The tumor-suppressor cholesterol metabolite, dendrogenin A, is a new class of LXR modulator activating lethal autophagy in cancers

Sandrine Silvente-Poirot, Marc Poirot

Review, Diamond Jubilee Special Issue

The tumor-suppressor cholesterol metabolite, dendrogenin A, is a new class of LXR modulator activating lethal autophagy in cancers.

Marc Poirot and Sandrine Silvente-Poirot

Team "cholesterol metabolism and therapeutic innovation", Cancer Research Center of Toulouse, UMR 1037 Inserm-university of Toulouse, Toulouse, France

Cancer Research Center of Toulouse, UMR 1037INSERM-University of Toulouse, Toulouse, France;

Address correspondence to these authors at the Department of Cholesterol Metabolism, Cancer Research Center of Toulouse, INSERM UMR 1037-University of Toulouse III, P.O. Box: 31017, Toulouse, France; Tel/Fax: +33-582741626; E-mail: marc.poirot@inserm.fr; sandrine.poirot@inserm.fr

ABSTRACT

Dendrogenin A (DDA) is a mammalian cholesterol metabolite recently identified that displays tumor suppressor properties. The discovery of DDA has revealed the existence in mammals of a new metabolic branch in the cholesterol pathway centered on 5,6α-epoxycholesterol and bridging cholesterol metabolism with histamine metabolism. Metabolic studies showed a drop in DDA levels in cancer cells and tumors compared to normal cells, suggesting a link between DDA metabolism deregulation and oncogenesis. Importantly, complementation of cancer cells with DDA induced 1) cancer cell re-differentiation , 2) blockade of 6-oxo-cholestan-3β,5α-diol (OCDO) production, an endogenous tumor promoter and 3) lethal autophagy in tumors. Importantly, by binding the liver X receptor (LXR), DDA activates the expression of genes controlling autophagy. These genes include *NR4A1*, *NR4A3*, *LC3* and *TFEB*. The canonical LXR ligands 22(R)hydroxycholesterol, TO901317 and GW3965 did not induce these effects indicating that DDA delineates a new class of selective LXR modulator (SLiM). The induction of lethal autophagy by DDA was associated with the accumulation in cancer cells of lysosomes and of the pro-lysosomal cholesterol precursor zymostenol due to the inhibition of the 3β-hydroxysteroid-$\Delta^8\Delta^7$-isomerase enzyme (D8D7I). The anti-cancer efficacy of DDA was established on different mouse and human cancers such as breast cancers, melanoma and acute myeloid leukemia, including patient derived xenografts, and did not discriminate bulk cancer cells from cancer cell progenitors. Together these data highlight that the mammalian metabolite DDA is a promising anticancer compound with a broad range of anticancer applications. In addition, DDA and LXR are new actors in the transcriptional control of autophagy and DDA being a “first in line” driver of lethal autophagy in cancers via the LXR.

Key words: Dendrogenin A; oxysterol; Nur77; lysosomes; cell death; OCDO

1. Introduction

Dendrogenin A (DDA) is the first cationic steroid discovered to date as an endogenous metabolite in mammals. DDA defines a new structural class of mammalian steroidal alkaloids [1-4]. DDA is the product of a stereo-selective condensation of 5,6α-epoxycholesterol (5,6α-EC) with histamine. DDA materializes the existence of a new metabolic branch at the crossroad between cholesterol and histamine metabolism. Cholesterol is a neutral semi rigid lipid necessary for mammalian life [5]. Cholesterol is important not only to the constitution of membranes, but also to the formation of various bioactive lipids such as steroid hormones, bile acids (BA) and oxysterols (OS). While the physiological role of steroid hormones is well established, BA and OS were first considered as catabolic products or side products of cholesterol. More recently, researchers discovered that several of these metabolites displayed biological properties [6-11] supporting a physiological function for BA and OS. These metabolites were found to act through nuclear receptors such as LXR, FXR, ER, ROR, GR or through G-protein coupled receptors [6-8, 11-15].

2. The dendrogenin hypothesis

The hypothesis on the existence of dendrogenins as new metabolites came from biochemical pharmacology studies on tamoxifen (Tam). Tam is an anticancer agent widely used for the treatment and the chemoprevention of estrogen receptor positive breast cancers [16-18]. Tam was initially defined as a selective estrogen receptor modulator (SERM), blocking the mitogenic action of 17β-estradiol by competition on estrogen receptor α (ERα) and displaying tissue selective agonist or antagonist properties [16-18]. Since the seventies, Tam remains the lead compound in the family of SERMs and has not yet been supplanted to date by any other SERM for anticancer applications. Extensive studies on the biochemical pharmacology of Tam,

showed that the mechanism of action mediating its anticancer action was more complex than first thought, and involved, in addition to ER, the "microsomal antiestrogen binding site" (AEBS) [19-31]. The AEBS is not a nuclear receptor, but is an intracellular membranous high affinity binding site for Tam[32]. The AEBS is a hetero-oligomeric proteinaceous complex located on the membranes of the endoplasmic reticulum from cancer cells and hepatocytes [24, 33-35]. The AEBS is made of two subunits: the 3β-hydroxysteroid-$\Delta^8\Delta^7$-isomerase (D8D7I) and the 3β-hydroxysteroid-Δ^7-reductase (DHCR7) [24, 33-35]. Tamoxifen inhibited the D8D7I and induced the accumulation of zymostenol in BC cells and in the blood of patients [19, 21, 23, 24, 36-39]. Importantly, zymostenol accumulation was associated with the induction of a protective autophagy [7, 19, 21, 22, 32, 36, 40], which impaired Tam efficacy and therefore constitutes a mechanism of resistance [19, 32]. It was next showed that the AEBS complex carried out a cholesterol-5,6-epoxide hydrolase (ChEH) enzymatic activity [41]. Biochemical studies established that the D8D7I was the catalytic subunit and the drug-binding site of the ChEH/AEBS complex [41, 42]. The AEBS is known as a promiscuous binding site interacting with different structural families of compounds including SERMs, diphenyl methane compounds like tesmilifene, phenothiazines, as well as endogenous metabolites such as ring-B oxysterols, unsaturated fatty acids, or histamine [3, 19, 25, 28, 29, 42-44]. The AEBS is present in BC cells and other cell lines representative of different cancers [23, 24, 26, 27, 45, 46]. Histamine was the only non-lipidic compound ever described as an AEBS ligand, and the AEBS was considered as an intracellular binding site for histamine (HIC) for a while [47, 48]. Histamine is involved in pleiotropic physiological functions such as neurotransmission, inflammation and gastric acid secretion [49] and was proposed to play a role in cell proliferation through the AEBS-HIC [47, 50-53]. Tam and selective AEBS ligands were considered as antagonists of histamine on HIC [46, 54, 55] before the molecular identification of the AEBS was made. High affinity AEBS

ligands induced breast cancer cell differentiation into lactating cells via binding to the AEBS and modulation of the cholesterol metabolism in cancer cells [20-23, 44]. Looking at the physiological importance of D8D7I and DHCR7, geneticists showed that null mutations on DHCR7 and D8D7I were associated with severe alteration of mammalian development, and these syndromes were not reversed by cholesterol complementation [56, 57]. Since DHCR7 and D8D7I carried out the AEBS/ChEH complex, this would suggest that 5,6-EC metabolism could play a role in developmental processes in mammals and may required the production of compounds that could activate pluripotent cell differentiation and may induce re-differentiation of de-differentiated cells like cancer cells. We thus postulated that 5,6-ECs or their putative metabolites could be involved in the control of cell differentiation. 5,6-EC exist as two diastereoisomers (5,6α-EC and 5,6β-EC) that display different biological properties and are prone to different metabolic fates [58]. In BC cells expressing the sulfotransferase SULT2B1b, it was found that the 3β-sulfated form of 5,6α-EC (but not 5,6β-EC) was the second messenger of AEBS ligands (including Tam) in their induction of BC cell differentiation and LXR-dependent cell death [19, 20]. Other metabolic transformations of 5,6-EC would be possible at the level of the epoxide group, which can make addition reaction of with nucleophiles (i.e. –SH or $–NH_2$ groups) in the presence of a catalyst [58, 59]. A member of the epoxide hydrolase family: the cholesterol-5,6-epoxide hydrolase (ChEH) is required for the hydration of the epoxide group of both 5,6-EC isomers in cells and tissues to produce cholestane-3β,5α,6β-triol (CT) [42, 60, 61]. CT was not found to display cell differentiation properties [42], but instead to inhibit osteoblastic differentiation of rat bone marrow stromal cells [62]. CT is prone to sulfation by SULT2B1b but not to fatty acid esterification by the AcylcoA-Cholesterol:Acyl Transferase (ACAT) on its 3β-hydroxyl group [20, 58]. The biological properties of the 3β-sulfated form of CT have never been investigated to date. In BC cells, CT was found extensively metabolized into 6-oxo-cholestan-

