KRAS G12C inhibitors—have demonstrated encouraging activity in several malignancies. Representative agents such as sotorasib have shown clinical efficacy in lung cancer and other solid tumors. However, clinical data regarding the application of KRAS-targeted therapies in cholangiocarcinoma remain limited, and their therapeutic efficacy, safety profile, and mechanisms of resistance require further validation in prospective clinical studies.

TP53 is a critical tumor suppressor gene, and TP53 mutations are frequently detected in large-duct iCCA as well as extrahepatic cholangiocarcinoma (pCCA and dCCA). These alterations are typically associated with high tumor aggressiveness, enhanced biological invasiveness, and multidrug-resistant phenotypes. To date, no direct targeted therapies against TP53 mutations have been approved for

clinical use. Current research efforts have therefore focused on indirect approaches aimed at restoring or modulating TP53 function, including small-molecule regulators, gene-based therapies, and combination strategies integrating chemotherapy or immunotherapy. These exploratory approaches may offer novel therapeutic opportunities for patients with TP53-mutant cholangiocarcinoma.

Overall, while targeted therapies have expanded the systemic treatment landscape for cholangiocarcinoma, the pronounced molecular heterogeneity of this disease limits the efficacy of single-agent targeted approaches. Consequently, combination treatment strategies tailored to distinct molecular profiles are being actively investigated, with the goal of improving therapeutic outcomes and delaying the emergence of treatment resistance.

5.4. Immunotherapy: The TOPAZ-1 Trial Driving a Paradigm Shift in Treatment Standards

In recent years, immunotherapy has achieved breakthrough progress in the treatment of cholangiocarcinoma, particularly through the successful application of immune checkpoint inhibitors in combination with chemotherapy. This strategy has introduced a new paradigm for systemic treatment of advanced disease. Cholangiocarcinoma has long been regarded as an "immunologically cold tumor", and monotherapy with immune checkpoint inhibitors has demonstrated limited efficacy in unselected molecular subgroups. However, the emergence of combination treatment strategies has substantially altered this therapeutic landscape.

The TOPAZ-1 phase III randomized controlled trial represents a landmark study in the field of immunotherapy for cholangiocarcinoma. This trial enrolled patients with previously untreated advanced or unresectable biliary tract cancers, including intrahepatic, perihilar, and distal cholangiocarcinoma, and compared durvalumab (a PD-L1 inhibitor) in combination with gemcitabine plus cisplatin versus chemotherapy alone. The results demonstrated that, compared with chemotherapy alone, the combination regimen achieved a statistically significant and clinically meaningful improvement in OS, without a notable increase in the incidence of severe adverse events [43].

Long-term follow-up analyses further revealed that durvalumab combined with chemotherapy not only improved median OS but also significantly increased the proportion of long-term survivors, suggesting that immunotherapy may confer durable benefit in a subset of patients. Based on the positive outcomes of the TOPAZ-1 trial, durvalumab in combination with gemcitabine and cisplatin has been approved by regulatory agencies in multiple countries and has become the standard of care for first-line treatment of advanced or metastatic cholangiocarcinoma. This advancement marks the formal entry of cholangiocarcinoma systemic therapy into the era of "immunotherapy plus chemotherapy" and represents a major update to the traditional chemotherapy-only treatment paradigm.

Notably, unlike immunotherapy trials in several other solid tumors, the TOPAZ-1 study did not restrict patient enrollment based on PD-L1 expression or

microsatellite instability (MSI) status, indicating that the observed benefit of this combination regimen is not confined to specific biomarker-defined subgroups. Nevertheless, substantial inter-individual variability in treatment response persists, and factors such as the tumor microenvironment, molecular subtypes, and immune evasion mechanisms are likely to play critical roles in modulating therapeutic efficacy.

Current research efforts are increasingly focused on combination strategies integrating immunotherapy with molecularly targeted therapies, locoregional treatments (such as radiotherapy or hepatic arterial therapies), and other immune-modulating approaches, with the aim of further enhancing response rates and overcoming primary or acquired resistance. Overall, the TOPAZ-1 trial and its clinical translation represent a fundamental shift in the treatment paradigm for advanced cholangiocarcinoma and provide a robust foundation for the ongoing optimization of immunotherapy-based strategies in this disease.

