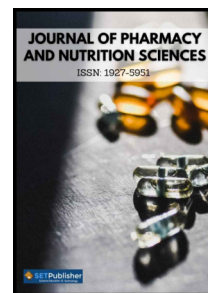




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Mode of Actions of Bile Acids in Avoidance of Colorectal Cancer Development; and their Therapeutic Applications in Cancers - A Narrative Review

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Abstract:

Bile Acids (BAs) possess a considerably significant part in the form of emulsifiers in digestion besides absorption of dietary lipids. BAs represent amphiphilic molecules, that are primary metabolites formed from cholesterol by the aid of enzymes acting on cholesterol. Earlier BAs were believed to be tumor repressors. The tumor repressive actions of BAs are correlated with programmed cell death (PCD). Furthermore, dependent on this observation different Synthetic BAs products have been generated along with their utilization regarding induction of PCD (in the form of apoptosis, autophagy or necroptosis in variable kinds of human cancers. Thus we conducted a narrative review till August 2022. The present article might form the basis of generation of such innovative therapies even for cancers/tumors that are cisplatin resistant. With time these therapies might be used for the treatment of neurodegenerative diseases (NDD), amyotrophic sclerosis, and numerous metabolic and haematological diseases as described for use of TUDCA.

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INTRODUCTION

Bile Acids (BA) are important constituents of Bile accounting for nearly 85% of the solid substances in bile [1]. The formation of bile acids takes place from cholesterol in the liver along with facilitates the absorption of fatty acids along with cholesterol from the dietary resources in the Gastrointestinal Tract (GIT), they further play a considerably significant part in sustenance of the signaling event besides organizational homeostasis [2]. As per their chemical structure, variable bile acids possess unique biological actions [3]. Subsequent to their generation in the liver along with expulsion into the Bile ducts along with the intestinal tract, primary bile acids like cholic acid (CA), chenodeoxycholic acid (CDCA) undergo metabolism by intestinal bacteria for the generation of secondary bile acids like lithocholic acid (LCA) as well as deoxycholic acid (DCA), as well as tertiary BA's like ursodeoxycholic acid (UDCA) (Figure 1) [rev in 4]. In general greater than 95% of the BA's remain in the enterohepatic circulation besides the rest 5% gets excreted via feces [5] (Figure 2). In case of healthy persons the full quantity of bile acids persistence in the hydrophobicity enterohepaticcirculation is determined to be about 3g comprising of 35% CA, 40% CDCA as well as 20% DCA [6]. The hydrophobicity of bile acids is based on besides the number, placement as well as the positioning of the hydroxyl groups as well as on amidation at the C24 position. The extent of hydrophobicity of bile acids is; $LCA > DCA > CDCA > CA > UDCA$ [7]. Bile acids conjugate with glycine (75%) along with taurine (25%) for production of a greater hydrophilic amidated kind with predominance of glycine binding [8]. On conjugation with glycine along with taurine bile acids lose their toxicity besides further provision of escalation of cell membrane permeability as well as water solubility [2]. Bile acids were illustrated to be implicated in cancer generation in rodents subsequent to subcutaneous administration of secondary bile acid DCA in 1940's [9]. This caused the generation of malignant spindle cell tumors whose manifestation was in the form of epitheliomas (benign growth or malignant carcinoma) based on the epithelial cell of origin). These prior findings were associated with the epidemiological studies that illustrated a correlation amongst bile acids as well as cancer in particular colorectal cancer. Subsequently variable studies have illustrated that bile acid works as a facilitator in different organs like stomach, esophagus, liver as well as colon [10]. Nevertheless, in the past decade different studies illustrated that bile acids work in the form of tumor

suppressors that resulted in reduction of proliferation along with migration of cancer cell kinds [11,12]. Furthermore, numerous studies have illustrated that the bile acid/gut microbiome (GM) axis causes considerably significant decrease in the canonical *in vitro* characteristics of malignancies (cell invasion, clonogenicity, cell migration, cell adhesion) [13]. Additionally, various laboratories have generated innovative Bile Acid obtained substance for evaluation of the tumor suppressive actions of bile acids. These substances were illustrated to result in induction of programmed cell death (PCD) in different cancer cells besides possess tumor repressive action [14-24].

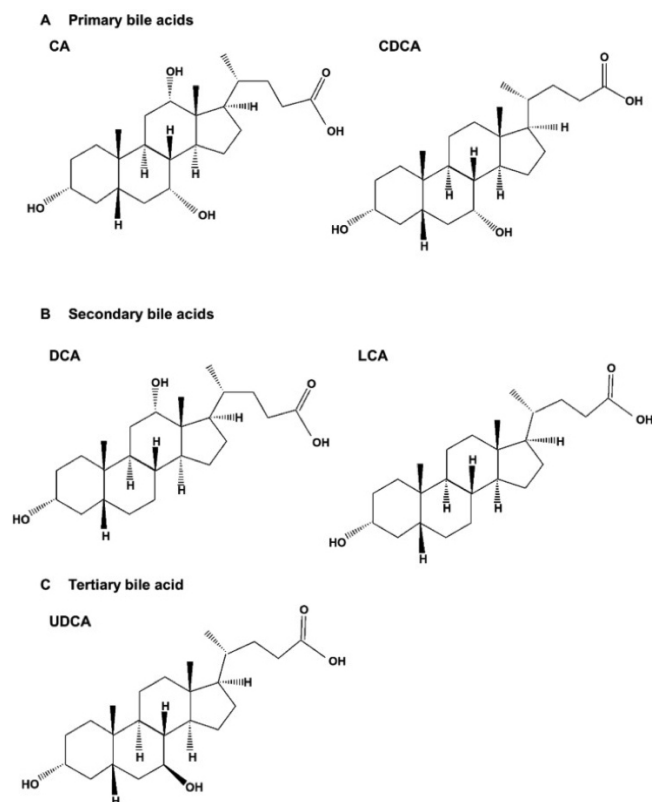


Figure 1: Courtesy ref no-4-Molecular structures of bile acids. (A) Molecular structures of primary bile acids such as cholic acid (CA) and chenodeoxycholic acid (CDCA). (B) Molecular structures of secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid (LCA), and (C) tertiary bile acids such as ursodeoxycholic acid (UDCA).

Cell death possesses a key part in embryonic formation, sustenance of homeostasis, as well as elimination of injured cells. Classification of Cell death can be as programmed cell death (PCD) or non PCD based on their signal dependence [25]. In the year 2018 the Nomenclature Committee on Cell death (NCCD) cell demise, described variable kinds of totally physiological kinds of controlled Cell death is in general labelled as PCD. Different kinds of PCD has been detailed which are inclusive of apoptosis (extrinsic

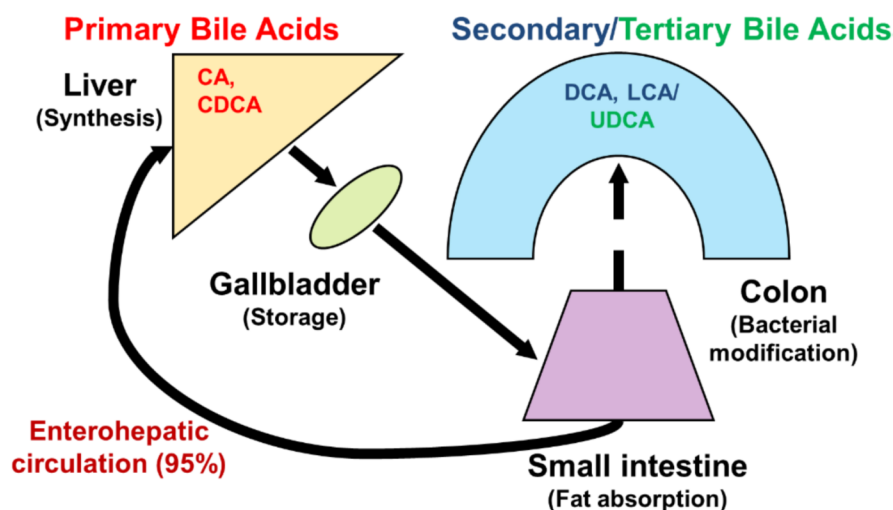


Figure 2: Courtesy ref no-4-Bile acid circulation. Primary bile acids are synthesized from cholesterol in the liver. Then, they bind to glycine or taurine, are discharged into the bile, and are stored in the gallbladder. After a meal, conjugated bile acids are released into the intestinal tract to facilitate the digestion of dietary lipids and fat-soluble vitamins. Bile acids are then efficiently reabsorbed in the ileum, and most (95%) are transported back to the liver via the hepatic portal vein, where they are re-secreted in the bile to prepare for new circulation. This process is called enterohepatic circulation. In the large intestine, some bile acids are deconjugated to free bile acids by bacterial bile salt hydrolases, and are converted to secondary and tertiary bile acids. They are then reabsorbed into colonocytes, returned to the liver for detoxification, and recycled. Only a small amount (approximately 5%) of bile acids is lost through the feces.

along with intrinsic), autophagy- based cell demise, mitochondrial permeability, transit guided necrosis, ferroptosis, pyroptosis, parthanotos,entotic cell demise, NETotic cell demise, lysosomal based cell demise, besides immunogenic cell demise [26].

