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Ursodeoxycholic acid and cancer: From chemoprevention to chemotherapy

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ABSTRACT

Ursodeoxycholic acid (UDCA) is a secondary bile acid issued from the transformation of (cheno)deoxycholic acid by intestinal bacteria, acting as a key regulator of the intestinal barrier integrity and essential for lipid metabolism. UDCA is also a long-established drug, largely used for the dissolution of cholesterol gallstones, the treatment of primary biliary cholangitis and other hepatobiliary disorders. The history of UDCA is briefly retraced here as well as its multifactorial mechanism of action, based on its anti-inflammatory, antioxidant and cytoprotective activities. The present review is centred around the anticancer properties of UDCA and synthetic antitumor derivatives designed over the past 20 years. Paradoxically, depending on the conditions, UDCA exhibits both pro- and anti-apoptotic properties toward different cell types. In particular, the UDCA drug can protect epithelial cells from damages and apoptosis while inducing inhibition of proliferation and apoptotic and/or autophagic death of cancer cells. The effects of UDCA on cancer cell migration, cancer stem cells and drug-induced dysbiosis are also evoked. The drug has revealed modest activities against colon and gastric cancers but may be useful to improve treatments of hepatocellular carcinoma, notably in combination with other drugs such as sorafenib. UDCA can also protect from damages induced by cancer chemotherapeutic agents. The potential of UDCA in cancer, as a chemo-protecting or chemotherapeutic agent, is highlighted here as well as the design of tumour-active derivatives, including UDCA-drug conjugates. A repurposing of UDCA in oncology should be further considered.

Contents

1. Bile acids

Bile acids form a class of molecules essential for lipid absorption and cholesterol metabolism, playing a major role in glucose regulation and energy homeostasis. The pool of bile acids includes the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) both biosynthesized in the liver from cholesterol, and secondary bile acids modified by intestinal bacteria such as deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA) (Fig. 1). The pool also includes the glycine- or taurine-conjugated bile salts. The history of bile acids and their main chemical and biological properties have been comprehensively reviewed (Fiorucci & Distrutti, 2019; Hegyi, Maléth, Walters, Hofmann, & Keely, 2018; Hofmann & Hagey, 2014).

Abbreviations: UDCA, ursodeoxycholic acid; HCC, hepatocellular carcinoma; PBC, primary biliary cholangitis; ROS, reactive oxygen species.

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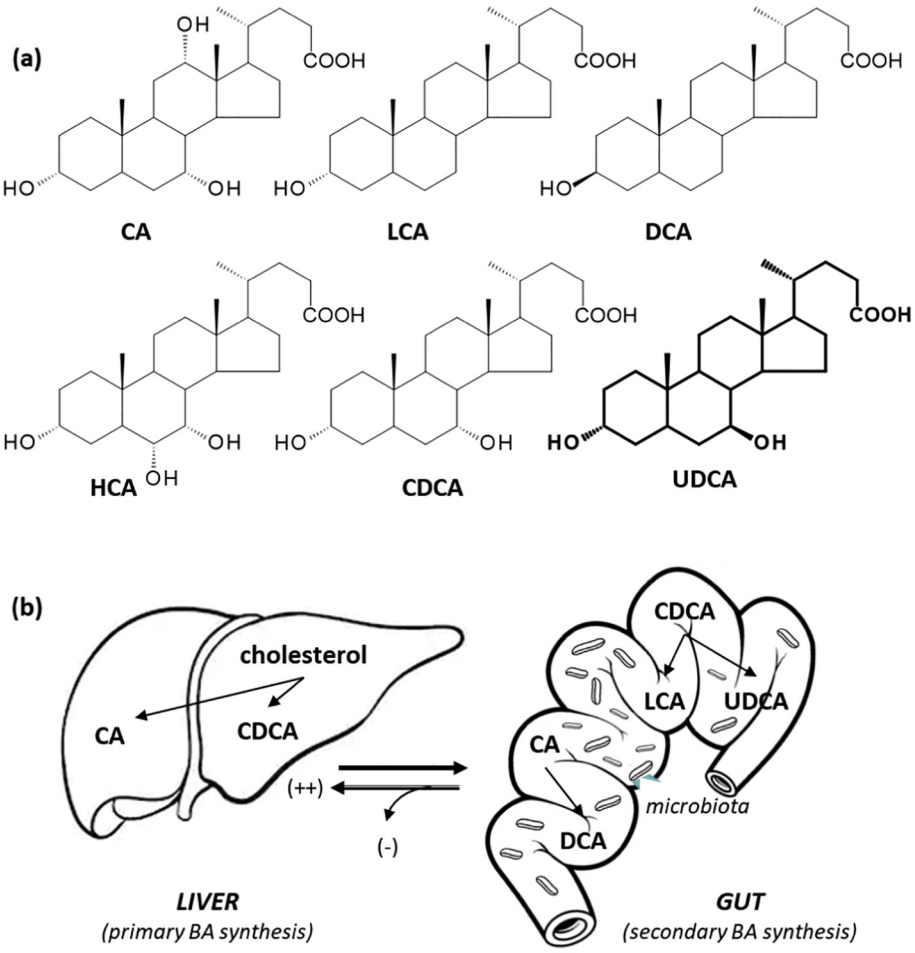

Fig. 1. (a) Chemical structures of selected bile acids (BA). CDCA (Chenodiol®) and UDCA (Ursodiol®) are approved drugs. UDCA (3α,7ß-dihydroxy-5ß-cholan-24-oic; $C_{24}H_{40}O_4$) is shown in bold. (b) Simplified BA biosynthetic pathway, with the formation of CA and CDCA in the liver and of DCA, LCA and UDCA in the intestine, via the gut microbiota. Their tauro- and glyco-conjugated forms are also biosynthesized. The pool of BA is largely reabsorbed via the enterohepatic circulation (++) to limit the fecal loss (−).

The most common bile acids DCA, UDCA and LCA are synthesized endogenously by the intestinal microbial flora (Ridlon, Harris, Bhowmik, Kang, & Hylemon, 2016). In Humans, UDCA accounts for up to 3–4% of the bile acid pool; it is formed by epimerization of 7α-hydroxy-CDCA in the gut by intestinal bacteria and represents the most hydrophilic and the least toxic bile acid. The pool of biliary acids circulates in human through the enterohepatic circulation, to reach the liver mainly, the gallbladder for the storage of the bile and the small intestine to contribute to digestion of fats and their excretion (Šarenac & Mikov, 2018). UDCA is more easily reabsorbed back into the enterohepatic circulation than LCA, the most hydrophobic unconjugated biliary acid. Very small quantities of bile acids are normally present in the systemic circulation. But the serum levels of DCA, UDCA and CDCA were found to be much higher in patients with non-small cell lung cancer than in healthy people (Liu et al., 2018). The different bile acids are considered as hormones with a major role in glucose and lipid metabolism, cholesterol biosynthesis and elimination, and to contribute to the functions of liver and other organs. The mechanism of action of UDCA is multifactorial (Fig. 2): it involves the displacement of endogenous toxic bile acids at the intestinal and liver levels, an increase of the secretion of bile acids from the liver (choleretic effect), a regulatory action on glucose metabolism, immune-modulatory and cytoprotective activities (Lazaridis, Gores, & Lindor, 2001). UDCA positively impacts glucose homeostasis by reducing fasting plasma glucose and insulin concentrations (Sánchez-García, Sahebkar, Simental-Mendía, & Simental-Mendía, 2018). UDCA also accelerates the enterohepatic circulation of bile acids (Zhang et al., 2019), exerts direct anti-secretory actions on colonic epithelial cells (Kelly et al., 2013) and promotes epithelial wound healing to protect the intestinal barrier (Mroz, Lajczak, Goggins, Keely, & Keely, 2018). In this short review, we will focus on UDCA and its potential applications for the prevention and treatment of cancers. The anticancer potential of UDCA has received little attention thus far; it is highlighted here.

