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Review

Research Progress in Interactions between Bile Acids, Gastrointestinal Microbes and GPBAR1 in Pancreatic Cancer

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ABSTRACT: Bile acids have traditionally been regarded as a contributing factor in the development of pancreatic cancer. However, the mechanisms by which they induce or promote the progression of pancreatic cancer remain controversial. Additional research is necessary to provide substantial evidence. There is evidence that gastrointestinal microbes involved in bile acid metabolism and thw G protein-coupled bile acid receptor (GPBAR1), which responds to bile acid signals, may collaborate with bile acids in the initiation and progression of pancreatic cancer. This review focuses on the relationships between bile acids, gastrointestinal microbes, GPBAR1 and pancreatic cancer. It aims to interpret the factors influencing the onset and development of pancreatic cancer from a holistic perspective and provide insights towards the treatment of pancreatic cancer.

Keywords: Bile acid; Gastrointestinal microbe; GPBAR1; Pancreatic cancer

1. Introduction

Pancreatic cancer is a lethal disease with high mortality rates. There is a consistent increase in the prevalence of pancreatic ductal adenocarcinoma (PDAC) yearly, yet the mortality rate remains high due to delayed diagnosis, early metastasis, and limited efficacy of chemotherapy or radiotherapy [1]. Clinically, pancreatic head cancer usually manifests as obstructive jaundice and elevated serum bile acid levels. Increasing evidence suggests that bile acids (BAs) have toxic effects on both normal and tumor cells in humans. Nevertheless, the impact of BAs on the development and progression of pancreatic cancer remains a controversial issue [2].

Bile acids (BAs) can be categorized based on their chemical structures as primary and secondary BAs, as well as conjugated and unconjugated BAs. The liver utilizes cholesterol to synthesize two primary BAs: cholic acid (CA) and chenodeoxycholic acid (CDCA). Before active hepatic secretion, the C-24 carboxyl group of BAs binds with either taurine or glycine to form conjugated BAs [3]. These are then secreted from hepatocytes into bile canaliculi and accumulated in the gallbladder. Following food ingestion, bile is released into the duodenum to facilitate the dissolution and digestion of fat-soluble nutrients [4]. BAs are absorbed through passive diffusion and active transport in the terminal ileum and then transported back to the liver *via* the portal vein in a process known as enterohepatic circulation (EHC) [5]. Some BAs, however, bypass the EHC and enter the colon. Colonic bacteria are capable of undergoing multiple biotransformations, converting primary BAs into various secondary BAs. These transformations consist of two critical steps: the hydrolysis of conjugated BAs by bile salt hydrolase (BSH) into unconjugated BAs and glycine or taurine [6]; and the conversion of unconjugated primary BAs CA and CDCA into deoxycholic acid (DCA) and lithocholic acid (LCA) *via* 7-dehydroxylation, respectively [7]. In fact, the microbial metabolism of BAs is widely believed to play a role in various human diseases [8].

BAs serve as lipid solubilizers, modulators of bile acid homeostasis, metabolic integrators, and signaling factors. Bile acid-regulated signaling pathways have emerged as promising targets for novel drug development in the treatment

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of common metabolic and liver diseases [4]. As signaling molecules, BAs exert biological effects through their corresponding receptors. Through extensive research, certain BAs receptors have gradually received more attention, including the G protein-coupled bile acid receptor (GPBAR1) [9], farnesol X receptor (FXR), neocarbonine-1-phosphate receptor 2 (S1PR2), pregnant X receptor (PXR), constitutive androstane receptor (CAR), and vitamin D receptor (VDR) [10]. These receptors have been identified to play significant roles in gastrointestinal inflammation [11], cancers [12], metabolic diseases [9], immune regulation [10], and neurodegenerative diseases [13]. Therefore, this review focuses on the connections between BAs, gut microbiota, and GPBAR1 and pancreatic cancer, seeking to analyze the progression of pancreatic cancer from a global perspective to provide novel insights and directions for the treatment of pancreatic cancer.