As per this review we detailed type 1 cell demise (apoptosis), type II cell demise (autophagy-based) as well as programmed necrosis (alias necroptosis) amongst the kinds of PCD whose induction takes place by the natural bile acids as well as synthetic products in Cancer cells.

METHODS

Here we conducted a narrative review utilizing search engine pubmed, google scholar; web of science; embase; Cochrane review library utilizing the MeSH terms like cell death modes; apoptosis; autophagy; necroptosis; BAs; colorectal cancer; leukaemia; breast cancer; thyroid carcinoma cells; CDCA; DCA; LCA; UDCA; GCDCA; TCDCA; synthetic bile acids; other GIT cancers; NFkB; Bcl2 family members from 1940 till August 2022.

RESULTS

We found a total of 7000 articles out of which we selected 140 articles for this review. No meta-analysis was done.

Part of Natural Bile Acids in Cancer

Bile acids represent the main signaling molecules which play a considerably significant part in the form of emulsifiers at the time of absorption besides digestion of dietary lipids [13]. The part of bile acids regarding cancer (stomach, esophagus, liver as well as colon) generation has been well appreciated in the literature [27]. Apparently a combination of modes is operative regarding the modulation of induction of cancer by bile in these particular organs with escalated bile acids quantities like oxidative injury (Reactive oxygen species (ROS)), epithelial proliferation, activation of signal, apart from instability of deoxy ribonucleic acid (DNA) [28]. Particular modes by which cancer formation by bile acids take place is; the activation of nuclear factor κB (NFκB), a transcription factor implicated in DNA transcription, production of proinflammatory cytokines, besides escalated cell survival in reaction to exposure of esophagus as well as colon to DCA [29]. Akin to that the expression of cyclooxygenase2 (COX2) as well as prostaglandin E2 are considerably significantly facilitated in pancreatic cancer cells (BxPC3 as well as SU 86.86) in reaction to DCA along with CDCA [30]. In patients of colorectal adenoma or carcinoma, the quantities of DCA along with LCA in the systemic circulation besides colon in the form of secondary bile acids are escalated [31]. Of the bile acids UDCA is maximum hydrophilic, whereas DCA along with LCA possess least polarity, besides

possessing maximum toxicity of the bile acid lot as well as in the generation of colorectal cancer [32].

Although above findings were found maximum *in vitro* morphological properties of cancer cells (like cell infiltration, cell migration, cell survival besides cell adhesion) represent the targets for bile acids for hampering the metastatic type of phenotype in certain kinds of cancer models. Actually CDCA, DCA along with LCA have been illustrated to hamper proliferation along with cause induction of cell differentiation of leukaemia HL60 cells through protein kinase C [33]. The proliferation capabilities of pancreatic cancer cells were evaluated in reaction to DCA. Reduction of cell proliferation besides structural alterations in microvilli were seen in certain cell lines pointing that DCA might restrict pancreatic cancer cells proliferation as well as invasion *in vitro* [34]. Akin to that DCA has been illustrated to result in reduction of migration, invasion along with proliferation of gastric carcinoma cells [35]. Additionally, UDCA has been demonstrated to be possessing anti proliferative besides anti apoptotic actions on M14 human melanoma cells. In case of M14 cells UDCA resulted in activation of the intrinsic apoptotic pathway as well as induction of G2/M cell cycle arrest. Simultaneously UDCA demonstrated minimum toxicity towards LO2 hepatocytes along with HaCaT keratinocytes, that are considered to be normal human cell lines [36]. Studies conducted on Hepatocellular carcinoma (HCC) besides colitis correlated with colon cancer have illustrated that the anti inflammatory action of tauroursodeoxycholic acid (TUDCA) can be the reason for its anti cancer characteristics. In case of carcinogen induction of liver impairment along with HCC, TUDCA has demonstrated chemo avoidance characteristics by hampering endoplasmic reticulum (ER) stress along with reduction of hepatic inflammation [37]. Additionally, in case of HCT-116- human colon cancer cells whose stimulation takes place by Tumor necrosis factor alpha (TNF α), TUDCA considerably significantly decreased interleukin-8 (IL-8), IL- α , besides resulted in reduction of TNF α stimulated nuclear factor κ B phosphorylation/ breakdown of I κ B α besides the hampering of nuclear factor κ B (NF κ B) DNA binding activity [38]. The suggestions provided by these studies was that TUDCA abrogated tumor generation basically by relieving NF κ B modulation of inflammatory reaction bile acids like DCA as well as CDCA, besides facilitation of destabilization of Hypoxia inducible factor 1(HIF 1), a significant transcription factor implicated in the tumor hypoxic shift besides hampering the pro cancer

phenotypes like invasion, migration, adhesion along with clonogenicity of DU145prostate cancer cells [39].

UDCA has been believed to be an attractive therapeutic substance regarding therapy of colorectal cancer as well as liver cancer. On enriching the rodents diet chronically with UDCA resulted in reduction of DCA quantities in the stool along with the incidence of benign as well as malignant tumors in Fischer 344 rats [40]. Hence UDCA apparently possesses chemotherapeutic action in humans. In a human phase III trials UDCA delivery was correlated with considerable decrease in the recurrence rates of colorectal adenoma with hyperplastic dysplasia in 1285 patients that is the main observation in patients that are prone towards invasive colorectal carcinoma [41]. Additionally, applying UDCA directly resulted in reduction of risk of colorectal cancer in patients with primary sclerosing cholangitis besides ulcerative colitis [42], patients with primary biliary cirrhosis [43], as well as patients with chronic liver disease [44], reduction of risk of recurrence of colorectal cancer subsequent to its surgical removal [41].

The working of bile acids signaling regarding cancer propagation does not represent a single process besides probiotics, aging, diet, drugs, as well as different kinds of bile acids/microbial axis which impact the bile acids quantities as well as profiles. A strategy of imbalanced consumption of diet, junk food, escalated medicine/probiotics, besides alcohol consumption can result in escalated bile acids quantities besides alterations in bile acids profile towards pathophysiological quantities. This resulted in cell membrane injury as well as DNA instability secondary to escalated ROS generation. The activation of downstream inflammatory pathways besides considerably significant controlling factors (NF κ B, PKC, EGFR, etc) can result in exaggeration of cancer epithelial cell proliferation in prone organs like stomach, esophagus, liver along with colon. Conversely a strategy of balanced consumption of diet, drug consumption along with other factors might result in physiological quantities of bile acids.

Action of natural Bile Acids on apoptosis

Apoptosis

This word apoptosis got coined by Kerr *et al.* [45], In 1972 for detailing a morphologically distinctive kind of cell demise. Apoptosis represents an event where there is stoppage of cell growth along with division, however rather instead of spillage of its constituents

into surrounding microenvironment however finally result in cell demise. Apoptosis is further known as programmed cell death (or' cellular suicide) [46]. Apoptosis is a foundational physiological event. Its decontrolling has been correlated with different pathologies along with diseases inclusive of immune reactions, toxicity of drugs, Infections, tumors besides metabolic conditions [47]. Apoptosis possesses the properties of cell shrinking, chromatin condensations, DNA fragmentation, nuclear fragmentation, as well as blebbing of cell membrane [48].

