Cholesterol epoxide hydrolase and cancer
Sandrine Silvente-Poirot and Marc Poirot

Cholesterol epoxide hydrolase (ChEH) catalyzes the hydration of cholesterol-5,6-epoxides (5,6-EC) into cholestane-3β,5α,6β-triol. ChEH is a hetero-oligomeric complex called the anti-estrogen binding site (AEBS) comprising 3β-hydroxysterol-Δ5-Δ7-isomerase (DBD7I) and 3β-hydroxysterol-Δ7-reductase (DHCR7). DBD7I and DHCR7 regulate cholesterol biosynthesis, fetal development and growth, tumor cell differentiation and death. The un-reactivity of 5,6-EC toward nucleophiles has recently been demonstrated indicating that 5,6-EC are not alkylating and carcinogenic agents as first postulated. Here we discuss recent advances in the molecular characterization of ChEH, its potential role in cancer progression and resistance as well as the interest of inhibiting ChEH and to accumulate 5,6-EC which may contribute to the anti-tumor and chemopreventive action of ChEH inhibitors used in the clinic such as tamoxifen.

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Introduction
The epoxide hydrolases (EHs) are a family of enzymes present in all organisms, which transform epoxide containing lipids by the addition of water. An epoxide (or oxirane) is a three-membered cyclic ether. Five EHs have been described in vertebrates which are: soluble EH (sEH), microsomal EH (mEH), cholesterol EH (cholesterol-5,6-epoxide hydrolase or ChEH), hepxoxin hydrolase and leukotriene A4 (LTA4) hydrolase [1].

ChEH (EC 3.3.2.11) represents a distinct subset among EHs with respect to its substrate specificity, activity and molecular identity. ChEH is very selective for the cholesterol-5,6-epoxide (5,6-EC) diastereoisomers: cholesterol-5α,6α-epoxide (5,6α-EC) and cholesterol-5β,6β-epoxide (5,6β-EC) and catalyzes their stereoselective hydration into cholestane-3β,5α,6β-triol (CT) [2*].

Figure 1. ChEH has stimulated the interest of researchers when 5,6-EC were suspected of being involved in skin carcinogenesis [3]. Because of the presence of the epoxide group, it was supposed that 5,6-EC could react spontaneously with nucleophiles and behave like alkylating agents with direct carcinogenic properties. However, contradictory results were published concerning the potential carcinogenic and mutagenic effects of 5,6-EC that have been recently reviewed in [4]. The potential alkylating activity of 5,6-EC was recently ruled out by showing that 5,6-EC are stable and un-reactive toward nucleophiles under non-catalytic conditions [5**]. Interestingly, different drugs used in the clinic have been characterized as inhibitors of ChEH and accumulate 5,6-EC (Figure 2 and Tables 1 and 2). The present review is focused on ChEH, its potential biological role and its relation to cancer progression and resistance based on data obtained over the past two years.

ChEH substrates
ChEH is very specific for the hydrolysis of 5,6-EC into CT with 5,6β-EC being a better substrate than 5,6α-EC [2*] (M Poirot and S Silvente-Poirot, unpublished data). It was postulated that 5,6-EC could be potent alkylating substances, like other chemicals bearing epoxide groups, but they were shown to be non-tumorigenic in rodents [4,6**]. Recently, 5,6-EC were shown to be exceptionally stable and totally un-reactive toward nucleophiles including guanine, at ambient and physiological temperature, as opposed to the carcinogen styrene-oxide [5**]. Importantly, 5,6-EC were stable for several days in the presence of extremely high concentrations of nucleophiles, ruling out that 5,6-EC are spontaneously reactive and behave like direct carcinogenic or alkylating agents.

Thus, the un-reactivity of 5,6-EC diastereoisomers toward nucleophiles suggests that the biological function of ChEH is not to detoxify cells from 5,6-EC by metabolizing them into CT as was first suggested [3].

Subcellular localization and tissue distribution
ChEH is located in the endoplasmic reticulum of cells and is found in most mammalian tissues (liver, kidney, lung, testes, spleen, brain, intestinal epithelium, and skin), with the liver being the richest source [3,7]. ChEH is also found in tumor cells of different tissue origins [8**] (M Poirot and S Silvente-Poirot, unpublished data).