2. A short history of UDCA

The discovery and applications of bile acids has been reviewed previously (Hofmann & Hagey, 2014). Only the main dates in the history of UDCA are briefly evoked here, as illustrated in Fig. 3. At the very beginning of the 20th century, after two expeditions to explore Greenland, Olof Hammarsten (Uppsala, Sweden) isolated a new bile acid from a polar bear (*Thalarctos maritimus*) and named it "ursocholeinsäure". More than twenty-five years later, Masato Shoda (Okayama, Japan) was able to crystallize this new bile acid from a preparation of black bear (*Ursus americanus*) bile and renamed the product "Ursodesoxycholsäure" in 1927. Keeping the *urso* root-word for bear (*ursus* in Latin); the history was enrooted. The chemical structure was determined in 1936. The first total synthesis of UDCA was published in 1954 and a few years later UDCA was identified as a minor constituent of human bile by Jan Sjövall in 1959 (Stockholm, Sweden). Since the 1950s, the pharmaceutical company Tokyo Tanabe had marketed UDCA as a liver tonic, based on the legendary therapeutic effects of bear bile. Testing in Human were initiated in the 1960s–70s and the first prospective therapeutic trials of gallstone dissolution with UDCA were

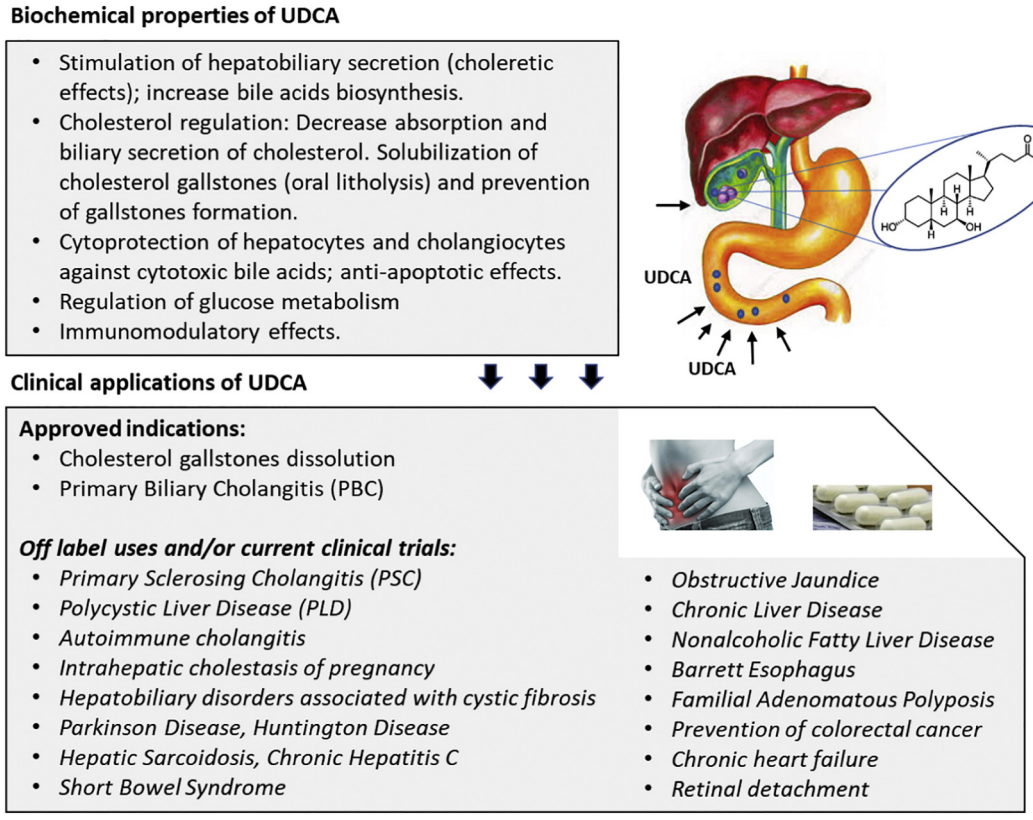

Fig. 2. Main biochemical characteristics of UDCA and its multiple clinical applications. Ongoing clinical trials with UDCA were identified via the US-FDA website (www.clinicaltrials.gov).

conducted around 1975 by Isao Makino (Makino, Shinozaki, Yoshino, & Nakagawa, 1975), confirmed by several studies in the late 1970s-early 1980s (Erlinger, Le Go, Husson, & Fevery, 1984). The drug is approved and marketed in France since 1980 and in the EU, as well as outside the EU for gallstone dissolution. The process for the preparation of UDCA was first patented in 1980 (Bonaldi & Molinari, 1983). In 1987, Ulrich Leuschner (Frankfurt, Germany) noted that UDCA ingestion improved biochemical parameters in patients with primary biliary cirrhosis (Leuschner & Kurtz, 1987) and the same year, Raoul Poupon (Paris, France) published the results of a landmark clinical study on the long-term use of UDCA for the treatment of patients with primary biliary cirrhosis (PBC, now designated primary biliary cholangitis) (Poupon et al., 1987; Poupon, Poupon, & Balkau, 1994). Many clinical studies will then confirm the medical benefit of UDCA and the US FDA approved the use of the drug for the treatment of PBC in 1998. For almost two decades, UDCA was the only approved treatment for PBC, until July 2016 when the FDA granted accelerated approval to obeticholic acid (Ocaliva®, a potent agonist of the farnesoid X receptor (FXR)) for the treatment of PBC, in combination with UDCA in adults with an inadequate response to UDCA or as monotherapy in adults unable to tolerate UDCA. The drug received a marketing authorization from the European Medical Agency in December 2016. The anticancer potential of UDCA was underlined in the late 1990s. The involvement of secondary bile acids, including UDCA, in colorectal carcinogenesis was noted in 1995 (Pongracz, Clark, Neoptolemos, & Lord, 1995) and the design of anticancer UDCA analogues started in 1997 (Park et al., 1997). Over the past 20 years, many studies have highlighted the hepato-protective properties of UDCA and its capacity to inhibit tumour cell proliferation. These studies are discussed below.

Ursidae (bears) are thus at the origin of the name of the UDCA bile acid, but however it is worth to "bear" in mind that despite the high level of UDCA in their bile, bears like other animals can suffer from cancer. One may recall the iconic polar bear Gus (1985–2013) of the Central Park Zoo in New York City which was euthanized after the discovery of a

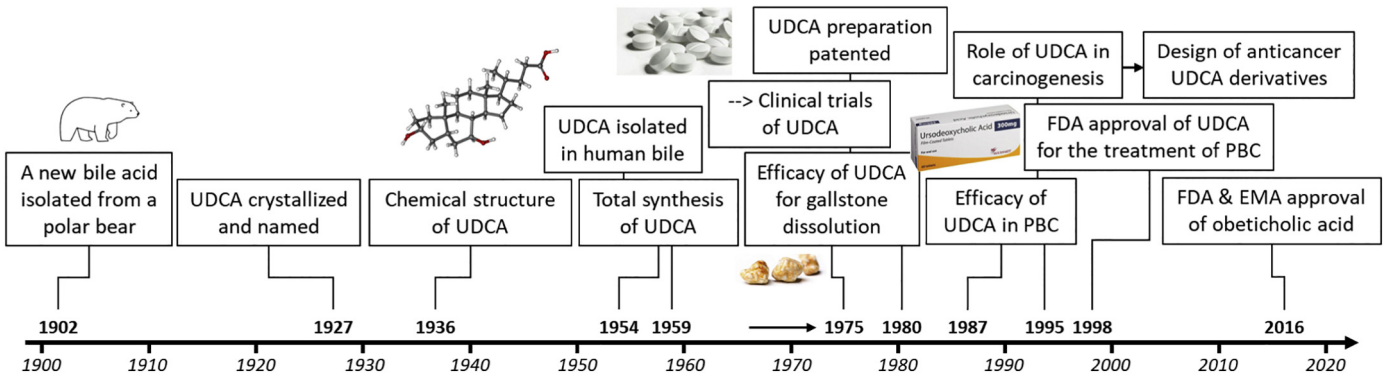

Fig. 3. History of UDCA discovery and development. Over a century of evolution of UDCA, to retrace the key dates of its discovery, biochemical properties and clinical applications.

large, inoperable thyroid tumour (Shuman, 2014). Different types of cancer have been observed in bears, including HCC (Matsuda et al., 2010).

3. The drug UDCA

As an endogenous natural product, UDCA is a non-toxic hydrophilic bile acid, which exhibits antioxidant, anti-inflammatory and cytoprotective properties. As a drug, UDCA (Ursodiol®, Ursosan®, Ursolvan®, Ursofalk® and many other brands) is commercially available as capsules and tablets, and a stable liquid dosage form has been recently described (Pramar et al., 2019). The drug is mainly used for the treatment of biliary tract diseases, in particular PBC, which is an autoimmune chronic cholestatic liver disease characterized by biliary destruction and progressive intrahepatic cholestasis (Onofrio, Hirschfield, & Gulamhusein, 2019). It is also approved for cholesterol gallstone dissolution (cholecystolithiasis) and is used (off label) in many other indications, such as for the treatment of enlarged polycystic liver disease, intrahepatic cholestasis of pregnancy, primary sclerosing cholangitis and other cholestatic hepatopathies, on the basis of its capacity to counteract inflammation and bile acids-induced liver damages (Fig. 2). As a protective agent, UDCA plays a role in the maintenance of intestinal barrier function. The drug may be useful to limit mucosal inflammation in patients with intestinal bowel disease (Mroz et al., 2018).