Kinds of Apoptosis

Apoptosis mainly takes place through the extrinsic cell demise receptor pathway or the intrinsic mitochondrial pathway [49]. The initiation of intrinsic apoptotic pathways takes place by variable intracellular stimuli, inclusive of growth factor, DNA injury, oxidative stress(OS) [50]. This is based on the generation of a complex known as apoptosome comprising of pro-caspase 1, apoptosis protease activating factor (Apaf-1), B cell lymphoma-2 (Bcl2) family members, like Bcl2 correlated X protein (Bax), Bcl2, Bcl2 antagonistic killer1 (Bak1), B cell lymphoma extra large (Bcl-xL), controlled mitochondrial membrane permeability, thus controlling the liberation of cytochrome-c [49]. The Bcl2) family can be subclassified in the form of proapoptotic besides antiapoptotic. The proteins belonging to the class proapoptotic are Bad, Bcl-xS, Bak, Bid, Bax, Bik, Bim along with Hrk, while those of antiapoptotic being Bcl2, Bcl-xL, Bcl-W, Bfl1, myeloid leukaemia 1(Mcl-1) [51]. In combination with Apaf-1, cytochrome-c generates an apoptosome, that result in the enrollment of pro-caspase-9. In the apoptosome, activation of caspase-9 takes place by autoproteolytic cleavage for the initiation of caspase processing cascade [52]. The initiation of extrinsic pathway of apoptosis can take place by crosstalk of a death ligand like TNF α , Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), as well as Fas ligand with the death receptor of the TNF α receptor super family. This crosstalk causes congregation of the death induced signaling complex (DISC) comprising of the Fas associated death domain (FADD) protein pro-caspase-8/10. Subsequent to that DISC results in activation of downstream effector caspases (caspase -3,-6 along with -7) for induction of cell demise or cleavage of the Bcl2 family member Bid to t Bid for the initiation of mitochondrial modulated intrinsic apoptosis pathway [51]. Several factors have been documented to be implicated in the controlling of apoptotic pathways like p53, NF κ B as well as cellular inhibitors of apoptosis

proteins (cIAPs). Numerous small molecule hampering agents have been generated regarding targeting apoptotic pathways for cancer treatment [48].

Bile Acids Associated Apoptosis

The hydrophobicity of Bile Acids (in the following order LCA > DCA > CDCA> CA, > UDCA) is intricately associated with the number, placement as well as the positioning of the hydroxyl groups that have attachment with bile acids, besides is the elemental factor that decides the biological action [53]. Cytotoxicity towards bile acids at apoptosis being induced always doesn't possess an association with hydrophobicity. Apoptosis being induced is based on the bile acids quantities or regarding their conjugation status [54].

Actions of Primary Bile Acids on Apoptosis

The generation of primary Bile Acids can be by 2 methods i) the Canonical (alias neutral) ii) alternate (alias acid) pathways [55]. The canonical pathway is implicated in about 90% of bile acids generation in the general situations is believed to be the main pathway regarding bile acids formation. Cholesterol-7 α hydroxylase (CYP7A1) starts the canonical pathway, that is the rate restricting enzyme regarding this pathway whose placement is within ER, Further its passage takes place via sterol 12 α hydroxylase (CYP8B1) along with the sterol 27 α hydroxylase (CYP27A1) to generate primary bile acids CA besides CDCA respectively. The initiation of alternate pathways takes place by CYP7A1 that results in transformation of cholesterol to 27 α hydroxy cholesterol through a hydroxylation reaction. 27 α hydroxy cholesterol persistently gets transformed to CDCA rather than CA, with oxysterol-7 α hydroxylase (CYP7B1). This alternate pathways represents the secondary pathway of bile acids generation that is implicated in about 10% of bile acids formation as well as in general is believed to be active in the pathological situations [53,56]. Subsequent to primary bile acids production taurine or glycine conjugation in a 1:3 ratio takes place through covalent modifications (labelled as Bile salts) which enhance solubility with simultaneous reduction in toxicity [55].

Numerous studies have documented that CDCA, a primary BA results in induction of apoptosis in human cancer cells [57,58]. Ignacio Barrasa *et al.* [57], assessed the actions of CA as well as CDCA on BCS-TC2 human colon cancer cells along with observed that both kinds of bile acids facilitated apoptosis along with that cell demise was greater with CDCA. Bile acids

where apoptosis results in OS, as well as ROS formation, This action causes elimination of mitochondrial potential besides liberation of cytoplasmic proapoptotic factors as corroborated by the activation of caspase-3 as well as 9. Nevertheless, caspase-8 activation did not occur. The early proapoptotic stage promotes the cleavage of Bcl2 aiding in the activation of Bax as well as pore generation in the mitochondrial membrane for augmentation of the apoptotic signals. Additionally, Shen *et al.* [58], demonstrated that the therapy of lung adenocarcinoma A549 along with H650 cells with CDCA caused induction of apoptosis along with hampered cell proliferation, invasion along with migration. On treating A549 along with H650 cells with CDCA the reduction in expression of the mesenchymal markers N-cadherin along with Snail took place whereas that of the epithelial markers E-cadherin escalated. These outcomes corroborated that CDCA hampered the epithelial –mesenchymal transition (EMT), migration along with invasion. Additionally, it was corroborated that CDCA hampered integrin β 1 along with phosphorylation of focal adhesion kinase (p-FAK) by hampering the integrin α -5. CDCA resulted in growth arrest along with enhanced DNA damage inducible 45 (GADD45) that is a downstream gene of -p53. Additionally, CDCA results in induction of apoptosis by enhancing expression of p21, P2xm, Mcl-1, along with Bax that are genes correlated with p53 upregulation besides reduction in expression of the Insulin like growth factor binding protein3 (IGFBP-3) besides Bcl2 that are genes correlated with p53 downregulation. Via the assessment of A549 cells xenografts in nude mice it was corroborated that CDCA results in repression of tumor volume along with weight besides results in reduction of p-FAK along with integrin α -5 quantities. Hence they illustrated that CDCA controlled EMT, migration, invasion along with apoptosis in lung adenocarcinoma cells through the integrin α -5 β 1 / FAK/ p53 axis.

Numerous studies illustrated that glycochenodeoxy cholic acid (GCDCA) which represents glycine conjugated to CDCA results in induction of apoptosis in HCC cells [59]. Iizaka *et al.* [59], documented that crosstalk amongst caspase-8 activation along with ER-stress in GCDCA resulted in induction of apoptosis in HepG2 HCC cells. They observed that GCDCA treated HepG2 cells illustrated escalated LDH leaking, cleavage of caspase3 protein, cytochrome-c liberation from the mitochondria, ER-resident molecular chaperone Bip mRNA expression along with ER- stress

reaction associated C/EBP-homologous protein (CHOP) mRNA. Additionally, GCDCA treatment enhanced the cleavage of BAP3, a membrane protein that is integral in ER along with pre treatment with caspase-8 hampering agent, Z-IETDFMK hampered an escalation of caspase-8 along with BAP31 cleavage. Hence they illustrated that activated caspase-8 facilitates ER- stress reaction by cleavage of BAP31 in cells with GCDCA induction of apoptosis.

Actions of Secondary along with Tertiary Bile Acids on Apoptosis

The conjugated bile acids in the intestine get transformed to the free primary bile acids. CA gets transformed to DCA along with CDCA is transformed to LCA along with UDCA [53]. DCA, a secondary bile acid has been illustrated to be implicated in the induction of apoptosis in various human gastric cancer cells [60]. As per the outcomes of Yang *et al.* [75], DCA result in apoptosis induction in BGC-823 cells via a p53 modulated pathway. On treatment of BGC-823 cells with DCA, there was an escalated expression of Bax besides p53 proteins enhanced along with expression of cyclinD1, Bcl2, cyclin dependent –kinase2 (Cdk2) decreased. These outcomes illustrated that DCA hampered cell growth along with arrests cells at the G1 phase of the cycle. Furthermore, they corroborated that DCA result in apoptosis induction that is correlated with the breakdown of mitochondrial membrane potential. It was corroborated that the activation of mitochondrial based pathways occurred by enhancing the ratio of Bax:Bcl2. Hence they confirmed that DCA result in apoptosis induction in gastric cancer cells via the mitochondrial based pathways by implicating p53. Numerous studies have illustrated that DCA causes apoptosis induction in colon cancer cells [57,61]. Yu *et al.* [61], conducted a study on Bax knockout (Bax $^{-/-}$) in HCT-116 cells on treatment with DCA for evaluation of the part of Bax that is a proapoptotic member of the Bcl2 family regarding apoptosis induction. Hence they illustrated that treatment of (Bax $^{-/-}$) along with Bax $+/+$ caused liberation of cytochrome-c along with activation of caspase 3,8 as well as 9 protein. Such outcomes illustrated that Bax is not necessary for DCA caused apoptosis induction in HCT-116 cells.

