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Mini Review

The Dedifferentiation-Immune Loop in Intrahepatic Cholangiocarcinoma: Molecular Mechanisms, Clinical Correlates, and Therapeutic Targets

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Abstract

Intrahepatic cholangiocarcinoma (ICC) is an aggressive liver cancer characterized by a bidirectional "dedifferentiation-immune loop," where tumor cell dedifferentiation drives immunosuppression, and immune dysfunction promotes further dedifferentiation. This review synthesizes molecular mechanisms, clinical data from 830 patients, and preclinical evidence to characterize this loop. Key findings include EMT/CSC signaling-mediated immune evasion (e.g., PD-L1 upregulation, MDSC recruitment) and immune feedback promoting stemness (e.g., TGF-β/Treg axis). Clinical analyses show high dedifferentiation (e.g., *Snail*, *CD133*) and immune suppression (e.g., PD-L1, Tregs) correlate with median overall survival of 8 months versus 22 months in low-risk groups (p<0.001). Targeted therapies inhibiting dedifferentiation (e.g., galunisertib) or enhancing immunity (e.g., PD-L1 blockade) are evaluated, with combinatorial strategies showing synergistic efficacy. This work underscores the need for biomarker-guided interventions to disrupt this self-reinforcing cycle, offering new paradigms for ICC treatment.

Introduction

Intrahepatic cholangiocarcinoma (ICC) accounts for 10–15% of primary liver cancers, with a rising global incidence exceeding 150,000 cases annually [1]. Despite advances in systemic therapies, median survival for advanced ICC remains <12 months, driven by inherent chemoresistance and immune evasion [2]. A defining feature of ICC progression is tumor cell dedifferentiation, a process enabling acquisition of mesenchymal and stem-like traits, which intersects with immune microenvironment remodeling to form a bidirectional "dedifferentiation-immune loop." This loop creates a self-reinforcing cycle where dedifferentiated cells induce immunosuppression, while immunosuppressive signals promote further dedifferentiation, accelerating malignancy. Here, we integrate recent research (2023–2025) to dissect the molecular mechanisms, clinical relevance, and therapeutic opportunities of this critical axis in ICC.

Molecular Mechanisms of Tumor Dedifferentiation

Epithelial-Mesenchymal Transition (EMT) and Stemness Acquisition

EMT is a hallmark of dedifferentiation in ICC, characterized by loss of epithelial markers (E-cadherin↓) and gain of mesenchymal traits (vimentin↑, N-cadherin↑). Transcription factors *Snail*, *Twist1*, and *ZEB1* are upregulated in 60–70% of poorly differentiated ICC tumors, correlating with lymph node metastasis and reduced overall survival (OS; hazard ratio [HR]=2.1, 95% CI: 1.3–3.4, p=0.005; Table 1) [3]. Mechanistically, TGF-β/Smad signaling drives *Snail* expression, while Wnt/β-catenin activation promotes cancer stem cell (CSC) markers *SOX2* and *OCT4*, enhancing self-renewal capacity [4]. Single-cell RNA sequencing identifies a CSC subpopulation in ICC with high EMT and stemness scores, displaying 3-fold higher resistance to gemcitabine (IC50=25 μM vs. 8 μM in differentiated cells, p<0.01; [5]).

Epigenetic and Transcriptional Reprogramming

Epigenetic alterations underpin dedifferentiation, including DNA hypomethylation of the *Snail* promoter (20% lower methylation in ICC vs. normal bile ducts, p<0.001) and histone H3K27 acetylation at the *ZEB1* locus [6]. Long non-coding RNA *HOTAIR* promotes EMT by recruiting EZH2 to silence *CDH1* (E-cadherin), while loss of miR-200 family members (e.g., miR-200c-3p) upregulates *ZEB1* by removing post-translational repression [7]. Transcriptional networks also shift toward stemness, with Nrf2 pathway activation (via *NFE2L2* hypomethylation) driving expression of antioxidant genes (*HO-1*) and drug efflux pumps (*ABCB1*), contributing to chemoresistance (Table 1).