Other indications have been proposed (Fig. 2). UDCA may be used for patients with hepatitis C virus infection to ameliorate elevated alanine aminotransferase levels (Ikegami & Matsuzaki, 2008). UDCA is also known to improve peripheral blood flow in patients with chronic heart failure and to protect the heart against reperfusion injury (Hanafi, Mohamed, Sheikh Abdul Kadir, & Othman, 2018). In addition, the potential use of UDCA (and Tauro-UDCA) has been suggested for the treatment of pathological conditions with deregulated levels of apoptosis, including neurological disorders, such as Alzheimer, Parkinson, and Huntington diseases (Amaral, Viana, Ramalho, Steer, & Rodrigues, 2009; Vang, Longley, Steer, & Low, 2014). Recently, UDCA has been proposed as a novel therapeutic agent for obesity (Chen, Liu, & Lee, 2019).

UDCA is generally well tolerated but long-term UDCA treatments can lead to adverse reactions, such as fever, bronchopneumonia, pharyngitis, nephritis, skin rash, nausea or diarrhea. Other unwanted effects have been reported (Kotb, 2012). The reflux of bile acid into the oesophagus can lead to esophagitis, inflammation-stimulated hyperplasia, metaplasia such as Barrett's oesophagus and ultimately oesophageal adenocarcinoma. UDCA plays a role in the regulation of this oesophageal inflammation-metaplasia-carcinoma sequence by decreasing the overall proportion of the toxic bile acids. The anticancer activities and antitumor mechanisms of UDCA are discussed below.

4. UDCA and cancer prevention

UDCA - and its taurine-conjugated form TUDCA - exhibits cytoprotective properties and as such it attenuates colon carcinogenesis in Human. The chemopreventive effects of UDCA have been evidenced in animal models and to some extent in Humans. Retrospective studies have indicated that patients with PSC and ulcerative colitis treated with UDCA have a reduced risk of developing colitis-associated cancer (Tung et al., 2001). Later it was shown that UDCA significantly reduced risk for advanced adenoma in men, not women (Thompson et al., 2009). A specific modulation of the gut microbiome by UDCA in men may be implicated (Pearson et al., 2019). It was shown that UDCA treatment prevents progression of dysplasia in intestinal bowel disease patients and the risk of recurrence of adenoma (Alberts et al., 2005; Serfaty et al., 2003). Patients with chronic liver diseases treated with UDCA have a reduced risk of colorectal cancer (Huang et al., 2016). However, it should be noted the chemo-preventive effect of UDCA has been challenged (Carey & Lindor, 2012; Serfaty, 2012). The long-term use of high-dose UDCA has been associated with an increased risk of colorectal cancer in patients with ulcerative colitis and primary sclerosing cholangitis (Eaton et al., 2011). At low doses, UDCA may reduce the risk of advanced colorectal cancer in those patients, but not at high doses (Singh, Khanna, Pardi, Loftus Jr, & Talwalkar, 2013). Another study indicated that the use of UDCA was not associated with a risk of colorectal cancer in PSC or IBD patients but the high or low dose of UDCA was a source of heterogeneity across studies (Hansen et al., 2013). A supplementation with high dose UDCA can result in favourable changes in gastric bile acid composition without modulating the levels of cell proliferation and apoptosis in the intestinal epithelium (Banerjee et al., 2016). Oral administration of UDCA produces a specific bile acids profile with high-abundance tauro-UDCA and glyco-UDCA (Zhang, Jiang, et al., 2019). The dose effect is a matter of discussion; it may also be influenced by the microbiome composition.

At the cellular level, UDCA functions as a potent inhibitor of apoptosis in different situations, although it can also promote apoptosis in other environments (see below). UDCA was shown to protect from the apoptotic effects of DCA in oesophageal cells (Abdel-Latif, Inoue, & Reynolds, 2016), colon cancer cells (Im & Martinez, 2004; Yui, Saeki, Kanamoto, & Iwami, 2005) and in differentiated hepatocytes derived from bone marrow mesenchymal cells (Ji, Qu, Jin, Zhao, & He, 2009). The effect is mediated, at least in part, by the modulation of the EGFR/ERK signalling pathway by UDCA and degradation of c-Myc protein thereby decreasing the expression of cell cycle regulators such as CDK4 and CDK6 (Krishna-Subramanian et al., 2012; Peiró-Jordán et al., 2012). The protective effect of UDCA implicates a number of effectors (Fig. 4) such as the regulation of substrates of the metalloproteinase ADAM17 (Buryova et al., 2013). In colon cancer cells, UDCA does not induce DNA binding of the transcription factors NFκB and AP-1, in contrast to DCA. Moreover, UDCA can block DCA-induced NFκB and AP-1 activation (Shah, Volkov, Arfin, Abdel-Latif, & Kelleher, 2006) and it inhibits DCA-induced intracellular translocation from cytosol to plasma membrane of protein kinase C (PKC) isoenzymes to its site of activity (Shah, Looby, Volkov, Long, & Kelleher, 2005). The mechanism would also implicate UDCA-mediated inhibition of the protein C/EBPβ, which is a transcriptional regulator of cyclooxygenase-2 (Khare et al., 2003, 2008). In general, UDCA increases expression of antioxidants that prevent toxic bile acids, such as DCA, from causing DNA damage and NFκB activation (Peng et al., 2014). The inhibition of NFκB activation and its nuclear translocation is coupled with a blockade of IκB degradation and inhibition of subsequent phosphorylation of proteins, such as ERK1/2 (Abdel-Latif et al., 2016). Several studies have pointed out the opposing cellular effects of DCA and UDCA (Fig. 4), and their capacity to activate unique signalling pathways (Fimognari, Lenzi, Cantelli-Forti, & Hrelia, 2009; Huo et al., 2011; Lim, Duong, Parajuli, & Han, 2012), although the two molecules have also overlapping signalling routes, since the majority of cell lines resistant to UDCA-induced growth arrest are also resistant to DCA-induced apoptosis (Powell et al., 2006). The distinctive effects of DCA and UDCA on oncogenic signalling pathways may explain, at least partially, the different effects of the two molecules on colon cancer progression (Fig. 4). DCA activates MAPK signalling, leading to activation of protooncogenes (e.g. AP-1), markers of inflammation and suppression of tumour suppressor genes (e.g. p53). On the opposite UDCA tends to suppress MAPK signalling and exerts globally negative regulatory effects on the EGFR-MAPK pathway (Centuori & Martinez, 2014). These differences may explain the pro- and anti-apoptotic effects of DCA and UDCA respectively and their opposite capacity to regulate epithelial wound healing (Mroz et al., 2018).

5. UDCA and cancer treatment

The anticancer potential of UDCA was suggested a few years ago, based on several studies evidencing the capacity of the drug to limit tumour cell growth and to modulate different molecular pathways implicated in tumour cell growth and/or cell death, as illustrated in Fig. 5. The mechanisms and drug effects are discussed below.