LCA, the maximum hydrophobic secondary bile acid has been documented to result in apoptosis induction in various studies [62-4]. As per the study of Luu *et al.* [62], where MCF7 cells breast cancer cells had treatment with LCA, expression of proapoptotic p53 protein along with antiapoptotic Bcl2 protein reduction

took place. Furthermore they illustrated that the treatment with LCA of both breast cancer cell lines resulted in reduction of the expression of sterol regulatory element binding protein 1c (SREBP1c), fatty acids synthase (FAS) in addition to acetylCoA Carboxylase (ACC), that resulted in lesser lipid droplet (LDs) in contrast to those control cells not treated. Moreover, it was corroborated that a reduction of ER- α expression occurred on treatment of MCF7 cells with LCA. They illustrated the probability of a therapeutic part of LCA in breast cancer cells by reverting the decontrolling of lipid metabolism. That LCA selectively resulted in apoptosis induction in androgen dependent LNCaP along with androgen independent PC3 prostate cancer cells was illustrated by Goldberg *et al.* [63]. LCA resulted in apoptosis induction in LNCaP along with PC3 cells through extrinsic apoptotic pathway besides activating caspase 3 as well as 8. It was further documented that LCA resulted in reduction of cleavage of Bid along with Bax, downregulation of Bcl2, reduction of mitochondrial membrane potential, along with enhanced the activation of caspase 9. Furthermore LCA has been revealed to result in apoptosis induction in neuroblastoma cells [84,5]. Trah *et al.* [64], illustrated that treating WT-CLS1 along with SK-NEP1 neuroblastoma cells with LCA escalated the quantities of G- protein coupled bile acids receptor (TGR5) without impacting the expression of NRF2, a downstream controlling protein of the TGR5 pathway besides resulted in cytotoxicity secondary to activation of caspase3 along with 7.

Numerous studies have documented that UDCA, a tertiary bile acid possessing lesser hydrophobicity resulted in apoptosis induction in various cancer cell lines [11,36,65-69]. As per the study by Yu *et al.* [36], UDCA resulted in apoptosis induction in M14 as well as A375 human melanoma cell lines through the mitochondrial pathway. Cell arrest took place in the G2/M phase of cell cycle besides resulted in reduction of Cdk1 along with Cyclin B. UDCA has been illustrated to cause apoptosis induction in human melanoma M14 cells through ROS stimulated mitochondrial correlated pathways leading to escalation of cleavage p21, p53, Bax/Bcl2 ratio, cytochrome-c liberation as well as Apaf1 along with caspase -3,-9 having been cleaved, besides PARP. Additionally, Z-VAD-FMK (a caspase hampering agent) considerably resulted in reduction of apoptosis rate. Furthermore UDCA decreased the expression of Matrix Metalloproteinases 2 (MMP2) along with MMP-9 that are implicated in migration. Yao *et al.* [87], documented that UDCA resulted in

apoptosis induction incase of glioblastoma A172 along with 7 LN229 cells. UDCA caused cell cycle arrest at the time of G1 phase of the cycle, resulted in reduction of the expression of Cdk2, Cdk4, Cdk6, cyclinD1, along with pRb besides escalated the expression of p21, as well as p53. Additionally, they illustrated that UDCA apoptosis induction occurred in a caspase independent way. Nevertheless, UDCA resulted in reduction of mitochondrial membrane potential, along with enhanced the ROS amounts. It was corroborated that the escalation of ROS was correlated with extracellular signal –regulated kinase (ERK1), a member of the mitogen activated protein kinase (MAPK) pathway. It was corroborated that the expression of CHOP, ATF6, along with IRE1 α that are correlated with ER stress were escalated following UDCA treatment. Furthermore, they illustrated that combination of UDCA with the related proteasome hampering agent bortezomib could enhance their quantities in a synergistic fashion by prolongation of the ERstress. Lee *et al.* [88], documented that UDCA resulted apoptosis induction occurred in DU145 prostate cancer cells. This took place by activation of caspase 8 illustrating that UDCA stimulated apoptosis is associated with the extrinsic pathway. Moreover UDCA escalated the expression of TRAIL associated with the death receptor 4 (DR4), DR5, TRAIL, Bax, besides cytochrome-c, along with resulted in downregulation of Bcl -xL, in DU145 cells via the extrinsic along with intrinsic pathway. Liu *et al.* [11], illustrated in a study that apoptosis induction in BEL 7402 Hepatocellular carcinoma (HCC) xenografts took place in mice. UDCA resulted in repression of tumor growth along with enhanced DNA fragmentation.

Additionally, the expression of Bax, Apaf1, besides cleaved caspase3 as well as 9 were escalated while that of Bcl2 was decreased. These outcomes illustrated that UDCA hampered the growth of HCC cells by apoptosis induction. Lim *et al.* [67], demonstrated that apoptosis induction occurred in SNU 601 gastric cancer cells via MEK/ERK pathways. The decrease in UDCA induction of apoptosis by the utilization of MEK1 hampering agent PD98059 besides MEK1/2 hampering agent U0126 caused UDCA apoptosis induction in gastric cancer cells was illustrated by an escalation of apoptotic body generation by caspase-3,-6 as well as-8 activity as well as PARP cleavage. U0126 caused reduction of UDCA induction of TRAIL-R/ DR5 expression. Jung *et al.* [69], demonstrated that apoptosis induction by UDCA occurred in FRO anaplastic thyroid cancer cells. UDCA escalated the

expression of proapoptotic (Bax, caspase-3, cytochrome-c as well as PARP) proteins besides hampered the expression of antiapoptotic (Bcl2) along with angiogenic (TGF β , VEGF, N-cadherin as well as SIRT) proteins. Additionally, UDCA treatment caused repression of mammalian target of rapamycin inhibitors (mTOR) phosphorylation. These outcomes pointed that UDCA resulted in apoptosis induction as well as hampered angiogenesis by controlling the Akt/ mTOR signaling pathway.

Action of Natural Bile Acids on Autophagy

Autophagy

Autophagy word comes from the Greek "auto"(self) as well as phagy (to eat). Type II cell demise represents necessary controlled along with conserved catabolic events which modulate recycling along with breakdown of various cytoplasmic constituents of the eukaryotic cell [5,70]. Autophagy plays a considerably significant part in cancer as well as autophagy correlated proteins (ATG) [71], Till now the double part of autophagy in cancer propagation along with hampering remains debatable. Autophagy possesses a dynamic tumor repressor or tumor facilitating part in different stages of cancer generation. At the time of early tumor generation autophagy results in avoidance of the tumor getting started as well as hampers cancer propagation through survival pathways along with quality regulatory modes. Nevertheless, once the tumor propagates along with forms an end stage besides exposure to environmental stress, autophagy works in the form of a dynamic recycling system that aids in the survival along with growth of generated tumors along with facilitates cancer acceleration via metastasis. Therefore this pointed that autophagy modulation might prove to be an efficacious interventional approach regarding cancer therapy [72].

