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REVIEW ARTICLE

Multifaceted roles of microbiota-derived deoxycholic acid in gastrointestinal cancers: from barrier disruption to therapeutic implications

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Abstract

Deoxycholic acid (DCA), a microbial-derived secondary bile acid, plays a multifunctional role in gastrointestinal (GI) carcinogenic through various molecular and cellular mechanisms. Mechanistically, DCA causes disruption of epithelial barrier function by occludin, downregulation of claudin-5, and disruption of ERK signaling, increasing permeability and inflammation. DCA initiates DNA damage by reactive oxygen species (ROS), production of hydroxyl radicals, and degradation of p53, triggering Poly (ADP-ribose) polymerase (PARP)-mediated DNA repair signals. DCA triggers pro-oncogenic signaling such as β-catenin, M3 muscarinic receptor (M3R) transactivation of Epidermal Growth Factor Receptor (EGFR), and Nuclear factor kappa B (NF-κB), promoting cell proliferation, synthesis of Mucin 2 (MUC2), and pro-inflammatory cytokine release (e.g., Interleukin-8 (IL-8), Interferon gamma (IFN-γ)). DCA also inhibits antitumor immunity by blocking Ca^{2+}-Nuclear factor of activated T-cell (NFAT) 2 signaling in $CD8^+$ T cells, thus disrupting cytotoxicity. DCA causes intestinal metaplasia and trans-differentiation in gastric and esophageal epithelial cells via KLF Transcription Factor 5 (KLF5)-caudal-related homeobox transcription factor 2 (CDX2) signaling. While acute levels of DCA induce apoptosis by mitochondrial membrane depolarization and caspase-9 activation, chronic accumulation leads to tumorigenesis through chronic inflammation, disruption of barrier function, and immune escape. DCA-heparin conjugates are antiangiogenic and chemo-sensitizing and offer new therapeutic windows. Taken together, these data provide evidence for the dualistic action of DCA and its central position as a microbial metabolite linking diet, barrier function, immunity, and GI carcinogenesis.

Keywords DCA · GI carcinogenesis · Barrier disruption · Apoptosis · Signaling · Immunomodulation · $CD8^+$ T cell inhibition · Therapeutic option

Introduction

The colon and the liver are in dialogue with each other by way of the portal vein, and microbes and microbial products from the intestine are transported directly to the liver, which affects the microenvironment of the liver [1]. Deoxycholic acid (DCA), a microbial product of the major bile acid cholic acid (CA) in the intestine, is known to be involved in the etiology of colorectal and liver cancers [2, 3]. DCA has been shown to induce intestinal metaplasia (IM) markers caudal-related homeobox transcription factor 2 (CDX2) and mucin 2 (MUC2) by activating the nuclear factor-κB (NF-κB) signaling pathway [4]. Subsequent studies have demonstrated that DCA induces the onset of Barrett's esophagus (BE) [5]. This metabolite is recognized as a promoter of colorectal cancer (CRC) by inducing DNA damage and disrupting the integrity of the intestinal mucosal barrier [6]. Clinical

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evidence has detected elevated concentrations of DCA in the serum and feces of CRC patients [7, 8]. Cong et al. [9] findings revealed DCA as a CD8 + T cell anti-cancer suppressor acting by blocking Ca2 + -Nuclear factor of activated T-cell (NFAT) 2 signaling via a plasma membrane Ca^{2+}-ATPase (PMCA)-mediated mechanism. Of particular interest, higher levels of DCA and expression of the bile acid-inducible (bai) operon genes were negatively associated with the proportion of granzyme B (GzmB), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) producing CD8 + T cells in patients with CRC [9]. In addition, mitogen-activated protein kinase (MAPK) signaling pathways, including p38 and Erk1/2, have been found to be involved in mediating IL-8 production induced by DCA in esophageal adenocarcinoma (EAC) cells [10]. Inhibition of p38 MAPK with SB203580 and Protein Kinase A (PKA) with H89 reduced DCA-induced increase of Interleukin (IL)−8 production in a model of human esophageal epithelial cells (HEEC) [11]. Some studies, such as Song et al. [12] and Barrasa et al. [13] found DCA to exhibit dose- and duration-dependent duality where short-term or high-dose exposure caused apoptosis through mitochondrial depolarization, reactive oxygen species (ROS)generation, and activation of caspase-9, but chronic low-to-moderate doses—characteristic of high-fat diet, obesity, or dysbiosis—promoting proliferation, epithelial-to-mesenchymal transition (EMT), and tumor growth through β-catenin–cyclin D1, epidermal growth factor receptor (EGFR)–MAPK, and NF-κB activation and reorganization of the tumor microenvironment [14–16]. Finally, high DCA concentrations—typically due to obesity, dysbiosis, or high-fat diets—have been implicated in CRC and put DCA on the shortlist of candidate biomarkers and therapeutic targets [17, 18]. Analysis of fecal, serum, and tissue samples highlights the role of DCA-producing microbiota and investigates methodologies for regulating DCA concentrations through microbial, dietary, or drug-mediated intervention [19]. These findings highlight the pivotal function of DCA as a microbial metabolite contributing substantially to barrier integrity, apoptosis, signaling cascades, metastasis, and immune regulation of gastrointestinal (GI) cancer progression.

Deoxycholic acid biogenesis and biological functions

Primary bile acids, CA and chenodeoxycholic acid (CDCA), are produced from cholesterol via a multi-step enzymic process that is localized predominantly in the liver [20]. The biosynthesis requires 17 different enzymes and occurs in more than one intracellular compartment, such as the cytosol, endoplasmic reticulum (ER), mitochondria, and peroxisomes [21]. Conjugation of the produced bile acids with glycine or taurine occurs before their secretion and storage in the gallbladder. In human beings, most of the bile acids are reabsorbed in the terminal ileum, but less than 5% of the bile acid pool is transported to the colon every day [20]. Once they arrive in the colon, bile acids are degraded by the colonic bacterial flora, which hydrolyzes CA to the secondary bile acid DCA [20]. DCA is absorbed in part in the colon and then enters the enterohepatic circulation, where it is conjugated in the liver prior to being excreted in bile [22]. Secondary bile acids like DCA are formed mainly in the colon but, via enterohepatic circulation and systemic transport, are found elsewhere in the GI tract, like the bile ducts, gallbladder, stomach, and esophagus; however, their tumorigenic activity is confirmed by experimental and clinical evidence only in some organs, like the colorectum, liver, and esophagus [23].

Cholesterol is an essential component of the plasma membrane, contributing to its function as a selective permeability barrier [24]. As cholesterol derivatives with a detergent nature, bile acids can affect the bilayer permeability of membrane lipids [25]. The hydrophobicity of bile acids increases with an increased ability to destabilize or degrade cell membranes in part [26]. DCA has been particularly recognized to elevate paracellular permeability in a dose-dependent manner [27]. At higher concentrations, DCA induces nonspecific damage to the cell membrane and thereby local damage to the intestinal epithelium [28]. The repair process that follows inflammation and hyperproliferation of undifferentiated cells elevates the risk of developing into precancerous lesions. This colorectal mucosal hyperplasia has been identified as an early occurrence of colorectal carcinogenesis [29]. DCA is generated through a tightly regulated host–microbiota process, but its detergent-like properties can compromise epithelial integrity.

Multifaceted roles of microbiota-derived deoxycholic acid in cancer

This chapter summarizes the multifaceted roles of microbiota-derived DCA in GI cancer with an emphasis on its duality (Table 1). Mechanistically, DCA compromises intestinal epithelial barrier integrity by altering the expression of tight junction proteins and inducing inflammation through NF-κB activation and ROS generation. These changes enhance the translocation of bacteria and chronic immune stimulation, and their resultant formation of a pro-tumorigenic microenvironment. In addition, DCA is a signaling molecule that plays a part in cell proliferation regulation, resistance to apoptosis, DNA damage, tumorigenesis, and tumor growth. We further address the tissue-specific action of DCA in liver cancer, CRC, and gastric cancer, and its cross-talk with bile

Table 1 Function of microbiota-derived deoxycholic acid in cancer

Cancer	Study setting	Role	Mechanism	Conclusion	Ref
Esophageal adenocarcinoma	In vitro	MUC2 overexpression induced by bile acids	NF-κB activation	Bile acids induce MUC2 mucin overexpression via PKC-dependent NF-κB activation; aspirin and NF-κB p65 siRNA inhibit this pathway, suggesting potential chemopreventive strategies	[46]
Human intestinal epithelial Caco-2 cell monolayers	In vitro	DCA impairs intestinal barrier function via gene suppression	DCA increases permeability, intracellular ROS, and reduces ERK1/2 signaling and occludin levels	DCA induces intestinal hyperpermeability by downregulating cell-junction-related genes and disrupting barrier integrity, potentially linking high-fat diets to barrier dysfunction and cancer-related inflammation	[40]
Colorectal cancer (CRC)	In vitro	DCA promotes intestinal tumorigenesis and mucosal barrier breakdown	DCA activates the NLRP3 inflammasome, increases proinflammatory cytokines, reduces ZO-1, goblet, and Paneth cells; decreases sIgA; promotes M2 macrophage polarization	DCA aggravates CRC development by disrupting mucosal physical and immune barriers, increasing inflammation, and promoting tumor progression in the intestine	[3]
CRC	In vitro and i*n vivo*	DCA damages intestinal stem cells and impairs barrier function via AHR suppression	DCA suppresses AHR signaling in ISCs via reduced IDO1 in Paneth cells → ↓kynurenine → ↓GCs and MUC2; reversed by AHR agonist (FICZ) or bile acid sequestrant (cholestyramine)	DCA impairs ISC differentiation and reduces goblet cells/MUC2 via AHR pathway inhibition; AHR ligand supplementation may restore function and barrier integrity under high-fat diet conditions	[106]
Barrett's Esophagus	In vivo and in vitro	DCA promotes squamous-to-columnar trans-differentiation	DCA increases KLF5 → upregulates intestinal markers CDX2, MUC2, villin; KLF5 knockdown reverses this effect; overexpression mimics DCA action	DCA promotes BE-associated metaplasia via KLF5-mediated reprogramming; KLF5 is a key factor in DCA-driven esophageal epithelial trans-differentiation	[107]
Barrett's Esophagus	In vitro	DCA and RA have limited cooperative effects	RA induces MUC2 expression; DCA does not enhance or suppress squamous differentiation; involucrin expression and morphology remain squamous	RA induces partial intestinal marker expression (MUC2), but neither RA nor DCA alters squamous phenotype, suggesting partial differentiation cues in BE pathogenesis	[108]
Colon cancer	In vivo	Dietary DCA and cholesterol as promoters of colonic epithelial cell proliferation	Elevated fecal bile acids and neutral steroids increase colonic crypt cell proliferation; AOM carcinogen induces proliferation; effects are additive	High fecal concentrations of neutral and acid steroids enhance carcinogen-induced colonic cell proliferation, suggesting a key role in colon cancer etiology	[59]

Table 1 (continued)

Cancer	Study setting	Role	Mechanism	Conclusion	Ref
Colon cancer	In vitro	Butyrate and DCA both inhibit proliferation and induce apoptosis, but via distinct mechanisms	DCA increases ROS, DNA breakage, activates ERK1/2, caspase-3, PARP; decreases activated Rb protein. Butyrate increases p21 expression; alters cell cycle (G1 & G2 increase)	Both inhibit proliferation and induce apoptosis; butyrate enhances tumor suppressor gene expression, while DCA reduces tumor suppressor activation, highlighting opposite effects in colon cancer risk modulation	[109]
CRC	Clinical study	Serum DCA correlates with colorectal mucosal proliferation	Serum DCA levels positively correlate with colorectal epithelial cell proliferation rate (S and G2/M phases) measured by flow cytometry	Serum deoxycholic acid levels correlate significantly with colorectal mucosal proliferation, supporting DCA's tumor-promoting role in colorectal carcinogenesis	[110]
Colon cancer	In vitro	DCA transiently promotes proliferation in HT29 cells	DCA increased proliferation at low doses (5–10 μM) only at 6 h; no effect on subclones; no change in PKC activity or isoform expression	DCA stimulates transient proliferation in HT29 cells independent of protein kinase C signaling; high doses cytotoxic to all cell lines	[111]
Colon cancer	In vitro	DCA promotes proliferation and polyamine accumulation	DCA (up to 20 mM) increased Caco-2 cell proliferation (threefold) and ornithine decarboxylase (ODC) activity; enhanced putrescine uptake by increasing transporter capacity without changing affinity	Physiological DCA concentrations stimulate colon cancer cell proliferation via increased polyamine synthesis and transport	[112]
General tumor models	In vitro and in vivo	Oral antiangiogenic agent	LHD (low molecular weight heparin + deoxycholic acid) inhibits angiogenesis by blocking blood vessel formation	LHD is orally absorbable, inhibits angiogenesis effectively, reduces tumor growth, and has an additive effect with doxorubicin; promising oral cancer therapy	[113]
Colon cancer	In vivo	DCA acts as a dietary tumor promoter that sensitizes resistant AKR/J mice to colon carcinogenesis	DCA exposure increases aberrant crypt foci (ACF) formation; associated with nuclear accumulation of β-catenin without loss of E-cadherin; loss of APC occurs in AOM-induced ACF	DCA exposure overcomes genetic resistance in AKR/J mice by increasing ACF and promoting nuclear β-catenin translocation, leading to high-grade dysplasia in the colon	[114]
Colon cancer	In vitro	Bile acids act as proliferative agents	Conjugated bile acids (tauro- and glyco-conjugated DCA/LCA) stimulate M3 muscarinic receptor-dependent EGFR transactivation → activates p44/42 MAPK → promotes proliferation; blocked by atropine and EGFR inhibitors; no apoptosis seen (no caspase-3 activation)	Bile acid-induced proliferation in colon cancer is dependent on M3R expression and mediated by EGFR transactivation. This explains the proliferative (rather than apoptotic) action of bile acids in certain colon cancer contexts	[58]