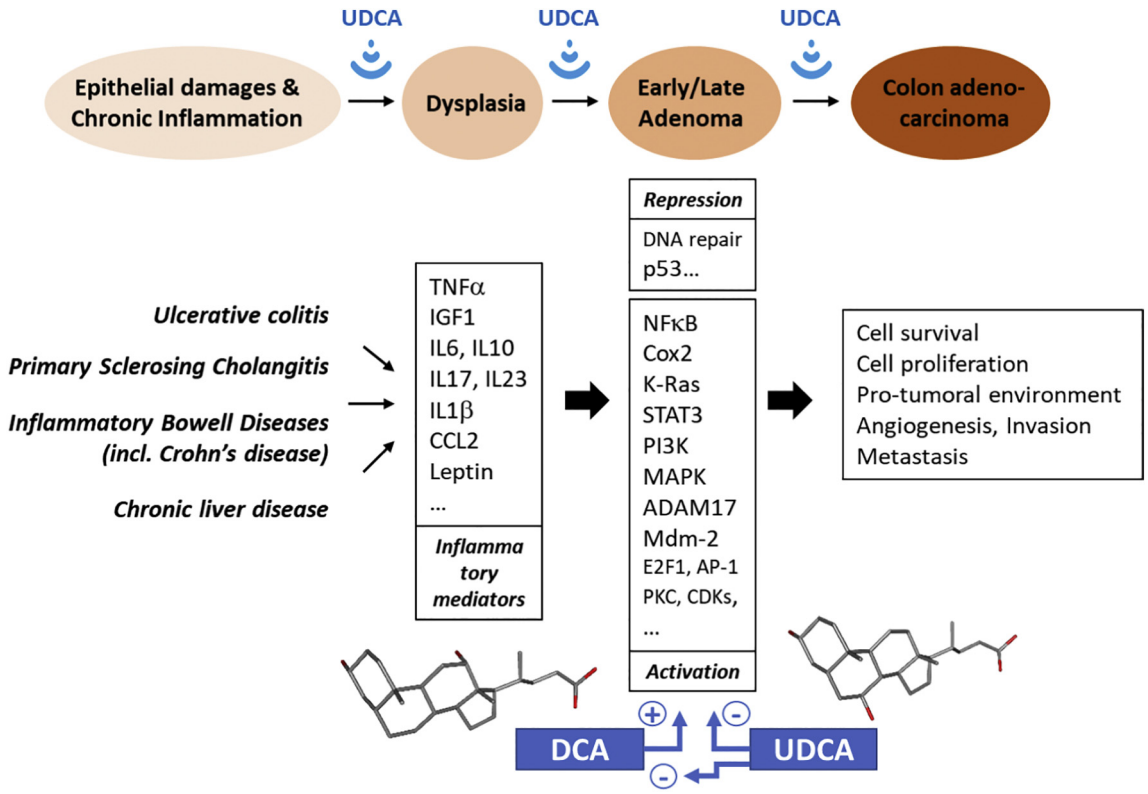

Fig. 4. UDCA would restrain colon progression in patients after gastro-intestinal diseases, by attenuating the deleterious effects of DCA or via specific modulation of signalling molecules.

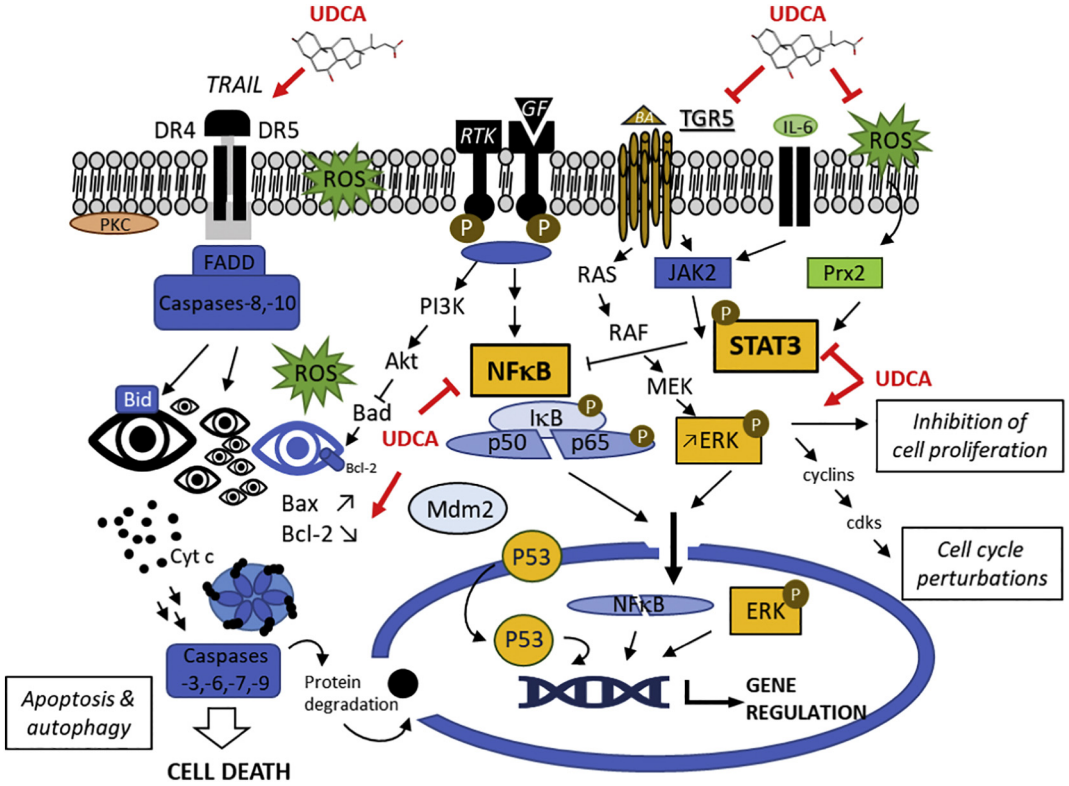

Fig. 5. Molecular pathways and signalling molecules implicated in the anticancer effects of UDCA. The drug activates or represses a complex and dynamic machinery, which varies from one cancer type to another. The main effectors, such as STAT3, NFκB, p53 and ERK are highlighted.

5.1. Cell growth inhibition and induction of cancer cell death

Several studies demonstrated that UDCA could inhibit the proliferation of human cancer cell lines in vitro (Table 1). The mechanism evidenced with melanoma cells implicates a drug-induced cell cycle arrest in the G2/M phase associated to a decrease of cyclin-dependent kinase 1 and cyclin B1 protein levels, followed by an induction of apoptosis through the ROS-triggered mitochondrial-associated pathway (Yu et al., 2019). But the mechanism is most likely cell-type dependent. With human DU145 prostate cancer cells, it was shown that UDCA-induced apoptosis is associated with extrinsic pathway, characterized by an increased expression of TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) receptor and the death receptors 4 and 5 (DR4, DR5) (Lee et al., 2017). The implication of cell surface death receptors in the mechanism of apoptosis induced by UDCA was also evidenced using a gastric cancer cell line. In this case, it was shown that UDCA induces both apoptotic and autophagic cell death depending on the intracellular signalling environment (Lim & Han, 2015). Interestingly this study showed that UDCA is active against the cisplatin-resistant SNU601 gastric cancer subline (SNU601/R), which is resistant to other drugs like oxaliplatin, etoposide, and death ligand TRAIL (Lim & Han, 2015). UDCA can induce apoptosis in a variety of cell lines (Table 1).

Hepatocellular carcinoma (HCC) has a high prevalence in Asia, representing one of the major leading causes for cancer-related death and the medical need remains high for this type of tumour. UDCA selectively inhibits proliferation and induces apoptosis of HCC cell lines in vitro. The drug-induced apoptotic process is accompanied by cell cycle perturbations and was characterized by a down-regulation of proteins Bcl-2 and Smac, and up-regulation of proteins Bax and Livin in HCC cells (Liu et al., 2007; Zhu et al., 2014). The regulation of Bax to Bcl-2 ratio is considered to play a key role in tumour progression or inhibition of intrinsic apoptotic pathway triggered by mitochondrial dysfunction. UDCA differentially activates initiator and effector caspases but caspases activation is not always translated into increased apoptosis (Tsagarakis, Drygiannakis, Batistakis, Kolios, & Kouroumalis, 2010). UDCA was shown to inhibit proliferation of BEL7402 HCC cells both in vitro and in vivo. In the mice model, the drug dose-dependently reduced BEL7402 cell growth and triggered apoptosis through a mitochondrial pathway (Liu et al., 2015). However, in these experiments the administration of UDCA was initiated 1-day before tumour cells implantation in nude mice; it therefore essentially represents a prevention model rather than a therapeutic model. Nevertheless, the anticancer potential of UDCA was evidenced in HCC in vivo. The molecular mechanism leading to HCC tumour cell growth is not totally clear but it seems to implicate an inhibition of the proteasomal degradation of the tumour suppressor protein DLC1 in an ubiquitin-independent manner (Chung et al., 2011). An in vivo activity has also been evidenced in a mice xenograft model of gastric cancer and in this case, the induction of apoptosis by UDCA was found to be mediated by an increased expression of DR5 (Lim et al., 2011). The extrinsic apoptotic pathway is clearly implicated in the cell death mechanism triggered by UDCA.