Kinds of Autophagy

Dependent on the administration approach 3 Kinds of Autophagy have been detected towards the lysosome; i) macroautophagy ii) microautophagy besides ii) chaperone modulated autophagy (CMA) [73]. I) Macroautophagy, the maximum functional along with properties of kinds of autophagy implicated in the generation of double membrane autophagosome that result in elimination of injured organelles or undesired cellular constituents by administration to lysosomes for breakdown along with recycling. Different studies have documented that Macroautophagy as well as Macroautophagic cell demise represent anti tumor

reactions [45,51]. In case of microautophagy, the cargo comprised of (organelles or cytoplasmic constituents) crosstalk directly undergoes fusion with lysosomes for breakdown along with recycling. Microautophagy is more particular in contrast to macroautophagy besides results in transmission of signals of molecules existent on the surface of injured small organelles like mitochondria, peroxisomes resulting in particular fusion amongst lysosomes as well as these organelles. Based on the organelles whose targeting takes place the produced autophagic vesicles are labelled by particular names. Like for mitochondria, peroxisomes, lipids, as well as RNA the labelling is mitophagy, peroxophagy, lipophagy as well as ribophagy respectively [74]. Intriguingly CMA reflects the chaperone based selection of cytoplasmic proteins meant for targeting by lysosomes their translocation takes place across the membranes of lysosomes for breakdown. A distinctive characteristic of this kind of autophagy is the direct transfer of these proteins without requirement of selectivity of the proteins broken down besides the generation of extra vesicles. The upregulation of CMA is correlated with cancer cells survival besides proliferation [75].

Bile Acids Associated Autophagy

Maximum studies regarding bile acids programmed cell death concentrate on apoptosis, nevertheless numerous studies associated with autophagy are present. Beclin1 possesses a central part regarding controlling autophagy in mammals [76]. The autophagic stimulus escalates Beclin1 along with binding to class III phosphatidylinositol 3 – kinase (PI3K) for activating autophagosome generation besides maturation. Beclin1 works in the form of a tumor repressor in mammals [76] besides results in reduction of Beclin1 protein in contrast to normal tissue was observed in breast cancer, colon cancer, renal cell cancer, cholangiosarcoma, non small cell lung cancer, besides gastric cancer [77].

Rosely *et al.* [78], evaluated the association amongst Beclin1 expression along with esophageal adenocarcinoma. Beclin1 expression was greater in normal esophagus epithelium HET1A cells (obtained from normal squamous epithelium) however was lesser in case of Barrett's esophagus along with esophageal adenocarcinoma cell lines (CP-A, CP-C, along with OE33). Acute exposure to DCA escalated Beclin1 expression besides autophagy. Nevertheless chronic DCA exposure had no impact on Beclin1 quantities or autophagy. Hence they illustrated that in the beginning

autophagy initiation takes place in reaction to DCA however chronic DCA exposure resulted in reduction of Beclin1 expression besides resistance to autophagy. Gafar *et al.* [79], documented that LCA a secondary BA resulted in autophagy induction in androgen dependent LNCaP along with androgen independent PC3 human prostate cancer cells. LCA resulted in induction of endoplasmic reticulum stress (ER)- correlated phosphorylation of eukaryotic initiation factor-2 alpha (p-eIF2 α), CHOP, c-Jun-N-terminal kinase (JNK) in LNCaP along with PC3 cells. Additionally, LCA resulted in induction of autophagy correlated protein ATG5, besides the autophagy correlated transformation of the microtubule correlated protein 1A/1B light chain 3B (LC3BILC3BII) in PC3 cells however not in DU145 cells. They illustrated that LCA resulted in ER stress induction, autophagy besides mitochondrial impairment in the PC3 cells along with DU145 cells. Furthermore they illustrated that LCA resulted in ER stress induction, autophagy besides mitochondrial impairment in human prostate cancer cells. Lim *et al.* [80], demonstrated that UDCA caused autophagy induction in the cisplatin resistant SNU 601 gastric cancer cell sub cell line (SNU 601/R). In these cancer cells other anti cancer drugs (etoposide, L-OH-P, along with rhTRAIL) influenced the survival of resistant cells, however these cancer cells possessed sensitivity to UDCA. The decrease in the viability of SNU 601/R cells subsequent to treatment with UDCA took place secondary to autophagy in contrast to cell demise in parent SNU 601/WT took place secondary to apoptosis. Prior studies have illustrated that UDCA caused apoptosis induction in gastric cancer cells is regulated by TRAIL-R2/DR5, besides in SNU 601/R cells, UDCA stimulation is controlled by TRAIL-R2/DR5 inspite of lack of apoptosis. Hence these outcomes illustrated that UDCA possesses benefits in getting over drug resistance via apoptosis impairment by induction of autophagy along with apoptosis cell demise, based on the intracellular signaling milieu.

Action of Natural Bile Acids on Necroptosis

Necroptosis alias Programmed necrosis possesses the properties of the activation of receptor interacting protein kinase (RIPK's) through numerous signaling pathway [25]. Other than apoptosis necroptosis is not based on caspase regarding cell demise [81]. Morphological alterations inclusive of organelles expansion, plasma membrane injury besides liberation of cellular constituents can result in the generation of secondary inflammation [82]. Activation of RIPK's takes place on enrollment of cell surface receptors, like DR's,

toll like receptors (TLRs) along with T cell receptors (TCR) into a macromolecular complex [83]. The crucial role of necroptosis mode is the controlling of the generation of the necrosome a complex comprised of receptor interacting serine/threonine protein kinase 1 (RIPK1 MLKL), RIPK3 along with mixed lineage kinase domain (MLKL) [84]. RIPK3 resulted in MLKL oligomerization domain by activation of the downstream molecule MLKL1 through phosphorylation [85]. Oligomerization of MLKL result in the insertion of MLKL into besides permeation of cell plasma membrane ultimately resulting in cell demise [86]. RIPK3 based necroptosis takes place by induction of a DNA based interferon activator (DAI modulator), a cytosolic sensor of DNA subsequent to the existence of a double stranded viral DNA or virus Infection [87]. Additionally, its part in cell demise induction necroptosis can result in adaptive immune reactions via induction of proinflammatory cytokines [88]. Necroptosis is correlated with generation of pathologies with the properties of unnecessary cell elimination besides inflammatory constituents.

Necroptosis in Cancer

Necroptosis possesses both procancer along with anti cancer actions [70]. This double actions of propagating as well as tumor growth reduction has been visualized in various cancer kinds [90]. In the form of cell demise that takes place in cells in which apoptosis induction does not takes place this is not a safe mode of cell demise, however it can result in avoidance of tumor generation. Induction of inflammatory reaction by necroptosis is that it is known to facilitate cancer metastasis along with cause immune repression [90]. Induction of apoptosis in cancer cells takes place for elimination of malignant cells [91]. Nevertheless, the decontrolling of apoptosis signals in cancer in particular the activation of antiapoptotic systems that aid cancer cells to dodge this program aiding in unregulated cell proliferation that aid in tumor survival. Hence, tumor necroptosis that is a caspase independent cell demise possesses greater therapeutic probability of cancer treatment. Several natural product, chemotherapeutic agents besides drugs causing necroptosis induction have been observed to result in induction of MLKL modulated necroptosis in cancer cells [92]. Like the natural substance shikonin along with its analogs have been documented to result in necroptosis in myeloma as well as glioma cells [93]. Chemotherapeutic agents inclusive of 5-FU, etoposide along with cisplatin can result in induction of tumor cell necroptosis on avoidance of caspase activity [94]. Second

mitochondrial activator of caspases (Smac) simulator BV6, a necroptosis inducer possessing the capacity of antagonism of the hampering agent of apoptosis protein (IAP), resulting in necroptic demise of cancer cells, hence is an alternate approach regarding anti cancer therapy [95]. Cekay *et al.* [96], illustrated that a synergistic crosstalk amongst interferon- γ (IFN- γ) as well as BV6 results in induction of interferon regulatory factor 1 (IRF 1) based necroptosis in variable kinds of apoptosis resistant cancer cells on blockade of caspase activity. In case of pancreatic cancer along with acute myeloid leukaemia cells BV6 results in induction of TNF- α generation besides the formation of necrosomes [95].