Table 1 (continued)

Cancer	Study setting	Role	Mechanism	Conclusion	Ref
Colon cancer	In vitro	Pro-tumorigenic (invasive, proliferative)	DCA activates COX-2 signaling via PKC in both normal and cancer-associated fibroblasts (CAF), with greater COX-2 induction in CAFs. DCA-pretreated CAFs enhance the proliferation and invasion of colon cancer cells in co-culture. COX-2 siRNA reverses these effects	DCA enhances tumor progression by activating stromal COX-2 in fibroblasts (especially CAFs), promoting epithelial proliferation and invasion through paracrine signaling	[71]
Colon cancer	In vitro	Pro-tumorigenic (β-catenin activation)	DCA increases tyrosine phosphorylation of β-catenin, upregulates uPAR and cyclin D1, and reduces E-cadherin–β-catenin binding. siRNA against β-catenin or neutralizing antibodies to uPAR suppress DCA-induced proliferation and invasion	DCA activates β-catenin signaling to enhance colon cancer cell growth and invasiveness, partly through uPAR upregulation and E-cadherin disengagement	[14]
CRC with liver metastasis	In vivo	Modulation of metastasis by gut microbiota-derived metabolites	Non-absorbable antibiotics reduced gut bacteria performing 7α-dehydroxylation, leading to decreased DCA levels, which in turn limited CRC proliferation and liver metastasis	Non-absorbable antibiotic treatment inhibits CRC liver metastasis by suppressing microbial DCA production, highlighting the gut-liver axis in cancer progression	[1]
Oesophageal adenocarcinoma (EAC)	In vitro	DCA promotes tumor invasiveness	DCA increased cancer cell invasion by upregulating MMP-10 expression at both mRNA and protein levels; MMP-10 was overexpressed in EAC tissue samples	DCA enhances the invasiveness of EAC via MMP-10 induction, offering insights into bile acid-induced cancer progression and potential therapeutic targets	[74]
Colon cancer	In vitro and in vivo	DCA promotes intestinal inflammation via Th17 induction	DCA enhances Th17 differentiation by upregulating cholesterol biosynthesis enzyme CYP51 via SREBP2 and TGR5 pathways, increasing endogenous RORγt agonists (zymosterol, desmosterol) that drive pathogenic Th17 responses	DCA links high-fat diet to Th17-mediated colonic inflammation through modulation of cholesterol metabolism; bile acid sequestration mitigates this inflammation, offering a therapeutic avenue	[115]
Lung cancer	Clinical	DCA promotes immune escape via TGR5/STAT3/PD-L1 signaling; inhibited by Yi-Fei-San-Jie Formula (YFSJF)	YFSJF modulates bile acid metabolism, especially reducing DCA levels, which inhibits TGR5 → STAT3 → PD-L1 signaling. This reactivates T cells by lowering PD-L1 expression and enhancing TNF-α, IFN-γ, and GzmB production	YFSJF suppresses lung tumor progression by reversing DCA-mediated immune escape, offering therapeutic value through modulation of immune checkpoints via TGR5/STAT3/PD-L1 axis	[116]

Table 1 (continued)

Cancer	Study setting	Role	Mechanism	Conclusion	Ref
Intestinal metaplasia (gastric precancerous lesion)	In vitro	DCA stimulates macrophages to release exosomes that promote intestinal metaplasia and suppress epithelial proliferation	DCA-stimulated macrophage-derived exosomes (D-Exos) are enriched in miR-30a-5p, which targets FOXD1 in gastric epithelial cells, upregulating CDX2 and inhibiting cell proliferation	DCA alters the macrophage-gastric epithelium crosstalk via exosomal miR-30a-5p, inducing intestinal metaplasia, a key event in gastric carcinogenesis	[77]
Spasmolytic polypeptide-expressing metaplasia (SPEM; a gastric cancer precursor)	In vivo	DCA stimulates macrophages to secrete exosomes that induce SPEM	DCA-induced macrophage-derived exosomes (D-Exos) are taken up by gastric organoids and upregulate SPEM markers (TFF2, GSII lectin); macrophage infiltration (F4/80+) increased after DCA exposure	DCA promotes SPEM via macrophage-derived exosomes, suggesting a novel inflammatory and metabolic link in gastric carcinogenesis	[78]
CRC	In vitro	Microbiota-derived DCA suppresses anti-tumor immunity and promotes tumor growth	DCA suppresses $CD8^+$ T cell effector function by targeting plasma membrane Ca^{2+} ATPase (PMCA), leading to inhibition of Ca^{2+}-NFAT2 signaling; $CD8^+$ dysfunction correlates with DCA levels and bacterial DCA biosynthesis genes	Microbial metabolism of bile acids, particularly DCA, impairs $CD8^+$ T cell responses and accelerates CRC progression, highlighting a microbiota-immune-metabolite axis as a therapeutic target	[76]
Gastric cancer	In vitro	Dual role: Pro-apoptotic and potential pro-carcinogenic agent	DCA induced significant apoptosis in GES-1 cells; proteomic analysis identified 134 upregulated and 214 downregulated proteins; bioinformatics analysis indicated altered signaling pathways and protein interactions linked to carcinogenesis	Microbiota-derived DCA induces apoptosis in normal gastric epithelial cells and alters proteomic networks, potentially triggering gastric carcinogenesis	[117]
Colon adenocarcinoma	In vitro	Pro-apoptotic	DCA and CDCA induced apoptosis via oxidative stress with increased ROS production (mainly through NAD(P)H oxidases and PLA2); triggered mitochondrial permeability transition, caspase-9 and −3 activation, Bax activation, and Bcl-2 cleavage; not dependent on caspase-8; prolonged exposure led to secondary necrosis due to ATP depletion	Microbiota-derived bile acids (DCA, CDCA) induce apoptosis in colon cancer cells through oxidative stress and mitochondrial pathways, potentially contributing to colon cancer pathogenesis and therapy	[13]
Colon cancer	In vitro	Cytoprotective (anti-apoptotic)	UDCA inhibited DCA-induced apoptosis by suppressing EGFR activation and downstream Raf-1/ERK signaling; also reduced AP-1 DNA binding activity	UDCA antagonizes the pro-apoptotic effects of DCA by modulating the EGFR/Raf-1/ERK signaling pathway, suggesting a protective mechanism in colon epithelial cells	[118]

Table 1 (continued)

Cancer	Study setting	Role	Mechanism	Conclusion	Ref
Colon cancer	In vitro	Cytoprotective (anti-apoptotic)	UDCA inhibited DCA-induced apoptosis by blocking Apaf-1/caspase-9 complex formation (apoptosome), without affecting upstream signals like cytochrome c release, Smac/DIABLO, XIAP, or PI3K/MAPK/cAMP signaling pathways	UDCA protects colon cancer cells from DCA-induced apoptosis by specifically inhibiting apoptosome formation, independently of major survival pathways	[119]
Colon cancer	In vitro	Pro-apoptotic (Bax-independent)	DCA induces cytochrome c release and activates caspase-9, −3, and −8 even in Bax–/– HCT116 cells. This suggests a Bax-independent pathway for DCA-induced mitochondrial apoptosis	DCA triggers apoptosis via the mitochondrial pathway independent of Bax, though still sensitive to UDCA protection	[64]
Hepatocellular carcinoma (SMMC-7721), colorectal cancer (HCT-116)	In vitro	Selective anticancer activity (A2)	A2 selectively induced apoptosis in SMMC-7721 cells via both apoptosis-inducing factor (AIF) and caspase-dependent mitochondrial pathways; uptake of A2 was mediated by OATP1B3 transporter; expression of OATP1B3 was increased, and its inhibition by rifampin blocked A2 uptake	A2 exhibited potent and selective cytotoxicity against hepatocellular carcinoma cells through OATP1B3-mediated uptake and dual apoptotic mechanisms; C2 and D2 lacked this selectivity	[120]
Colon cancer	In vitro	Pro-apoptotic (via GADD153)	DCA strongly induces GADD153, a pro-apoptotic gene. Both AP-1 and C/EBP transcription factors mediate its upregulation. Antisense GADD153 suppresses DCA-induced apoptosis, indicating its essential role	GADD153 is critical for DCA-induced apoptosis; multiple transcription factors (AP-1, C/EBP) regulate its expression in response to DCA	[121]
Colon cancer	In vitro	Apoptosis-to-necrosis switch	DCA induces caspase-3-dependent apoptosis; Bcl-2 overexpression or PKC inhibition (via calphostin C) blocks apoptosis but switches cell death mode to necrosis instead of promoting survival	DCA-induced apoptosis can be shifted to necrosis by Bcl-2 or PKC inhibition; cell death still occurs, suggesting DCA cytotoxicity is unavoidable via either pathway	[122]
Esophageal cancer	In vitro	Pro-apoptotic/ionic disruption	DCA induces lysosomal perturbation, cytoplasmic acidification, Na^+ influx (via Na^+/H^+ exchanger), K^+ loss, Ca^{2+} increase, and caspase-3/7 activation. Inhibition of NHE by EIPA prevents ion imbalance and apoptosis; low Na^+ media also inhibits cell death	NHE-mediated Na^+ influx is essential for DCA-induced apoptosis; its inhibition confers apoptosis resistance, relevant to tumor adaptation during GI tumorigenesis	[123]

Table 1 (continued)

Cancer	Study setting	Role	Mechanism	Conclusion	Ref
Colon cancer	In vitro	Anti-apoptotic (via UDCA)	UDCA protects cells from DCA-induced apoptosis by activating PI3K/Akt survival signaling. Blocking PI3K or Akt (pharmacologically or genetically) abolishes UDCA's protective effect	UDCA prevents DCA-induced apoptosis via Akt/PKB signaling, confirming a central role of survival signaling in bile acid cytotoxicity modulation	[124]
Gastric cancer	In vitro	Pro-apoptotic	DCA suppresses cell growth and induces G0/G1 arrest. Apoptosis features include mitochondrial membrane potential collapse, DNA fragmentation (laddering), increased Bax/Bcl-2 ratio, and altered p53, cyclin D1, and c-Myc expression	DCA induces intrinsic apoptosis in SGC-7901 gastric cancer cells through mitochondrial depolarization and Bax/Bcl-2 modulation, implicating mitochondrial and cell cycle regulators	[12]

acid receptors like Farnesoid X Receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5).

Disruption of barrier function by DCA and its oncogenic consequences

There has been increasing and more evidence in recent times to point towards the harmful effects of DCA on intestinal barrier integrity and its cancer-promoting effect [30]. A study proved that exposure to deoxynivalenol (DON) increased DCA levels, and as a result, gut barrier dysfunction was linked with DON toxicity. Proof of barrier disruption caused by DON consisted of activated proinflammatory cytokines like IFN-γ, Chemokine (C-X-C motif) ligand (CXCL) 9, CXCL10, and CXCR3, histological changes, and a decrease in the gut barrier-maintaining tight junction proteins Claudin 5 and E-Cadherin [31, 32]. Nevertheless, the combination of DON exposure, extreme metabolomics changes, and enterotoxicity has not yet been thoroughly documented. By employing an untargeted metabolomic footprint, He et al. [30] identified plasma DCA to be significantly increased in DON-treated mice, and this result was further validated with quantitative assays. At the same time, DON exposure was accompanied by alteration of the gut microbiota composition, including the one recently discovered to regulate secondary bile acid production [30]. Secondary bile acids, DCA and lithocholic acid (LCA), are produced in the colon due to the conversion of primary bile acids by microbial [33]. These findings indicate that DON-induced dysbiosis of gut microbial increases DCA. Confirming previous studies [34, 35], in vitro models of colonic epithelial cells revealed that DCA induced inflammation and decreased expression of Claudin-5 [30]. More accurate studies with germ-free animal models or with antimicrobial therapy can determine the pathogenic action of higher DCA in DON-induced mucosal damage [3, 36]. Increased levels of DCA in the colonic lumen can promote intestinal permeability, leading to unregulated movement of antigens through the colonic epithelium [37]. These results show that DCA regulates gene expression responsible for various cell junction pathways, as well as promoting intestinal permeability.

ERK1/2 signaling is important for mediating physiological reactions to external insults like oxidative stress, and modulates cell proliferation and intestinal barrier function [38, 39]. Zeng et al. [40] found that DCA decreases ERK1/2 phosphorylation, which impairs tight junction integrity and increases intestinal permeability. In this setting, ERK activation supports barrier maintenance, so inhibition by DCA is detrimental. Supporting this, polyphenol extracts have been shown to increase intestinal barrier function by activating ERK signaling in human intestinal cells [41]. Consequently, DCA inhibition of ERK1/2 phosphorylation would thus weaken tight junction integrity and lead to disruption

of the epithelial barrier. In addition, proto-oncogene product c-Myc plays a role in oxidative stress, cell proliferation, and permeability of the intestine, which are signs of signaling pathways involved [42, 43].