5.2. Effects on cancer cell migration/invasion and cancer stem cells

There is limited information on the antimetastatic potential of UDCA. However, it has been reported that by perturbing the bile acids pool, UDCA impedes tumour invasiveness of gastric cancer MKN-74 cells induced by CDA, a process implicating PKC activation and COX-2 induction (Wu, Chiu, Hsueh, & Hsueh, 2018). DCA can promote non-small cell lung cancer (NSCLC) cell migration and invasion through a mechanism implicating the membrane bile acid receptor TGR5, which is aberrantly expressed in NSCLC and positively correlated with an advanced clinical stage in NSCLC patients (Liu et al., 2018). TGR5 knockdown prevented JAK2 and STAT3 phosphorylation and repressed the expression of STAT3 target genes, thus inhibiting cell proliferation, migration and invasion in NSCLC. UDCA, which is able to decrease the phosphorylation of STAT3 (Chen et al., 2019; Kim, Cho, Kim, & Kim, 2017), could also reduce cell migration and invasion. Its conjugated analogue tauro-UDCA has been shown to reduce the invasion of metastatic breast cancer cells in vitro, via a process implicating a decreased expression of matrix metalloproteases MMP-7 and MMP-13, which play important roles in metastasis (Park, Han, Han, & Lee, 2016).

UDCA can activate hepatic stem cells, which contribute to the regenerative response of the liver. In cancer, UDCA was found to inhibit formation of cancer stem-like cells. This was shown in colon and pancreatic cancers. In both cases, the effects was accompanied by a drug-induced reduction of the intracellular level of ROS, and modulation of specific signalling pathways, such as activation of Erk1/2 in colon cancer cells (Kim, Cho, et al., 2017) and inhibition of the phosphorylation of STAT3 and expression of peroxiredoxin II (Prx2) in pancreatic cancer cells. Prx2 is a redox regulatory protein that plays a key role in maintaining ROS homeostasis in the tumour microenvironment. The drug-induced reduction of STAT3 activation increases the expression of E-cadherin and decreases the expression of N-cadherin, thereby affecting the formation of cancer stem-like cells generated by tumorsphere culture (Kim, Cho, et al., 2017; Kim, Jeong, et al., 2017). Thus, UDCA seems to inhibit cancer stemness. In sharp contrast, DCA and LCA have been shown to induce cancer stemness (measured by the drug-induced elevation of cancer stem cell markers such as ALDHA1 and colonosphere formation) in colonic epithelial cells, a

Table 1
UDCA-induced cancer cell death.

Tumour type	Cancer cells	UDCA effects and cell death mechanism and/or pathways implicated	References
Melanoma	M14, A375	ROS-triggered mitochondrial-associated pathway leading to apoptosis	Yu et al., 2019
Gastric carcinoma	SNU601 MKN-74	Apoptosis via downregulation of ATG5, preventing the autophagic pathway	Lim et al., 2011, 2012; Lim & Han, 2015
Oral squamous carcinoma	HSC-3	Apoptosis through caspase activation. Induction of TRAIL, DR4, DR5, and IκB-α expression.	Pang et al., 2015
Hepatocellular carcinoma	BEL7402 SNU761,	Tumour growth inhibition and apoptosis induction in vivo. Regulation of Bax/Bcl-2 ratio. Inhibition of Dlc1 protein proteasomal degradation	Chung et al., 2011; Liu et al., 2015; Zhu et al., 2014
Ovarian carcinoma	A2780	Reduces PKC activity, minimal cytotoxic effect	Horowitz et al., 2007
Prostate carcinoma	DU145	Inhibition of cell growth. Apoptosis via extrinsic and intrinsic pathways.	Choi et al., 2003; Lee et al., 2017
Pancreatic carcinoma	HPAC, Capan-1	Reduces levels of intracellular ROS and Prx2. Reduced formation of pancreatic cancer stem cells	Kim, Jeong, Kim, Kim, & Cho, 2017
Colon carcinoma	HT29, HCT8, HCT116	Regulation of intracellular ROS generation, activation of Erk1/2, inhibition of colon cancer stem-like cells formation. Suppresses expression of c-Myc, thereby decreasing the expression of the cell cycle regulators CDKs	Peiró-Jordán et al., 2012; Kim et al., 2018
Leukaemia	Jurkat HL-60	Inhibition of T cell proliferation, cell cycle arrest, necrosis. Enhanced intracellular Ca^{2+} release in HL60 cells leading to apoptosis.	Baek et al., 1997; Fimognari et al., 2009

mechanism possibly contributing to the promotion of colon cancer (Farhana et al., 2016).

5.3. UDCA-induced dysbiosis and cancer

Another interesting and emerging aspect of the mechanism of action UDCA is its ability to influence the gut microbiome and the relationship with cancer development. The microbiome changes induced by UDCA may be associated with a reduced risk for the development of adenoma in men (Pearson et al., 2019). Recently, changes of the microbiota profiles have been observed also after UDCA treatment in patients with primary biliary cholangitis (Chen, Liu, & Lee, 2019). Dysbiosis was observed in the gut microbiome in PBC and partially relieved by UDCA (Tang et al., 2018). It is also known that the microbial metabolism of UDCA is necessary for the expression of its protective effects against colonic inflammation (Ward et al., 2017). Considering the important role of the intestinal microbiota in cancer chemotherapy, at least as for certain anticancer drugs such as methotrexate and 5-fluorouracil, it is conceivable that UDCA-induced impacts on the intestinal bacterial flora contribute to the efficacy of cancer chemotherapy or its tolerance. For example, UDCA treatment was found to be associated with a decrease in *Fusobacterium* in the patient population (Pearson et al., 2019) and a high abundance of certain *Fusobacterium* species, notably *Fusobacterium nucleatum*, has been correlated with chemoresistance in advanced colorectal cancer patients (Zhang et al., 2019). Similarly, the long-term UDCA treatment has been associated with an over-representation of *Faecalibacterium prausnitzii* (Pearson et al., 2019), a bacterium known to mitigate 5-fluorouracil-induced mucositis (Wang, Jatmiko, Bastian, Mashtoub, & Howarth, 2017). The microbiota can modulate the host response to chemotherapy via numerous mechanisms, including immunomodulation and xeno-metabolism, which is essential for biliary acids. The gut microbiota may thus represent a bridge between UDCA bioactivity and anticancer effects. UDCA effects on microbiota may also contribute to reduce the gastrointestinal toxicities of chemotherapeutic drugs. As mentioned above, UDCA can prevent methotrexate-induced liver injury and can attenuate intestinal inflammation induced by 5-fluorouracil. The gut microbiota is known to be implicated in the mechanism of action of these two cytotoxic drugs (Alexander et al., 2017).

5.4. Protection from damages induced by anticancer drugs

A few studies have underlined the potential use of UDCA to protect from damages induced by anticancer drugs, usually conventional cytotoxic agents. Three different cases can be cited. First, UDCA has been shown to attenuate gastro-intestinal mucositis induced by 5-fluorouracil in a rat model (Kim et al., 2018). The effect can be linked, at least in part, to the UDCA-induced decrease of inflammatory cytokine levels. Through the modulation of intestinal barrier dysfunction and oxidative stress, UDCA exerts a local protective effect to attenuate intestinal inflammation (Bernardes-Silva et al., 2004). Second, UDCA has a neuroprotective effect on cisplatin-induced cell death of sensory neurons via a down-regulation of the p53 signalling pathway (Park, Kim, & Kim, 2008). Similarly, UDCA was shown to switch oxaliplatin-induced necrosis to apoptosis via inhibition of ROS production and activation of the p53-caspase 8 pathway in HepG2 cells (Lim, Choi, Kang, & Han, 2010). Third, UDCA may be used to prevent methotrexate-induced liver injury. In a rat model, it was shown that hepatocyte necrosis induced by methotrexate could be prevented by UDCA treatment (Uraz et al., 2008). In a paediatric population of children with acute lymphoblastic leukaemia (ALL), the UDCA treatment was associated with a relative decrease of hepatic transaminases when concomitantly administered with chemotherapy (Mohammed Saif, Farid, Khaleel, Sabry, & El-Sayed, 2012). But a more recent study using UDCA did not support hepatoprotective effects of UDCA in ALL paediatric patients (Bordbar, Shakibazad, Fattahi, Haghpanah, & Honar, 2018). UDCA can be useful to alleviate drug-induced liver injury but also damages potentially caused by radiotherapy. The post-therapeutic application of UDCA combined with pentoxifylline and low-dose low molecular weight heparin was found to significantly reduce the extent and incidence of radiation-induced liver injury (Seidensticker et al., 2014).

5.5. Combinations with anticancer drugs

A few studies indicated that UDCA could be advantageously combined with known anticancer agents. For example, UDCA promotes the apoptotic response induced by the topoisomerase I inhibitor SN38 (the active metabolite of the anticancer drug irinotecan) in different cancer cell lines, by increasing SN38-induced DNA damages (Ikegami et al., 2006). It was also observed that irinotecan-induced neutropenia can be reduced when given in combination with oral alkalization drug mixture of UDCA, magnesium oxide, and sodium hydrogen carbonate (Hamano et al., 2019).