3β,5α-diol (OCDO), which was shown to be a tumor promoter [6, 63]. Looking at 5,6-EC chemical reactivity, 5,6α-EC (but not 5,6β-EC) was found prone to conjugation reactions with nucleophilic groups on its epoxide ring in the presence of a catalyst [3, 58, 59, 64]. Because of the positioning of the epoxide group at the junction of ring A and B on the steroid backbone and for thermodynamic reasons, this reaction gave a single product of addition with nucleophiles [58, 59]. The product of this conjugation reaction resulted from a trans-diaxial epoxide ring opening with the concerted engrafting of the nucleophilic group at C6 in a β orientation, and the formation of a hydroxyl group on C5 in an α orientation [58, 59]. The facts that:1) 5,6α-EC was the only isomer to react, 2) 5,6α-EC gave a single product of condensation with nucleophiles, and 3) the reaction needed a catalyst to occur, suggested a metabolic transformation pathway of 5,6-EC [3]. Because histamine was known as a ligand of the AEBS/ChEH complex [47, 48], it was postulated that biogenic amines such as histamine or polyamines (spermine, spermidine or putrescine) could produce compounds with potent cancer cell differentiation properties at low concentrations. A series of conjugation products were synthesized and screened for their capacities to induce cell differentiation.

2. DDA is an inductor of cell differentiation and a mammalian metabolite

The conjugation of 5,6α-EC with histamine gave as single product of transformation identified as 5α-hydroxy-6β-[2-(1H-imidazol-4-yl)-ethylamino]-cholestan-3β-ol also named dendrogenin A (DDA) (Figure 1A) [64]. DDA induced on cancer cells, *in vitro* and *in vivo*, the appearance of a "normal-like" phenotype at sub-nM concentrations evidencing a re-differentiation of cancer cells. 5,6-EC or histamine had no effects in the same conditions [1-4, 64]. As an example, DDA induced the differentiation of melanoma cells into melanocytes, and of breast cancer cells of ductal origin into lactating cells [4, 64]. In addition, DDA was shown to

induce the differentiation of pluripotent cells and normal neural stem cells into neurons [64, 65]. Dendrogenin A (DDA) was confirmed as a mammalian metabolite in 2013, and was detected in several mammalian tissues including breast, brain, liver, spleen and blood [4]. Evidences were obtained that DDA can be produced endogenously by an enzyme [4], and we have recently succeeded in our quest on the molecular identification of this enzyme confirming the enzymatic nature of DDA production. (Poirot M & Silvente-Poirot S et al, unpublished observations). The presence of DDA in serum indicated that DDA circulates in the body and could eventually feed tissues and organs. Quantification of DDA in tissues were done by reverse phase HPLC purification and mass fingerprint of fractions of interest to discriminate DDA from its inactive isomer C17 (Figure 1B) [4]. A new method of dosage is being developed using liquid chromatography tandem mass spectrometry. This new method is fast, resolving, has a weak carryover, and efficiently separates DDA from its C17 isomer and other steroidal alkaloids from the same family [66]. The use of this method will make possible to analyze large cohort of samples. Despite a high expression of the AEBS/ChEH in cancer cells, DDA was not detected and DDA levels were found strongly decreased in breast cancers samples from patients compared to “normal” adjacent tissues, indicating a deregulation of DDA metabolism in cancers [4]. These observations ruled out the hypothesis that the AEBS/ChEH, even if it could concentrate 5,6α-EC and histamine at their ligand binding site, was not sufficient to contribute to DDA biosynthesis and to catalyze the reaction by a proximity effect in cancer cells[67-69]. The recent molecular identification of the DDA synthase showed that this enzyme was different from the AEBS/ChEH and that a loss in DDA synthase expression on breast tumors explained the decrease in the DDA level (M Poirot Silvente-Poirot et al, unpublished results). Pharmacological studies showed that DDA induced cancer cell differentiation and death on cells of murine and human origins (Figure 2) [4, 64]. It was shown that DDA was active *in vivo* at low concentrations (0.015 µg/kg) to

prevent and inhibit the development of BC and melanoma on immunocompetent mice [4]. This effect was accompanied by cancer cell differentiation and tumor infiltration by T lymphocytes and dendritic cells, suggesting a possible activation of an immune response by DDA against the tumor [4]. DDA was found to be a potent and selective inhibitor of the ChEH, with no impact on other known epoxide hydrolases, and DDA did not display any ER modulatory activity [4]. DDA is, to date, the most potent endogenous inhibitor of ChEH compared to other ring-B oxysterols [4, 41, 42]. Importantly, the Ki of DDA for ChEH was 120 nM, which is the range of its endogenous concentration in healthy tissues [4]. To determine whether DDA might be of clinical interest, the efficacy of DDA was tested on melanoma and leukemia human cancers and the mechanisms mediating DDA-induced cell death were studied.

3. DDA induces lethal autophagy in cancer cells.

It was shown that DDA was cytotoxic on human and mouse cancer cells of various tissue origins including melanoma and leukemia cells [7, 64, 70]. DDA induced a dose- and time-dependent cytotoxicity in both melanoma and acute myeloid leukemia (AML) cell lines indicating that DDA effects were receptor-mediated, while the C17 compound (Figure 1B), a geometric isomer of DDA, was inactive. It was shown that DDA induced characteristics of apoptosis, but caspase inhibitors failed to protect cells against DDA showing that apoptosis was not the driver of DDA cytotoxicity. DDA cytotoxicity was shown to require transcription and translation of new genes. The screening on the expression of a panel of transcription factors stimulated by DDA revealed a huge increase in NR4A1 (Nur77) and NR4A3 (NOR1) mRNAs expression in melanoma cells. This stimulation was confirmed at the protein level [7]. Knock down experiments in cancer cells showed that these transcription factors were required in DDA cytotoxicity [7]. The contribution of Nur77 to cell death was mainly related to caspase