Although the majority of the studies have been conducted on tight junctions, less attention has been paid to the action of DCA on other cell junctions [40]. Based on Zeng et al. [40] findings, DCA reduces the expression of genes in not only the tight junction but also focal adhesion, gap junction, and adherens junction pathways. Protein–protein interaction analysis demonstrated that genes involved in tight junction clustered together mainly, while genes involved in the focal adhesion, gap junction, and adherens junction pathways exhibited another cluster separately [40]. Five of the genes—*CAV1*, *CDH1*, *GJB1*, *JAM3*, and *PVRL3*—also acted as bridges between the two clusters, and they may be implicated in functional interactions among all kinds of junctions [40]. Generally, the homologous relationship between focal adhesion, gap junction, and adherens junction pathways is more pronounced than with tight junctions alone, even though functional crosstalk among all of them occurs. Global downregulation of junction gene expression by DCA is demonstrated as an important mechanism for mediating elevated colonic permeability in epithelial barriers [40]. Additionally, mRNA and protein levels of occludin, an important tight junction protein, decreased after treatment with DCA. Notably, not only is the expression level of occludin in the epithelial barrier is required, but also trafficking and subcellular localization [44, 45]. In addition to tight junctions, occludin is localized in non-junctional compartments like the nucleus and centrosome, where it regulates gene transcription (mRNA export) and cell cycle in renal, neuronal, and other cells [44, 45]. Zeng et al. [40] found that DCA reduced occludin expression in tight junctions and the nucleus of Caco-2 epithelial cells. Given that occludin has an assumed impact on transcriptional regulation, it is important to further study how DCA affects global gene expression, which can reveal information about mechanisms that connect DCA to colon carcinogenesis.

In another study, Wu et al. [46] showed that bile acids elevate the expression of MUC2 in SEG-1 EAC cells. That is, the transcriptional activity of a MUC2 promoter-reporter construct transiently transfected into SEG-1 cells was elevated by DCA in a dose-dependent fashion, suggesting bile acid-induced upregulation of MUC2 to be at the transcriptional level [46]. Wu et al. [46] results also indicate that caffeic acid phenethyl ester (CAPE), a blocker of NF-κB nuclear translocation, markedly inhibited basal and bile acid-evoked MUC2 transcription. NF-κB expression and transcription activity were also markedly associated with MUC2 induction. Blocking NF-κB expression and activity significantly inhibited bile acid-evoked MUC2 upregulation, indicating an important role for NF-κB in the signaling pathway. They further noted that DCA induces NF-κB p65 expression, which points towards degradation of its inhibitor IκB as the reason for this induction [46]. NF-κB binding sites within the MUC2 promoter region between bases 1528 and 1307 have been confirmed in other studies in support of the suggestion that NF-κB expression induced by bile acids will induce transcription of MUC2 [47–49]. Further, the PKC inhibitor calphostin C blocked NF-κB activation and MUC2 induction by DCA profoundly, suggesting the participation of PKC in bile acid regulation of MUC2 [46]. The PKA inhibitor H-8, however, was incapable of blocking bile acid-stimulated MUC2 or NF-κB activity, suggesting the process does not involve PKA. Unlike previous studies in which phorbol 12-myristate 13-acetate (PMA) induction of MUC2 was through the ERK/MAP kinase cascade, our findings show that bile acid-stimulated MUC2 expression in SEG-1 cells occurs independently of MAP kinase signaling [46]. Elucidation of the mechanisms through which bile acids regulate MUC2 expression could assist in establishing chemopreventive interventions that will mitigate the risk of carcinogenesis and metastasis, especially in esophageal cancer.

Finally, Liu et al. [3] findings indicate that DCA perturbs the integrity of Caco-2 intestinal epithelial cells and induces the release of proinflammatory cytokines in intestinal tumor cells. To determine whether these effects are enhanced in vivo, they used Apc $^{min/+}$ mice as a model of human intestinal cancer to investigate DCA's effect on the intestinal barrier. Their results proved that DCA treatment caused chronic low-grade intestinal inflammation, compromised intestinal mucosal barrier function, increased mucosal permeability, and promoted carcinogenesis in these mice. Liu et al. [3] suggested a novel view that the intestinal barrier is of fundamental importance to DCA-induced intestinal carcinogenesis. Namely, DCA triggered mRNA expression of NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) and initiated activation of the NLRP3 inflammasome, leading to chronic low-grade intestinal inflammation during tumor growth in Apc $^{min/+}$ mice. DCA enhanced epithelial permeability and evoked proinflammatory cytokine release in vitro. Supportive animal experiments verified that DCA disrupts intestinal barrier integrity and increases permeability. In addition, DCA was also reported to induce inflammatory responses, modulate immune regulation, and enhance tumor formation in the intestine. Liu et al. [3] proposed that DCA-induced loss of intestinal barrier function results in microbial dysbiosis, contributing to tumorigenesis in the intestine. Overall, these findings demonstrate that DCA stimulates intestinal carcinogenesis by compromising the physical and functional integrity of the intestinal mucosal barrier. Hence, the elucidation of mechanisms by which DCA modulates the intestinal mucosal barrier is pivotal to the generation of prevention and treatment strategies

for dietary high-fat intake-associated CRC. These observations place DCA in a pivotal regulatory position as a critical mediator between gut barrier dysfunction, microbial dysbiosis, inflammatory signaling, and tumorigenesis, and possibly as a therapeutic target in GI disease and cancer. In summary, DCA mediates its oncogenic actions through a multi-potent attack on the intestinal barrier—suppression of junctional proteins, inhibition of ERK1/2 signaling, disruption of mucin expression, and induction of chronic inflammation through NLRP3 activation. These alterations induce microbial dysbiosis and chronic immune activation, making the colon cancer-preventive niche pro-tumorigenic. Overall, evidence implicates DCA as a critical mediator of diet-induced microbiota alterations, disruption of epithelial barrier function, and CRC pathogenesis, and thus a prime candidate for prevention and therapeutic intervention.

Growth and proliferation induced by DCA: mechanisms and oncogenic outcomes

DCA was found to be a strong mitogen for tumor cell proliferation and growth, especially in the GI region. Numerous mechanistic explanations have been presented on how DCA affects mitogenic and oncogenic functions through various means. An investigation by Pai et al. [14] showed that physiologically attainable concentrations of DCA stimulate the β-catenin signaling pathway and induce urokinase plasminogen activator (uPA), its receptor (uPAR), and cyclin D1 expression in colon cancer cells. Silencing β-catenin with siRNA significantly decreased DCA-stimulated uPAR and cyclin D1 expression. Furthermore, blocking uPAR with a neutralizing antibody significantly eliminated DCA-stimulated Matrigel colony formation and suppressed cell invasiveness [14]. DCA induced the tyrosine phosphorylation of β-catenin, which stabilizes and allows it to translocate to the nucleus and activate uPA, uPAR, and cyclin D1 expression. Phosphorylation inhibited the β-catenin from binding with E-cadherin, causing loss of cell–cell adhesion. Both proteolysis through uPA/uPAR and cyclin D1-mediated proliferation are implicated in colon cancer tumor formation [14]. The accumulation of cytosolic β-catenin and its activation are recognized to be growth and metastasis inducers of tumors [50]. Inhibited E-cadherin binding is associated with decreased cell–cell adhesion and an invasive mode of growth [51]. The evidence supports that DCA reduces E-cadherin/β-catenin binding, with enhanced migratory cancer cell activity and elevated, denser Matrigel colonies. Blocking uPAR also inhibited DCA-mediated proliferation, evidencing the contribution of uPAR to the invasiveness and proliferation of cancer cells [14]. Other bile acids, such as LCA, have also been reported to cause colon cancer invasiveness and proliferation through mechanisms including RhoA/Rho-kinase signaling and matrix metalloproteinases [52, 53].

Cyclin D1, a key cell cycle progression regulator, is important in carcinogenesis [54]. Pai et al. [14] results indicate that DCA markedly increases cyclin D1 expression, whose knockdown significantly suppresses, indicating that DCA promotes colon cancer cell growth through the β–catenin–cyclin D1 pathway. DCA-induced enhancement of β-catenin tyrosine phosphorylation and nuclear translocation enhances tumor cell proliferation and invasiveness, characterizing β-catenin as a critical molecular target of the oncogenic effects of DCA. These actions were found at DCA concentrations that are encountered physiologically within the human colonic lumen and portal circulation [14]. Of special note, at higher doses, DCA inhibited β-catenin tyrosine phosphorylation, blocked cancer cell proliferation, and effectively eliminated colony formation in Matrigel. The previous research on primary hepatocytes and cholangiocarcinoma cells had indicated that DCA was activating the EGFR [55]. Growth factor receptors like EGFR and c-Met (hepatocyte growth factor receptor) have been shown to induce β-catenin tyrosine phosphorylation [56, 57]. DCA-induced activation of β-catenin in colon cancer cells could therefore be via EGFR and/or c-Met pathways. In conclusion, these findings show that secondary bile acids such as DCA increase the proliferation of colon cancer cells by activating the β–catenin–cyclin D1 and uPAR signaling pathways.

In a quantitative investigation of the interaction between the primary human bile acids and CHO cells bearing the five muscarinic receptors (M1–M5) subtypes, Cheng et al. [58] found that glycine and taurine conjugates of DCA also interact with the M3R. This is relevant because conjugates of DCA are present in the gut at far higher concentrations than LCA. In addition, equimolar concentrations of DCA conjugates are less lipophilic and therefore less toxic than LCA conjugates [58]. Receptor-mediated effects of DCA conjugates are, therefore, likely to be of physiological significance rather than the lower concentration, potentially more toxic LCA conjugates. Initial experiments in M3R-transfected CHO cells revealed that DCA conjugates are muscarinic receptor antagonists [58]. The agents decreased maximal muscarinic receptor radioligand binding by 60% at maximum doses, but equivalent concentrations did not modulate basal inositol phosphate production or p44/42 MAPK activation [58]. As predicted by antagonists, dose-dependent inhibitions of ACh-stimulated inositol phosphate production and MAPK activation were obtained with increasing doses of DCA conjugates [58].

Cheng et al. [58] data emphasize the importance of the cellular context in which the activity of DCA conjugates is expressed, i.e., co-expression of EGFR and M3R. In co-expressing EGFR/M3R H508 colon carcinoma cells, DCA conjugates induce signaling and proliferation. Yet, in CHO cells that co-express M3R alone or SNU-C4 cells that co-express EGFR alone, an effect is not seen. The proliferative

response is thought to be due to EGFR transactivation induced by M3R-stimulated signal pathways, introducing an additional layer of regulation that broadens cellular responses to stimuli like cholinergic agonists and bile acids [58]. In most cell types, mitogenic signaling via G-protein coupled receptor (GPCR) ligands—e.g., M3R ligands—is EGFR transactivation-dependent, a receptor tyrosine kinase [58]. This is the mechanism of proliferative responses by other GPCR ligands, including endothelin-1, lysophosphatidic acid, thrombin, and ACh [58]. The interaction of receptor tyrosine kinases and GPCRs adds another checkpoint to limit cell proliferation without regulation, thus limiting neoplastic transformation.

As far as colon cancer development is concerned, receptor tyrosine kinase activation is a leading inducer of tumor cell growth and also a key target for chemotherapy. Recent evidence, consistent with observations on bile acids, has shown ligand-specific phosphorylation on EGFR [58]. Particularly, EGFR tyrosine 992 (Tyr992), a constitutively and strongly phosphorylated point after incubation with deoxycholyltaurine (DCT), is a high-affinity phospholipase C-γ SH2 domain-binding point and is required for Phospholipase C-gamma (PLC-γ) activation by EGF [58]. Suppression of DCT-induced EGFR Tyr992 phosphorylation by an EGFR inhibitor verifies bile acid-induced EGFR transactivation in H508 cells, implicating PLC activation and calcium mobilization in downstream signaling [58]. Together, the results confirm that EGFR transactivation is a key mediator of bile acid-stimulated proliferation of colon cancer cells. Bile acids induce EGFR tyrosine phosphorylation, activation of the respective cascades of downstream signaling, and enhancement of cell growth—events that are inhibited by EGFR-specific antibodies and pharmacologic inhibitors. Notably, the inhibitors are unable to stimulate MAPK signaling or growth in EGFR single-expressing SNU-C4 cells, indicating that bile acid growth requires both co-expression of M3R and EGFR. Thus, conjugated bile acids appear to promote the growth of colon cancer cells through coordinated muscarinic and EGFR plasma membrane activation.

Of note, hyper-proliferation of the colonic epithelium is normal in colon carcinogen-exposed animals and in humans at high risk for colon cancer [59]. Bile acid-induced hyperproliferation is believed to be the result of the activation of luminal lytic activity, leading to injury to epithelial cells and provoking a compensatory elevation in proliferative activity [59]. Steroid-induced hyperproliferation also has cytotoxic actions on the colonic epithelium, and this gives a net gain in intestinal cell proliferation [59]. Hori et al. [59] revealed a very dramatic increase in proliferative activity among the morphologically normal crypts of azoxymethane (AOM)-treated animals versus saline-treated controls. Briefly, DCA is identified as a primary colon carcinogen because it has the potential to initiate aberrant growth and proliferation of normal colon epithelial cells as well as cancer cells. Mechanistically, DCA activates several oncogenic pathways, such as the β-catenin–cyclin D1 pathway and uPA/uPAR system, and EGFR transactivation by M3R cross-talk [59]. These signal cascades together propel cell cycle movement, impair cell adhesion, damage the extracellular matrix, and increase cellular invasiveness—characteristics of cancer progression. These observations indicate that DCA induces colorectal carcinogenesis by multimodal stimulation of growth. The critical mechanisms are activation of the β-catenin–cyclin D1 pathway, induction of the uPA/uPAR cascade, and EGFR transactivation by M3R signaling. All these pathways cooperatively facilitate cell cycle promotion, cell–cell adhesion loss, extracellular matrix remodeling, and invasiveness.