UDCA can also be combined with targeted anticancer drugs. The combination of low dose UDCA and the COX-2 inhibitor celecoxib has revealed growth inhibitory effects in colon cancer cells (van Heumen et al., 2012) but high-dose UDCA *co*-treatment was found to counteract the beneficial effects of celecoxib in patients with familial adenomatous polyposis (van Heumen et al., 2013). A very interesting recent study demonstrated a synergistic effect of UDCA on the antitumor activity of the oral multi-kinase inhibitor sorafenib in hepatocellular carcinoma cells. UDCA was found to increase the production of ROS by activating the mitogen-activated protein kinase ERK and subsequently inhibiting STAT3 phosphorylation (Fig. 5). As such, UDCA reinforces the down-regulation of phospho-STAT3 by sorafenib, amplifying the tumour growth inhibitory and antimetastatic effects in HCC (Lee et al., 2018). It will be useful to determine if the synergy observed in vitro also occurs in vivo because both UDCA and sorafenib undergo major enterohepatic recycling. The drug transporter OATP1B1 plays a role in the elimination of the sorafenib-β-D-glucuronide metabolite (Edginton, Zimmerman, Vasilyeva, Baker, & Panetta, 2016) but UDCA inhibits the expression of this transporter by repressing the hepatocyte nuclear factor 1α (HNF1α) (Lee et al., 2014). UDCA plus sorafenib might represent a new attractive combination for the treatment of HCC.

6. Design of anticancer UDCA derivatives

The potential use of UDCA in cancer has prompted the design of analogues with superior anticancer activities. This field is still emerging and a limited number of UDCA analogues have been designed thus far. There are two categories: UDCA conjugates and synthetic derivatives (Figs. 6 and 7).

The first approach consists in the coupling of UDCA to anticancer agents, taking advantage of the pentanoic acid side chain in particular. For example, the coupling of UDCA to cytarabine provided a conjugate with a 2-fold increased oral bioavailability. This UDCA-cytarabine conjugate (Fig. 6) enhanced the in vitro stability and significantly prolonged the in vivo half-life of cytarabine (Zhang, Li, Shang, He, & Sun, 2016). Similarly, bile acid-tamoxifen conjugates can afford interesting anticancer agents, active against both oestrogen receptor positive and negative breast cancer cell lines. In this case, the most interesting compounds were obtained using cholic acid (UDCA was not tested) and one conjugate bearing three tamoxifen molecules was found to be potent in a tumour model in vivo (Sreekanth et al., 2013). Bile acids-chlorambucil conjugates were also designed to change the normal route of elimination of chlorambucil (kidney excretion) to a hepatobiliary one. A chlorambucil-taurocholate conjugate was shown to maintain the pharmacological activity of the parent DNA alkylating drug (Kullak-Ublick et al., 1997). Very recently, bile acids were conjugated to dihydroartemisinin (DHA) to afford a series of cytotoxic hybrid molecules (Marchesi et al., 2019). The coupling of the DHA unit to the C-3 or C-24 positions of the bile acid core provided potent hybrid

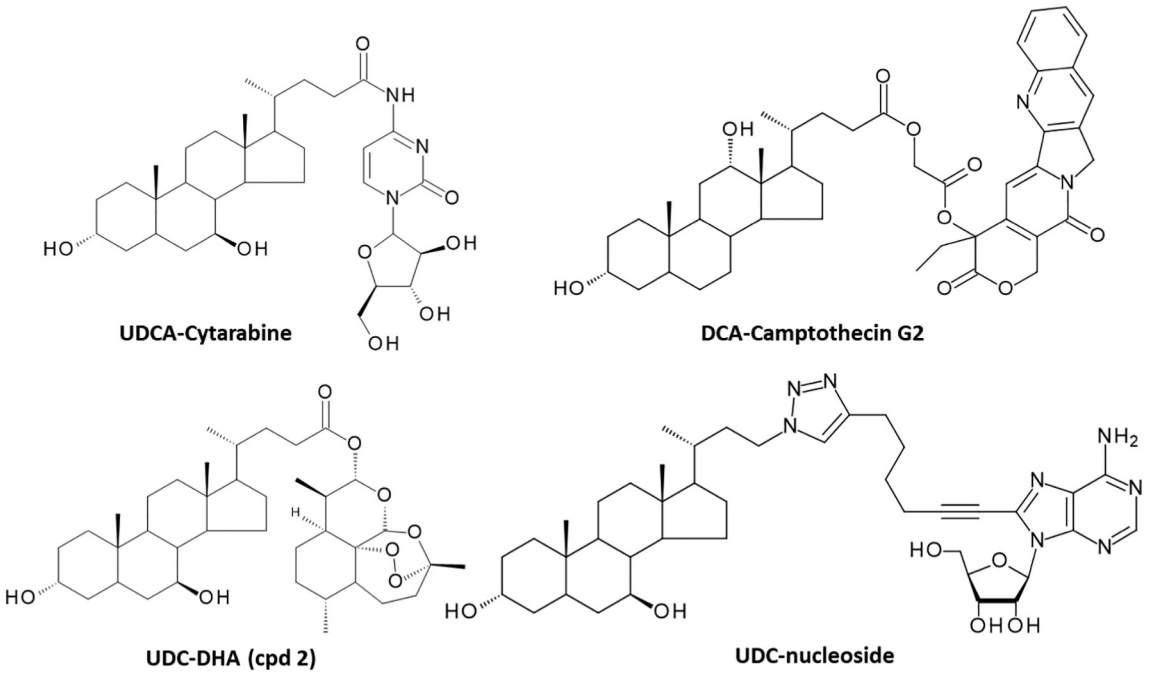

Fig. 6. Chemical structures of selected anticancer UDCA-drug conjugates: UDCA-Cytarabine (Zhang et al., 2016), DCA-Camptothecin G2 (Xiao et al., 2019), UDC-DHA compound 2 (Marchesi et al., 2019) and an UDCA-nucleoside hybrid (Navacchia et al., 2017).

compounds, 10 to 15 times more cytotoxic to HL60 leukaemia cells compared to DHA alone. The UDC-DHA hybrid compound 2 (Fig. 6) triggered the production of ROS and induced apoptosis in HL60 and HepG2 cells (Marchesi et al., 2019). The bile acid moiety can be used as a drug delivery vector. Coupling with a bile acid entity to an anticancer agent is an interesting approach for liver-targeted drug delivery, taking advantage of bile acid receptors on hepatocytes. The deoxycholic acid-camptothecin conjugate G2 was found to better target the liver that