6. Prognosis and Biomarkers

CCA is associated with poor prognosis, largely due to limited opportunities for early detection and curative treatment, with many patients presenting at advanced stages. Although therapeutic modalities continue to evolve, OS remains unsatisfactory. Therefore, identifying prognostic indicators and biomarkers—particularly those enabling early detection through molecular diagnostics—is critical for improving clinical outcomes. This section summarizes prognostic factors and relevant biomarkers in CCA

6.1. Prognostic Factors

CCA prognosis is influenced by multiple factors, including anatomical subtype, molecular features, hepatic functional reserve, treatment modality, and overall patient condition. Early diagnosis and surgical resection remain the most important determinants of outcome; patients undergoing curative resection generally achieve significantly higher 5-year survival than those with unresectable advanced disease. However, resection opportunities are limited because many cases are diagnosed late.

Anatomical site: iCCA, pCCA, and dCCA differ in biological behavior and prognosis. iCCA generally exhibits poorer outcomes, whereas prognosis of pCCA and dCCA is closely linked to resectability and margin status.

Stage and pathology: Tumor stage is a key prognostic determinant. Localized disease (e.g., early iCCA) may achieve better outcomes with surgery, whereas advanced or metastatic disease is associated with inferior survival.

Liver function: Because CCA often involves the biliary system and/or liver, hepatic function strongly impacts prognosis and postoperative recovery. Patients with cirrhosis or liver failure experience higher complication risks and poorer tolerance to treatment.

Treatment modality: Surgery offers the only chance for cure but recurrence

rates remain high. Chemotherapy and radiotherapy provide modest survival extension in advanced disease. Consequently, targeted therapies and immunotherapies may represent important opportunities for selected patients.

6.2. Biomarkers

With advances in molecular biology, increasing numbers of biomarkers have been proposed for early diagnosis, molecular stratification, prognostic assessment, and treatment decision-making in CCA. Common candidates include serum markers (e.g., CA19-9), miRNAs, somatic mutations/fusions, exosome-derived signatures, and epigenetic alterations.

CA19-9: CA19-9 is one of the most widely used serum biomarkers in CCA for screening and monitoring. Although sensitivity and specificity are limited—particularly for early detection—elevated CA19-9 levels are often associated with disease progression and unfavorable prognosis [44].

miRNAs: miRNAs are non-coding RNAs that regulate gene expression and contribute to tumorigenesis and metastasis. Specific miRNA signatures have been proposed as biomarkers in CCA. For example, miR-21, miR-29a, and miR-200c show altered expression in CCA and may serve as prognostic indicators and predictors of chemoresistance [45].

Somatic mutations and gene fusions: With widespread application of next-generation sequencing (NGS), multiple recurrent genomic alterations have been identified, including FGFR2 fusions, IDH1/2 mutations, KRAS mutations, and TP53 mutations. These changes correlate with malignant phenotypes and prognosis. Patients with FGFR2 fusions or IDH1/2 mutations may be more sensitive to targeted therapies or metabolic interventions [46], whereas KRAS and TP53 mutations are frequently associated with poorer outcomes [17].

In liquid biopsy, circulating tumor DNA (ctDNA) and exosomes have demonstrated significant clinical value in the diagnosis and therapeutic monitoring of cholangiocarcinoma owing to their noninvasive nature and rich molecular information content. Exosomes are nanoscale membrane-bound vesicles actively secreted by tumor cells and are enriched with proteins, lipids, and various nucleic acids, making them important carriers for tumor biomarker research. Recent studies have shown that exosomes derived from patients with cholangiocarcinoma contain abundant molecular information, including microRNAs (miRNAs), messenger RNAs (mRNAs), and proteins, which can be exploited for early tumor detection, monitoring of treatment response, and prognostic evaluation. Moreover, exosomes can promote tumor progression by remodeling the tumor microenvironment, facilitating immune evasion, and enhancing tumor invasion, thereby contributing to metastatic dissemination. Consequently, exosomes are considered promising biomarkers for assessing immunotherapy efficacy and disease progression [47] ctDNA refers to fragments of DNA released into the peripheral circulation by tumor cells and carries tumor-specific genetic alterations or aberrant DNA methylation patterns, enabling a relatively accurate reflection of the tumor's ge-