2. Bile Acids and Pancreatic Cancer

In pancreatic cells (Figure 1), BAs function as both detergents and signaling molecules. On one hand, exposure to high concentrations of BAs primarily leads to cell death, with minimal activation of signaling pathways [14]. On the other hand, BAs activate transporters or specific receptors to damage cells through downstream pathways.

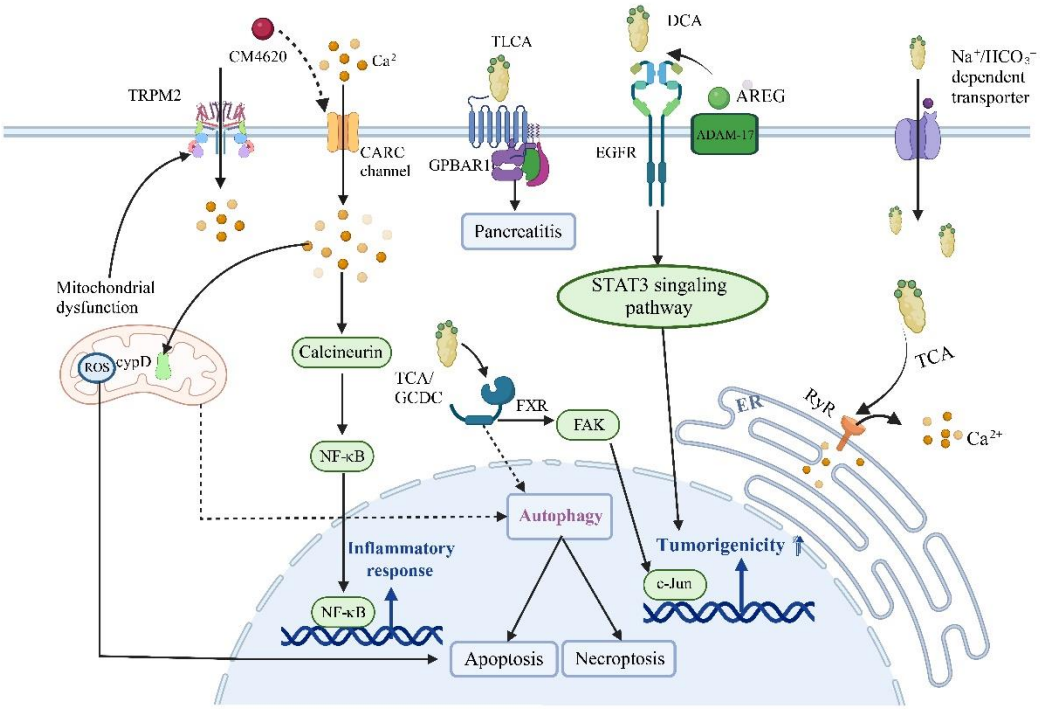

Figure 1. Relationship between BAs and pancreatic cancer. Solid arrows represent facilitation and dashed arrows represent inhibition.

BAs were initially identified as carcinogens in the 1940s. BAs, especially secondary BAs, were reported to play an important role in the development of gastrointestinal cancers [15] and breast cancers [14]. There is a substantial evidence indicating that BAs are necessary in the development of pancreatic cancer. Conjugated and free BAs differ in hydrophilicity, influencing their capability to cross cell membranes [14]. Taurine-conjugated BAs are soluble and come into contact with cells frequently, contributing to increased carcinogenicity of BAs. The gastrointestinal inflammation and tumorigenicity caused by glycine conjugation, taurine conjugation, or free BAs vary, with unconjugated BAs exhibiting a more pronounced carcinogenic effect [16]. Hence, it is essential to comprehend the diversity in bile acid composition and concentration among patients with pancreatic cancer [14].