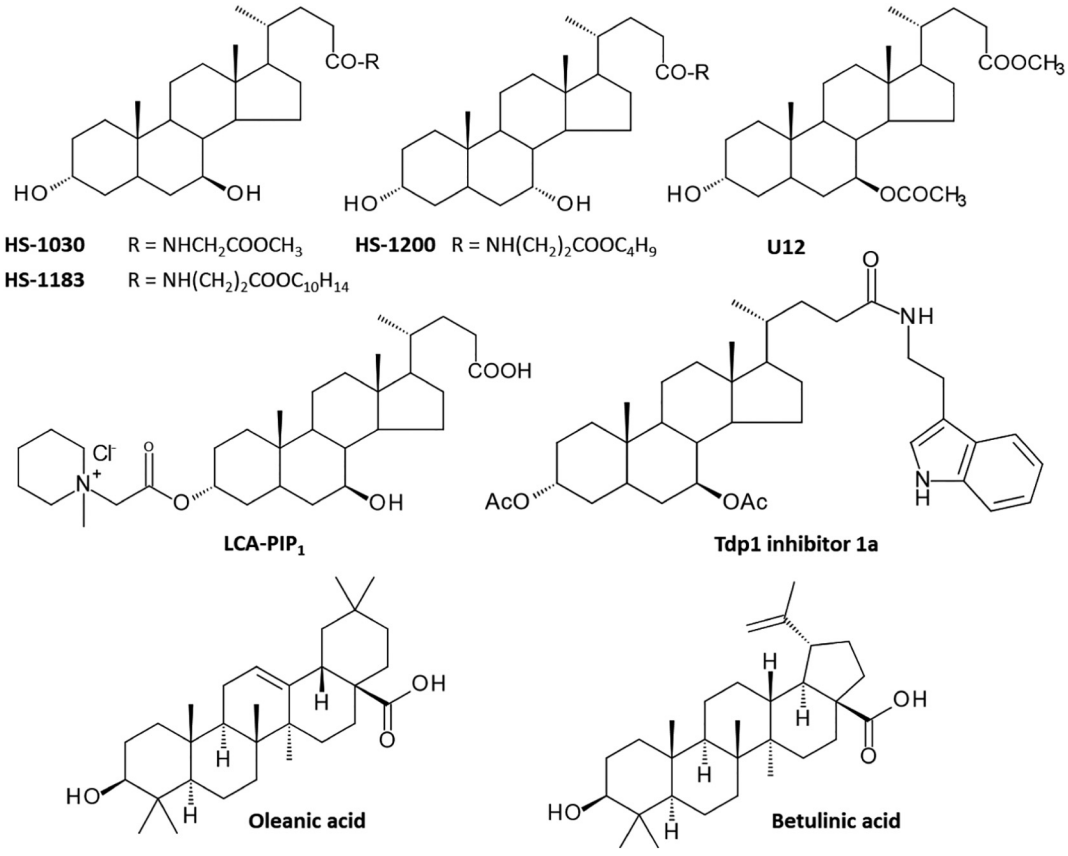

Fig. 7. Chemical structures of selected anticancer UDCA analogues: HS-1030, HS-1183 and HS-1200 (Choi et al., 2001; Park, Lee, et al., 2008), derivative U12 (Xu et al., 2014); compounds LCA-PIP1 (Singh et al., 2015) and a TdP1 inhibitor (Salomatina et al., 2018). The structures of the related natural products oleanic acid and betulinic acid are also shown.

unconjugated camptothecin (Xiao, Yu, Yue, & Li, 2019). Other examples of UDCA bioconjugates could be cited, including photosensitive derivatives (Navacchia et al., 2016) and nucleoside-bile acid conjugates, such as the UDC-nucleoside hybrid represented in Fig. 6 (Agarwal, Siva Krishna, Sriram, Yogeeswari, & Sakhuja, 2018; Navacchia et al., 2017).

The second approach refers to modifications of the UDCA skeleton with the aim to improve the pharmacological profiles of the drugs. UDCA can be readily modified by substitution of the OH groups at positions 3 and 7, or via its acid side chain, to generate anti- or pro-apoptotic derivatives (Dosa, Ward, Castro, Rodrigues, & Steer, 2013). Over the past 20 years, scientists at Pusan University (South Korea) have designed series of UDCA and CDCA derivatives endowed with interesting anticancer properties. They started with a glycine methyl ester conjugate of UDCA, designated HS-1030 (Fig. 7), which inhibited proliferation and induced apoptosis of HepG2 cells (Park et al., 1997). This compound also revealed anti-angiogenic activities in vitro (Suh et al., 1997). The L-leucine methyl ester analogue, HS-1133, was found to significantly reduce the DNA cleavage activity of topoisomerase I, which is a well-established anticancer target (Kim et al., 1999). Many other UDCA and CDCA derivatives were synthesized and tested. The most interesting compounds are probably the UDCA derivative HS-1183 and CDCA derivative HS-1200 (Fig. 7) active against breast cancer cells, inducing apoptosis via a p53-independent pathway (Im et al., 2001). Unsurprisingly, CDCA derivatives are more cytotoxic than the UDCA analogues. HS-1200 was found to completely inhibit the proliferation of cell proliferation of human leukemic T cells in (Jurkat cell line) whereas HS-1183 showed a weak inhibitory activity and both UDCA and CDCA had no significant effects (Choi et al., 2001). The mechanism of drug-induced cell cycle perturbation and apoptosis implicated inhibition of pRB phosphorylation and suppression of Cdk/cyclin complex kinase activity, coupled with a decreased expression of different cyclins and Cdk proteins (Choi et al., 2003; Park et al., 2004). In human hepatoma cells, HS-1200 decreased the levels of cyclooxygenase-2 mRNA and protein expression and markedly induced the Egr-1 expression (Park et al., 2008). The drugs also inhibited the proliferation of breast cancer cell lines in vitro and induced apoptosis through a p53-independent pathway (Im et al., 2001). They also inhibited the growth of cervical carcinoma cells, inducing apoptosis through activation of both NFκB and c-Jun N-terminal kinase (JNK) pathways (Im et al., 2005). Interestingly, the CDCA derivative HS-1200 was found to inhibit tumour growth and to increase life span of immunodeficient mice bearing U87MG glioblastoma tumours (Yee et al., 2005). The drug can also delay the growth of TE671 medulloblastoma cells in vivo (Kim, Im, Yoo, & Choi, 2006). HS-1200 induced apoptosis in a variety of tumour cell lines (Liu et al., 2008; Moon, Kim, & Park, 2004; Park et al., 2004; Seo et al., 2003) and the level of drug-induced apoptosis can be augmented when HS-1200 is combined with a proteasome inhibitor (Seo et al., 2003). This synthetic bile acid derivative was also found to sensitize radiation-induced apoptosis in MCF-7 human breast carcinoma cells (Yee et al., 2007). Oral administration of HS-1200 suppressed tumorigenesis, attenuated pathological changes in liver tissues, and decreased serum levels of liver enzymes in a rat model of hepatocellular carcinoma (Xu et al., 2017). HS-1200 might be an interesting anticancer candidate for Human clinical trials. Other UDCA derivatives modified at the C-24 position have been reported. For example, a series of UDCA amide derivatives designed to induce glucocorticoid receptor nuclear translocation has been described (Sharma et al., 2011).

A thorough investigation of the anticancer activities of a series of 20 UDCA derivatives led to the identification of derivative U12 as a potent antitumor compound (Fig. 7). This 7-acetyl-UDCA-methyl ester derivative has revealed potent activities against hepatocarcinoma cells triggering apoptosis in vitro and inhibiting tumour growth in vivo (Xu et al., 2014). Other examples can be cited, including anticancer derivatives of lithocholic acid. Compound LCA-PIP$_1$ bearing a piperidine head group was found to be about 10 times more cytotoxic than its lithocholic acid precursor against colon cancer cells, to induce apoptosis and to inhibit tumour growth in vivo (Singh et al., 2015). It is reasonable to believe that it is now possible to create new drugs useful for cancer treatment from bile acid derivatives as lead compounds.

A markedly distinct approach consists to use the bile acid scaffold and virtual screening approaches to find novel anticancer agents targeting specific enzymes or signalling pathways implicated in tumorigenesis. A recent example is the discovery of potent semi-synthetic derivatives of bile acids as effective inhibitors of tyrosyl-DNA phosphodiesterase 1 (Tdp1), a DNA repair enzyme considered as an important cancer target. Just to cite one example, the UDCA derivative 1a in Fig. 7 was found to markedly inhibit Tdp1 but there was no major difference in the UDCA, CDCA and DCA series (Salomatina et al., 2018).

7. Discussion

UDCA is increasingly used for the treatment of chronic cholestatic liver diseases. The prevalence of cystic fibrosis liver disease is high, affecting about 30,000 people in the US in 2015 and about 1000 new cases are diagnosed every year. UDCA is the 1st-line (lifetime) treatment for primary biliary cholangitis (PBC) which cost about $3000/year, much less than the 2nd-line treatment with the farnesoid X receptor agonist obeticholic acid (Ocaliva®, $70,000/year) for patients intolerant to UDCA or who do not respond to UDCA (Samur et al., 2017). Gallstones is affecting 10–15% of the US population annually, corresponding to about 25 million people. The global UDCA market represented about 350 million US$ in 2017 and is estimated to grow by about 6–7% annually for the next 7 years. Another study cited a global market size of US$ 530 million in 2019 and an estimate of US$ >1 billion by 2024. The annual production of cholic acid was estimated to about 1000 metric tons in 2014, the majority of which is used for the production of UDCA (Hofmann & Hagey, 2014). It must be significantly higher today, with the development of UDCA therapies. Of course, the market could grow considerably if in the future UDCA is used to treat other pathologies, in the field of CNS or cancer for example.

UDCA is usually obtained by hemi-synthesis from cholic acid, extracted from bovine bile. It can also be prepared starting from CDCA extracted from chicken, goose or bovine bile. It requires the use of specific bacterial enzymes (7α- and 7β-hydroxysteroid dehydrogenases) to achieve the conversion of the hydroxyl group at C-7 from α- into β-position to generate UDCA (Tonin & Arends, 2018). An additional step, the removal of the hydroxyl group at C-12, is required when starting from cholic acid. The process is usually long and costly. The compound may also be obtained from hyodeoxycholic acid, which is less expensive and more easily obtained than CDCA from pig bile (Dou & Jiang, 2016). More economical synthetic routes are being developed to facilitate the large-scale production of UDCA (He, Wang, Gu, Xiao, & Qiu, 2018; Tonin & Arends, 2018; Zheng, Chen, Li, Li, & Xu, 2018).