independent cell death [71] and lethal autophagy [72-76] when over expressed. Because DDA was shown to be a potent inhibitor of ChEH [4], and because the inhibition of ChEH was associated to the accumulation of pro-autophagic sterol zymostenol [19, 40], the impact of DDA on cholesterol metabolism in cancer cells was studied. *In vitro* and *in vivo* studies showed that DDA did not induce 5,6-EC accumulation. As opposed to other known ChEH inhibitors, cholesterol epoxidation was not stimulated by DDA, even if DDA induced ROS production. The explanation was that DDA induced catalase expression, the enzyme that destroys H_2O_2. This mechanism impairs the cholesterol epoxidation process. By comparison the ChEH inhibitors Tam and PBPE stimulated H_2O_2 production but had no impact on catalase expression [7], and they induced 5,6-EC formation and accumulation [7, 19]. DDA induced the accumulation of the cholesterol precursor zymostenol in cancer cells as observed with other high affinity ChEH inhibitors [7, 19]. Because zymostenol accumulation [19, 21, 22, 36, 44] and NR4A1 [73, 75, 76] have been linked to autophagy, the impact of DDA on autophagy was studied. It was found that DDA induced various characteristics of autophagy such as the formation and the accumulation of autophagosomes, lysosomes, and autolysosomes in melanoma and acute myeloid leukemia cells [7]. DDA did not induce a blockage of autophagy as observed for several compounds [77, 78] but on the contrary stimulated the autophagic flux [7]. Pharmacologic and genetic inhibitions of autophagy and NR4A protein expression inhibited DDA-induced tumor cell death, evidencing that DDA induced a lethal autophagy (Figure 2). This is the opposite to what was found with ChEH inhibitors such as Tam or PBPE which induced a protective autophagy in cancer cells [7, 19, 21, 22, 70]. Because DDA is not a SERM like Tam, but is an AEBS ligand and a ChEH inhibitor like PBPE, and because DDA induces a lethal autophagy and not a survival autophagy like Tam and PBPE, the existence of another molecular target for DDA that could explain this effect was investigated.

4. DDA is a ligand and modulator of the nuclear receptors LXR.

Microscopic autoradiography of cancer cells treated with ^{14}C-labelled DDA showed that DDA was mainly present into the nucleus of cells [7], suggesting that DDA could activate a ligand-dependent transcription factor. DDA being an OS and NOR1 which expression is stimulated by DDA being under the transcriptional control of the OS receptor [20, 79-82] liver-X-receptor (LXR) [83], it was investigated whether the LXR could be a DDA target. LXR belong to the nuclear receptor superfamily and exist as two isoforms, LXRα (NR1H3) and LXRβ (NR1H2) [84]. OS are oxygenation products of cholesterol [85], and while side chain OS were found to be agonists on LXR, ring B-OS were reported to exert cell and gene context-dependent antagonist, agonist, or inverse agonist activities on the LXR [20, 79, 80]. Tests using LXR-dependent gene reporter assays showed that DDA inhibited dose-dependently the stimulation of the reporter gene by the agonist 22(R)hydroxycholesterol with an IC_{50} of 76±12 nM for LXRβ and 362±52 nM for LXRα [7]. This established a potent regulatory activity on the LXR different from side chain-OS that displayed only agonist activity on such assays [8]. A similar approach was used to established that DDA did not modulate other ligand-dependent nuclear receptors such as steroid hormone receptors or SXR, AHR, VDR, RAR and PPAR[7]. The direct targeting of LXR by DDA was supported by DDA binding experiments performed on purified ligand binding domain LXR isoforms through both a ligand competition assay and surface plasmon resonance assays. Molecular modeling and docking experiments of DDA-ligand-binding domain of LXR isoforms complexes revealed the importance of specific chemical groups of DDA in their interaction with specific amino acids of LXRs. Structure-activity studies with DDA analogues modified on specific chemical groups of DDA such as the imidazol ring (C41, Fig 1B), the hydroxyl in C5 (C31, Fig 1B) or the sterol side chain (C51, Fig 1B), as well as the DDA isomer

in which histamine was grafted on 5,6α-EC by the imidazol ring (C17, Fig 1B), induced a loss or an inversion in the LXR modulatory activities of DDA. Although it was shown on the gene reporter assay that DDA was an antagonist. DDA treatment of cancer cells increased in an LXRβ-dependent manner the levels of mRNAs encoding NR4A1, NR4A3, LC3A and LC3B, while the prototypical LXR ligands 22(R)HC, GW3965 and T0901317 had little or no effects [7] (Table 1). Looking at the LXR-dependent gene expression at the mRNA level. It was found that DDA repressed ABCA1 mRNA expression while it stimulated the expression of SCD1, two LXR-dependent genes. DDA stimulated the expression of the LDLR at the mRNA level. LDLR is known as indirectly regulated through LXR-Idol pathway at the protein level [86], but no regulation of Idol mRNA expression by DDA was found at least on mouse embryonic fibroblast even if 22(R)HC increased Idol expression in an LXRβ-dependent manner [7]. *LDLR* is known to be primarily under the transcriptional control of the transcription factor SREBP2, which is activated when the intracellular cholesterol decreases [87]. However, chromatin immunoprecipitation assays showed that DDA repressed the binding of LXRβ on an enhancer region close to the *LDLR* gene suggesting a direct regulation of LDLR by LXRβ, while it was found on the same study that 22(R)HC increased LXRβ binding [7]. Thus, it is probable that DDA cholesterol efflux and influx in cancer cells and a detailed analysis will required further investigations. Altogether, these data showed that DDA was an LXR modulator rather than an antagonist. The use of a gene ontology (GO) that could be regulated by LXR led to the identification of several genes regulating autophagy and lysosome biogenesis, in particular the transcription factor EB (TFEB), known as a master regulator of both pathways [88-90]. DDA decreased LXRβ-binding to a TFEB enhancer and stimulates TFEB expression in a concentration-dependent manner, while 22(R)HC did the opposite, evidencing that the LXRβ and LXR agonists act as repressors of TFEB, while DDA de-repressed this gene. In accordance with

this, DDA increased the activity of a TFEB-dependent gene reporter assay dose-dependently showing that DDA stimulated the transcriptional activity of TFEB [7]. The targeting of LXR by DDA was further confirmed through knockdown experiments showing that LXRβ was necessary for DDA-induced NR4A1, NR4A3, LC3 expression and lethal autophagy (Figure 3). Moreover, it was shown that LXR agonists partially reversed DDA cytotoxicity. Altogether, these data indicated that DDA triggered an LXR-dependent tumor cell death. This effect was specific of DDA and was not observed with other LXR ligands such as 22(R)HC, GW3965 and T091317 and thus DDA appears as "first in line" on this effect (Table 1). These data revealed that DDA and LXRβ are new players in the control of autophagy and lysosomes biogenesis [70].

5. DDA triggers anticancer action *in vivo* on human cancer cells, including patient-derived AML.

It was shown that DDA displayed chemopreventive and anticancer effects at low doses on syngeneic tumors implanted on immunocompetent mice. These affects were associated with cancer cell re-differentiation and T lymphocyte and dendritic cell infiltration suggesting the a contribution of the immune system on the anticancer action of DDA [4]. DDA also killed human cancer cells *in vitro* at micromolar concentrations [64] suggesting that DDA could be active against human tumors *in vivo*. To investigate the efficacy of DDA on human cancers, DDA was tested on human cell lines or patient tumors implanted on immunodefficient mice. A new formulation of DDA was required to achieve higher concentration, and a lactate salt form of DDA with improve water solubility was produced [91]. Experiments were conducted by implanting melanoma cells lines B16F10 and SKMEL-28 on nude mice, acute myeloid leukemia cell lines HL-60 and KG1 on NOD/SCID mice. Administration of DDA to mice bearing established tumors showed a blockade in the tumor growth. Tumor analyses showed that DDA

treatment induced an over-expression of Nur77 and NOR1 and a stimulation of LC3-II expression and a punctation of LC3 was observed by immunohistochemistry. All these characteristics are those that were measured *in vitro.* The LXR-dependence of DDA effects *in vivo* was demonstrated both by genetic and pharmacologic approaches. The knock down of LXRβ in cancer cells using small hairpin RNA targeting LXRβ, induced a loss of sensitivity to DDA in these cells on *in vitro* and *in vivo* assays and the synthetic LXR agonist T0901317 partially reversed DDA anticancer action [7]. Because DDA is not a modulator of most other nuclear receptor, DDA could be consider at the moment as a selective liver X receptor modulator (SLiM) [92], confirming that developing modulator of such compounds is of interest for therapeutic application as earlier suggested [93].