Bile acids such as cholic acid (CA), deoxycholic acid (DCA), lithocholic acid (LCA), taurocholic acid (TCDC), taurodeoxycholic acid (TDCA), and glycodeoxycholic acid (GCA) significantly inhibited the proliferation of pancreatic cancer cells when administered at different concentrations and durations of incubation. DCA and CA increased the percentage of G0 + G1 phase in the cell cycle, while GCA and TDCA promoted an increase in cells during the S phase. Bile acids also demonstrated direct cytotoxic effects on pancreatic cancer cells, as evidenced by structural damage in pancreatic cancer cells (PANC-1) following a 48-hour incubation with DCA, including loss of their microvilli and

vacuolization of organelles in the cytoplasm [16]. The mechanisms by which deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and glycodeoxycholic acid (GCDCA) inhibited PANC-1 involved changes in mitochondrial membrane potential and the generation of reactive oxygen species (ROS), potentially leading to apoptosis. Additionally, they inhibited the epithelial-mesenchymal transition (EMT) pathway, thereby impeding the migration of pancreatic cancer cells [2]. Bile acids also exhibit a dose-dependent effect on the damage to pancreatic cancer cells. Cyclooxygenase-2 (COX-2), an enzyme that catalyzes prostaglandin synthesis, is an early response gene that is rapidly induced by growth factors, tumor promoters, oncogenes, and carcinogens and is overexpressed in human pancreatic adenocarcinoma [17]. Research indicated that the induction of two human pancreatic cancer cell lines, BxPC-3 and SU86.86, with 12.5 to 100 μM of CDCA or DCA, resulted in a dose-dependent increase in COX-2 protein expression. The same effect was also observed with TDCA although it required higher concentrations (200–1200 μM) [18].

Interestingly, bile acids were also found to enhance the tumorigenicity of pancreatic cells. Increased levels of BAs were found to enhance the tumorigenic potential of pancreatic cells by inducing the FXR/FAK/c-Jun axis to up-regulate MUC4 expression [19]. Bile acids may also promote apoptosis and necroptosis in acinar cells by inhibiting autophagy through the local accumulation of FXR. Pancreatic cell lines exposed to GCDC and taurocholic acid (TCA) exhibited increased FXR expression along with decreased expression of the essential autophagy-related protein Atg7, resulting in apoptosis, necroptosis, inflammation, and fibrosis [20]. Furthermore, the EGFR ligand amphiregulin (AREG) participated in DCA-induced EGFR and STAT3 signaling, which was reliant on cell surface proteases (TACE/ADAM-17) and affected the cell cycle and tumorigenicity of human colorectal cancer and pancreatic ductal adenocarcinoma (PDAC) [21].

Pancreatitis and pancreatic cancer were once considered two distinct diseases due to different cell sources; pancreatitis predominantly impacts pancreatic acinar cells, while pancreatic cancer originates from ductal cells [22]. Nevertheless, a lineage tracing study proposed a different view that chronic inflammation induced dedifferentiation of acinar cells into progenitor tube-like cells, which may be a source of pancreatic cancer [23]. Kim et al. [24] identified two potential mechanisms of bile acid uptake. The first involves Na^+ taurocholate cotransporting polypeptide (NTCP), which primarily operates at the luminal membrane and accounts for approximately 25% of bile acid uptake. The second mechanism involves an HCO_3^- dependent exchanger, which acts on the surface of basolateral acinar cells and can provide serum or interstitial BAs. Regardless of the underlying cause, persistent intracellular Ca^{2+} overload is an essential marker of acute pancreatitis (AP). Taurocholic acid (TCA) opens the ryanodine receptor (RyR) *via* an allosteric mechanism, resulting in pathological Ca^{2+} leak from the endoplasmic reticulum of acinar cells [25]. TRPM2 channels (metabolic and/or oxidative stress-sensitive Ca^{2+} -permeable nonselective ion channels) are expressed in both acinar and ductal cells of the pancreas. Bile acid-induced AP triggers TRPM2-mediated Ca^{2+} overload, partially attributed to mitochondrial dysfunction [26]. Mitochondrial dysfunction also causes pancreatic ER stress, impaired autophagy, and dysregulation of lipid metabolism [27], promoting apoptosis rather than necrosis [28].