DCA induced apoptosis and DNA damage: tumor-suppressive or tumor-promoting?

Several research studies have shown that DCA may cause apoptosis in different types of cancer, such as hepatocytes and colon cancer cells [60, 61]. The hydrophobicity of bile acids has been seen to be strongly associated with apoptosis induction and/or growth arrest [62]. Although DCA has been found to cause oxidative stress, DNA damage, and mitochondrial membrane instability in cancer cells, the exact mechanisms underlying apoptosis are unclear. In a study by Song et al. [12] DCA inhibited the proliferation well in various tumor cell lines in a dose-dependent manner, and SGC-7901 cells were more susceptible to its antiproliferative effect. Cell cycle arrest is a normal response of proliferating eukaryotic cells to DNA-damaging agents, and DCA could achieve G0/G1 phase arrest, in accordance with the results in Mz-ChA-1, BGC-823, and QBC939 cell lines [12]. Apoptosis, originally demarcated by typical morphological changes, occurs with cell shrinking, condensation and margination of the chromatin, nuclear fragmentation, and apoptotic body formation [12]. After DCA treatment, SGC-7901 cells formed these typical apoptotic morphologies. These results are in line with previous work to show that DCA was cytotoxic to colon cancer cells by inducing apoptosis [12]. Mechanistically, DCA induced the apoptosis of colon cancer cells primarily through the mitochondrial pathway by triggering caspase-9 and caspase-8. Dose-dependent mitochondrial membrane potential decrease, indicated by rhodamine staining, was observed in Song et al. [12] study. The balance of Bcl-2 family proteins, which regulate mitochondrial integrity, was disrupted by DCA by downregulating the anti-apoptotic Bcl-2 and upregulating the pro-apoptotic Bax, thereby increasing the ratio of Bax/Bcl-2 in SGC-7901 cells. In addition, the p53 tumor suppressor gene, responsible for regulating *Bax*, *Bcl-2, c-Myc*, and *cyclin D1* genes, seems to mediate DCA-induced apoptosis through the mitochondrial pathway in these cells [12]. In summary,

the results demonstrate that DCA arrests proliferation and induces apoptosis in human gastric carcinoma (HGC) cells and that the mitochondrial pathway is a significant pathway to engage in this process.

Barrasa et al. [13] demonstrated that DCA selectively triggered the activation of caspase-9 but not the activation of caspase-8. Activation of caspase-9, in turn, triggers activation of effector caspase-3, thus completing the entire apoptotic cascade. This was set out by the degradation of some well-characterized caspase substrates from nuclear extracts of BCS-TC2 cells, such as Poly (ADP-ribose) polymerase (PARP), Lamin B1, p21, and, less dramatically, p53. These findings set out to demonstrate that apoptosis of BCS-TC2 cells by DCA is mediated by the intrinsic mitochondrial pathway [13]. This receptor-independent apoptosis is observed in other colon cancer cell lines, both with (Caco-2 and SW620) and without (HT-29 and SW480) the death receptor CD95/Fas [13]. In contrast to this, it has been shown in HCT-116 cells that DCA first causes the activation of caspase-9 and then the subsequent activation of caspase-8. With the use of selective inhibitors, Barrasa et al. [13] observed that DCA and CDCA caused ROS production in BCS-TC2 cells through the activation of NAD(P)H oxidase, with a minor role being represented by phospholipase A2 (PLA2). Earlier studies revealed that bile acids induce PLA2 activity in Caco-2 cells via modulation of transepithelial permeability [63]. Activation of NAD(P)H oxidase and ROS production is essential for bile acid-induced apoptosis in hepatocytes [13]. Nonetheless, PLA2 inhibition and NAD(P)H oxidase blockade partially saved BCS-TC2 cells from bile acid-induced cytotoxicity, implying that there are alternative minor pathways involved in cell injury. Enhanced ROS generation caused by bile acids is adequate to induce mitochondrial permeability transition (MPT). MPT causes pro-apoptotic factor release, like cytochrome c and SMAC/Diablo, into the cytosol, which activates initiator caspase-9 [13]. These findings demonstrate that DCA increases ROS levels in BCS-TC2 cells, in association with the decrease in mitochondrial membrane potential, consistent with bile acid-induced MPT. Together, these findings confirm that DCA triggers apoptosis in BCS-TC2 cells via ROS-dependent mitochondrial damage.

Yui et al. [64] to ascertain if DCA activates caspase-9 directly or not, HCT116 cells' cytoplasmic fractions were incubated with DCA. Upon incubation with purified mitochondrial lysate, however, there was detectable activation of caspase-9. From these results, it can be concluded that DCA does not activate caspase-9 directly but needs mitochondrial components—e.g., cytochrome c—for the activation of caspase-9 by DCA [63]. In the human colon cancer cell line HT-29, DCA has been reported to cause severe apoptosis by elevating intracellular Ca^{2+} levels. Yui et al. [64] also investigated the involvement of Bax in DCA-induced cytochrome c release in *Bax*–/– and *Bax*+/– HCT116 cells. Both these cell lines underwent apoptosis in a dose-dependent fashion upon treatment with DCA, with no discernible difference in apoptotic frequency between the two. This indicates that the mitochondrial pathway by cytochrome c release is crucial for DCA-induced apoptosis in HCT116 cells, and Bax is not required for this purpose [64]. Together, these studies show that acute DCA effects on tumor cells are largely mitochondrial intrinsic pathway-dependent, with ROS generation, MPT, and caspase activation as key features. Although the evidence promotes a tumor-suppressive effect mediated through cancer cell apoptosis, the parallel triggering of oxidative and DNA damage forms the foundation for the potential that prolonged exposure to the agent could lead to tumorigenesis through the maintenance of genomic instability. This duality underscores the context-dependent character of DCA's action on cancer biology.

Deoxycholic acid in metastasis and invasion: driver of EMT and cell motility

Nguyen et al. [65] found that DCA treatment of hepatic stellate cells (HSCs) resulted in suppressed cell proliferation, which was coupled with simultaneous DNA damage and G0/G1 phase cell cycle arrest (Fig. 1). HCC cells that were cultured in a conditioned medium from LX2 cells treated with DCA (CM-LX2-DCA) were more activated in the MAPK/ERK signaling pathway. Aberrant MAPK/ERK signaling has also been implicated in the facilitation of HCC development by suppressing apoptosis and promoting migratory and invasive capabilities of cancer cells [66, 67]. Interestingly, inhibition of transforming growth factor-β (TGF-β) or IL-8 resulted in a striking decrease in migration and invasion of CM-LX2-DCA cultured HCC cells, underlining the pivotal role of these mediators in promoting HCC. TGF-β, an extracellularly secreted cytokine, controls the EMT process, which plays a pivotal role in cancer development [68]. TGF-β activates both the canonical Smad signaling pathway and the non-Smad signaling pathways like Phosphoinositide 3-kinase (PI3K)/Protein kinase B (PKB), MAPK, and Rac1, which trigger EMT [69]. IL-8 is also a critical immune modulator in the tumor microenvironment, creating a pro-tumor microenvironment and promoting metastasis and tumor development [70]. Nguyen et al. [65] showed the activation of MAPK and Smad pathways in HCC cells, which were accompanied by upregulation of EMT markers N-cadherin, vimentin, and Snail2, as well as downregulation of the epithelial marker E-cadherin. These modifications were properly abrogated by TGF-β neutralizing antibodies. These findings indicate that DCA induces HSC senescence by activating DNA damage response signaling pathways. Senescent HSCs secreted SASP factors, which promoted the migration, invasion, and EMT of HCC cells. In addition,

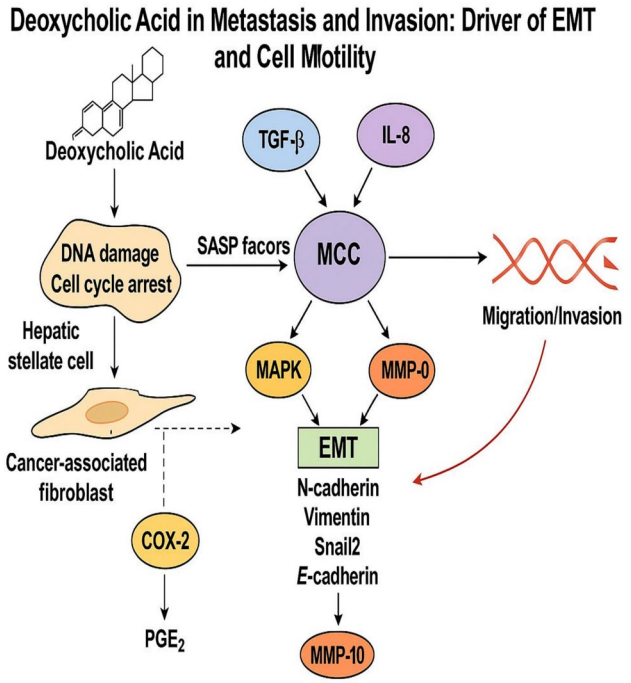

Fig. 1 Deoxycholic acid in cancer metastasis and invasion. Deoxycholic acid (DCA) promotes cancer metastasis and invasion through multiple mechanisms across various tumor microenvironments. In hepatocellular carcinoma (HCC), DCA induces senescence in hepatic stellate cells (HSCs), triggering DNA damage and G0/G1 cell cycle arrest. Senescent HSCs adopt a senescence-associated secretory phenotype (SASP), releasing pro-tumorigenic cytokines such as IL-8 and TGF-β, which activate MAPK/ERK and Smad signaling in adjacent HCC cells. These pathways upregulate EMT markers (N-cadherin, vimentin, Snail2) and downregulate E-cadherin, promoting cancer cell migration and invasion. In colorectal cancer (CRC), DCA enhances COX-2 expression and prostaglandin E2 (PGE2) production in cancer-associated fibroblasts (CAFs) more strongly than in normal fibroblasts (NFs), stimulating paracrine signaling that supports epithelial carcinoma cell proliferation and invasiveness. COX-2 silencing significantly reduces these effects, highlighting the pivotal role of DCA-stimulated stromal COX-2 signaling in tumor progression. In esophageal adenocarcinoma (EAC), DCA promotes cancer cell invasion by upregulating MMPs, particularly MMP-10, which facilitates extracellular matrix degradation and migration. Collectively, these findings implicate DCA as a critical modulator of the tumor microenvironment, enhancing invasive and metastatic potential through cytokine secretion, fibroblast activation, EMT induction, and MMP expression

neutralization of IL-8 and TGF-β significantly suppresses these activities, indicating the predominant role of these mediators in HCC invasion and metastasis.

In another study, Zhu et al. [71] confirmed that DCA treatment dramatically increased Cyclooxygenase-2 (COX-2) expression in normal-associated fibroblasts (NFs) and even higher levels in cancer-associated fibroblasts (CAFs). In addition to the identification of COX-2 in resting NFs and CAFs, further studies demonstrated that DCA highly upregulated COX-2 mRNA in both NFs and CAFs, validating the fact that stromal cells play a significant role in COX-2 expression. As anticipated, resting CAFs contained higher levels of COX-2 protein than resting NFs, and DCA treatment also upregulated COX-2 protein expression, particularly in CAFs [71]. These observations are consistent with the previous publications demonstrating that pro-inflammatory cytokines TNF-α and IL-1β induce colonic fibroblast COX-2 expression [72, 73]. In addition, treatment with DCA increased the level of PGE2 production in CAFs than in NFs, further demonstrating that CAFs are major producers of PGE2. Next, Zhu et al. [71] investigated whether and how DCA-treated fibroblasts affect the growth and invasion of human colonic epithelial carcinoma cells. Interestingly, DCA pre-treated CAFs significantly promoted the proliferative capacity of cancer cells over NFs, suggesting that paracrine signaling factors secreted from CAFs trigger tumor cell growth [71]. Interestingly, COX-2 silencing with siRNA in DCA-treated fibroblasts notably affects the proliferative and invasive activities of colonic epithelial cancer cells, proving the reality of the fact that activation of COX-2 signaling in stromal fibroblasts is essentially accountable for these cancer-promoting activities [71]. In conclusion, these findings indicate that CAFs are the primary source of COX-2 expression, DCA further augments COX-2 expression, and stromal COX-2 signaling plays a pivotal role in mediating the improved invasiveness and cell growth of human colonic epithelial cancer cells.

Besides, to determine whether DCA's effect on EAC invasion involved direct enhancement of cellular migration, Quilty et al. [74] considered two-dimensional migration, an integrin-dependent process involving actin cytoskeleton remodeling. Matrix metalloproteinases (MMPs) profiling in esophageal epithelial cells revealed basal MMP-1 mRNA as the most abundant in SKGT-4 cells, followed by MMP-9 and MMP-10 [74]. This aligns with previous studies linking elevated MMP-1 levels to positive lymph node metastases in EAC [75]. Notably, SKGT-4 cells overexpress MMP-1, MMP-9, and MMP-10 relative to non-cancerous Het-1A cells, reflecting MMP alterations during EAC development [74]. Quilty et al. [74] investigation confirmed that DCA upregulates MMP-10 at both gene and protein levels in SKGT-4 cells. In conclusion, these findings demonstrate that DCA promotes invasion in EAC cells, partly by inducing MMP-10 expression. This highlights the importance of developing therapies targeting bile acid pathways to inhibit cancer initiation, progression, and metastasis.