To further confirm the clinical interest of DDA, tests were conducted on primary tumors from patients with AML. Theses cancer cells were found sensitive to DDA independently to their molecular and cytogenetic classification and thus AML cells with intrinsic resistance to cytarabin (AraC) or anthracyclins were found sensitive to DDA as well as cancer stem cells [7]. DDA induced *in vitro* and *in vivo* an anticancer action of these cells blocking bone colonization by cancer cells. No toxicity was observed at active doses of 20 to 40 mg/kg and DDA met the important expectation for an LXR ligand that is candidate for a clinical evaluation [94]; DDA did not induced steatosis and hypertrigliceridemia in mice[7]. Importantly, DDA treatment led to the accumulation of DDA in tumors to reach an active anticancer concentration. Together these data established the strong anticancer potency of DDA *in vivo* on melanoma and AML.

Conclusion

If genomic and proteomic approaches led to an extensive characterization of the existing genes and proteins, the metabolome is still an underexplored universe [95] in which the immerged and hidden part of this iceberg remains to be characterized [96]. Lipids account for more than 30,000 different species and specialists estimated that this number could grow up to 200,000 different species [97]. Among them, sterol lipids constitute an emerging class defining the sterolomics [98, 99]. Cholesterol-5,6-epoxides (5,6-EC) was found at the center of a new branch [58] in the cholesterol pathway thanks to the recent identification of DDA [4]. The identification of DDA as a metabolite was possible because DDA was first chemically synthesized, chemical tools were produced (labeled precursors and DDA), specific analytical methods were set up, and because synthetic DDA displayed cancer cell re-differentiation properties at low concentrations *in vitro*. Studies on DDA metabolism led to the discovery that DDA was present in various mammalian tissues. The analyses of cancer cells and tumors revealed a deficiency in DDA presence highlighting a deregulation in DDA metabolism during carcinogenesis. This suggested that a DDA complementation therapy may constitute a possible therapeutic approach against cancer. This hypothesis was validated on tumor implanted in mice showing that DDA displayed anticancer properties against various cancers. This was exemplified for breast cancer, melanoma and acute myeloid leukemia [4, 7]. The analysis of the cellular effects of DDA showed that it activated cancer cell differentiation and death. The nature of cell death was lethal autophagy as opposed to most chemotherapeutic drugs that induced apoptosis and protective autophagy [40, 100, 101]. DDA does not affect normal cell fate in mice, and thus DDA targets specifically cancer cells *in vivo*, deserving further clinical evaluations. Analyses of the molecular mechanism involved in the effect of DDA revealed that autophagic genes stimulated by DDA were under the transcriptional control of LXRβ showing for the first time that this nuclear receptor controlled the expression of genes involved in autophagy and lysosome biogenesis. LXRβ was found necessary

to mediate DDA anticancer effects and the presence of LXRβ in cancer cells will condition a response to DDA. LXRβ is widely expressed in cancer cells of different origins and is up regulated in cancer cells compared to normal tissues [7, 102], strongly suggesting a possible efficacy on other cancers in addition to breast cancers, melanoma and AML as already shown in vitro on cancer cell lines [64]. Both LXR and autophagy have been shown to be involved in the control of immunity [93, 103-105], thus it would be of interest to determine if DDA have a positive impact on antitumor immunity which could strengthen its anticancer potency. In conclusion, DDA is a molecule from the self, with tumor suppressor and anticancer properties that induces lethal autophagy through an original mechanism making of DDA a "first in line" anticancer drug candidate. DDA is well tolerated in mice at therapeutic consentrations and exhibits the expected properties of a drug candidate for further application in LXR-targeted anticancer therapy.

List of abbreviations

AEBS, antiestrogen binding site; ChEH, cholesterol-5,6-epoxide hydrolase; dendrogenin A/DDA, 5α-hydroxy-6β-[2-(1H-imidazol-4-yl)-ethylamino]-cholestan-3β-ol; DHCR24, 3β-hydroxysteroid-Δ^{24}-reductase; DHCR7, 3β-hydroxysteroid-Δ^{7}-reductase; D8D7I/EBP, 3β-hydroxysteroid-Δ^{8},Δ^{7}-isomerase; cholesterol, cholest-5-en-3β-ol; desmosterol, cholest-5,24-dien-3β-ol; zymostenol, 5α-cholest-8-ene-3β-ol; zymosterol, 5α-cholesta-8,24-dien-3β-ol; 7-dehydrocholesterol, cholest-5,7-dien-3β-ol; lanosterol, lanosta-8,24-dien-3β-ol; 5,6α-EC, 5,6α-epoxycholesterol; 5,6β-EC, 5,6β-epoxycholesterol; 5,6α-ECS, HA, histamine; ER, estrogen receptor; BC, breast cancer.

Chemical compounds:

dendrogenin A (PubChem CID: 9806490); tamoxifen (PubChem CID: 2733526); zymostenol (PubChem CID: 101770); 5,6α-epoxycholesterol (PubChem CID: 227037); histamine (PubChem CID: 774).

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

Both authors have contributed to the writing of this manuscript. This work was funded by the Institut National de la Santé et de la Recherche Médicale, the university of Toulouse III, the Fondation de France (R11166BB), the INCA (PRTK-K15-118), the association « pour Céline » and the association « Flo ».

References

[1] Silvente-Poirot S, de Medina P, Record M, Poirot M. From tamoxifen to dendrogenin A: The discovery of a mammalian tumor suppressor and cholesterol metabolite. Biochimie. 2016;130:109-14.
[2] Poirot M, Silvente-Poirot S. When cholesterol meets histamine, it gives rise to dendrogenin A: a tumour suppressor metabolite. Biochem Soc Trans. 2016;44:631-7.
[3] Dalenc F, Poirot M, Silvente-Poirot S. Dendrogenin A: A Mammalian Metabolite of Cholesterol with Tumor Suppressor and Neurostimulating Properties. Curr Med Chem. 2015;22:3533-49.
[4] de Medina P, Paillasse MR, Segala G, Voisin M, Mhamdi L, Dalenc F, et al. Dendrogenin A arises from cholesterol and histamine metabolism and shows cell differentiation and anti-tumour properties. Nat Commun. 2013;4:1840.
[5] Silvente-Poirot S, Poirot M. Cholesterol metabolism and cancer: the good, the bad and the ugly. Curr Opin Pharmacol. 2012;12:673-6.
[6] Voisin M, de Medina P, Mallinger A, Dalenc F, Huc-Claustre E, Leignadier J, et al. Identification of a tumor-promoter cholesterol metabolite in human breast cancers acting through the glucocorticoid receptor. Proc Natl Acad Sci U S A. 2017;114:E9346-E55.