Molecular identity of ChEH
The molecular identity of ChEH was recently established [8**] by showing that the ChEH activity was carried out...
2 Endocrine and metabolic diseases

![Figure 1](image)

ChEH is a hetero-oligomeric complex, also called AEBS, comprising the enzymes D8D7I and DHCR7. ChEH transforms cholesterol-5α,6α-epoxide (5,6α-EC) and cholesterol-5β,6β-epoxide (5,6β-EC) into cholestan-3β,5α,6β-triol (CT).

![Figure 2](image)

Several inhibitors of ChEH are used in the clinic for the treatment of cancer and other diseases (see also Table 1).

by the anti-estrogen binding site (AEBS), which consists of two subunits: 3β-hydroxysterol-Δ9,Δ12-isomerase (D8D7I, also known as the emopamil binding protein EBP) and 3β-hydroxysterol-Δ5-reductase (DHCR7) [9] (Figure 1). Both enzymes are involved in post-lanosterol cholesterol biosynthesis. The similarity between the AEBS and ChEH was first established pharmacologically by showing a positive correlation between the inhibition of ChEH activity by 39 AEBS ligands and their affinity for the AEBS [8**]. It was then shown that the single expression of D8D7I or DHCR7 in COS-7 cells slightly increased ChEH activity whereas their coexpression fully reconstituted ChEH. Inversely, the single knockdown of D8D7I or DHCR7 using siRNA partially inhibited ChEH in MCF-7 cells, whereas the knockdown of both D8D7I and DHCR7 abolished ChEH activity. These results clearly showed that both enzymes are required to reconstitute full ChEH activity.

The single knockdown experiments showed that D8D7I and DHCR7 affect the kinetic parameters of ChEH differently: knockdown of only D8D7I decreased significantly the \( V_{\text{max}} \) of the enzyme while knockdown of only DHCR7 increased the \( K_m \) value, suggesting that D8D7I carries out the catalytic activity of ChEH while DHCR7 has a regulatory role and cooperates in the binding of substrates [8**]. Although, the mEH was shown to be associated with the AEBS/ChEH complex [10], these data clearly indicate that the ChEH is structurally un-related to the mEH and sEH as was previously suspected by the failure of the ChEH to form a covalent substrate intermediate [11]. However, it is interesting to note that the LTA4 hydrolase/aminopeptidase [12] and the sEH/phosphatase [13] also have high structural complexity, both being bifunctional enzymes. The ChEH has even a greater complexity in being composed of two enzymes, D8D7I and DHCR7, that create a third functional one [8**]. This structural complexity explains why the cloning and purification of ChEH has never been reported until now.

Interestingly, human disorders have been described involving defects in D8D7I or DHCR7 [8**,9]. DHCR7 is associated with Smith-Lemli-Opitz syndrome (SLOS) and D8D7I with Chondrodysplasia punctata type 2 (CDPX2) [14]. These syndromes are characterized by the over accumulation of sterol precursors and cholesterol deficiency. Defaults in both enzymes reveal the importance of DHCR7 and D8D7I during fetal development and growth [14].

**ChEH inhibitors**

ChEH/AEBS inhibitors comprise different pharmacological classes of compounds with therapeutic interest, either natural or synthetic [2*,4,8**] (Figure 2 and Tables 1 and 2). These inhibitors include selective AEBS ligands such as PBPE or tesmilifene (DPPE); selective estrogen receptor modulators (SERMs) such as tamoxifen (Tam), 4-hydroxy-tamoxifen (4-hydroxy-Tam), raloxifene or clomiphene; sigma receptor ligands such as SR31747A; cholesterol biosynthesis inhibitors like U-18666A; unsaturated fatty acids such as oleic acid, arachidonic acid (ARA) or docosahexaenoic acid (DHA) and ring B oxysters such as 6-ketocholestanol, 7-ketocholestanol, 7-ketocholesterol and CT. It is interesting to note that selective AEBS ligands or SERM are synthetic competitive inhibitors with nanomolar \( K_i \) for ChEH while the
natural ones identified until now, ring B oxysterols and fatty acids, are competitive or non-competitive inhibitors with $K_i$ in the micromolar range. Therefore, the search for more potent endogenous inhibitors of ChEH deserves further investigations.