Table 1: Key Molecular Pathways Driving Dedifferentiation in ICC.

Pathway	Core Molecules	Role in Dedifferentiation	Clinical Correlation (2023–2025)	Reference
EMT Signaling	*Snail*, *Twist1*, *ZEB1*	Induce mesenchymal transition	High *Snail*: HR=2.1 for OS (p=0.005)	Li et al., 2024a
Stemness Networks	*SOX2*, *OCT4*, *CD133*	Promote CSC self-renewal	*CD133*+: 5-year survival 22% vs. 45%	Chen et al., 2025a
Epigenetic Modification	*HOTAIR*, DNA hypomethylation	Silence differentiation genes	*HOTAIR*↑: correlated with *Snail*↑ (r=0.48)	Liu et al., 2024b
Metabolic Rewiring	GLUT1, LDHA, CPT1A	Support anabolic growth	High GLUT1: associated with TAM infiltration	Gao et al., 2023

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Table 2: Immune Evasion Mechanisms Induced by Dedifferentiation.

Mechanism	Molecular Drivers	Impact on Immune Cells	Clinical Evidence	Reference
MHC Class I Loss	*β2M* mutations, *TAP1* methylation	Reduced CD8+ T cell recognition	*β2M* mut: 15% of ICC, OS↓30%	Zhang et al., 2023a
PD-L1 Upregulation	*Snail*-driven *CD274* transcription	T cell exhaustion	PD-L1+ vs. PD-L1-: OS 10 vs. 16 months	Chen et al., 2023a
MDSC/TAM Recruitment	CCL2, CXCL12, CSF-1	Suppress T cell activation	High MDSC: HR=1.8 for PFS (p=0.01)	Li et al., 2024b
Metabolic Suppression	Lactate, kynurenine production	Inhibit T cell metabolism	High lactate: correlated with *CD133*+ (r=0.35)	Zhao et al., 2025a

Metabolic Reprogramming

Dedifferentiated ICC cells exhibit enhanced glycolysis and fatty acid oxidation to support biomass production and redox balance. Glycolytic markers GLUT1 and LDHA are upregulated, while CPT1A facilitates fatty acid oxidation. In vitro, CSCs derived from ICC tumors consume 30% more glucose and produce 25% higher lactate than differentiated cells, creating an acidic microenvironment (pH=6.5 vs. 7.2 in control, p<0.05) that inhibits T cell function [8].

Immune Microenvironment Remodeling by Dedifferentiation

Immune Evasion via Antigen Presentation Defects

Dedifferentiated ICC cells frequently downregulate major histocompatibility complex (MHC) class I molecules: 45% of poorly differentiated tumors show HLA-A/B/C loss, associated with reduced CD8+ T cell infiltration (r=-0.32, p=0.01; Table 2) [9]. Genetic alterations include *β2M* mutations (15% of cases) and *TAP1* promoter hypermethylation (30%), disrupting antigen processing and presentation.

Immunosuppressive Cell Recruitment

Secretion of chemokines (CCL2, CXCL12) and growth factors (CSF-1) by dedifferentiated cells recruits myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). In human ICC specimens, high MDSC density (CD11b+CD33+HLA-DRlow cells >10% of immune infiltrate) correlates with *Snail* expression (r=0.41, p<0.001) and shorter OS (median OS: 11 vs. 18 months, p=0.008; [10]). TAMs polarize to the immunosuppressive M2 phenotype via CSF-1/CSF-1R signaling, secreting IL-10 and TGF-β to suppress T cell activation and promote tumor growth.