[7] Segala G, David M, de Medina P, Poirot MC, Serhan N, Vergez F, et al. Dendrogenin A drives LXR to trigger lethal autophagy in cancers. Nat Commun. 2017;8:1903.
[8] Mutemberezi V, Guillemot-Legris O, Muccioli GG. Oxysterols: From cholesterol metabolites to key mediators. Prog Lipid Res. 2016;64:152-69.
[9] Theofilopoulos S, Griffiths WJ, Crick PJ, Yang S, Meljon A, Ogundare M, et al. Cholestenoic acids regulate motor neuron survival via liver X receptors. J Clin Invest. 2014;124:4829-42.
[10] Theofilopoulos S, Wang Y, Kitambi SS, Sacchetti P, Sousa KM, Bodin K, et al. Brain endogenous liver X receptor ligands selectively promote midbrain neurogenesis. Nat Chem Biol. 2013;9:126-33.
[11] Hannedouche S, Zhang J, Yi T, Shen W, Nguyen D, Pereira JP, et al. Oxysterols direct immune cell migration via EBI2. Nature. 2011;475:524-7.
[12] Baek AE, Yu YA, He S, Wardell SE, Chang CY, Kwon S, et al. The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. Nat Commun. 2017;8:864.
[13] Rutkowska A, Dev KK, Sailer AW. The Role of the Oxysterol/EBI2 Pathway in the Immune and Central Nervous Systems. Curr Drug Targets. 2016;17:1851-60.
[14] Theofilopoulos S, Arenas E. Liver X receptors and cholesterol metabolism: role in ventral midbrain development and neurodegeneration. F1000Prime Rep. 2015;7:37.
[15] Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, et al. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. Science. 2013;342:1094-8.
[16] Poirot M. Four decades of discovery in breast cancer research and treatment - an interview with V. Craig Jordan. Int J Dev Biol. 2011;55:703-12.
[17] Jordan VC. Chemoprevention of breast cancer with selective oestrogen-receptor modulators. Nat Rev Cancer. 2007;7:46-53.
[18] Jordan VC. Tamoxifen: a most unlikely pioneering medicine. Nat Rev Drug Discov. 2003;2:205-13.
[19] Leignadier J, Dalenc F, Poirot M, Silvente-Poirot S. Improving the efficacy of hormone therapy in breast cancer: The role of cholesterol metabolism in SERM-mediated autophagy, cell differentiation and death. Biochem Pharmacol. 2017;144:18-28.
[20] Segala G, de Medina P, Iuliano L, Zerbinati C, Paillasse MR, Noguer E, et al. 5,6-Epoxy-cholesterols contribute to the anticancer pharmacology of tamoxifen in breast cancer cells. Biochem Pharmacol. 2013;86:175-89.
[21] de Medina P, Payre B, Boubekeur N, Bertrand-Michel J, Terce F, Silvente-Poirot S, et al. Ligands of the antiestrogen-binding site induce active cell death and autophagy in human breast cancer cells through the modulation of cholesterol metabolism. Cell Death Differ. 2009;16:1372-84.
[22] de Medina P, Silvente-Poirot S, Poirot M. Tamoxifen and AEBS ligands induced apoptosis and autophagy in breast cancer cells through the stimulation of sterol accumulation. Autophagy. 2009;5:1066-7.
[23] Payre B, de Medina P, Boubekeur N, Mhamdi L, Bertrand-Michel J, Terce F, et al. Microsomal antiestrogen-binding site ligands induce growth control and differentiation of human breast cancer cells through the modulation of cholesterol metabolism. Mol Cancer Ther. 2008;7:3707-18.
[24] Kedjouar B, de Medina P, Oulad-Abdelghani M, Payre B, Silvente-Poirot S, Favre G, et al. Molecular characterization of the microsomal tamoxifen binding site. J Biol Chem. 2004;279:34048-61.

[25] de Medina P, Favre G, Poirot M. Multiple targeting by the antitumor drug tamoxifen: a structure-activity study. Curr Med Chem Anticancer Agents. 2004;4:491-508.
[26] Kedjouar B, Daunes S, Vilner BJ, Bowen WD, Klaebe A, Faye JC, et al. Structural similitudes between cytotoxic antiestrogen-binding site (AEBS) ligands and cytotoxic sigma receptor ligands. Evidence for a relationship between cytotoxicity and affinity for AEBS or sigma-2 receptor but not for sigma-1 receptor. Biochem Pharmacol. 1999;58:1927-39.
[27] Delarue F, Kedjouar B, Mesange F, Bayard F, Faye JC, Poirot M. Modifications of benzylphenoxy ethanamine antiestrogen molecules: influence affinity for antiestrogen binding site (AEBS) and cell cytotoxicity. Biochem Pharmacol. 1999;57:657-61.
[28] van den Koedijk CD, Vis van Heemst C, Elsendoorn GM, Thijssen JH, Blankenstein MA. Comparative affinity of steroidal and non-steroidal antioestrogens, cholesterol derivatives and compounds with a dialkylamino side chain for the rat liver antioestrogen binding site. Biochem Pharmacol. 1992;43:2511-8.
[29] Poirot M, Garnier M, Bayard F, Riviere I, Traore M, Wilson M, et al. The anti-proliferative properties of 4-benzylphenoxy ethanamine derivatives are mediated by the anti-estrogen binding site (ABS), whereas the anti-estrogenic effects of trifluopromazine are not. Biochem Pharmacol. 1990;40:425-9.
[30] Fargin A, Bayard F, Faye JC, Traore M, Poirot M, Klaebe A, et al. Further evidence for a biological role of anti-estrogen-binding sites in mediating the growth inhibitory action of diphenylmethane derivatives. Chem Biol Interact. 1988;66:101-9.
[31] Lyman SD, Jordan VC. Possible mechanisms for the agonist actions of tamoxifen and the antagonist actions of MER-25 (ethamoxytriphetol) in the mouse uterus. Biochem Pharmacol. 1985;34:2795-806.
[32] Poirot M, Silvente-Poirot S, Weichselbaum RR. Cholesterol metabolism and resistance to tamoxifen. Curr Opin Pharmacol. 2012;12:683-9.
[33] Mesange F, Sebbar M, Capdevielle J, Guillemot JC, Ferrara P, Bayard F, et al. Identification of two tamoxifen target proteins by photolabeling with 4-(2-morpholinoethoxy)benzophenone. Bioconjug Chem. 2002;13:766-72.
[34] Mesange F, Sebbar M, Kedjouar B, Capdevielle J, Guillemot JC, Ferrara P, et al. Microsomal epoxide hydrolase of rat liver is a subunit of theanti-oestrogen-binding site. Biochem J. 1998;334 (Pt 1):107-12.
[35] Poirot M, Chailleux C, Fargin A, Bayard F, Faye JC. A potent and selective photoaffinity probe for the anti-estrogen binding site of rat liver. J Biol Chem. 1990;265:17039-43.
[36] Sola B, Poirot M, de Medina P, Bustany S, Marsaud V, Silvente-Poirot S, et al. Antiestrogen-binding site ligands induce autophagy in myeloma cells that proceeds through alteration of cholesterol metabolism. Oncotarget. 2013;4:911-22.
[37] Holleran AL, Lindenthal B, Aldaghlas TA, Kelleher JK. Effect of tamoxifen on cholesterol synthesis in HepG2 cells and cultured rat hepatocytes. Metabolism. 1998;47:1504-13.
[38] Gylling H, Pyrhonen S, Mantyla E, Maenpaa H, Kangas L, Miettinen TA. Tamoxifen and toremifene lower serum cholesterol by inhibition of delta 8-cholesterol conversion to lathosterol in women with breast cancer. J Clin Oncol. 1995;13:2900-5.
[39] Gylling H, Mantyla E, Miettinen TA. Tamoxifen decreases serum cholesterol by inhibiting cholesterol synthesis. Atherosclerosis. 1992;96:245-7.
[40] Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy. 2016;12:1-222.