Immunomodulatory and pro-inflammatory roles of DCA in the tumor microenvironment

DCA's pro-inflammatory and immunomodulatory actions involve multiple mechanisms that regulate immune cell recruitment, polarization, and cytokine production (Fig. 2)

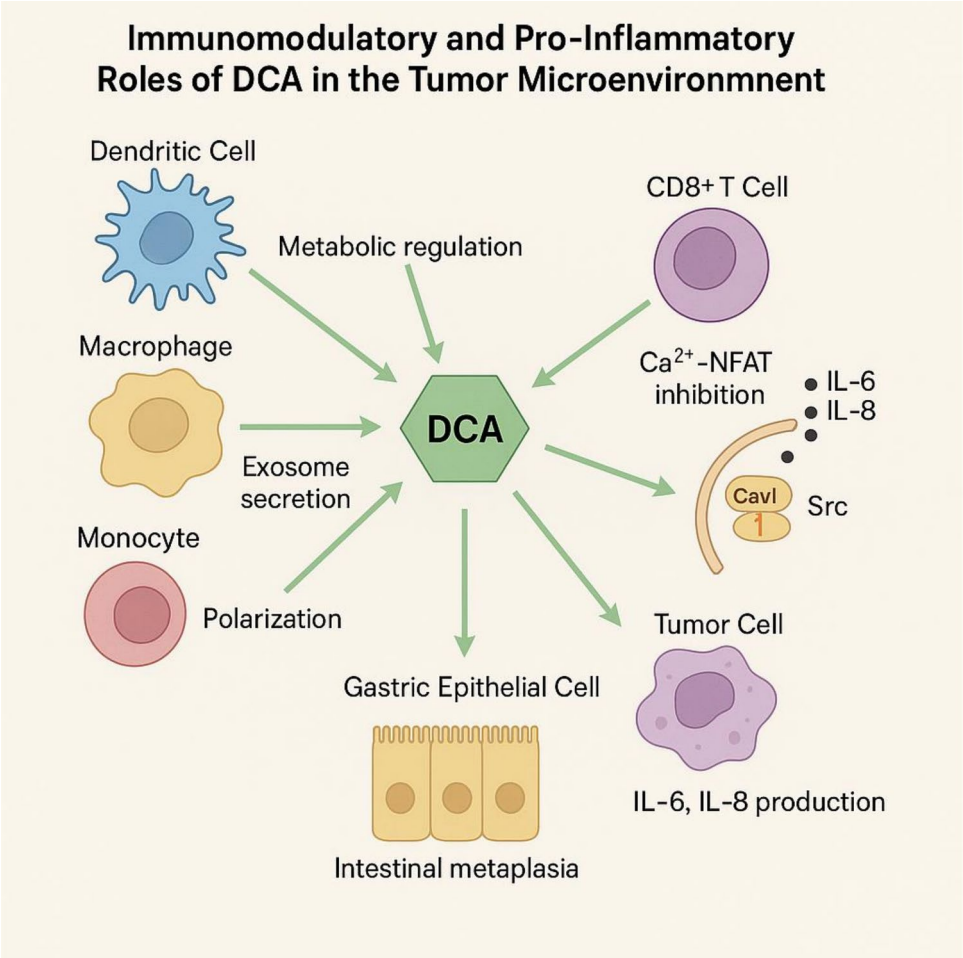

Fig. 2 Immunomodulatory and pro-inflammatory roles of DCA in cancer. DCA acts through both receptor-dependent (e.g., GPBAR1, FXR, VDR) and receptor-independent pathways to regulate immune cell function, promote inflammation, and facilitate carcinogenesis. In colorectal cancer (CRC), DCA suppresses $CD8^+$ T cell effector function by enhancing plasma membrane Ca^{2+}-ATPase (PMCA) activity, thereby reducing Ca^{2+}-NFAT2 signaling independently of surface receptors, ultimately weakening antitumor immunity. Elevated fecal DCA and increased expression of its biosynthetic genes in CRC patients contribute to an immunosuppressive environment that supports tumor progression. In gastric metaplasia, DCA-activated macrophages secrete exosomes enriched in miR-30a-5p, which target FOXD1 to inhibit proliferation and promote CDX2-driven intestinal metaplasia. DCA also induces spasmolytic polypeptide-expressing metaplasia (SPEM) by stimulating macrophage-derived exosomal signaling that enhances the expression of markers like TFF2, GSII, Wfdc2, Olfm4, and Cftr in gastric epithelium. In esophageal epithelial cells, DCA promotes the secretion of pro-inflammatory cytokines IL-6 and IL-8 through lipid raft/caveolin-1 (Cav1)-mediated signaling involving Src kinase, PKC isoforms, and the MAPK cascade. These actions collectively foster a tumor-permissive milieu by reprogramming immune cells, disrupting epithelial integrity, inducing chronic inflammation, and suppressing adaptive immune responses

[76]. Bile acids control metabolism and regulate the immune system in dendritic cells, macrophages, CD4 + T cells, and monocytes [76]. Cong et al. [76] demonstrated that DCA directly suppresses $CD8^+$ T cell effector function, revealing a mechanism by which DCA can promote CRC progression and highlighting the broad immunomodulatory influence of bile acids across diverse immune cell types. DCA generally acts by targeting receptors like G protein-coupled bile acid receptor 1 (GPBAR1), FXR, and vitamin D receptor (VDR), but Cong et al. [76] established that DCA inhibits Ca2 + -NFAT2 signaling by enhancing the activity of PMCA to regulate CD8 + T cell effector function. It is an immunomodulatory effect of DCA that does not involve receptors. When T cells are activated, PMCA-driven Ca2 + efflux is frequently inhibited by binding of PMCA to STIM1 and PMCA re-localization under mitochondria that facilitates increased Ca2 + signaling for effective T cell activation and NFAT-mediated transcription [76]. Inhibition of PMCA failed to further enhance CD8 + T effector function when DCA was absent. However, upon the presence of DCA, PMCA-mediated Ca2 + efflux was enhanced, which was inhibited by LaCl3 or shPMCA knockdown, thus removing

DCA's suppressive activity on CD8 + T cell function [76]. DCA is capable of sustaining immune homeostasis in the colon by regulating counteractive signals. An imbalance in the form of dominance by DCA in a CRC patient elevated fecal DCA levels, and the induction of its biosynthetic genes has been found. The conversion enables an immunosuppressive environment to persist to support CRC development. In summary, these data show that microbial DCA suppresses cytotoxic CD8 + T cell-induced anti-tumor immunity through the activation of PMCA and suggest the inhibition of DCA-producing bacteria as an excellent therapeutic and preventive strategy for CRC.

In another research, Xu et al. [77] investigated the function of exosomes released by bile acid-activated macrophages in IM by examining intercellular communication via coculture of gastric epithelial cells (GES-1) and DCA-induced macrophage-derived exosomes. Growth of GES-1 cells was suppressed by DCA-activated macrophage-derived exosomes (D-Exos), and this was verified by CCK-8 and EdU assays [77]. Activation of CDX2 is a critical event in gastric IM and is the most reliable molecular marker for its diagnosis. Exosomes were also found to harbor a high number of microRNAs (miRNAs), which are exploited for their use in delivering these regulatory particles to target cells [77]. There is evidence mounting that the dysregulation of miRNA plays a central role in the pathogenesis of gastric intestinal metaplasia. miRNA sequencing showed that hsa-miR-30a-5p was highly enriched in D-Exos versus exosomes from non-treated macrophages (M-Exos). To explore the implication of the function of hsa-miR-30a-5p and its target Forkhead Box D1 (FOXD1), Xu et al. [77] used mimics, inhibitors, and siRNAs to investigate the effect on cell proliferation with CCK-8 and EdU assays, and IM by CDX2 expression. In conclusion, the work proves that DCA-activated macrophage-released exosomal hsa-miR-30a-5p promotes IM and suppresses gastric epithelial cell proliferation by targeting FOXD1. The results expose a novel model of intercellular communication between DCA-activated macrophages and gastric epithelial cells in bile acid–associated chronic inflammatory disease.

Another study by Xu et al. [78] proved that DCA induces the development of gastric spasmolytic polypeptide-expressing metaplasia (SPEM) by modulating macrophage secretions in mice. The importance here is that it's the first proof to show macrophage-derived exosomes as a significant mediator through which DCA regulates the interaction between macrophages and gastric organoids, leading to the acceleration of SPEM development [78]. Recent evidence indicates that IM could develop from SPEM, and DCA is a driving force in this process. In an in vivo model, DCA treatment with subsequent immunofluorescence examination detected enhanced SPEM marker Trefoil Factor 2 (TFF2) and GSII expression in the mouse mucosae [78]. These data were confirmed by enhanced mRNA SPEM-related gene expression, *WAP four-disulfide core domain 2 (Wfdc2)*, Olfactomedin 4 (*Olfm4)*, and cystic fibrosis transmembrane conductance regulator (*Cftr)*. Gene expression and immunostaining also detected enhanced macrophage marker F4/80 in DCA-treated mice, confirming macrophage participation in SPEM development [78]. In addition, in vitro results indicated that the expression levels of *Wfdc2*, *Olfm4*, and *Cftr* were considerably higher in DCA-stimulated macrophage exosome-treated (D-Exos) gastric epithelial cells than those treated with exosomes from control macrophages (M-Exos) [78]. These results suggest that DCA can induce SPEM at least partially by activating macrophages to secrete exosomes. Together, these findings indicate that macrophage-derived exosomes mediate intercellular communication within the DCA microenvironment and thus contribute to the induction of SPEM.

Quilty et al. [79] found that DCA exposure promotes the secretion of pro-inflammatory cytokines IL-8 and IL-6 from normal esophageal epithelial cells. They established a mechanistic connection between DCA-induced cell membrane disruption and lipid raft-mediated signaling by caveolin-1 (Cav1) and Src kinase, which regulates transcription and secretion of IL-6 and IL-8. Particularly, DCA treatment also increased IL-6 and IL-8 mRNA levels and secretion in normal esophageal cells. The lower doses of DCA and TDCA did not remarkably increase IL-8 levels in Het-1A cells, suggesting that DCA needs to be above a certain concentration to cause this action. It has been demonstrated that bile acids have also been reported to induce IL-8 release in squamous esophageal cells, such as CA, taurocholic acid (TCA), CDCA, and taurochenodeoxycholic acid (TCDCA) [79]. Pharmacological inhibition studies defined key signaling pathways involved in this response. Inhibitors of p38 MAPK, SB203580, and MEK inhibitor PD98059 in Het-1A cells reduced DCA-induced IL-8 and IL-6 mRNA and protein significantly. Bisindolylmaleimide inhibition of PKC inhibited cytokine synthesis, also very significantly, on stimulation with DCA [79].

Supportive of these observations, studies on BHK-21 and colonic cells showed that DCA induces activation of phospholipase C in the plasma membrane, followed by calcium-dependent translocation of PKCα and PKCβ1 isoforms, thus validating the role of various PKC isoforms in bile acid signaling [79]. Furthermore, while DCA has been found to indirectly transactivate the EGFR, Quilty et al. [79] genistein experiments demonstrated that IL-6 and IL-8 induction by DCA were EGFR-independent but dependent upon associated downstream signaling pathways, specifically the MAPK cascade. PD98059 inhibition of cytokine production further demonstrates MAPK activation downstream of DCA signaling. Disruption of membrane cholesterol with nystatin, whether in the presence or absence of DCA,

enhanced release of IL-6 and IL-8, indicating that disruption of membrane cholesterol by itself can trigger pro-inflammatory signaling pathways like those triggered by DCA. This supports the inference that the disruption of caveolae represents the hub mechanism of the activity of DCA. Quilty et al. [79] also demonstrated that lowering Cav1 expression blocks DCA-induced IL-8 production, confirming the involvement of caveolae and Cav1 in bile acid-stimulated signaling. Methyl-β-cyclodextrin (MCD) treatment, which removes cholesterol from membranes, competitively inhibited DCA-induced secretion of IL-6 and IL-8. This indicates that cholesterol-rich lipid raft microdomains are required for effective cytokine induction. While DCA can also form into non-raft membrane domains to activate MAPK pathways, the effect of cholesterol depletion on raft and non-raft domains—and their subsequent inhibition of Src kinase activity—can potentially decrease inflammatory signaling. Cumulatively, Quilty et al. [79] evidence shows that DCA causes IL-6 and IL-8 secretion by esophageal cells via lipid raft-dependent signaling pathways involving Cav1, Src kinase, PKC isoforms, and downstream MAPK activation. These pro-inflammatory and immunomodulatory effects of DCA play a role in the establishment of a tumor-permissive microenvironment through the induction of chronic inflammation, immune cell phenotype reprogramming, and suppression of adaptive immunity.