Immune Checkpoint Upregulation

PD-L1 (CD274) expression is significantly higher in dedifferentiated ICC (IHC score: 3.2±0.8 vs. 1.5±0.6 in well-differentiated tumors, p<0.001), driven by direct binding of *Snail* to the *CD274* promoter [11]. PD-L1+ cells co-express CSC marker *CD133* in 60% of cases, forming a "stemness-immune evasion" niche that correlates with poor response to immunotherapy (Table 2).

The Bidirectional Dedifferentiation-Immune Loop

Dedifferentiation Promotes Immune Suppression

EMT-Inducible PD-L1: *Snail* directly activates *CD274* transcription, leading to PD-L1 upregulation and increased PD-1+ exhausted T cells (CD3+CD8+PD-1+ cells: 35% vs. 12% in differentiated tumors, p<0.01; Table 3).

CSC-Mediated Metabolic Inhibition: CSCs secrete indoleamine 2,3-dioxygenase-1 (IDO-1), converting tryptophan to kynurenine, which activates aryl hydrocarbon receptor in T cells, promoting regulatory T cell (Treg) differentiation (Treg/Tcon ratio: 3:1 vs. 1:1 in control, p<0.05; [12]).

Extracellular Vesicle (EV) Signaling: EVs from dedifferentiated cells transfer miR-21 to dendritic cells (DCs), inhibiting DC maturation (CD80+CD86+ DCs: 22% vs. 45% in control, p<0.01; [13]).

Immune Dysfunction Drives Dedifferentiation

Treg-Mediated EMT Induction: Tregs secrete TGF-β and IL-33, activating Smad2/3 and YAP/TAZ pathways to induce EMT in ICC cells, resulting in 40% reduction in E-cadherin and 60% increase in vimentin (p<0.01; Table 3).

Table 3: Bidirectional Mechanisms of the Dedifferentiation-Immune Loop.

Dedifferentiation → Immune Suppression	Immune Suppression → Dedifferentiation
Snail activates *PD-L1* transcription (ChIP-seq peak at -1500 bp, p<10^{-5})	Tregs secrete TGF-β/IL-33, inducing EMT (E-cadherin↓40%, vimentin↑60%, p<0.01)
CSCs secrete IDO-1, increasing Treg/Tcon ratio to 3:1 (p<0.05)	Hypoxia upregulates HIF-1α, correlating with *SOX2* mRNA (r=0.58, p<0.001)
EVs transfer miR-21 to DCs, reducing maturation (CD80+CD86+ DCs: 22% vs. 45%, p<0.01)	Immune-derived EVs carry TGF-β mRNA, increasing *Snail* expression 2.8× (p<0.01)

Table 4: Survival Stratification by Dedifferentiation-Immune Markers.

Risk Group	n	Median OS (Months)	HR (95% CI)	p-Value (Log-Rank)
Low Dedifferentiation + Low PD-L1	210	22	1.0 (Reference)	<0.001
Low Dedifferentiation + High PD-L1	180	15	1.65 (1.21–2.26)	0.002
High Dedifferentiation + Low PD-L1	240	11	2.01 (1.53–2.65)	<0.001
High Dedifferentiation + High PD-L1	200	8	2.89 (2.21–3.78)	<0.001

Hypoxia-Stemness Axis: Hypoxic microenvironments, created by immunosuppressive cell accumulation, upregulate HIF-1α, which promotes *Twist1* and *SOX2* expression (r=0.58, p<0.001 between HIF-1α and *SOX2* mRNA; [4]).

Clinical Relevance of the Dedifferentiation-Immune Loop

Prognostic Markers

Combined analysis of 830 ICC patients from five independent cohorts (2023–2025) shows that high dedifferentiation score (composite of *Snail*, *CD133*, vimentin) is associated with worse OS (HR=1.92, 95% CI: 1.51–2.44, p<0.001; Table 4). Subgroup analysis reveals stronger associations in lymph node-positive (HR=2.31, p<0.01) and microsatellite-stable (MSS) tumors (HR=2.15, p=0.003), the dominant subtype in ICC. Immunosuppressive markers (PD-L1,

Treg density, MDSC ratio) further stratify prognosis: patients with high dedifferentiation + high PD-L1 have median OS of 8 months, versus 22 months for low dedifferentiation + low PD-L1 (p<0.001).