[41] de Medina P, Paillasse MR, Segala G, Poirot M, Silvente-Poirot S. Identification and pharmacological characterization of cholesterol-5,6-epoxide hydrolase as a target for tamoxifen and AEBS ligands. Proc Natl Acad Sci U S A. 2010;107:13520-5.
[42] Silvente-Poirot S, Poirot M. Cholesterol epoxide hydrolase and cancer. Curr Opin Pharmacol. 2012;12:696-703.
[43] Watts CK, Sutherland RL. Studies on the ligand specificity and potential identity of microsomal antiestrogen-binding sites. Mol Pharmacol. 1987;31:541-51.
[44] de Medina P, Paillasse MR, Segala G, Khallouki F, Brillouet S, Dalenc F, et al. Importance of cholesterol and oxysterols metabolism in the pharmacology of tamoxifen and other AEBS ligands. Chem Phys Lipids. 2011;164:432-7.
[45] Jones JA, Albright KD, Christen RD, Howell SB, McClay EF. Synergy between tamoxifen and cisplatin in human melanoma cells is dependent on the presence of antiestrogen-binding sites. Cancer Res. 1997;57:2657-60.
[46] Brandes LJ, Davie JR, Paraskevas F, Sukhu B, Bogdanovic RP, LaBella FS. The antiproliferative potency of histamine antagonists correlates with inhibition of binding of [3H]-histamine to novel intracellular receptors (HIC) in microsomal and nuclear fractions of rat liver. Agents Actions Suppl. 1991;33:325-42.
[47] Brandes LJ, LaBella FS, Glavin GB, Paraskevas F, Saxena SP, McNicol A, et al. Histamine as an intracellular messenger. Biochem Pharmacol. 1990;40:1677-81.
[48] Brandes LJ, Macdonald LM, Bogdanovic RP. Evidence that the antiestrogen binding site is a histamine or histamine-like receptor. Biochem Biophys Res Commun. 1985;126:905-10.
[49] Panula P, Chazot PL, Cowart M, Gutzmer R, Leurs R, Liu WL, et al. International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors. Pharmacol Rev. 2015;67:601-55.
[50] Brandes LJ. Hormetic effects of hormones, antihormones, and antidepressants on cancer cell growth in culture: in vivo correlates. Crit Rev Toxicol. 2005;35:587-92.
[51] Brandes LJ, Bogdanovic RP, Tong J, Davie JR, LaBella FS. Intracellular histamine and liver regeneration: high affinity binding of histamine to chromatin, low affinity binding to matrix, and depletion of a nuclear storage pool following partial hepatectomy. Biochem Biophys Res Commun. 1992;184:840-7.
[52] Brandes LJ, Bogdanovic RP, Cawker MD, LaBella FS. Histamine and growth: interaction of antiestrogen binding site ligands with a novel histamine site that may be associated with calcium channels. Cancer Res. 1987;47:4025-31.
[53] Brandes LJ, Bogdanovic RP. New evidence that the antiestrogen binding site may be a novel growth-promoting histamine receptor (?H3) which mediates the antiestrogenic and antiproliferative effects of tamoxifen. Biochem Biophys Res Commun. 1986;134:601-8.
[54] Brandes LJ, Gerrard JM, Bogdanovic RP, Lint DW, Reid RE, LaBella FS. Correlation of the antiproliferative action of diphenylmethane-derivative antiestrogen binding site ligands with antagonism of histamine binding but not of protein kinase C-mediated phosphorylation. Cancer Res. 1988;48:3954-8.
[55] Kroeger EA, Brandes LJ. Evidence that tamoxifen is a histamine antagonist. Biochem Biophys Res Commun. 1985;131:750-5.
[56] Porter FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. J Lipid Res. 2011;52:6-34.
[57] Herman GE. Disorders of cholesterol biosynthesis: prototypic metabolic malformation syndromes. Hum Mol Genet. 2003;12 Spec No 1:R75-88.

[58] Poirot M, Silvente-Poirot S. Cholesterol-5,6-epoxides: chemistry, biochemistry, metabolic fate and cancer. Biochimie. 2013;95:622-31.
[59] Paillasse MR, Saffon N, Gornitzka H, Silvente-Poirot S, Poirot M, de Medina P. Surprising unreactivity of cholesterol-5,6-epoxides towards nucleophiles. J Lipid Res. 2012;53:718-25.
[60] Kodani SD, Hammock BD. The 2014 Bernard B. Brodie award lecture-epoxide hydrolases: drug metabolism to therapeutics for chronic pain. Drug Metab Dispos. 2015;43:788-802.
[61] Morisseau C. Role of epoxide hydrolases in lipid metabolism. Biochimie. 2013;95:91-5.
[62] Liu H, Yuan L, Xu S, Wang K, Zhang T. Cholestane-3beta,5alpha,6beta-triol inhibits osteoblastic differentiation and promotes apoptosis of rat bone marrow stromal cells. J Cell Biochem. 2005;96:198-208.
[63] Poirot M, Soules R, Silvente-Poirot S. Chemistry, biochemistry, metabolic fate and carcinogenicity of 6-oxo-cholestan-3β,5α-diol, a cholesterol metabolite. Biochimie. 2018;in preparation.
[64] de Medina P, Paillasse MR, Payre B, Silvente-Poirot S, Poirot M. Synthesis of new alkylaminooxysterols with potent cell differentiating activities: identification of leads for the treatment of cancer and neurodegenerative diseases. J Med Chem. 2009;52:7765-77.
[65] Khalifa SA, de Medina P, Erlandsson A, El-Seedi HR, Silvente-Poirot S, Poirot M. The novel steroidal alkaloids dendrogenin A and B promote proliferation of adult neural stem cells. Biochem Biophys Res Commun. 2014;446:681-6.
[66] Noguer E, Soules R, Netter C, Nagarathinam C, Leignadier J, Huc-Claustre E, et al. Quantitative analysis of the tumor suppressor dendrogenin A using liquid chromatography tandem mass spectrometry. Chem Phys Lipids. 2017;207:81-6.
[67] Motta A, Fragala IL, Marks TJ. Proximity and cooperativity effects in binuclear d(0) olefin polymerization catalysis. theoretical analysis of structure and reaction mechanism. J Am Chem Soc. 2009;131:3974-84.
[68] Rainey MA, Callaway K, Barnes R, Wilson B, Dalby KN. Proximity-induced catalysis by the protein kinase ERK2. J Am Chem Soc. 2005;127:10494-5.
[69] Li H, Li L, Marks TJ. Polynuclear olefin polymerization catalysis: proximity and cocatalyst effects lead to significantly increased polyethylene molecular weight and comonomer enchainment levels. Angew Chem Int Ed Engl. 2004;43:4937-40.
[70] Silvente-Poirot S, Segala G, Poirot MC, Poirot M. Ligand-dependent transcriptional induction of lethal autophagy: a new perspective for cancer treatment. Autophagy. 2018:1-6. in press doi: 10.1080/15548627.2018.1425059.
[71] Xu Y, Kim SO, Li Y, Han J. Autophagy contributes to caspase-independent macrophage cell death. J Biol Chem. 2006;281:19179-87.
[72] Yin H, Zhang S, Sun Y, Li S, Ning Y, Dong Y, et al. MicroRNA-34/449 targets IGFBP-3 and attenuates airway remodeling by suppressing Nur77-mediated autophagy. Cell Death Dis. 2017;8:e2998.
[73] Hu M, Luo Q, Alitongbieke G, Chong S, Xu C, Xie L, et al. Celastrol-Induced Nur77 Interaction with TRAF2 Alleviates Inflammation by Promoting Mitochondrial Ubiquitination and Autophagy. Mol Cell. 2017;66:141-53 e6.
[74] Gao H, Chen Z, Fu Y, Yang X, Weng R, Wang R, et al. Nur77 exacerbates PC12 cellular injury in vitro by aggravating mitochondrial impairment and endoplasmic reticulum stress. Sci Rep. 2016;6:34403.
[75] Wang WJ, Wang Y, Chen HZ, Xing YZ, Li FW, Zhang Q, et al. Orphan nuclear receptor TR3 acts in autophagic cell death via mitochondrial signaling pathway. Nat Chem Biol. 2014;10:133-40.