Other roles of DCA in cancer: angiogenic effects, autophagy, drug resistance, and miRNA induction

Besides its well-established activities in apoptosis, metastasis, inflammation, and epithelial barrier dysfunction, DCA also significantly influences the tumor microenvironment to further enhance cancer growth [16]. These activities comprise the induction of angiogenesis, modulation of autophagy, augmentation of chemoresistance, and modulation of oncogenic and tumor suppressor miRNAs. Song et al. [16] concluded that a high-fat diet (HFD) greatly enhanced vasculogenic mimicry (VM) and EMT of patients with CRC, establishing a special link between diet, VM, and CRC development. Current research attests that HFD plays a role in colorectal carcinogenesis by triggering gut microbiota dysbiosis and changing microbiota-derived metabolites. Recurrently, they observed HFD-fed Apc $^{min/+}$ mice to have changed gut microbiota structure and diversity, thus affecting the amount of microbial metabolite production in the GI tract. In contrast to tumor angiogenesis, VM is the creation of tumor cell-lined, non-endothelial, channel-like conduits that perfuse the tumor with blood. The current research reveals, for the first time, direct proof that the microbial metabolite DCA induces VM formation in intestinal carcinogenesis. Identification of DCA as a potent risk factor for VM induction underscores the significance of elucidating the specific molecular processes involved in this action. Song et al. [16] found that HFD and DCA individually reduced epithelial markers but enhanced mesenchymal markers, indicative of EMT induction. Notably, EMT inhibitors U0126EtOH and SB431542 powerfully abolished the capability of CRC cell lines HCT-116 and HCT-8 to establish vascular mimicry structures, and this strongly demonstrates that EMT is indeed crucial in VM formation in intestinal cancers [16]. In addition, DCA highly upregulated vascular endothelial growth factor (VEGF) production, a central pro-angiogenic factor in tumor metastasis and angiogenesis. Song et al. [16] findings revealed that VEGF receptor 2 (VEGFR2) was a key mediator of VM formation induced by DCA, thus indicating this pathway as a promising target for therapeutic VM-based interventions. In this sense, sorafenib, a recognized VEGFR2 inhibitor, has been reported to inhibit VM in canine mammary gland cancer [80]. Therefore, early inhibition of VEGFR2 could thus represent an effective therapeutic regimen to prevent VM formation in intestinal cancers by inhibiting deleterious tumor microenvironment effects. In summary, Song et al. [16] findings show that HFD causes visceral obesity and disturbs gut microbiota and bile acid homeostasis to increase the levels of DCA, thus promoting intestinal initiation and tumor progression. DCA markedly promotes VM and EMT through the regulation of VEGFR2 signaling. The identification of VM as an emergent DCA-induced carcinogenic pathway provides valuable information and a promising target for individualized treatment of CRC.

Autophagy is an evolutionarily conserved process of cellular self-digestion of organelles and macromolecules, maintaining the viability of stressed and deprived cells, including cancer cells [81]. Autophagy is also involved in type II programmed cell death and is involved in many physiological processes, and autophagy defects are implicated in various types of malignancies, which implies that autophagy serves as a cellular transformation barrier [82]. Beclin-1, a principal autophagy regulator, is present in decreased levels in dysplastic BE and EAC patient biopsies and yet remains in increased levels in nondysplastic BE, squamous epithelium, and colon epithelium. Also, it has been found a drastic reduction in the mRNA expression of Beclin-1 has been found in EAC tissues in comparison to BE tissues. Roesly et al. [81] results show that acute exposure to DCA induces increased expression of Beclin-1 and enhances autophagy, whereas chronic exposure lowers the expression of Beclin-1 and inhibits autophagy. The ensuing inflammatory response is accompanied by the recruitment of proinflammatory cytokines TNF-α, IL-1β, and IL-6, which are all directly involved in carcinogenesis. Bile acids are implicated in the etiology of GI cancers such as EAC, colon cancer, and pancreatic cancer. In the current experiments, Roesly et al. [81] employed 0.2 mM DCA, a physiological

concentration. Short-term exposure to bile acids initiated a normal cellular response involving the induction and strengthening of Beclin-1 expression. Long-term exposure was not, however, able to maintain Beclin-1 levels, resulting in inhibition of autophagy. Particularly in CP-A cells (derived from nondysplastic BE), acute 4 h treatment with 0.2 mM DCA, a duration with minimal cytotoxicity, caused augmented expression of Beclin-1, as validated by immunoblotting, immunocytochemistry, and confocal microscopy [81]. However, sustained treatment with this dose of DCA caused cell apoptosis and decreased Beclin-1 expression. Globally, reduced levels of Beclin-1 and the resultant loss of autophagy after chronic bile acid exposure could be the etiology for enhanced genomic instability and cancer progression [81]. In the face of the prevailing increasing incidence of EAC due to as yet unknown causes, it recommends the regulation of bile acid reflux in BE patients to avoid cell changes secondary to the chronic action of such deleterious stimuli.

Another study by Kong et al. [17] found that the carcinogenic activities of DCA could be mediated by miRNA expression regulation. They examined miRNA microarray profiling in DCA-treated PCEC cells and identified extensive changes in miRNA expression profiles. miRNAs with over- or under-expression greater than 1.3-fold were deemed likely to contribute to CRC pathogenesis in this situation. Interestingly, miR-199a-5p was reduced in the majority of CRC samples, whereas its putative target CAC1 was increased, indicating a causal relationship between the molecules. Kong et al. [17] findings showed that miR-199a-5p targets the 3′-untranslated region (3′-UTR) of CAC1 mRNA, thus suppressing its translation. Furthermore, suppressing miR-199a-5p using anti-miR-199a-5p reversed this suppressive effect, resulting in enhanced expression of CAC1. These data indicate that miR-199a-5p is a tumor suppressor that represses cell proliferation and causes cell cycle arrest in CRC cells at least in part by downregulating CAC1 [17]. In summary, these findings demonstrate the prominent role of miRNAs in mediating hydrophobic bile acids like DCA-induced carcinogenic effects, with abnormal miRNA expression being the prevalent mechanism. Since miRNAs control oncogenes as well as tumor suppressors, their abnormally induced expression by DCA would significantly impact colorectal carcinogenesis. These observations have significant implications for the mechanism of miRNA deregulation in tumorigenesis associated with bile acids and could offer new therapeutic strategies.

Lin et al. [83] discovered that serum DCA levels were lowered in patients with gallbladder cancer (GBC) and are most likely tumorigenic via elevated m^6A methylation modification of pri-miR-92b. This miRNA selectively downregulates the tumor suppressor Phosphatase and tensin homolog (PTEN) and leads to activation of the PI3K/AKT signaling pathway. Importantly, GBC patients with lowered serum DCA levels exhibited significantly lower overall survival, which supports the clinical significance of this bile acid imbalance. Lin et al. [83] also established DCA treatment of GBC cells to cause differential expression of miRNAs at substantial levels, with downregulation of the most prominent miR-92b-3p. The oncogenic miR-17–92 cluster on chromosome 13 member miR-92b-3p has been reported to drive tumorigenesis in a majority of human cancers. This was further confirmed by the finding of higher expression of miR-92b-3p in tumor tissues of GBC and its ectopic overexpression-mediated increased proliferation of GBC cells [83]. Xenograft models also established the pro-tumorigenic function of miR-92b-3p. Notably, miR-92b-3p also partially regained the growth inhibitory action of DCA against GBC cell growth and tumorigenesis, indicating that DCA controls GBC growth at least in part by targeting this oncogenic miRNA. Furthermore, Lin et al. [83] also identified PTEN as a tumor suppressor under negative PI3K/AKT pathway control, as a direct miR-92b-3p target. The downregulation of miR-92b-3p resulted in the upregulation of PTEN in GBC cells. In this context, these results established that DCA treatment increases levels of PTEN by inhibiting miR-92b-3p and thereby interrupting PI3K/AKT signaling in GBC. Mechanistically, DCA significantly decreased pri-miR-92b m^6A methylation in GBC cells. It is achieved by preventing the formation of the METTL3/METTL14/WTAP methyltransferase complex that catalyzes m^6A modification on diverse mammalian RNAs, by direct binding to METTL3 without altering the protein levels. The decreased m^6A methylation decreased pri-miR-92b maturation and thus produced lower levels of miR-92b-3p after DCA treatment. In total, DCA enhances tumor development by direct cytotoxic effects and inflammation and by remodeling the tumor microenvironment by increased vasculogenic mimicry and angiogenesis, dysregulation of autophagy, and modulation of miRNA expression. Such mechanisms by DCA and particularly VEGFR2 signaling and modulation of miRNA are viable targets for the development of anticancer treatments, particularly in CRC and GBC.

Key signaling pathways modulated by DCA in carcinogenesis

Several studies have shown that bile acids regulate intracellular cascades of signals, and initiation of cascades is a determinant function in apoptosis and/or mitogenesis (Table 2) [84]. A close look at the MAPK pathway demonstrates that DCA potentiates signaling by activation of the EGFR (Fig. 3) [84]. Interestingly, EGFR has been reported to be localized in caveolae and lipid raft membrane microdomains, and DCA is claimed to be capable of activating

Table 2 Signaling pathways modulated by DCA in cancer

Cancer type	Study setting	Signaling pathway	Description	Ref
Colon cancer (colitis-associated)	In vivo and in vitro	Wnt/β-Catenin signaling (via FXR downregulation)	DCA levels increased after cholecystectomy, promoting colon carcinogenesis by enhancing cell proliferation and migration through activation of the Wnt/β-Catenin pathway; FXR downregulation leads to upregulation of β-Catenin and c-Myc	[125]
Gastric cancer (intestinal metaplasia stage)	In vivo and in vitro	TGR5/STAT3/KLF5 signaling	DCA induces intestinal metaplasia and dysplasia by activating STAT3 signaling and upregulating KLF5; also alters gastric bile acid metabolism and promotes microbial dysbiosis (e.g., Lactobacillus enrichment)	[92]
Esophageal adenocarcinoma	In vitro	IL-6/STAT3 signaling	DCA promotes cancer stem cell reprogramming factors KLF4 and OCT4 via the IL-6/STAT3 pathway, enhancing anti-apoptotic ability and malignancy; targeting this pathway may improve therapy	[88]
Colon cancer	In vitro	NF-κB and AP-1 signaling	UDCA does not activate NF-κB or AP-1 but inhibits DCA- and IL-1β-induced activation of these transcription factors via a PKC-dependent pathway, suggesting UDCA antagonizes DCA's pro-carcinogenic effects	[126]
Colon cancer	In vitro	MAPK pathways (SAPK/JNK, p38, ERK1/2), Cell cycle regulation	DCA and methylselenol both inhibit cell proliferation and promote apoptosis, but DCA induces activation of MAPK pathways (SAPK/JNK, p38, ERK1/2) while methylselenol does not; they have distinct molecular effects on colon tumorigenesis	[127]
Colon cancer	In vitro	MAPK pathways (SAPK/JNK, p38, ERK1/2), Cell cycle regulation	DCA and methylselenol both inhibit cell proliferation and promote apoptosis, but DCA induces activation of MAPK pathways (SAPK/JNK, p38, ERK1/2) while methylselenol does not; they have distinct molecular effects on colon tumorigenesis	[128]
Colon cancer (HCA-7 cells)	In vitro	Focal adhesion kinase (FAK) phosphorylation; ShP2 tyrosine phosphatase	DCA differentially regulates site-specific phosphorylation of FAK by dephosphorylating Tyr-576/577 and Tyr-925 but not Tyr-397; DCA activates Src kinase and increases association of tyrosine phosphatase ShP2 with FAK, promoting FAK dephosphorylation and reducing cell adhesion. ShP2 is critical in DCA-mediated signaling affecting focal adhesion and cell adhesion in colon cancer cells	[129]
Colon cancer	In vitro	ERK1/2 MAPK pathway	DCA induces EphA2 up-regulation via ERK1/2 activation independently of p53 status, contributing to carcinogenesis	[130]
Colon cancer	In vitro and in vivo	ADAM17-dependent EGFR/Akt pathway	DCA activates EGFR via ADAM17-mediated amphiregulin release, promoting intestinal tumor development and progression	[2]

Table 2 (continued)

Cancer type	Study setting	Signaling pathway	Description	Ref
Colon cancer	In vitro	Non-canonical EGFR-MAPK via Ca^{2+}/CAMKII/c-Src	DCA induces prolonged ERK1/2 activation through calcium signaling, preventing EGFR degradation and promoting tumorigenesis	[131]

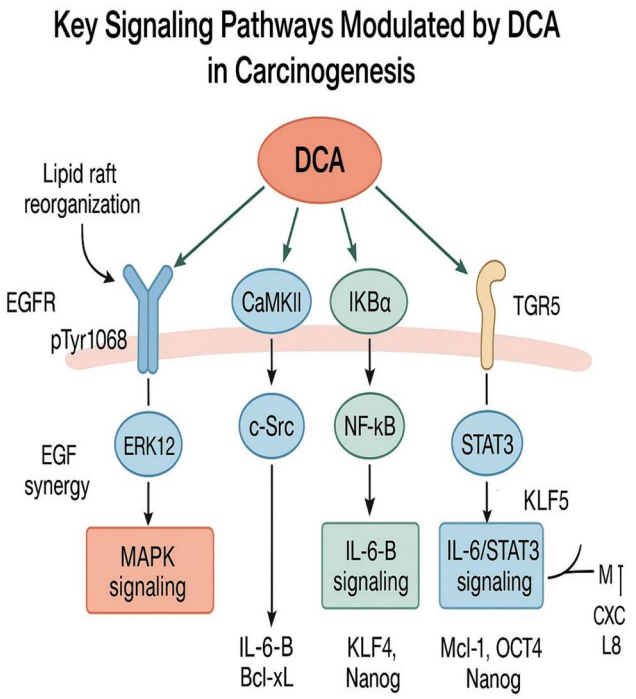

Fig. 3 Signaling pathways modulated by deoxycholic acid (DCA) in carcinogenesis. DCA alters the biophysical properties of the plasma membrane, reorganizing lipid rafts and caveolae by translocating caveolin and enriching cholesterol, which in turn enables ligand-independent activation of epidermal growth factor receptor (EGFR). EGFR phosphorylation at Tyr1068 and Tyr845, via calcium/CaMKII/c-Src signaling, triggers downstream mitogen-activated protein kinase (MAPK) signaling (ERK1/2), promoting cell survival and proliferation. DCA synergizes with epidermal growth factor (EGF) to enhance MAPK activation and tumor-promoting signals. Additionally, DCA activates the NF-κB pathway in Barrett's epithelial cells via ROS/RNS-mediated phosphorylation of IκBα and p65, promoting resistance to apoptosis and inducing pro-inflammatory and antiapoptotic genes such as IL-8, Bcl-2, and Bcl-xL. In esophageal adenocarcinoma (EAC) cells, DCA also induces IL-6/STAT3 signaling, driving the expression of stemness markers (KLF4, OCT4, Nanog) and antiapoptotic proteins (Bcl-xL), contributing to cancer stem cell (CSC) phenotypes and therapeutic resistance. In gastric epithelial cells, DCA activates the TGR5–STAT3–KLF5 axis, leading to intestinal metaplasia (IM), inflammation, and the expression of genes such as Mcl-1, IL-6, and CXCL8, creating a pro-tumorigenic microenvironment

the receptor by changing the physical state of the cell membrane [84]. DCA causes translocation of the major structural protein of caveolae, caveolin, into the cytoplasm from the plasma membrane, thus influencing both the number and the location of caveolae in the membrane. Further, membrane fractionation of DCA-treated cells using sucrose gradient centrifugation also showed a marked enrichment of cholesterol in caveolin-enriched fractions [84]. This suggests that DCA not only reorganizes the spatial structure of membrane microdomains but also controls their lipid and protein composition. Consistent with this, Cuijuan et al. [85] showed that

cholesterol displacement results after ceramide treatment, which is known to be an apoptosis inducer. It thus becomes plausible that DCA, as ceramide, controls cholesterol composition and related membrane lipids and proteins, possibly intracellular signal modulation.