Bile Acids Associated Necroptosis

As the invention of necroptosis is remarkably recent in the form of a PCD studies regarding BA are occasional hence besides cancer bile acid associated necroptosis was correlated with cholestasis as well as pancreatitis were reviewed as well. In necroptosis, the generation of the necroptosis characteristic necrosomes needs the phosphorylation of RIPK1, along with the activation of MLKL, resulting in pore generation along with cell demise [97]. The initial step regarding induction of necroptosis gets shared with the apoptotic pathways. RIPK1, has been documented to get enrolled to the active cytokines receptors like Tumor necrosis factor receptor type 1 (TNFR1) that is phosphorylated (p-RIPK1) in a multi protein complex alias complex 1 [98]. Activation of caspase-8 takes place via p- RIPK1 that results in apoptosis induction by crosstalk with Fas associated death domain (FADD) protein. On hampering caspase-8, RIPK3 gets phosphorylated hence initiating necroptosis pathway [99]. Binding of p-RIPK3 takes place to MLKL in a multi protein complex that causes stimulation of its phosphorylation MLKL (p-MLKL). This p- RIPK3/ p- MLKL complex gets engulfed into the membrane where oligomerization of various p-MLKL proteins takes place along with generation of pores ultimately resulting in cell breakdown [100]. Hoff *et al.* [101], revealed the controlling modes of RIPK 3, a considerably significant molecule regarding necroptosis, subsequent to treatment of hepatocyte along with HepG2 Hepatocellular carcinoma cells with BAs. They verified the expression of RIPK1, RIPK3, along with MLKL, considerably significant molecules regarding necroptosis in HepG2 Hepatocellular carcinoma cells, primary human hepatocytes (pHep) along with human primary macrophages (pM ϕ). In case of hepatocytes RIPK3 expression was lacking, while greater expression of RIPK3 along with MLKL

expression were observed in the 3 kinds of cells HepG2, pHep along with pM ϕ . HepG2 cells which were over expressing an N terminal FLAG tagged human RIPK3 construct with the treatment of unconjugated hydrophobic -BA's CA along with UDCA resulted in upregulation of RIPK3- FLAG phosphorylation along with of RIPK3- FLAG quantities Additionally, CDCA possessed minimum influence on RIPK3- FLAG phosphorylation without impacting RIPK3- FLAG quantities. Conversely LCA resulted in reduction in RIPK3- FLAG phosphorylation along with quantities. TCA, GCA- taurine conjugated CDCA (TCDCA) besides glycine conjugated CDCA (GCDCA) resulted in reduction in the stimulation actions of CA along with UDCA on RIPK3- FLAG phosphorylation however did not avoid them. Intriguingly TCDCA LCA resulted in reduction in RIPK3- FLAG quantities along with RIPK3- FLAG phosphorylation without any impact on toxicity; nevertheless GCDCA lacked any actions. The actions of GLCA along with taurine conjugated LCA (LCA) were akin to those of the unconjugated ones. The proinflammatory cytokine IL-8 escalated CA stimulation of RIPK3- FLAG activation in HepG2 cells with maximum robust RIPK3- FLAG activation along with phosphorylation at the protein level. Additionally, just the hydrophobic bile acid CDCA had a considerably significantly enhanced IL-8 expression. Regulation of IL-8 liberation can take place by c-Jun-N-terminal kinase (JNK) signaling pathway [102]. Furthermore, JNK phosphorylation was further stimulated by hydrophilic Bas (CA along with UDCA). These outcomes illustrated that RIPK3 expression along with phosphorylation resulted in necroptosis that in turn resulted in controlling the IL-8 liberation by JNK.

In a study regarding bile acids - associated cholestasis resulted in necroptosis was illustrated by Alfonso *et al.* [103]. Primary biliary cholangitis (PBC) represents chronic cholestatic liver disease possessing properties of breakdown of small intrahepatic bile ducts [104]. GCDCA is a main constituent of human serum as well as bile acids [105]. These studies illustrated escalated expression of RIPK3 along with MLKL phosphorylation in liver samples from human PBC patients. Bile duct ligation (BDL) represents a model regarding robust obstructive cholestasis that results in considerably significant jaundice as well as hepatocellular injury [106]. Their observation was that mRNA along with proteins expression of RIPK3 along with MLKL as well as MLKL phosphorylation got robustly escalated in the liver of BDL mice. These outcomes pointed that targeting necroptosis might be a therapeutic approach regarding acute cholestasis treatment.

Zhou *et al.* [107], illustrated that BAs induced necroptosis in chronic pancreatitis. Furthermore bile acids have been illustrated to result in pancreatic acinar cell demise via reduction in mitochondrial membrane potential, enhanced ROS, along with energy elimination [108]. We possess information regarding facilitation of acinar cell apoptosis by all of these bile acids, besides necrosis in pancreatitis. Maximum quantities of primary bile acids are those of GCDCA as well as TCA in humans [107]. Hence they corroborated that pancreatic cell lines that received exposure to GCDCA as well as TCA escalated the expression of the nuclear bile acid receptor called Farnesoid X nuclear receptor (FXR) as well as resulted in reduction of the expression of the necessary autophagy- correlated protein ATG 7. Furthermore bile acids were escalated in case of pancreatic tissue in human patients with chronic pancreatitis, that associated with enhanced expression of ATG 7 besides FXR that was correlated with local reduction of autophagic action. These outcomes illustrated that a stepwise process where local BAs accrual result in signaling taking place via FXR hampered autophagy in pancreatic acinar cells hence causing stimulation of acinar cell apoptosis along with necroptosis.

Cell Death Modes of Synthetic Bile Acids Prototypical Agents in Cancer

Various Synthetic bile acids prototypical agents have been documented to result in PCD. Figure 3A-D illustrates their structure.

Numerous investigators have illustrated that HS-1030 as well as HS1183 which are obtained from UCDA, along with HS1199, as well as HS1200 which are obtained from CDCA resulted in the apoptosis induction in different human cancer cells [14,109,116]. HS-1030 is conjugated with glycine methyl esters (Figure 3A, B). HS1183 is conjugated with L phenyl alanine benzyl ester. HS1199 is conjugated with β -alanine benzyl ester [14,117]. It was illustrated that these agents hamper the growth of different cancer cells via apoptosis. Out of the 4 synthetic bile acids prototypical agents evaluated HS1199, as well as HS1200 significantly escalated apoptosis. Of these HS1200 possesses maximum robust action [14,114].

The tumor suppressor gene TP53 gets mutated in about half of all human cancers. Besides its part in the form of a tumor suppressor p53 plays a considerably significant role in reaction of numerous anti cancer agents particularly in malignant cells along with cells

that have not undergone modifications resulting in DNA injury. p53 generates homodimers which directly control about 500 target genes hence regulating a broad range of cellular events inclusive of cell cycle, cell senescence, DNA healing, metabolism adaptation as well as cell demise [119]. Numerous laboratories have evaluated synthetic bile acids prototypical agents in MCF7 in human breast cancer cells (wild kind -p53), MDA-MB-231 (mutant p53), PC3 human prostate cancer cells (null type p53) besides human colon cancer cells (mutant p53), Their observation was that these bile acids prototypical substances resulted in arrest in cell cycle propagation in the G1 phase with escalated quantities of the Cdk hampering agent p21^{WAF1/CIP1}, as well as resulted in the apoptosis induction irrespective of p53 status [14,111,112].

Apoptosis gets modulated via a caspase (cysteine aspartyl specific protease) which result in the cleavage of target proteins [119]. Caspase protease results in cleavage of over 100's of various proteins, besides is necessary for apoptosis [118]. Four kinds of initiating caspases (caspase-2,-8,-9,-10) besides three caspases performing execution function (caspase-3,-6, as well as -7) [121]. Executioning caspases cleave target proteins finally resulting in cell demise. This pathway complex is strictly controlled besides takes place subsequent to apoptotic signaling. Treatment with the utilization of synthetic bile acids prototypical agents (HS1183, HS1199, as well as HS1200) resulted in reduction of the quantities of pro caspase-3 along with 8 decreased in Jurkat human leukaemic cells [109]. Additionally, the quantities of pro caspase-3 were decreased in SNU1 gastric cancer cells [113] besides malignant glioblastoma (U118MG, U-87 MG, U373MG) [114]. Additionally, HS1200 resulted in reduction of the quantities of pro caspase-3 along with pro caspase7 in KAT 18 thyroid carcinoma cells [116]. Caspase modulated signal transduction might aid in synthetic bile acids prototypical agents (HS1183, HS1199, as well as HS1200) modulated apoptosis.