[76] Bouzas-Rodriguez J, Zarraga-Granados G, Sanchez-Carbente Mdel R, Rodriguez-Valentin R, Gracida X, Anell-Rendon D, et al. The nuclear receptor NR4A1 induces a form of cell death dependent on autophagy in mammalian cells. PLoS One. 2012;7:e46422.
[77] Rios-Marco P, Rios A, Jimenez-Lopez JM, Carrasco MP, Marco C. Cholesterol homeostasis and autophagic flux in perifosine-treated human hepatoblastoma HepG2 and glioblastoma U-87 MG cell lines. Biochem Pharmacol. 2015;96:10-9.
[78] Lau A, Zheng Y, Tao S, Wang H, Whitman SA, White E, et al. Arsenic inhibits autophagic flux, activating the Nrf2-Keap1 pathway in a p62-dependent manner. Mol Cell Biol. 2013;33:2436-46.
[79] Berrodin TJ, Shen Q, Quinet EM, Yudt MR, Freedman LP, Nagpal S. Identification of 5alpha, 6alpha-epoxycholesterol as a novel modulator of liver X receptor activity. Mol Pharmacol. 2010;78:1046-58.
[80] Song C, Hiipakka RA, Liao S. Auto-oxidized cholesterol sulfates are antagonistic ligands of liver X receptors: implications for the development and treatment of atherosclerosis. Steroids. 2001;66:473-9.
[81] Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, et al. Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. Proc Natl Acad Sci U S A. 1999;96:266-71.
[82] Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature. 1996;383:728-31.
[83] Kumar N, Wang H, Liu D, Collins S. Liver X receptor is a regulator of orphan nuclear receptor NOR-1 gene transcription in adipocytes. Int J Obes (Lond). 2009;33:519-24.
[84] Moore DD, Kato S, Xie W, Mangelsdorf DJ, Schmidt DR, Xiao R, et al. International Union of Pharmacology. LXII. The NR1H and NR1I receptors: constitutive androstane receptor, pregnene X receptor, farnesoid X receptor alpha, farnesoid X receptor beta, liver X receptor alpha, liver X receptor beta, and vitamin D receptor. Pharmacol Rev. 2006;58:742-59.
[85] Schroepfer GJ, Jr. Oxysterols: modulators of cholesterol metabolism and other processes. Physiol Rev. 2000;80:361-554.
[86] Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR Regulates Cholesterol Uptake Through Idol-Dependent Ubiquitination of the LDL Receptor. Science. 2009;325:100.
[87] Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell. 1997;89:331-40.
[88] Shin HJ, Kim H, Oh S, Lee JG, Kee M, Ko HJ, et al. AMPK-SKP2-CARM1 signalling cascade in transcriptional regulation of autophagy. Nature. 2016;534:553-7.
[89] Raben N, Puertollano R. TFEB and TFE3: Linking Lysosomes to Cellular Adaptation to Stress. Annu Rev Cell Dev Biol. 2016;32:255-78.
[90] Napolitano G, Ballabio A. TFEB at a glance. J Cell Sci. 2016;129:2475-81.
[91] Poirot M, de Medina P, Paillasse M, Poirot S. New acid addition salt of 5alpha-hydroxy-6beta-(2-(1H-imidazol-4-yl)ethylamino)cholestan-3beta-ol formed with acid e.g. inorganic acid, acyclic aliphatic carboxylic, and aromatic carboxylic, useful to treat e.g. neurodegenerative diseases. World Patent (2013) WO2013/0776257.
[92] Viennois E, Mouzat K, Dufour J, Morel L, Lobaccaro JM, Baron S. Selective liver X receptor modulators (SLiMs): what use in human health? Mol Cell Endocrinol. 2012;351:129-41.
[93] Viennois E, Pommier AJ, Mouzat K, Oumeddour A, El Hajjaji FZ, Dufour J, et al. Targeting liver X receptors in human health: deadlock or promising trail? Expert Opin Ther Targets. 2011;15:219-32.

[94] Hong C, Tontonoz P. Liver X receptors in lipid metabolism: opportunities for drug discovery. Nat Rev Drug Discov. 2014;13:433-44.
[95] Schroeder F, Pohnert G. Editorial overview: Omics techniques to map the chemistry of life. Curr Opin Chem Biol. 2017;36:v-vi.
[96] Allard PM, Genta-Jouve G, Wolfender JL. Deep metabolome annotation in natural products research: towards a virtuous cycle in metabolite identification. Curr Opin Chem Biol. 2017;36:40-9.
[97] Fahy E, Cotter D, Sud M, Subramaniam S. Lipid classification, structures and tools. Biochim Biophys Acta. 2011;1811:637-47.
[98] Griffiths WJ, Wang Y. Sterolomics: State of the art, developments, limitations and challenges. Biochim Biophys Acta. 2017.
[99] Griffiths WJ, Abdel-Khalik J, Yutuc E, Morgan AH, Gilmore I, Hearn T, et al. Cholesterolomics: An update. Anal Biochem. 2017;524:56-67.
[100] Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cecconi F, et al. Molecular definitions of autophagy and related processes. EMBO J. 2017;36:1811-36.
[101] Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ. 2015;22:58-73.
[102] Holbeck S, Chang J, Best AM, Bookout AL, Mangelsdorf DJ, Martinez ED. Expression profiling of nuclear receptors in the NCI60 cancer cell panel reveals receptor-drug and receptor-gene interactions. Mol Endocrinol. 2010;24:1287-96.
[103] Zhong Z, Sanchez-Lopez E, Karin M. Autophagy, Inflammation, and Immunity: A Troika Governing Cancer and Its Treatment. Cell. 2016;166:288-98.
[104] Kidani Y, Bensinger SJ. Liver X receptor and peroxisome proliferator-activated receptor as integrators of lipid homeostasis and immunity. Immunol Rev. 2012;249:72-83.
[105] Jakobsson T, Treuter E, Gustafsson JA, Steffensen KR. Liver X receptor biology and pharmacology: new pathways, challenges and opportunities. Trends Pharmacol Sci. 2012;33:394-404.

Figure legends

Figure 1. A) scheme presenting the biosynthesis of dendrogenin A from 5,6α-epoxy-cholesterol and histamine. B) chemical structure of DDA inactive analogues.

Figure 2: Anti-cancer properties of DDA. DDA induces cancer cell differentiation at nM concentration and lethal autophagy at µM concentration.

Figure 3: Scheme describing the mechanism of lethal autophagy induced by DDA. The binding of DDA to the LXRβ and the transcriptional activation of LC3, TFEB, Nur77 and NOR1 genes are essential for DDA to mediate lethal autophagy in cancers *in vitro* and *in vivo*. The inhibition of the cholesterol-5,6-epoxide hydrolase (ChEH) and of its D8D7 subunit by DDA leading to sterol accumulation participates to DDA-induced increase lysosome formation, an essential component of the autophagy machinery. This dual targeting may explain the sustained lethal autophagy induced by DDA. LY: lysosome; AP: autophagosome; AM: amphisome; AL: autolysosome.

table 1

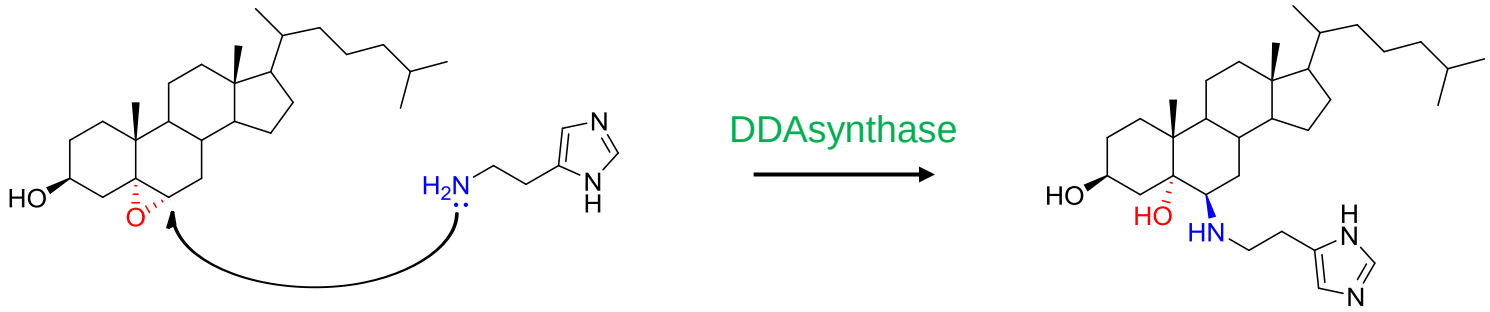
Table 1: Comparison between DDA and canonical LXR ligands on LXR-dependent genes expression and cancer cell fate.