Jean-Louis et al. [84] had previously shown DCA to activate the MAPK pathway; they thus investigated the activation of EGFR at tyrosine residue (Y1068) necessary for MAPK signaling. Notably, phosphorylation of tyrosine 1068 of EGFR in cells treated with DCA was not influenced by an EGF-neutralizing antibody, which suggests that DCA activates EGFR in an EGF-independent fashion. This finding is consistent with earlier research by Qiao et al. [55], which demonstrated that bile acids can stimulate the c-Jun N-terminal kinase (JNK) cascade in hepatocytes in a ligand-independent fashion. Jean-Louis et al. [84] results clearly demonstrate that DCA-induced changes in membrane structure and composition are responsible for the activation of EGFR. They also found that other transmembrane proteins are induced by DCA treatment, and additional studies are attempting to map the targeted signaling pathways of these transmembrane proteins [84]. Since receptors, such as EGFR and insulin-like growth factor 1 receptor (IGF-1R), reside in lipid microdomains, it is expected that DCA modulates membrane microenvironment dynamics to activate a variety of signaling receptors.

Other studies, such as Centuori et al. [15], and Nguyen et al. [65] show that DCA can activate the MAPK/ERK pathway—often via EGFR transactivation, calcium–calmodulin (CaM)-dependent protein kinase II (CaMKII)–Src signaling, or cytokine-mediated mechanisms—promoting proliferation, migration, invasion, and EMT. These effects are generally observed in transformed or tumor-associated cells, sometimes in the presence of additional cofactors (e.g., TGF-β, IL-8). Centuori et al. [15] found that exposure of colon cancer cells to DCA at levels equivalent to those seen with a Western diet causes calcium-dependent activation of phosphorylated CAMKII (p-CAMKII), c-Src, and EGFR phosphorylation at the c-Src-specific site Tyr845. Activation of these pathways leads to downstream MAPK signaling, which leads to ERK1/2 activation. Most notably, co-treatment with DCA and EGF elicited a more robust and sustained MAPK activation than either drug alone, suggesting that non-canonical as well as canonical EGFR signaling pathways may be synergistic [15]. The current research explains a novel, previously unknown mechanism through which DCA activates the pro-tumorigenic EGFR-MAPK signaling pathway in colon cancer, demonstrating DCA's redundancy as a pathway activator. Colon cancer cells treated with physiologically relevant DCA concentrations show robust EGFR-MAPK activation. To our knowledge, this is the first evidence that DCA stimulates EGFR-MAPK signaling in a ligand-independent mode and synergizes with the natural ligand EGFR. Centuori et al. [15] results negate earlier controversy by demonstrating that DCA modulates ERK1/2 activity in both ligand-dependent and ligand-independent manners. Significantly, DCA activates EGFR without enhancing its degradation, a finding supportive of the hypothesis that DCA amplifies EGFR-mediated signaling, which might be accountable for its tumor-promoting activity. It is suggested that DCA stimulates the mobilization of calcium, which in turn stimulates calcium-dependent CAMKII kinase activation. Activated CAMKII also activates c-Src, leading to alternative EGFR activation at Tyr845 and downstream MAPK signaling [15]. The signaling pathway offers a reasonable molecular mechanism for the clinical correlation of elevated levels of DCA and increased adenoma formation. Accordingly, the suppression of components of this non-canonical EGFR activation pathway, for example, calcium signaling, is a potential therapeutic strategy through which colon cancer risk in patients with elevated DCA levels can be reduced [15].

Huo et al. [86] showed that DCA causes NF-κB pathway master protein phosphorylation in Barrett's epithelial cells. They showed, with two Barrett's epithelial cell lines, that DCA-induced NF-κB activation and DNA damage are regulated by ROS/Reactive nitrogen species (RNS) production. The previous findings showed that Barrett's epithelial cells are resistant to apoptosis after Ultraviolet B (UV-B) induced DNA damage due to the activation of the NF-κB pathway [87]. In the current study, Huo et al. [86] exposed Barrett's cells to physiologic levels of DCA to simulate gastric reflux conditions. In Barrett's cancer cell lines, DCA induces the generation of ROS/RNS, DNA damage, and NF-κB activation, all of which are reversible by antioxidant treatment. In the current study, they determined that DCA elevates ROS/RNS in non-neoplastic Barrett epithelial cells. N-acetylcysteine (NAC) blockade of ROS/RNS scavenging inhibited DCA-mediated DNA damage and p65 phosphorylation in BAR-T cells, suggesting ROS/RNS-dependent activation [86]. Huo et al. [86] findings corroborate that, as in cancer cells, DCA triggers DNA damage and NF-κB activation through ROS/RNS production in benign Barrett's cells. DCA has been reported to trigger NF-κB activation and NF-κB target gene IL-8 and IκB overexpression in Barrett-associated adenocarcinoma cells. They further established that NF-κB activation enables benign Barrett's cells to evade UV-B-induced DNA damage-induced apoptosis. Huo et al. [86] demonstrate that DCA induces p-IκBα and p-p65 levels in Barrett's epithelial cells in vitro and biopsy samples in vivo from patients with BE. DCA elevated nuclear total p65 and p-p65 levels in Barrett's cell lines with increased Bcl-2 levels, justifying NF-κB pathway activation. Notably, NF-κB pathway blockade by BAY 11–7085 or an IκB super-repressor reversed apoptosis resistance in BAR-T cells exposed to DCA. Conversely, UDCA failed to elevate

p-H2AX, p-IκBα, or p-p65 levels in vitro and in vivo [86]. In general, DCA induces genotoxic stress in non-neoplastic Barrett's epithelial cells, which are apoptosis-resistant via activation of the NF-κB pathway. This was confirmed by showing DNA damage and NF-κB phosphorylation in BE biopsies of patients who underwent transient DCA perfusion during endoscopy.

Chen et al. [88] findings further show that DCA triggers the IL-6/Signal transducer and activator of transcription 3 (STAT3) signaling pathway in EAC cells. After DCA exposure, resulting pSTAT3 phosphorylation promotes expression of KLF Transcription Factor (KLF) 4, Octamer-binding transcription factor 4 (OCT4), and Nanog, which induces Cancer stem cell (CSC)-like features as well as reprogramming of EAC cells into CSCs. Decreased STAT3 phosphorylation has also been involved with curcumin's anticancer potential in esophageal cancer [88]. STAT3 activation also enhances Bcl-xL antiapoptotic protein expression, thereby favoring EAC cell survival and apoptosis resistance. Bcl-xL is a Bcl-2 family member and an important antiapoptotic effector, and its overexpression results in a higher apoptotic threshold of the tumor cells. DCA-promoted inflammation increases intracellular ROS, which is associated with the upregulation of circular RNA (circRNA) derived from miR-21, a transcriptionally regulated process by NF-κB. Activation of the NF-κB pathway further increases the expression of antiapoptotic proteins Bcl-2, allowing DNA-damaged cells to resist apoptosis [88]. In agreement with this mechanism, previous research has shown that Panax notoginseng saponins (PVN) suppress JAK2/STAT3 signaling, thereby decreasing the expression of Bcl-xL and Bcl-2 [89]. Liu et al. [90] found that the co-activation of NF-κB and STAT3 in chronic lymphoma cells enhances the expression of IL-6, Bcl-xL, and Mcl-1 and chemoresistance. Zaanan et al. [89] also showed that STAT3 mediates resistance of Kirsten rat sarcoma virus (KRAS)-mutated tumors to apoptosis by inducing Bcl-xL upregulation. Matsumoto et al. [91] also recognized that *Helicobacter pylori* vacuolating cytotoxin induces gastric cancer cell apoptosis by inhibiting STAT3 activity and reducing Bcl-xL and Bcl-2. These data validate Chen et al. [88] conclusion that activation of STAT3 is the most crucial factor for promoting EAC cells' antiapoptotic phenotype through upregulation of Bcl-xL. DCA induces malignant transformation by activating the IL-6/STAT3 pathway and overexpressing central pluripotency and survival genes, such as KLF4, OCT4, Nanog, and Bcl-xL [88]. This induction of CSC-like characteristics not only increases the antiapoptotic features of the EAC cells but also results in increased tumor aggressiveness [91]. Since CSC formation is inherently associated with therapeutic resistance, recurrence, invasion, and metastasis, the IL-6/STAT3 pathway is a promising therapeutic target. Small molecule pathway inhibitors and DCA-neutralizing activities may enhance therapeutic benefits by inhibiting CSC formation and optimizing the efficacy of treatment in esophageal adenocarcinoma.

Jin et al. [92] found that stimulation of gastric epithelial cells by DCA, the most common secondary bile acid in the stomach, triggers a new signaling pathway with bile acid receptors TGR5, STAT3, and KLF5. These findings showed that pathologic molecular signaling after bile acid exposure makes it permissive for the gastric microenvironment to develop IM. Sustained elevation in the levels of DCA significantly disrupts bile acid metabolism and changes gastric microbiota composition with marked enrichment of bacterial genera, such as *Lactobacillus,* that are implicated in carcinogenesis. Mechanistically, Jin et al. [92] data showed that in DCA-induced inflammation and IM, STAT3 is constitutively phosphorylated and found in the nucleus, where it binds directly to two sites on the KLF5 promoter to initiate its transcription. In addition, DCA stimulation also initiates a number of STAT3 downstream target genes, including Mcl-1, Bcl-2, IL-6, and CXCL8, thereby endowing gastric epithelial cells with heightened inflammatory responses and anti-apoptosis properties that are commonly associated with a pre-neoplastic phenotype [92]. These findings indicate that STAT3 is an important mediator of bile acid-induced carcinogenesis in IM tissue. The TGR5 bile acid receptor is presumably an upstream regulator conveying DCA exposure with STAT3 activation. Additional studies will need to be performed to identify other STAT3 downstream effectors of IM development. More physiologically appropriate animal models must also be developed to more accurately model duodenogastric reflux (DGR) and confirm these mechanistic observations [92]. In conclusion, the findings incriminate the TGR5–STAT3–KLF5 signaling pathway as an important etiological mechanism for bile acid-induced IM [92]. Pharmacologic inhibition of TGR5 might be an effective therapy in preventing gastric IM formation in patients with bile reflux. In addition, phosphorylated STAT3 may be a useful early biomarker for gastric carcinogenesis, and the occurrence of KLF5 in IM lesions can predict the risk of progression to intestinal-type gastric cancer. Finally, DCA-induced enrichment of targeted bile acids is uncovered with the proliferation of *Lactobacillus* species with oncogenic activity, indicating the correlation between bile acids, microbial dysbiosis, and gastric epithelial transformation.

Diagnostic and therapeutic perspectives: DCA as a diagnostic biomarker and therapeutic target

Mounting evidence suggests the multistep function of gut microbiota in cancerogenesis through the direct action of certain pathogenic microorganisms, as well as by bioactive

metabolites synthesized by them [93]. Obesity-induced dysbiosis results in increased levels of DCA synthesized by Clostridium species, recycled by enterohepatic circulation [93]. Yoshimoto et al. [94] illustrated that DCA liver accumulation causes a senescence-associated secretory phenotype (SASP) of hepatic stellate cells, leading to the secretion of proinflammatory and tumor-promoting factors involved in hepatocarcinogenesis. Aside from GI cancers, DCA has contributed to the pathogenesis of other types of cancer [93, 95–97].