Intrinsic pathways of apoptosis are in particular regulated by Bcl2 protein family which is constituted of proapoptotic BH3 -only proteins as well as the antiapoptotic protein Bcl2 proteins [120]. Antiapoptotic Bcl2 proteins hamper apoptosis by hampering the proapoptotic Bcl2 proteins BAX BAK [122]. In case of MCF cells that received treatment with synthetic bile acid prototypical agents (HS1183, HS1199, as well as HS1200), the expression of Bcl2 factors was not significantly changed, however Bax expression was

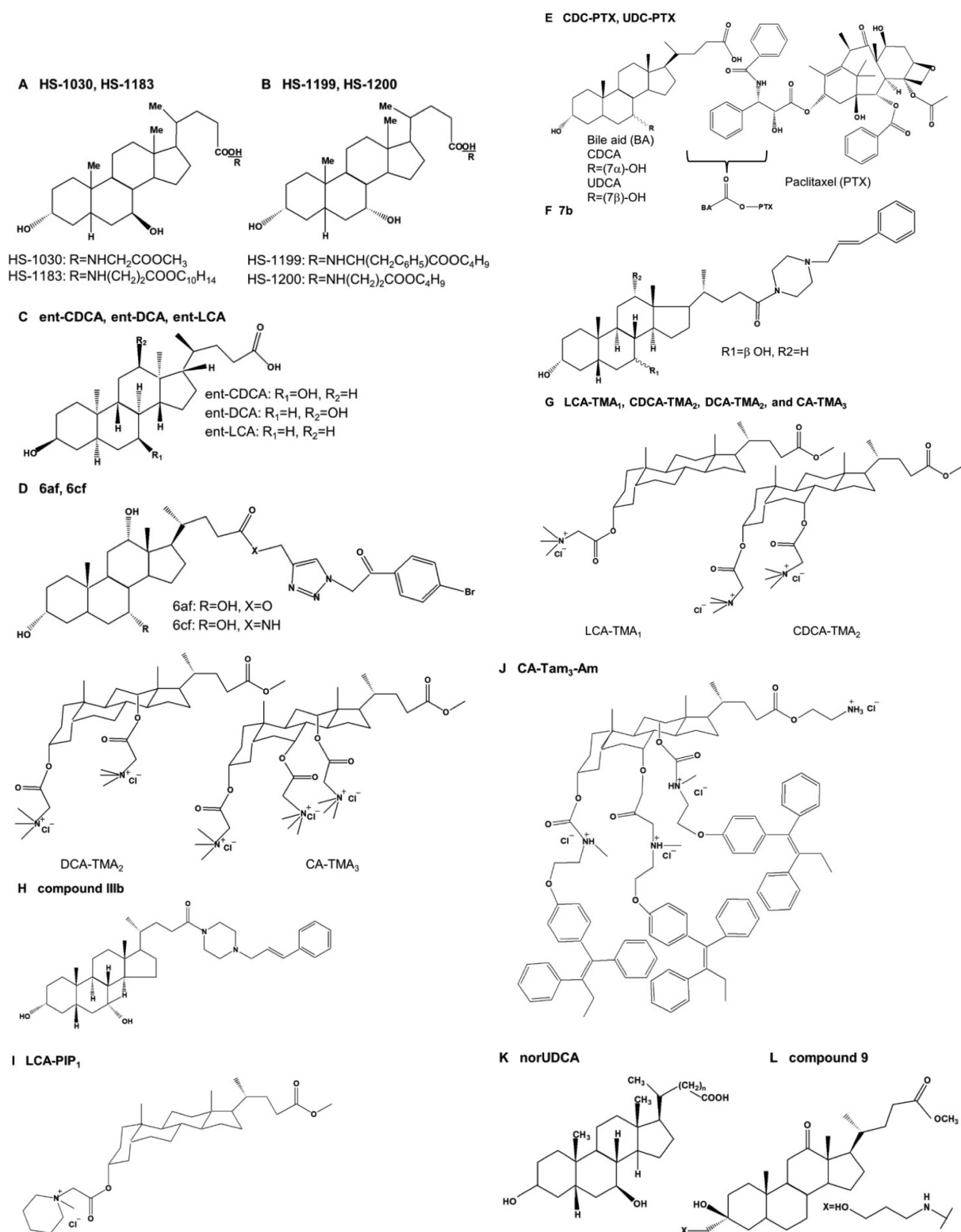


Figure 3: Courtesy ref no-4-Structures of synthetic bile acid derivatives. CA, cholic acid; CA-TMA₃, cholic acid based amphiphile; CA-Tam₃-Am, cholic acid-tamoxifen conjugate; CDC-PTX, chenodeoxycholic-paclitaxel hybrid; CDCA, chenodeoxycholic acid; CDCA-TMA₂, chenodeoxycholic acid based amphiphiles; compound IIIb, chenodeoxycholic acid-substituted piperazine conjugate; compound 9, chenodeoxycholic acid derivative; ent-CDCA, enantiomers of chenodeoxycholic acid; ent-DCA, enantiomers of deoxycholic acid; ent-LCA, enantiomers of lithocholic acid; DCA, deoxycholic acid; DCA-TMA₂, deoxycholic acid based amphiphiles; HS-1030 and HS-1183, ursodeoxycholic acid derivatives; HS-1199 and HS-1200, chenodeoxycholic acid derivatives; LCA, lithocholic acid; LCA-PIP₁, lithocholic acid-piperidine; LCA-TMA₁, lithocholic acid based amphiphile; norUDCA, nor-ursodeoxycholic acid; UDCA, ursodeoxycholic acid; UDC-PTX, ursodeoxycholic-paclitaxel hybrid; 6af and 6cf; bile acid-added triazolyl aryl ketones; 7b, piperazinyl bile acid derivative.

significantly escalated [14,111]. Conversely, Bax expression was escalated as well as the Bcl2 expression quantities were significantly decreased in MDA-MB231 HCT-116 cells (wild type, null kind p21, along with null kind p53) [111]. Early growth response1 (Egr¹) alias Zif268, Krox24, NGFI, TSI8rep a zinc finger transcription factor which controls transcription via a GC consensus sequence of 5'GGG(T/G)GGGCG-3 [123]. Egr¹ is referred to an immediate early response gene in view of its rapid kinetics that get induced by different signals inclusive of growth factors, cytokines besides stress DNA injury [124]. Egr might be implicated in cell proliferation, differentiation along with apoptosis. In case of cancer Egr might work in the form of tumor repressor by facilitation of apoptosis in reaction to stress DNA injury [125]. Currently there are numerous probable applications regarding Egr treatment. Prior studies have documented that BA result in upregulation of Egr in gastric cancer cells via the MAPK signaling pathway [126]. One more study illustrated that the BA DCA enhanced Egr¹ protein quantities in primary mouse hepatocyte. This pointed that the upregulation of Egr¹ in liver at the time of cholestasis might be bile based [127]. Park *et al.* [115], further observed that the treatment with the synthetic BA CNDA derivative HS1200 considerably significantly resulted in the induction of Egr1 expression at the time of an earlier stage. The expression of proteins p53, p21^{WAF1/CIP1}, p21^{KIP1}, as well as COX2 downregulation occurred by silencing Egr¹. As per Park *et al.* [115], Egr1 possessed a considerably significant part in the form of a gene controller in case of Hepatocellular carcinoma cells that received HS1200 treatment besides being implicated in significant cellular events like apoptosis as well as cell cycle control.

JNK, alias stress activating protein kinase belonging to the MAPK family has been taken into account in numerous cellular processes inclusive of reaction to different stress signals, apoptosis besides autophagy [128]. Numerous proofs are existent regarding BAs possessing a part in controlling cell growth besides apoptosis induction via the activation of JNK/ activator protein (AP1) signaling pathway [129]. CDCA, LCA, along with UDCA result in the activation of AP1 in HT29 cells, as well as HCT116 cells [130]. Additionally, CDCA results in the modification of AP1 in hepatic stellate cells [131]. Furthermore, we already possess information that AP1 induction by BAs needs the activation of ERK along with PKC [132]. More recently BAs were illustrated to result in the upregulation of the death receptor 5/TRAIL receptor 2 expression through

a JNK based pathway inclusive of Sp1 [188]. Im *et al.* [110], observation was that HS1199 as well as HS1200 resulted in significant upregulation of JNK phosphorylation. Additionally, HS1183 that resulted in lesser apoptosis in SiHa cells in contrast to HS1199 as well as HS1200 was further seen to result in lesser JNK phosphorylation.

NFκB protein sustenance takes place in the cytoplasm in an inactive state via a hampering subunit alias IκB. NFκB is constituted by a DNA binding domain (p50) along with transactivating domain (p65); which, might escalate or facilitate induction of gene or apoptosis [134].