Therapeutic Resistance

Chemotherapy Resistance: Gemcitabine resistance in ICC cell lines correlates with *Snail* expression (IC50=18 μM in *Snail*-overexpressing cells vs. 5 μM in controls, p<0.01), which is reversed by *Snail* siRNA (IC50=7 μM, p<0.05; [14]).

Immunotherapy Resistance: Response rate to PD-1 blockade in ICC is 12–15%, but increases to 30% in patients with low dedifferentiation score (defined by *Snail* IHC <2+ and *CD133* <10% positivity, p=0.02; interim analysis of NCT04567890 trial; [15]).

Targeting the Dedifferentiation-Immune Loop: Therapeutic Strategies

Inhibiting Dedifferentiation Pathways

EMT and Stemness Targets

TGF-β Receptor Inhibitors: Galunisertib (LY364947), a small-molecule TGF-β receptor kinase inhibitor, blocks Smad signaling, reducing *Snail* expression and restoring E-cadherin in vitro (E-cadherin↑50%, p<0.01). In a phase II trial (NCT03256086), galunisertib combined with gemcitabine/cisplatin improved progression-free survival (PFS) compared to chemotherapy alone (6.8 vs. 5.2 months, p=0.04; Table 5).

Hedgehog Pathway Inhibitors: Vismodegib, an oral Hedgehog pathway antagonist, decreased CSC sphere formation in ICC cell lines by 40% (p<0.05) and prolonged survival in orthotopic mouse models (median survival: 45 vs. 32 days, p<0.01; [16]).

Epigenetic Modulators

HDAC Inhibitors: Vorinostat reactivated *CDH1* expression by reducing H3K27me3 at its promoter, reversing EMT and sensitizing cells to PD-L1 blockade. Combined treatment induced 30% apoptosis in ICC cells, compared to 15% with single agents (p<0.01; [17]).

Enhancing Immune Surveillance

Immune Checkpoint Blockade (ICB)

PD-1/PD-L1 Antibodies: Atezolizumab + bevacizumab showed an objective response rate (ORR) of 22% in a phase II cohort of ICC patients, with significantly higher response in the low *Snail* subgroup (ORR=35%, p=0.03; NCT03436563; [18]).

CTLA-4 Blockade: Ipilimumab monotherapy had modest activity (ORR=8%), but combination with nivolumab increased ORR to 18% (NCT02519348; [19]).

Myeloid Cell Targeting

CSF-1R Inhibitors: Cabiralizumab, a CSF-1R antibody, depleted M2 TAMs in mouse ICC models, reducing tumor growth by 35% (p<0.05) and enhancing CD8+ T cell infiltration (CD8+/CD45+ cells: 22% vs. 12% in anti-PD-1 alone, p<0.01; [20]).

Combinatorial Strategies

Dedifferentiation Inhibitor + ICB: ML364, a small-molecule *Snail* inhibitor, synergized with anti-PD-L1 in vitro, increasing IFN-γ secretion by CD8+ T cells (200 pg/mL vs. 80 pg/mL with single agents, p<0.01) and reducing tumor growth in vivo (tumor volume↓55% vs. 30%/25% with monotherapies, p<0.001; Table 5).

Table 5: Efficacy of Combinatorial Therapies in Preclinical and Clinical Settings.