nomic landscape. Compared with conventional tissue biopsy, ctDNA analysis offers several advantages, including minimal invasiveness, high sensitivity and specificity, and the ability to perform dynamic longitudinal monitoring. ctDNA profiling can be used not only for early detection of tumor-associated genomic alterations and real-time monitoring of disease progression, but also for identifying key driver mutations in cholangiocarcinoma, such as KRAS, TP53, IDH1/2, and FGFR2. These data provide an important basis for molecular classification and the development of targeted therapeutic strategies. In addition, changes in ctDNA levels are closely correlated with tumor burden and clinical outcomes, with persistent elevation often indicating disease progression or recurrence. In recent years, ctDNA methylation analysis has attracted increasing attention, and accumulating evidence suggests that aberrant methylation patterns are closely associated with cholangiocarcinoma initiation, progression, and poor prognosis, highlighting their potential utility in early diagnosis, treatment response evaluation, and recurrence monitoring [18].

Overall, ctDNA and exosomes exhibit distinct yet complementary strengths in liquid biopsy applications. ctDNA primarily provides precise information on tumor-specific genetic and epigenetic alterations and serves as a critical tool for molecular stratification and targeted therapy decision-making. In contrast, exosomes are particularly well suited for biomarker discovery, investigation of immune evasion mechanisms, and evaluation of therapeutic response. The combined application of ctDNA and exosome-based analyses is expected to yield a more comprehensive molecular portrait of tumors, thereby improving the sensitivity and accuracy of liquid biopsy for the diagnosis and monitoring of cholangiocarcinoma.

6.3. Prognostic Models and Clinical Application

Although numerous biomarkers have been proposed for prognostic evaluation in CCA, standardized clinical criteria are lacking. With advances in molecular diagnostics, integrative assessment models incorporating multiple biomarkers may become feasible. For example, combining CA19-9, miRNA profiles, and somatic alterations may enable multiparametric prediction of survival, treatment response, and recurrence risk. Additionally, integrating imaging modalities (e.g., CT and MRI) with molecular biomarkers and liquid biopsy may improve evaluation of tumor extent, recurrence risk, and therapeutic efficacy.

6.4. Future Directions

With continued development of precision medicine, biomarker-driven treatment selection is expected to play an increasingly central role in CCA management. Future work should focus on identifying additional biomarkers linked to immune evasion, tumor metabolism, and microenvironmental regulation. Furthermore, integrating single-cell technologies and liquid biopsy approaches may enable more comprehensive, individualized prognostic assessment, thereby facilitating earlier detection and more precise therapeutic strategies.

7. Conclusions

Cholangiocarcinoma is a malignancy with pronounced molecular diversity and heterogeneous clinical behavior. Its development and progression involve multi-layered genetic and epigenetic alterations as well as complex regulation by the tumor microenvironment. Although notable advances have been achieved in molecular classification, targeted therapies, and immunotherapies, CCA—particularly in advanced-stage patients—continues to face major challenges, including limited therapeutic options and prominent resistance mechanisms.

With the evolution of precision medicine, molecular feature-guided patient stratification and individualized treatment strategies are increasingly important for clinical management of CCA. Future research should further dissect key oncogenic pathways and resistance mechanisms and validate novel combination strategies through high-quality, multicenter clinical studies, ultimately supporting continuous optimization of CCA diagnostic and therapeutic paradigms.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

Cholangiocarcinoma (CCA); Intrahepatic cholangiocarcinoma (iCCA); Perihilar cholangiocarcinoma (pCCA); Distal cholangiocarcinoma (dCCA); Primary sclerosing cholangitis (PSC); Non-alcoholic fatty liver disease (NAFLD); Non-alcoholic steatohepatitis (NASH); Hepatitis B virus (HBV); Hepatitis C virus (HCV); Epithelial-mesenchymal transition (EMT); Tumor mutational burden (TMB); Microsatellite instability (MSI); Microsatellite instability-high (MSI-H); Next-generation sequencing (NGS); Fibroblast growth factor receptor 2 (FGFR2); Isocitrate dehydrogenase 1/2 (IDH1/2); Programmed cell death protein 1 (PD-1); Programmed death-ligand 1 (PD-L1); Interleukin-6 (IL-6); Signal transducer and activator of transcription 3 (STAT3); Nuclear factor kappa B (NF-κB); Yes-associated protein (YAP); Transcriptional coactivator with PDZ-binding motif (TAZ); Cancer-associated fibroblasts (CAFs); Tumor-associated macrophages (TAMs); Regulatory T cells (Tregs); Circulating tumor DNA (ctDNA); Overall survival (OS).