3. Gastrointestinal Microbes and Pancreatic Cancer

It is estimated that the gastrointestinal tract contains over 10^{14} microbes, and the genomic content (microbiome) of these microbes surpasses that of the human genome by more than 100 times [29]. The main commensal bacteria in the human gastrointestinal tract belong to the *Firmicutes* and *Bacteroidetes*, which account for approximately 80%–90% of the total intestinal microbiota [30]. The intestinal microbiota plays a crucial role in coordinating human physiological activities by influencing the mucosal immune system, regulating intestinal structure, and participating in digestion and metabolism. However, there is ongoing debate concerning the presence of microbes in the normal pancreas, although the potential for translocation of intestinal flora is considered high [31].

Numerous studies have demonstrated that the relationship between intestinal flora and the occurrence and development of pancreatic cancer is not one-sided, but rather bidirectional. That is, there are both protective effects and damage mechanisms (Table 1).

On the one hand, the presence of gut microbiota contributes to alleviating the effects of pancreatic cancer. For example, probiotics seem to exhibit an inhibitory effect on pancreatic cancer. *Aspergillus oryzae* can activate the p38MAPK signaling pathway and induce apoptosis in pancreatic cancer cells [32]. In the analysis of the tumor microbiome composition in PDAC patients with short-term survival (STS) and long-term survival (LTS), higher α diversity was found in the LTS group, and the intratumor microbiome signature (*Pseudoxanthois-Streptomyces glycopolysporus-Bacillus claustrii*) was identified. Human-to-mouse fecal microbiota transplantation (FMT) experiments from STS, LTS, or control donors showed that tumor growth and tumor immune infiltration can be

influenced by differential regulation of tumor microbiome [33]. Besides the impact of intestinal flora itself on the progression of pancreatic cancer, its metabolites also play a significant role that cannot be ignored. For example, Urolycin A (Uro A), a metabolite of gut microbiota in mammals following the consumption of foods containing ellagic tannin and ellagic acid, can inhibit the proliferation and migration of PDAC cells and enhance apoptosis by regulating the PI3K/AKT/mTOR signaling axis [34]. Similarly, metabolites of the gut microbiota have the capacity to induce apoptosis in tumor cells as well as decrease tumor burden through immune modulation. Delivery of the gut microbial metabolite trimethylamine N-oxide (TMAO) to PDAC mice enhanced the immune-stimulated tumor-associated macrophage (TAM) type I interferon (IFN) pathway and activated effector T cell reactions within the tumor microenvironment. These findings suggest that TMAO can impede tumor growth through alterations in the tumor immune microenvironment. This was also demonstrated by the significant reduction in tumor burden and improved survival in PDAC mice when TMAO was combined with immune checkpoint blockade (ICB) (anti-PD1 and/or anti-TIM3) [35].

On the other hand, the presence of gut microbiota may also aggravate the damage of pancreatic cancer. In the research on pancreatic cancer and gut microbiota depletion, the proportion of poorly differentiated tumors decreased in antibiotic-treated mice in comparison to microbiota-intact $Kras^{G12D}/PTEN^{lox/+}$ mice. Meanwhile, PDAC xenografts in microbiologically depleted Nod-SCID mice showed increased tumor formation duration and diminished tumor size [36]. Depletion of the gut microbiome by oral antibiotics significantly reduces the burden of pancreatic malignancy, and this effect requires the participation of adaptive immunity [37]. Moreover, the anti-tumor effect of bile samples was reduced following contamination, although all bile samples (sterile, contaminated, and sterile bile incubated with isolated live bacteria) exhibited a substantial reduction in peritoneal metastasis of mouse Panc02 cells [38]. Fungi, as members of the gut microbiota, are also involved in the progression of pancreatic cancer. There was a significant increase in fungi in human PDCA and mouse models, approximately 3000 times higher compared to normal levels. According to the α and β diversity indices, the mycobiome composition of the PDCA was different from that of the gut or normal pancreas, showing an enrichment of *Malassezia* species in the PDCA. Mannose-binding lectin (MBL) binds to glycans in the fungal wall to activate the complement cascade, and depletion of MBL or complement C3 in the extraluminal compartment or knockdown of C3aR in tumor cells prevents tumor growth [39].