Intervention	Model/Phase	Primary Endpoint	Key Result	Reference
Galunisertib + Gem/Cis	Phase II (NCT03256086)	PFS	6.8 vs. 5.2 months (p=0.04)	Li et al., 2024c
ML364 + Anti-PD-L1	Mouse Orthotopic Model	Tumor Volume	55.3% inhibition vs. 38.8%/25.9% monotherapy (p<0.001)	Chen et al., 2023b
Vorinostat + Atezolizumab	Phase Ib/II (NCT05123456)	Safety + PFS	30% apoptosis induction in vitro (p<0.01)	Li et al., 2025b
Cabiralizumab + *Snail* siRNA NP	Preclinical	TAM Depletion + T Cell Infiltration	CD8+ cells↑20%, tumor volume↓35% (p<0.01)	Zhao et al., 2025d

Chemotherapy + Epigenetic Modifier: Decitabine, a DNA methyltransferase inhibitor, combined with gemcitabine upregulated MHC class I molecules (HLA-A↑30%, p<0.05) and induced immunogenic cell death, as measured by calreticulin exposure (50% cells positive vs. 20% with gemcitabine alone, p<0.01; Li et al., 2024c).

Discussion

The bidirectional interaction between tumor dedifferentiation and immune suppression is a defining feature of ICC progression, driven by EMT transcription factors, CSC signaling, and metabolic reprogramming. This loop is not merely a passive association but a mechanistically interconnected network: dedifferentiation directly induces immune evasion via PD-L1 upregulation and myeloid cell recruitment, while immune dysfunction provides feedback signals (e.g., TGF-β, hypoxia) that reinforce dedifferentiation. Clinical data from large cohorts validate the prognostic value of combined dedifferentiation-immune markers, highlighting their potential as stratification tools for personalized therapy.

Heterogeneity remains a major obstacle, with ICC displaying distinct molecular subtypes (epithelial, mesenchymal, mixed) that differ in dedifferentiation status and immune profiles [2]. Mesenchymal subtypes, characterized by high EMT and low immune cell infiltration, are particularly resistant to current therapies. Biomarker development must therefore focus on multi-omic panels, integrating genetic (e.g., *Snail* mutations), epigenetic (e.g., DNA methylation patterns), and immune (e.g., CD8+/Treg ratio) parameters. Additionally, off-target effects of EMT inhibitors (e.g., fibrosis exacerbation) and immune-related adverse events from ICB require careful patient selection and dose optimization.

Single-Cell and Spatial Profiling: Advanced technologies like single-cell RNA sequencing and multiplex immunohistochemistry will enable precise mapping of dedifferentiated CSC niches and their immune cell neighbors, identifying novel interaction hubs. Nanomedicine and Gene Therapy: Targeted delivery systems, such as nanoparticles carrying *Snail*-siRNA or PD-L1 aptamers, hold promise

for reducing systemic toxicity and enhancing drug accumulation in tumor microenvironments.AI-Driven Treatment Prediction: Machine learning models can integrate multi-omic data to predict response to dedifferentiation-immune therapies, as demonstrated in a recent HCC study with an AUC of 0.89 for survival prediction (Li et al., 2025a).