UDCA has revealed chemopreventive effects, in particular to reduce the occurrence of colorectal cancer in patients with chronic liver diseases (Huang et al., 2016). But it is difficult to envision the regular, chronic use of UDCA in asymptomatic individuals, not only because the tumour-preventive effect of UDCA is not definitively established, but also because the drug is not devoid of any side effects, even if it is globally well tolerated. The development of drugs for the chemoprevention of cancer remains a significant and costly challenge. Moreover, as highlighted here, UDCA exhibits both anti- and pro-apoptotic properties and inadequate long-term use of the drug may lead to cell damages and unwanted effects. UDCA is certainly more promising as a therapeutic anticancer agent, in combination with other drugs. There is much experimental work confirming a beneficial role of UDCA in various types of cancer. The drug may be useful to combat lung cancer cells migration and invasion, or to inhibit proliferation of colon cancer cells, but the most appropriate indication maybe liver cancer for several reasons.

The medical need remains high for the treatment of HCC, which is one of the most common cancers, with a high mortality rate in some countries, such as China for example. HCCs are heterogeneous tumours,

comprising different molecular subtypes with distinct molecular scoring (Nishida et al., 2018). There is a major need for new treatments in particular for HCC with a high metastatic recurrence. UDCA has revealed interesting anticancer effects in HCC models, mainly due to its anti-inflammatory and anti-oxidant properties. HCCs are resistant to conventional chemotherapy and the multi-kinases inhibitor sorafenib is one of the few agents that has shown clinical efficacy. Sorafenib is a standard of care for patients with advanced unresectable HCC since 2007. It is therefore interesting that UDCA synergizes the anticancer activity of sorafenib in HCC cell lines. The synergy was found to implicate drugs-induced apoptosis through ROS-dependent activation of ERK and dephosphorylation of STAT3 (Lee et al., 2018).

The mode of action of UDCA in HCC cells is multifactorial but we can underline different levels of activities (Fig. 5). First, UDCA functions as an inhibitor of STAT3 phosphorylation; the inhibition may be driven by different mechanisms, including an agonist effect of UDCA on the bile acids membrane receptor TGR5 (also known as Gpbar1). In non-small cell lung cancer, TGR5 drives cell growth and migration via activation of the JAK2/STAT3 signalling pathway (Liu et al., 2018). TGR5 activation antagonizes NFκB and STAT3 signalling pathways through suppressing phosphorylation of IκBα, translocation of p65 and phosphorylation of STAT3 (Su et al., 2017). It has been reported also that TGR5 activation suppresses phosphorylation of STAT3 and this effect contributes directly to the inhibition of gastric cancer cell proliferation and migration (Guo et al., 2015). In other words, in HCC cells UDCA may act as an agonist of TGR5, which then functions as a negative regulator of STAT3 signalling. UDCA has been shown to be a potent agonist for TGR5 in neonatal mouse ventricular cardiomyocytes (Ibrahim et al., 2018) but not in CHO cells stably expressing hTGR5 (Sato et al., 2008). TGR5 is expressed in advanced gastric cancers and its expression correlates with markers of the epithelial-mesenchymal transition (Carino et al., 2016). The effects of UDCA on TGR5 in HCC have not been reported.

In this context, it is interesting to mention that the plant natural product oleanic acid (triterpenoid extracted from *Olea europaea* leaves) and betulinic acid (triterpenoid found in leaves of white birch), both structurally close to UDCA are also potent TGR5 agonists and display significant anticancer activities (Genet et al., 2010). These two pentacyclic triterpenoids have been shown to synergize with sorafenib to induce oxidative-induced cell death of cancer cells, notably HCC (Kutkowska, Strzadala, & Rapak, 2018; Lange, Abhari, Hinrichs, Fulda, & Liese, 2016; Liese, Hinrichs, Lange, & Fulda, 2019; Wang et al., 2019).

Another level of activity of UDCA in cancer cells concerns the activation of the extrinsic apoptotic pathway. In cancer cells, UDCA can up-regulate the cell surface death receptors DR4 and DR5, leading to apoptotic and/or autophagic cell death, depending on the intracellular signalling environment (Lim & Han, 2015; Lee et al., 2018). A third level of UDCA activity refers to its anti-inflammatory properties. UDCA can decrease the expression of pro-inflammatory cytokines (TNF-α and IL-1β) and increase the level of anti-inflammatory cytokines such as IL-10, in macrophages. This type of effect can be linked to the inhibition of the expression of inflammatory transcription factor NFκB and associated inhibition of phosphorylation of IκBα, ERK, and p38 signals. Inhibition of nuclear NFκB activation by UDCA may explain the observed down-regulation of Bcl2, Smac and up-regulation of Bax. A fourth level of activity of UDCA concerns the inhibition of the degradation of specific proteins, notably tumour suppressors. DLC1 (deleted in liver cancer 1) is a tumour suppressor which contributes to the progression of HCC (Wu et al., 2018) and the inhibition of the degradation of the protein DLC1 by UDCA has been associated with inhibition of HCC growth (Chung et al., 2011). Similarly, UDCA has been shown to protect cell from apoptosis mediated by the tumour suppressor p53, by promoting its degradation via the Mdm2-ubiquitin-proteasome pathway (Amaral, Castro, Solá, Steer, & Rodrigues, 2010). UDCA also suppresses cell growth by inhibiting the mitogenic activity the EGF receptor (Feldman & Martinez, 2009).

The precise mechanism of action of UDCA in cancer cells is not well defined. The five pathways cited above play a role but other molecular signalling pathways are implicated with no doubt. For example, it is likely that UDCA-mediated AMPK activation also plays a role in the anticancer activity in HCC. AMPK activators offer interesting perspectives for the treatment of HCC (Jiang et al., 2019) and UDCA is known to activate AMPK in rheumatoid arthritis model (Lee, Kwon, et al., 2017), probably also in cancer. A more precise picture of the mechanism of action of UDCA in cancer is certainly needed to guide the selection of the most adapted tumour indications and drug combination therapies. Nevertheless, a rational for the testing of UDCA for the treatment of HCC is emerging.

Note that there are also different Chinese publications about the anticancer activity of "bear bile powder" that contains UDCA, including reports of anti-HCC activities. But the farming of bears, practiced in the 1980s in China, to extract UDCA being extremely inhuman (hopefully stopped today), we prefer not to refer extensively to these works (Feng et al., 2009). Bear bile has been used in traditional Chinese medicine (TCM) for thousands of years, but considering the imperative to protect populations of wild or farmed bears from overexploitation, practitioners of TCM should recommend the use of anti-inflammatory and hepatoprotective medicinal herbs as substitutes for bear bile (Appiah et al., 2017).

A better knowledge of the mechanism of action of UDCA will also be helpful to guide both the potential repositioning of UDCA in oncology and the design of new and more potent analogues active against tumour growth. A few interesting drug candidates are emerging, such as compounds U12 and HS-1200 cited here. Notably, the orally available analogue HS-1200 has revealed interesting activities in various cell lines and models, including HCC. U12 also showed a notable in vivo activity in a HCC model, insufficient to warrant a clinical development but encouraging to design more potent analogues. The synthesis of anticancer drugs derived from UDCA and the other bile acids should be encouraged. Bile acids are pleotropic signalling metabolites - and drugs for CDCA and UDCA - that regulate several metabolic and inflammatory pathways in different cell types and tissues. They should be further considered to propose novel anticancer treatments.

To conclude, UDCA is a well-established and well-tolerated drug used for the treatment of cholestatic hepatopathies. The therapeutic benefit of the drug may be further exploited in oncology, to improve cancer treatment and/or prevention. The dual pro- and anti-apoptotic activities of UDCA are not surprising; it is a characteristic trait of bile acids. Cholesterol itself also displays opposing functions in hepatitis and HCC, which define its paradoxical role in cell death as a pro- and anti-apoptotic factor (García-Ruiz, Ribas, Baulies, & Fernández-Checa, 2017). With no doubt, UDCA merits further attention as a potential anticancer agent and as a candidate for drug repurposing in oncology.

Declaration of Competing Interest

The authors declare no conflict of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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