Table 1. Bidirectional effects of gut microbiome in pancreatic cancer.

Gastrointestinal microbes	Metabolites	Effects	Mechanisms
Aspergillus oryzae		Protect	Activating p38MAPK signaling pathway and inducing apoptosis of pancreatic cancer cells [32]
Long-term Survival (LTS) PDAC Patient's Intratumor Microbiome (*Pseudoxanthois-Streptomyces glycopolysporus-Bacillus claustrii*)			Affecting tumor growth and tumor immune infiltration
	Urolycin A (Uro A)		Inhibiting the proliferation and migration of PDAC cells and enhancing apoptosis through the PI3K/AKT/mTOR signaling axis [34]
	Trimethylamine N-oxide (TMAO)		Inhibiting tumor growth by changing the tumor immune microenvironment [35]
Malassezia species		Damage	MBL binds to glycans in the fungal wall to activate the complement cascade, and depletion of MBL or complement C3 in the extraluminal compartment or knockdown of C3aR in tumor cells prevents tumor growth [39].

4. GPBAR1 and Pancreatic Cancer

G protein-coupled receptors (GPCRs) form a superfamily of receptors that are essential in a variety of biological effector pathways [40] (Figure 2). Due to the substantial amount of GPCRs involved in human pathophysiological processes and their pharmacological traceability, GPCRs have become the most extensively researched drug targets [41] and are potential drug targets for the treatment of numerous diseases [42].

The G protein-coupled bile acid receptor 1 (GPBAR1), also known as TGR5 (Takeda G protein coupled receptor 5), M-BAR, GPR131, GPCR19, BG37 and MGC40597 [4], is widely expressed in the human body, and TGR5 mRNA has been detected in many organs such as intestine, stomach, liver, and lung, placenta, and spleen [43]. Justyna M et al. found that GPBAR1 was expressed in Capan-1 cells (a pancreatic ductal adenocarcinoma cell line) by RT-PCR and immunoblotting [44]. GPBAR1 is potentially implicated in the development of pancreatic cancer. GPBAR1 expression

was significantly higher in cancer tissues compared to that in adjacent normal tissues (81.6% *vs*. 36.8%). Simultaneously, univariate analysis indicated that high expression of GPBAR1, lymph node metastasis and advanced tumor stage were associated with reduced overall survival rates in patients with pancreatic cancer, suggesting that GPBAR1 could serve as an important prognostic predictor for the disease [45].

RNA interference-mediated silencing of GPBAR1 or EGFR ligand double regulatory protein (AREG) inhibited DCA-induced EGFR, MAPK and STAT3 signaling, blunted cyclin D1 expression and cell cycle, and attenuated DCA-induced PDAC tumorigenicity [21]. Similarly, SBI-115, a GPBAR1 antagonist, was observed to limit the proliferation of pancreatic cancer. For example, the TUNEL assay showed that SBI-115 significantly induced cell death in PANC-1 and BXPC3 cells. Transmission electron microscope (TEM) analysis demonstrated that SBI-115 treatment could destroy the morphology of mitochondria in most of the cells. The metabolic profile of PANC-1 cells was found to be altered *in vitro* through liquid chromatography-mass spectrometry (LC-MS)-based metabolomics analysis [46]. Furthermore, a transcriptome analysis of a group of pancreatic ductal adenocarcinoma patients revealed that the expressions of leukemia inhibitory factor (LIF) and its receptor (LIFR) in tumor tissues were increased compared to their corresponding non-tumor tissues [47]. BAR502, when acting as a ligand for GPBAR1, effectively hinders the interaction between LIF to LIFR at inhibitory concentrations (IC), and can even reverse the LIF-induced signaling pattern, suggesting the potential role of BAR502 in the treatment of LIFR-overexpressing PDAC. Research on the downstream pathways of GPBAR1 activation by BAs found that DCA induced apoptosis and inflammatory response in AR42J cells, as shown by increased levels of caspase 8, caspase 9, calpain, p-RIP3, NF-kB p65, TNF-α and cAMP [48].