		LXR-dependent gene expression						D8D7I inhibition	Cell death	Autophagy
	LXRα/β	ABCA1	SCD1	LDLR	NR4A1	LC3A	TFEB			
DDA	yes	-	+	+	++	+	+	+	+	+
22(R)HC	yes	+	0	0	+	0	0	0	0	0
TO901317	yes	+	+	0	0	0	n.d.	0	0	0
GW3965	yes	+	+	0	0	0	n.d.	0	0	0

yes: LXRα/β ligand; -: down regulation; +: up regulation; 0: no effect; n.d. not determined.

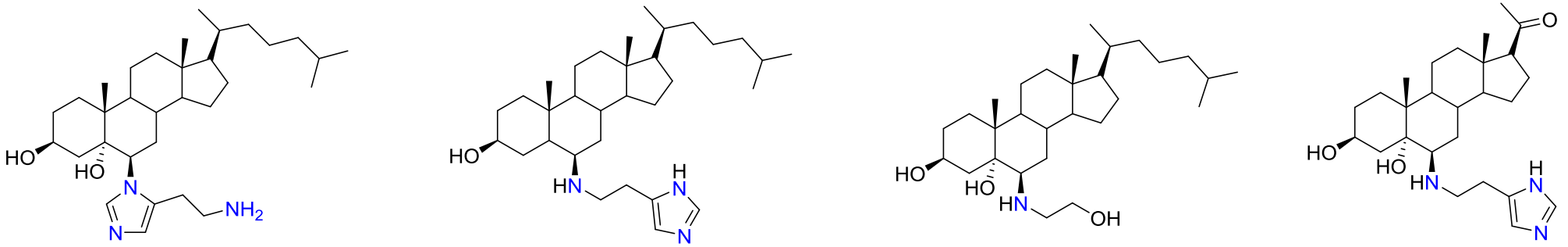
Figure 1

A



5,6α-epoxycholesterol histamine dendrogenin A (DDA)

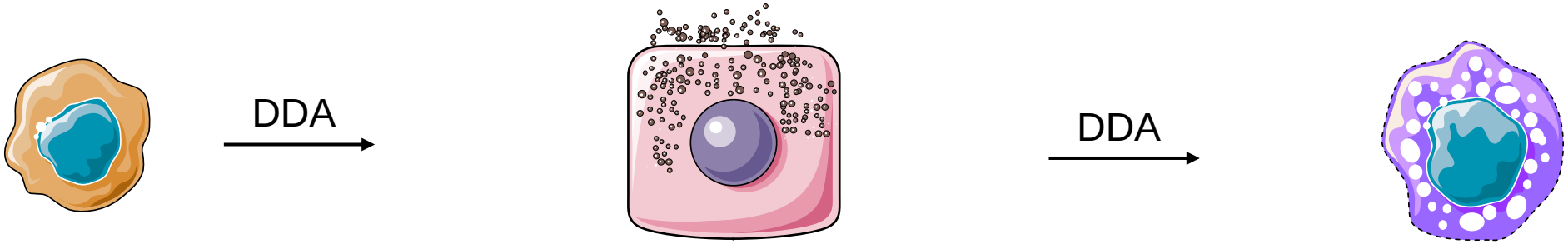
B



C17 C31 C41 C51

Fig 1 Poirot M

Figure 2



Cancer cell **Re-differentiated cancer cell** **cancer cell dying from autophagy**

Fig 2 Poirot

Figure 3

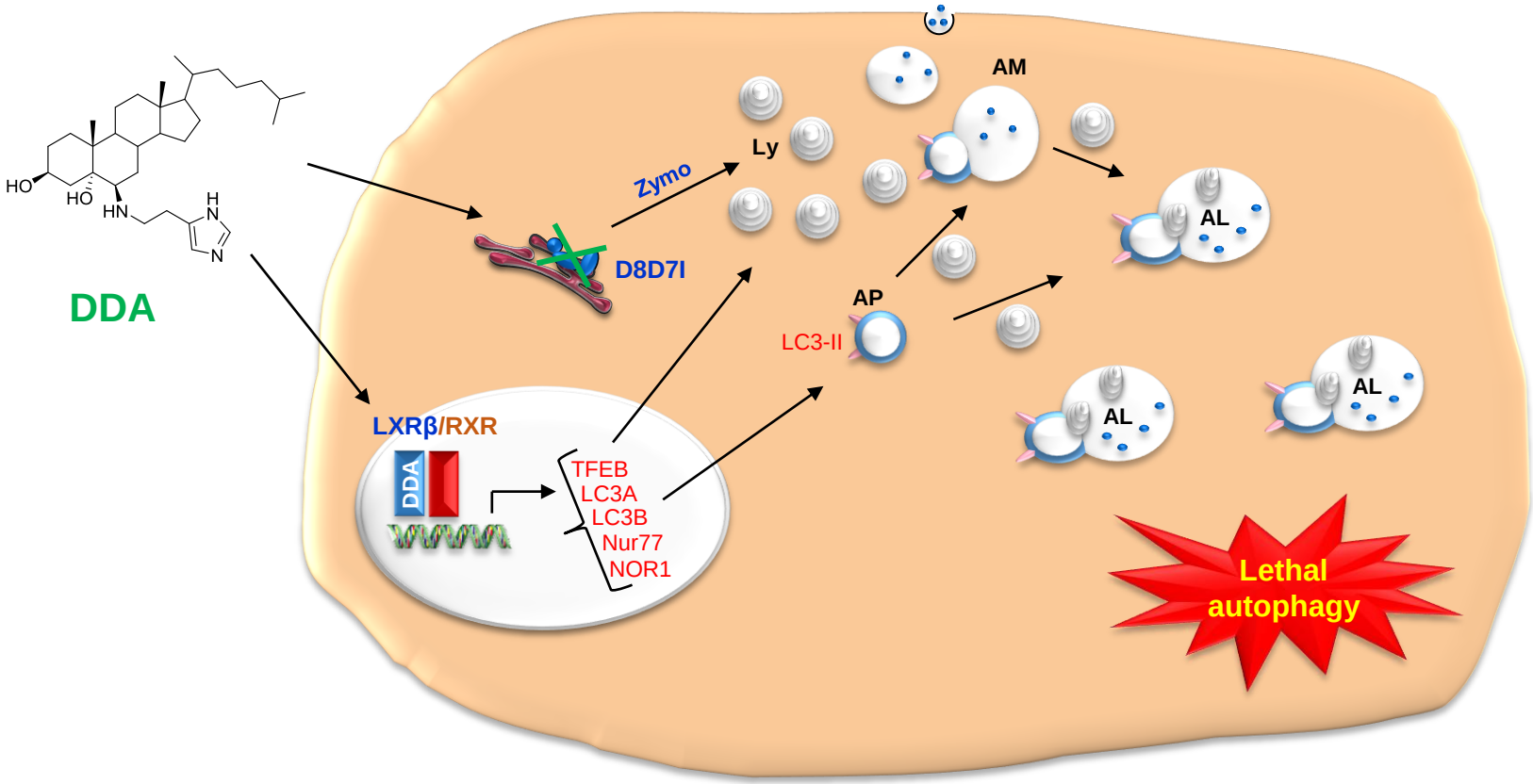


Fig 3 Poirot M

Graphical Abstract (for review)