Ma et al. [18] measured bile acids in malignant and nearby non-malignant colorectal tissues and found that DCA was markedly increased in malignant tissue, while CA and CDCA were drastically decreased. Ex vivo coculture experiments illustrated that mucosal microbiota in patients with CRC and chronic gastritis exhibited increased DCA-synthesizing activity, indicating enrichment of DCA-producing bacteria within these tissues [18]. As a hydrophobic secondary bile acid, DCA also presents a certain level of danger by virtue of the capacity to abrogate intestinal barrier function and trigger genotoxic stress—characteristics involved in colorectal tumorigenesis [18]. Ma et al. [18] results concur with earlier accounts of increased DCA levels with the development of multiple polypoid adenomas, once again contradicting the proposal that secondary bile acids, and DCA in particular, are tumor-aggressors in the colon. Hyperproliferation and enhanced migratory ability of a range of CRC cell lines by DCA were reported in accordance with cell-based observations [18]. It is the first study that compares directly mucosa-associated microbiota and tissue-associated bile acid profiles in malignant and matched non-malignant locations from CRC patients. Ma et al. [18] found striking differences in the microbial communities, with the malignant tissues harboring a higher percentage of bacteria with the ability to metabolize primary bile acids to secondary species or produce DNA-damaging metabolites. The noncancerous tissues had relative enrichment of commensal or protective microbes. Mechanistically, they discovered that high DCA in tumor tissue interacts with the FXR and represses its expression [18]. Since decreased FXR expression has been strongly implicated in colorectal carcinogenesis, this axis is a primary molecular pathway that links microbial dysbiosis, dysregulated bile acid homeostasis, and oncogenesis. In summary, these discoveries establish the interaction between mucosal microbiota composition, increased levels of DCA, and dysfunctional FXR signaling in tumor-bearing CRC tissues. These findings highlight the therapeutic potential of modulating the microbiota–bile acid–FXR pathway as a strategy to prevent and treat colorectal neoplasia.

Zhong et al. [98] explored the dynamic interactions of gut microbiota and bile acid metabolism by employing the DCA to taurocholic acid (TCA) ratio (DCA/TCA) as a marker for bile salt hydrolase (BSH)-catalyzed deconjugation and 7α-dehydroxylation activities. Elevated DCA composition and DCA/TCA ratio were observed in the high post-implantation pain neuropathy (PIPN) group. Notably, the DCA/TCA ratio was the most discriminatory between the high- and low-PIPN groups. Furthermore, in asymptomatic individuals, reduced glycohyocholic acid (GHCA) levels combined with a raised DCA/TCA ratio predicted the development of PIPN strongly [98]. These results reveal a distinct serum bile acid pattern in PIPN patients and corresponding rodent models that differs from pruritus models. Concurrent with these events, the chemokine receptor C–C chemokine receptor type 5 (CCR5) has surfaced as a therapeutic target against human immunodeficiency virus (HIV)-related neuropathy, including distal symmetrical polyneuropathy. Ligand-receptor interactions of CCL5 with CCR5 within dorsal root ganglia have been recognized to regulate neuronal excitability and play a role in the pathogenesis of PIPN. Pharmacologic or genetic inhibition of CCR5—through genetic knockout, maraviroc (CCR5 antagonist), or CCL5-neutralizing antibodies—alleviated paclitaxel-induced neuropathic pain in preclinical models [98]. These findings substantiate the idea that inhibition of CCR5 may confer protection against toxic bile acid actions, like those of secondary bile acids like DCA. The pro-inflammatory potential of DCA, especially to modulate chemokine signaling, is further augmented. Sun et al. [99] reported that DCA induced TGR5-mediated signaling and thus induced the expression of CCL28 by colon cancer cells. Likewise, Allen et al. [100] found that exposure to DCA increased mRNA expression of CCL2, CCL5, CCL7, and CCL20 in hepatocytes.

Nutritional high fats and gallbladder disease have previously been demonstrated to increase intestinal luminal DCA concentrations, thus enhancing the development of CRC [101]. In addition to disturbing intestinal metabolic activities, DCA disrupts gut microbiota composition, as reported by Su et al. [102]. Additionally, derivatives of DCA also act to modulate the immune system by affecting regulatory T cells (Treg cells) and hence driving CRC development [103]. In Liu et al. [101], treatment with DCA induced a remarkable increase in the size and number of intestinal tumors, which was associated with impaired intestinal barrier function. Treatment with Huangqin decoction (HQD) suppressed CRC tumor cell growth, restored goblet cell numbers, and augmented intestinal mucus secretion, all resulting in better barrier function [101]. Microbiologically, treatment with DCA induced significantly increased *Eggerthellales* abundance at the species level. *Eggerthellales* are involved in primary bile acid biotransformation to secondary bile acids—compounds implicated in carcinogenesis [101]. Enrichment of *Eggerthellales* would thus enhance GI tract accumulation of tumor-fostering secondary bile acids. In contrast, HQD

treatment decreased *Eggerthellales* dramatically and the concentration of secondary bile acids, thus preventing CRC formation [101]. In addition, DCA treatment was correlated with decreased abundance of health-sustaining bacterial phyla such as *Lachnospiraceae*, *Firmicutes*, *Fusobacteria*, and *Clostridium* [101]. HQD therapy reestablished this dysbiosis through the reversal of these microbial communities. *Lachnospiraceae*, in fact, is reported to be responsible for the maintenance of intestinal and carbohydrate metabolism [101]. The augmentation of *Lachnospiraceae* upon HQD therapy can be attributed to better glucose metabolism and energy homeostasis, and thereby the avoidance of DCA-induced weight loss in CRC mice. Together, these findings indicate that DCA induces colorectal carcinogenesis via microbiota dysbiosis, immune modulation, and barrier dysfunction, and that HQD therapy may inhibit these processes by restoring microbial homeostasis, intestinal integrity, and inhibiting tumor development.

Xu et al. [104], pH-responsive DCA dimer was reported as a nanocarrier for augmenting the efficacy of chemotherapeutic agents. The DCA dimer was prepared through ortho ester linkage and further co-self-assembled with doxorubicin (DOX) into nanoparticles (DCA-OE/DOX NPs). These nanoparticles showed greater drug encapsulation efficiency and physiological stability, but allowed for the effective release of drugs under acidic conditions like the tumor microenvironment. Antitumor activity and apoptosis in vitro experiments also demonstrated that DCA-OE/DOX NPs possessed equivalent antitumor effectiveness with respect to free DOX [104]. The nanoparticles were also able to effectively accumulate inside HepG2 multicellular tumor spheroids and H22 tumor tissues in vivo with greater inhibition of tumor growth. Notably, the DCA-based nanocarrier was highly biocompatible and reduced systemic toxicity considerably. Such results are indicative of the enormous promise of pH-sensitive DCA dimers as potential small-molecule carriers for targeted anticancer drug delivery [104].

A prodrug approach with bile acids as targeting ligands has been suggested in previous research to allow hepatocyte-targeted drug delivery via the use of bile acid transporter-facilitated uptake [105]. The method takes advantage of the high-affinity binding of bile acid moieties with the Na^+-dependent taurocholate co-transporting polypeptide (NTCP), which is highly and selectively expressed on the hepatocyte membrane [105]. In addition, under the enterohepatic circulation, where the bile acids are recycled 6 to 15 times daily, bile acids have a very high transport efficiency [105]. Thus, chemical conjugation of chemotherapeutic agents like camptothecin (CPT) with bile acids like DCA can potentially enhance hepatic uptake and lower systemic toxicity. A DCA-conjugated CPT prodrug, G2, was synthesized in this research. The efficiency of liver targeting and stability is examined in both in vivo and in vitro conditions [105]. Competitive inhibition experiments in HepG2 cells indicate that G2 uptake most likely occurs via bile acid transporters, which implies that conjugation with DCA is effective. Cellular uptake experiments in two-dimensional (2D) monolayer and three-dimensional (3D) spheroid models validated that DCA conjugation alters the route of internalization of CPT such that bile acid transporters have significant roles in G2's improved uptake [105]. Finally, these results justified the use of DCA as a bioactive moiety to ensure the most effective liver-targeting and drug-likeness stability of CPT and presented a solid theoretical foundation for the future preclinical development of G2 as a hepatotropic anticancer prodrug (Table 3).

Concluding remark and future direction

DCA, a secondary bile acid derived from gut microbiota, was identified as a major regulator of GI carcinogenesis by multiple mechanisms. There is robust evidence in numerous studies to conclude that DCA exerts pleiotropic effects on intestinal epithelial cells, immune cells, and oncogenic signaling. DCA also impairs epithelial barrier integrity by downregulation of occludin and claudin-5 tight junction proteins, blockade of ERK signaling, and enhanced transcellular and paracellular permeability. These disturbances cause chronic inflammation, increased luminal antigen presentation, and tumor initiation and development susceptibility. DCA also alters host immune response, most notably by suppressing $CD8^+$ T cell-mediated antitumor immunity by downregulation via calcium-NFAT2 signaling that is PMCA-dependent. In addition, DCA triggers the transcription of mucin genes like MUC2 by NF-κB pathway activation, promotes metaplastic transition of the stomach and esophagus by KLF5 and CDX2 pathways, and stimulates proliferation of colon cancer cells by EGFR transactivation and β-catenin pathways. At higher doses, DCA can induce mitochondrial apoptosis by triggering loss of membrane potential, activating caspase-9, and modulating Bcl-2 family proteins, though such dosing is generally cytotoxic and non-physiologic. Though the tumor-promoting effects of DCA are well known, there exist some very important lacunae. The bimodal action of DCA as an oncogenic and cytotoxic compound needs further elucidation, especially in terms of concentration range, exposure duration, and host genetic background. In addition, how diet, GI microbiota, and DCA biosynthesis interact is also not very clear, especially how variables interact to modulate susceptibility to cancer.

Follow-up studies should aim to clarify the DCA–microbiota–immune axis to further explore the mechanisms by which DCA modulates the tumor microenvironment and immune evasion processes. DCA production or activity-interfering enzymes—e.g., diet, bile acid sequestrant

Table 3 Therapeutic role of deoxycholic acid in cancer

Cancer type	Study setting	Therapeutic function	Outcome	Ref
Colorectal cancer (CRC)	In vitro	Targeting DCA-producing microbiota or restoring FXR expression as a therapeutic/preventive strategy	DCA accumulation due to tumor-enriched microbiota promotes CRC cell proliferation, migration, and invasion via FXR downregulation. FXR restoration or microbiota modulation suggested	[132]
Various cancers (with thrombosis)	In vitro and in vivo	DOCA used in redox-sensitive nanoparticles to deliver paclitaxel and inhibit cancer-associated thrombosis	Redox-sensitive PTX/heparin-DOCA nanoparticles reduced thrombosis in cancer-bearing mice by inducing apoptosis and downregulating coagulation pathways	[133]
Potential peptide-based cancer therapies	In vitro	DOCA conjugation to therapeutic peptides (e.g., calcitonin) to enhance intestinal absorption for oral delivery	DOCA conjugation significantly increased permeability of calcitonin across Caco-2 cells (up to 2.5-fold) when formulated in DMSO, supporting potential oral delivery strategies	[134]
CRC prevention	In vivo	Dietary polyphenols (e.g., curcumin, caffeic acid) reduce levels of DCA and LCA, colon cancer-promoting bile acids	Curcumin and other polyphenols significantly reduced fecal deoxycholic and lithocholic acids in rats fed high-fat diets, suggesting a protective effect against colon cancer	[135]
Lung cancer	In vitro and in vivo	DOCA-modified polymeric micelles (COS-DOCA/mPEG-PDLLA) used to improve paclitaxel delivery and antitumor efficacy	PTX-loaded COS-DOCA/mPEG-PDLLA micelles improved drug uptake in tumor cells, reduced systemic toxicity, prolonged circulation, and enhanced antitumor efficacy	[136]
Colon carcinoma (DOX-resistant)	In vitro	DOCA-conjugated dextran (DexDA) nanoparticles for doxorubicin delivery	DOX-loaded DexDA nanoparticles enhanced cytotoxicity against resistant colon cancer cells, promoted intracellular retention, and outperformed free DOX in antitumor activity	[137]
CRC	In vivo	Indirect: Huangqin decoction (HQD) alleviates DCA-induced tumorigenesis via microbiota regulation	HQD reduced tumor formation, increased goblet cells, modulated bile acid metabolism and energy pathways, and restored gut microbial balance (↑ Firmicutes, ↓ Eggerthellales)	[101]
Liver cancer (HepG2)	In vitro and in vivo	DCA-dimer (DCA-OE) as pH-sensitive nanocarrier for doxorubicin delivery	DCA-OE/DOX NPs showed improved drug stability, enhanced tumor accumulation, reduced systemic toxicity, and a significantly higher tumor inhibition rate (84.1%) vs. DOX	[138]
Hepatocellular carcinoma	In vitro	Pectin-deoxycholic acid (P-DOCA) micelles for targeted delivery of sorafenib (SF) to hepatocytes	SF-loaded micelles increased cytotoxicity by 30%, enhanced cellular uptake, and reduced HepG2 cell migration to 6.67% vs. 26.67% for free SF	[139]
Liver cancer (HepG2 cells)	In vitro and in vivo	Liver-targeted delivery of camptothecin (CPT) via conjugation with deoxycholic acid; improved stability and enhanced liver uptake	DCA-CPT conjugate showed better stability, increased liver targeting index by 1.67-fold vs CPT, and reduced cellular uptake upon bile acid transporter inhibition	[140]

treatment, inhibition of microbial bile acid-altering enzymes (e.g., the bai operon), or administration of engineered probiotics—may provide valuable new approaches to cancer prevention and cancer treatment. Also, investigation of epigenetic and transcription changes promoted by repeated DCA dosing may also identify early biomarkers for the risk of colorectal and liver cancers. Engineering of DCA-drug delivery systems, e.g., the DCA-heparin conjugate, with documented antiangiogenic activity, also points to the therapeutic potential of bile acid pathway manipulation. Lastly, in summary, DCA constitutes a crucial mechanistic link between gut microbiota, intestinal barrier integrity, immune modularity, and cancer initiation. Additional exploration of its pleiotropic action can lead to pioneering action for countering cancer risk and enhancing treatment with the application of microbiota-guided approaches.

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Declarations

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