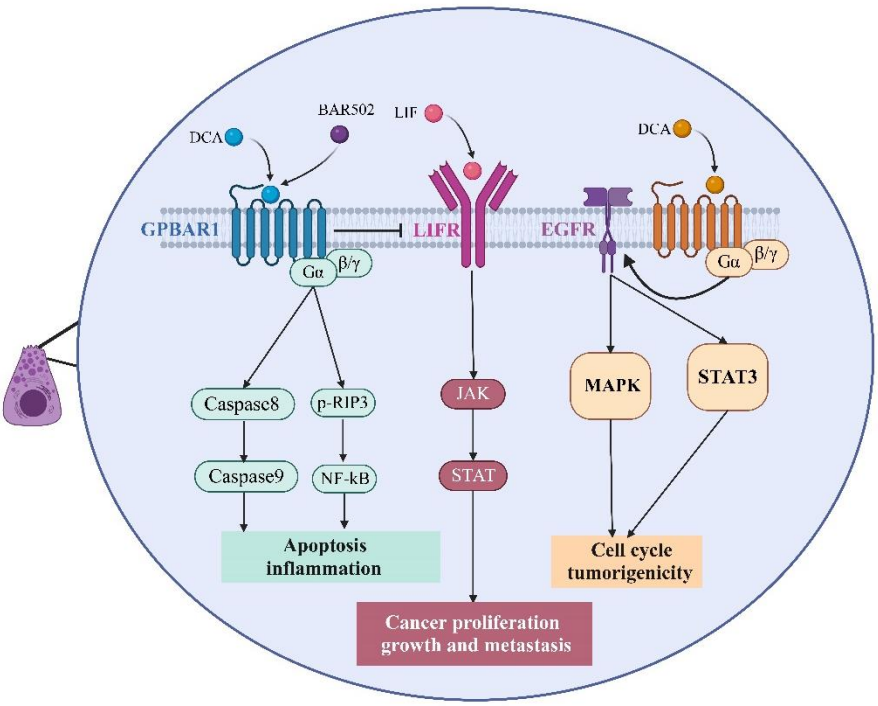

Figure 2. GPBAR1 effector pathways in pancreatic cancer. Solid arrows represent facilitation and T-lines represent inhibition.

5. Interactions between Bile Acids, Gastrointestinal Microbes and GPBAR1

5.1. Interaction between Bile Acids and Gastrointestinal Microbes

The maintenance of an appropriate BAs pool *in vivo* depends on hepatic BAs synthesis, secretion by the biliary system, gallbladder concentration, microbial biotransformation, enterohepatic circulation, and fecal excretion [49]. Gut bacteria are responsible for the transformation of primary BAs into secondary BAs. The steps involved include decoupling, oxidation of hydroxyl groups at positions 3, 7 and 12, 7-dehydroxylation [50], which significantly enhances the hydrophobicity of the BAs pool and therefore the risk of potential carcinogenic effects [51]. Bile salt lyases (BSLs) deconjugate BAs, while hydroxysteroid dehydrogenases (HSDs) act on the 3, 7, and 12 hydroxyl groups of BAs to catalyze epimerization and oxidation/reduction. The bacteria containing both enzymes can modify the hydrophobicity and toxicity of BAs. The 7α-hydroxylation activity in the cecum was significantly higher in gallstone-insensitive AKR/J