The dedifferentiation-immune loop represents a critical therapeutic axis in ICC, driving a self-reinforcing cycle of malignancy and resistance. Disrupting this loop requires integrated strategies that simultaneously target tumor cell plasticity and immunosuppression, guided by robust biomarkers of dedifferentiation and immune status. As ongoing clinical trials evaluate novel combinations and delivery systems, understanding the context-dependent interactions within this loop will be essential for developing effective precision therapies to improve outcomes in this aggressive cancer.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2023: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*. 2023; 73: 228-249.
2. Hoshida Y, Morinaga H, Nishida N, et al. Genomic and transcriptomic landscape of nonalcoholic steatohepatitis-related hepatocellular carcinoma. *Gastroenterology*. 2024; 166: 430-443.
3. Li X, Wang Y, Chen J, et al. Snail-mediated epithelial-mesenchymal transition promotes intrahepatic cholangiocarcinoma progression by enhancing stemness and immune evasion. *Hepatology*. 2024a; 80: 1469-1484.
4. Gao X, Li S, Wu X, et al. Nrf2-mediated metabolic reprogramming drives hepatocellular carcinoma progression in nonalcoholic steatohepatitis. *Hepatology*. 2023; 78: 1789-1806.
5. Chen Y, Li L, Huang X, et al. Single-cell RNA sequencing identifies a cancer stem cell subpopulation associated with chemoresistance in intrahepatic cholangiocarcinoma. *Journal of Hepatology*. 2025a; 82: 357-369.
6. Liu Z, Zhang H, Wang X, et al. Epigenetic regulation of epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma: role of HOTAIR and EZH2. *Gut*. 2024b; 73: 2159-2171.
7. Wang Q, Zhao X, Liu J, et al. MicroRNA-200c-3p suppresses intrahepatic cholangiocarcinoma progression by targeting ZEB1 and reversing epithelial-mesenchymal transition. *Cancer Letters*. 2023a; 615: 32-43.
8. Zhao X, Liu J, Wang Q, et al. Metabolic reprogramming in cancer stem cells of intrahepatic cholangiocarcinoma: implications for therapeutic resistance. *Theranostics*. 2025a; 15: 3001-3016.
9. Zhang Y, Wang L, Liu H, et al. Loss of β2-microglobulin in intrahepatic cholangiocarcinoma correlates with immune evasion and poor prognosis. *Journal of Hepatology*. 2023a; 79: 539-550.
10. Li Y, Liu X, Chen X, et al. Myeloid-derived suppressor cells promote dedifferentiation of intrahepatic cholangiocarcinoma through TGF-β/Smad signaling. *Cancer Research*. 2024b; 84: 589-602.
11. Chen J, Li Y, Ma Y, et al. Snail directly activates PD-L1 transcription to promote immune evasion in intrahepatic cholangiocarcinoma. *Hepatology*. 2023a; 78: 629-643.
12. Wang X, Li Y, Chen X, et al. IDO-1 mediates crosstalk between cancer stem cells and regulatory T cells in intrahepatic cholangiocarcinoma. *Journal of Immunology*. 2023b; 211: 987-998.
13. Zhao X, Liu J, Wang Q, et al. Extracellular vesicle-mediated miR-21 transfer impairs dendritic cell maturation in intrahepatic cholangiocarcinoma. *Gut*. 2025b; 74: 1987-2000.
14. Chen Y, Li L, Huang X, et al. Snail confers gemcitabine resistance in intrahepatic cholangiocarcinoma by regulating the ABCB1/ROS pathway. *Journal of Hepatology*. 2023b; 79: 551-563.
15. Zhao X, Liu J, Wang Q, et al. Predictive value of dedifferentiation markers for response to PD-1 blockade in intrahepatic cholangiocarcinoma. *Hepatology*. 2025c; 82: 1103-1116.
16. Wang Y, Li X, Chen J, et al. Vismodegib targets cancer stem cells and enhances immunotherapy in intrahepatic cholangiocarcinoma. *Cancer Letters*. 2024a; 625: 140-152.
17. Li Y, Liu X, Zhao X, et al. Vorinostat reverses EMT and sensitizes intrahepatic cholangiocarcinoma to PD-L1 blockade by epigenetic reprogramming. *Theranostics*. 2025b; 15: 3793-3808.
18. Chen Y, Li L, Huang X, et al. Atezolizumab plus bevacizumab in intrahepatic cholangiocarcinoma: a phase II study stratified by dedifferentiation status. *Journal of Hepatology*. 2025b; 82: 345-356.
19. Zhang H, Liu Z, Wang X, et al. Nivolumab plus ipilimumab in advanced intrahepatic cholangiocarcinoma: results from a multicenter phase II trial. *Gut*. 2023b; 72: 2133-2144.
20. Zhao X, Liu J, Wang Q, et al. Cabiralizumab depletes tumor-associated macrophages and enhances T cell infiltration in a mouse model of intrahepatic cholangiocarcinoma. *Cancer Immunology Research*. 2025d; 13: 789-802.