Firstly, Im *et al.* [110], illustrated that the HS1200 treatment of human cervical carcinoma cells with HS1200 resulted in the reduction of p65, p50 as well as IκB in a dose based fashion. These outcomes corroborated that HS1200 transiently enhanced NFκB actions along with the translocation of the active NFκB complex towards the nucleus is implicated in the controlling of transcription of other apoptosis correlated genes in human cervical carcinoma cells.

Secondly Katona *et al.* [15], found that the synthetic enantiomers of Lithocholic acid (ent LCA), chenodeoxycholic acid (ent CDCA) as well as deoxycholic acid (ent DCA) resulted in toxicity besides apoptosis in HT29 cells, as well as HCT116 in colorectal cancer cells (Figure 3C). Greater apoptosis besides cleavage of caspase-3 as well as -9 induction occurred with the utilization of natural BAs in contrast to enantiomeric BAs. Nevertheless both native along with enantiomeric BAs possessed akin actions on cell proliferation. Of these LCA as well as ent LCA avoidance of apoptosis induction occurred by pan caspases, besides selective caspase 8 hampering agent, while none protection got conferred by selective caspase 2 hampering agent. The induction of BAs modulated caspase 8 activation in hepatocyte occurred by CD95 oligomerization along with translocation to the cell membrane [68]. This illustrated that LCA as well as LCA caused apoptosis induction by CD95 activation that resulted in procaspase 8 cleavage secondary to escalated ROS generation.

Thirdly Agarwal *et al.* [16], illustrated that Bile acid appended triazolyl aryl ketones 6af as well as 6cf resulted in apoptosis induction in MCF7 cells breast cancer cells (Figure 3D). Specifically substance 6cf led to apoptosis induction by 46.09% in MCF7 cells whereas substance 6af led to apoptosis induction by

33.89%, illustrating that 6cf possesses greater effectiveness regarding apoptosis induction.

Fourthly Melloni *et al.* [17], illustrated that CDC-PTX along with UDC-PTX in combination with paclitaxel, an anti cancer agent led to apoptosis induction in leukaemia HL60 cells along with NB4 acute promyelocytic leukaemia cells via a high output condensation reaction of CDCA along with UDCA (Figure 3E). Additionally, it was illustrated that CDC-PTX along with UDC-PTX RKO led to apoptosis induction in HCT-116 colonic carcinoma cells. Specifically in all the 4 cell lines HL60 cells, NB4 human leukaemic cell lines, RKO along with HCT-116 colon colon cancer cells), CDC-PTX led to greater apoptosis in contrast to UDC-PTX. Additionally, Pacific blue (PB) conjugated agents obtained from CDC-PTX along with UDC-PTX (CDC-PTX- PB along with UDC-PTX- PB) were generated via multiple step generation for assessment of their capacity of crossing the plasma membrane in contrast to UDC-PTX- PB.

Fifthly Brossard *et al.* [18], illustrated that 7b,a newer piperaziny BA obtained substances led to apoptosis induction in KMS-11 multiple myeloma cells in contrast to HCT-116 colon cancer cells. Furthermore, substance 7b was illustrated to lead to DNA fragmentation as well, a property of apoptosis in KMS-11 cells.

Sixly Singh *et al.* [19], illustrated that four cationic BAs dependent facial amphiphilic agents LCA-TMA, CDCA-TMA, DCA-TMA, along with CA-TMA possessing the properties of trimethyl ammonium head groups led to apoptosis induction in HCT-116 besides DLD colon cancer cell (Figure 3G). LCA-TMA caused the maximum apoptosis induction in both cells.

Sevently Kihel *et al.* [20], generated six innovative Bas (LCA as well as CDCA)- substituted piperazine conjugates lithocholic acid besides chenodeoxycholic acid piperazine carboxamides illustrated that substance IIIB resulted in apoptosis induction in KMS-11 multiple myeloma cells (Figure 3H). Out of the six substances, IIIB illustrated the maximum apoptotic action in KMS-11 multiple myeloma cells. This illustrated that apoptosis is implicated in Mcl1 along with PARP cleavage, hampering of NFkB signaling along with DNA fragmentation.

Eighthly Singh *et al.* [21], generated cationic amphiphilic substances with different cationic charge head group properties with the utilization of KCA. Out of these it got corroborated that the Lithocholic acid -dependent

amphiphilic substances that possessed the piperidine head group (LCA-PIP1) possessed 10 fold greater cytotoxicity to colorectal cancer cells in contrast to their precursors (Figure 3I). This corroborated that the LCA-PIP1 resulted in greater quantities of apoptosis in HCT-116 colorectal cancer cells in contrast to LCA. LCA-PIP1 resulted in induction of sub-G0-arrest along with cleavage of caspase-3,-7,-8. These actions of LCA-PIP1 were further corroborated in a tumor xenograft model of HCT-116 cells reduction in volume took place by about 75%.

Ninethly Srekanth *et al.* [22], generated a conjugate of tamoxifen with utilization of 3 Bas (LCA, DCA, along with CA (Figure 3J). Of these, the free amine head groups dependent CA- tamoxifen conjugate (CA-tam₃ Am resulted in greater apoptosis in contrast to tamoxifen in 4T1, MCF-7, T47D, MDA-MB 231 breast cancer cells along with illustrated cell arrest in G0phase. Additionally, the MCF-7 cells, that are estrogen receptor positive, with CA-tam₃ Am induction of apoptosis via extrinsic along with intrinsic pathways while treatment of MDA-MB 231 cells which are estrogen receptor negative had apoptosis induction via intrinsic pathway.

Tenthly, Tang *et al.* [23], illustrated that nor UDCA that represents a UDCA analog with side chain shortening at C23 resulted in autophagy in case of HTOZ cervical cancer cells (Figure 3K). α 1 antitrypsin deficiency (α 1AT) deficiency represents a genetic condition that results in accrual of the α 1 mutant Z (α 1ATZ) protein in the ER of hepatocytes, resulting in chronic liver injury, liver fibrosis along with HCC [135]. nor UDCA hampered the accrual of α 1ATZ via autophagy modulated breakdown of α 1ATZ in HTOZ cells. Moreover, they illustrated that AMPK activation of protein mammalian target of rapamycin inhibitors (mTOR)/ULK1 pathway was implicated in nor UDCA induction of AMPK activation besides autophagy in HTOZ cells.

Elevently Markov *et al.* [24], illustrated that compound 9 in a series of innovative DCA obtained substances possessing an aliphatic diamine and aminoalcohol/morpholine moiety present at the C3 region, led to apoptosis along with autophagy in HuTu80 duodenal carcinoma cells (Figure 3L). They illustrated that compound 9 results in ROS based cell demise by activation of intrinsic caspase based pathway of apoptosis along with cell destroyer autophagy in HuTu80 duodenal carcinoma cells.

Caliceti *et al.* [137], illustrated that besides their utilization regarding therapy for cancer BA's possess a role regarding BAs signaling as a part in the initiation of colorectal cancer (CRC) along with detailed the modes of their association with membrane along with nuclear receptors with bad nutrition for avoidance of CRC generation [137].

CONCLUSIONS

Here we tried to find the further advances regarding use of bile acids for cancer patients of after reviewing the role of bile acids in NAFLD and use of probiotics [138-40]. Here we provided an overview of the modes of PCD in form of apoptosis, autophagy as well as necroptosis subsequent to therapy with natural along with bile acid obtained synthetic substances in the form of tumor repressors in cancer. The further dilemma that has plagued the medical fraternity is whether BAs are pro-cancer or anti-cancer in action. Many of the publications have revealed a correlation amongst bile acids along with certain cancers that corroborated with the precancerous phenotype of BAs. Nevertheless, certain bile acids might possess anti-cancer phenotype subsequent to prior malignant conversion. This is correlated with their amphiphilic structure along with extra target pathways not stimulated at physiological quantities. Additionally, the association of bile acids with cancer might get impacted by the bile acids/gut microbiome axis. Synthetic BAs substances resulted in the induction of programmed cell death in various human cancer cell lines. Hence these innovative bile acids related substances might turn out to be chemicals that are advantageous for the generation of innovative anti-cancer agents dependent on the structure of bile acids.

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