(GS-R) mice co-housed with gallstone-sensitive C57BL/6J (GS-S) mice. DCA, the product of bacterial 7a-dehydroxylation and the major component of cecal cholic acid, exhibited elevated levels in GS-S mice as well as GS-R mice that were co-housed with them [52]. A study investigating whether gut microbes modify the effect of bile on pancreatic cancer cell survival found that contaminated bile samples reduced anti-tumor effects compared with sterile bile, and preincubation of sterile bile with *Enterococcus faecalis* or *Streptococcus oralis* changed the anti-tumor effects of sterile bile. Moreover, the synergy of BAs and secondary BAs (DCA) had greater anti-tumor effect [38]. The majority of gram-positive symbiotic intestinal microorganisms, such as *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Clostridium*, contain bile salt hydrolases (BSHs) that are uncoupled to BAs [53]. In addition to biotransformation of BAs, gut microbiota also alters the BAs pool by regulating BAs synthesis. Gut microbiota can regulate the expression of certain enzymes involved in BAs synthesis, including CYP7A1, CYP7B1 and CYP27A1 [54].

Furthermore, the detergent effect of BAs on bacterial cell membranes disrupts bacterial integrity [55] and affects the composition of the gut microbiota by activating innate immunity genes in the small intestine [11]. The results of Principal Component Analysis (PCA) and β-diversity indicated a significant disparity between the intestinal flora of CA-fed mice and those in the control group. The relative abundance of Bacteroides and Verrucomicrobia in the CA group increased to 57.6% and 22.0%, respectively, while *Firmicutes* experienced a significant decrease [56]. In addition, short-chain fatty acids (SCFAs) levels decreased with alterations in gut microbiota, and the bacteria that produce the most SCFAs are firmicutes in *Clostridium* clusters IV and XI [57]. It has been reported that the increased risk of colon cancer may be attributed to a decrease in the production of anti-tumoral SCFAs [58].

5.2. Gastrointestinal Microbes-Bile Acids-GPBAR1 Axis

Given the intricate interactions between gut microbiota, the host, and the bile acid signaling network, a system biology perspective is necessary to understand the cholic acid-microbiota crosstalk and its effects on target organs [11] (Figure 3). The natural endogenous agonist of GPBAR1 is BAs, which activate GPBAR1 in a dose-dependent manner. In CHO cells transfected with human GPBAR1 induced by BAs, the order of cAMP production (EC_{50}) was as follows: TLCA (0.33 μmol/L) > LCA (0.53 μmol/L) > DCA (1.01 μmol/L) > CDCA (4.43 μmol/L) > CA (7.72 μmol/L) [59]. Yang F *et al.* first analyzed the high-resolution structure of complexes formed by GPBAR1 binding to P395 or INT-777 (GPBAR1 agonist) by using single-particle cryo-electron microscopy reconstruction technology. It was observed that the recognition of GPBAR1 ligand may occur through the triple leucine cluster ($L244^{6.55}$, $P < 0.05$, $L263^{7.36}$, and $L266^{7.39}$), and the potential role of $S247^{6.58}$ [60].

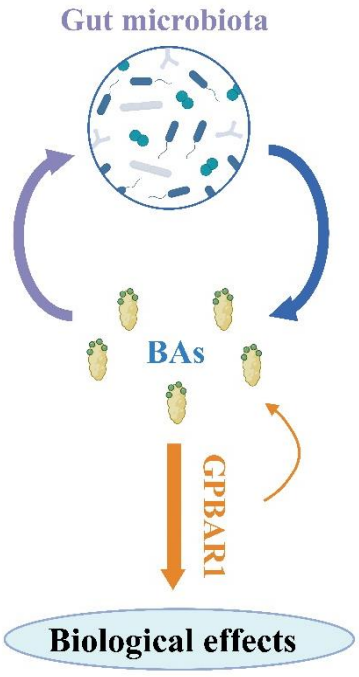

Figure 3. Gastrointestinal microbes-bile acids-GPBAR1 axis.

BAs, acting as a signaling molecule, can mediate the occurrence and development of various diseases through a complex network of signaling pathways upon activation of GPBAR1. After the gut-limiting FXR agonist fexofenadine (FEX) acts on FXR, there is an elevation in the number of bacteria *Acetatifactor* and *Bacteroides* that generate lithocholic acid, leading to an increase in LCA levels. The increase subsequently stimulates the GPBAR1/GLP-1 pathway, thereby improving hepatic glucose and insulin sensitivity while promoting adipose tissue browning. Moreover, FEX-induced metabolic phenotype could be reversed through antibiotic treatment [61]. The gut microbiota-bile acid axis also plays an important role in the development of inflammatory bowel disease (IBD), and Eucommia ulmoides leaf extract (ELE) can facilitate this process. ELE decreases the abundance of *Bacteroidetes* and elevates the serum content of DCA and TUDCA, and this increase exhibited a strong positive correlation with rises in *Ackermansia* and *Ruminants*.

In addition, there was a corresponding increase in GPBAR1 expression in the colon. In the Caco-2 cell model, it was confirmed that GPBAR1 activation improved the reduced transepithelial electrical resistance (TEER) and reduced FITC dextran permeability in LPS-induced monolayer cells, thereby improving IBD [62]. There have been limited studies regarding the role of GPBAR1 in regulating the body's bile acid pool in turn, unlike the effect of FXR on the bile acid pool in the gut microbiota-bile acid axis. GPBAR1 activation induces gallbladder dilation, which in turn protects the liver under obstructive conditions and reconstructs BAs to be more hydrophilic [63]. The relationship between GPBAR1 and gut microbiota has not been well-documented, with data suggesting that compared to the fecal microbiota of WT mice, GPBAR1 deficiency may result in higher abundance of Bacteroides and lower firmicutes, without leading to the establishment of a more hydrophobic BAs pool [63].

Despite the various hypotheses on the mechanism by which bile acids enter the pancreas, whether *via* the common pathway or as a result of biliary pancreatic ductal obstruction, substantial evidence links bile acids closely to pancreatic cancer [64]. Bile acids, at different concentrations and types, exhibit distinct effects on the occurrence and development of diseases through different pathways. High concentrations of bile acids can serve as a form of detergent that can harm cells [14], while low concentrations of bile acids can be used as signaling molecules to activate different downstream signaling pathways through interactions with corresponding receptors or uptake into the cells *via* transporters. Gut microbiota and its metabolites have both protective effects and harmful mechanisms on the pancreatic cancer. Further exploration is required the relationship between these two aspects whether they are cause and effect, effect and cause, or reciprocal causation. Furthermore, GPBAR1 is expected to act as a drug target for the treatment of pancreatic cancer, thus warranting further investigation into the role and mechanism of GPBAR1 in pancreatic cancer. It is certain that gut microbiota and GPBAR1 may play an indispensable role in the process of bile acid-induced and/or aggravation of pancreatic cancer. Unfortunately, there are few studies on the interplay between intestinal flora, bile acids and GPBAR1 as an axis from a holistic perspective, which may help to better understand the occurrence and progression of pancreatic cancer and provide effective interventions to improve the cure rate and reduce mortality.

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Author Contributions

Writing—Original Draft Preparation: L.C. and Y.Z. (Yi Zhu); Literature Collection: L.C.; Writing—Review & Editing: Y.Z. (Yi Zhu), J.Z., L.C. and Y.Z. (Yawen Zhuang).

Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

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Declaration of Competing Interest

The authors declared no conflict of interests. Figures were created with biorender.com.

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