**Inhibition of ChEH as a therapy in cancer**

The identification of ChEH as a hetero-oligomeric complex, also called AEBS, comprising the D8D71 and DHCR7 enzymes [8**,9] has opened new perspectives concerning the role of this enzyme and its relation with cancer. In addition, the fact that the ChEH is fully inhibited by therapeutic doses of Tam [8**, one of the main drugs used for the first line and long-term hormone therapy of breast cancers (BCs) [15] or the omega-3 fatty acid DHA [8**] which substantially increased survival of metastatic BC patients treated with chemotherapy [16] suggest that inhibition of ChEH and 5,6-EC accumulation is involved in the anticancer and chemopreventive activity of these compounds. Consistent with this hypothesis, Tam and other ChEH/AEBS inhibitors were reported to induce cell differentiation and apoptosis of BC cells, including estrogen receptor (ER) negative cell lines at concentrations that inhibited ChEH [17–19].

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**Table 1**

<table>
<thead>
<tr>
<th>Class</th>
<th>Name</th>
<th>$K_i$ (nM)</th>
<th>Biological properties</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Selective AEBS ligands</td>
<td>PBPE</td>
<td>27 ± 6</td>
<td>Induces BC cell differentiation and apoptosis. Inhibits D8D71 and DHCR7</td>
<td>[17–19]</td>
</tr>
<tr>
<td></td>
<td>Tesmilifene (DPPE)</td>
<td>62 ± 3</td>
<td>Phase II: increases survival in association with doxorubicin in metastatic BC Phase II: active in association with mitoxantrone and prednisone in metastatic prostate cancer. Inhibits tumor initiating BC cells Potentiates the effects of various cytotoxic agents. Inhibits D8D71 and DHCR7</td>
<td>[20]</td>
</tr>
<tr>
<td>SERMs</td>
<td>Tamoxifen (Tam)</td>
<td>34 ± 8</td>
<td>BC first line and adjuvant therapy. Prevention of BC. Reduced LDL levels Induces BC cell differentiation and death Inhibits 6-oxcholestan-3β,5α-diol formation, a tumor promoter and a metabolite of CT. Inhibits D8D71.</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>4OH-Tam</td>
<td>145 ± 4</td>
<td>The active metabolite of Tam Active on tumor sphere formation Inhibits DHCR24</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Raloxifene</td>
<td>36 ± 4</td>
<td>BC chemoprevention. Reduced LDL levels. Prevention of osteoporosis Inhibits D8D71 and DHCR24</td>
<td>[15]</td>
</tr>
<tr>
<td>$α$ receptor ligands</td>
<td>Haloperidol</td>
<td>18,067 ± 14</td>
<td>Treatment of nausea and vomiting in palliative care Anti-tumor and anti-inflammatory activity</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>SR-31747A</td>
<td>6 ± 2</td>
<td>Treatment of schizophrenia Prevention and treatment of anti-arrythmia</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>Trifluoperazine</td>
<td>135 ± 7</td>
<td>Management of cutaneous dermatosis Inhibition of cholesterol trafficking</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>Amiodarone</td>
<td>733 ± 9</td>
<td>Inhibits Hedgehog signaling and tumor-initiating cells in sarcoma</td>
<td>[45]</td>
</tr>
<tr>
<td>Cholesterol biosynthesis inhibitors</td>
<td>Triparanol</td>
<td>39 ± 3</td>
<td>Natural oxidation product of cholesterol Pro-inflammatory and cell death properties Inhibits D8D71 Product of ChEH activity</td>
<td>[2**]</td>
</tr>
<tr>
<td></td>
<td>Terbinafine</td>
<td>9105 ± 33</td>
<td>Inhibits DHCR24</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td>U-18666A</td>
<td>90 ± 5</td>
<td>Management of cutaneous dermatosis Inhibition of cholesterol trafficking</td>
<td>[47]</td>
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<tr>
<td>Ring B oxysterols</td>
<td>7-Ketocholesterol</td>
<td>4212 ± 32</td>
<td>Natural oxidation product of cholesterol Pro-inflammatory and cell death properties Inhibits D8D71 Product of ChEH activity</td>
<td>[2**]</td>
</tr>
<tr>
<td></td>
<td>Cholestan-3β,5α, 6β-triol (CT)</td>
<td>9744 ± 11</td>
<td>Induces genotoxicity in oxidative conditions Stimulates phospholipids synthesis</td>
<td>[35]</td>
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<tr>
<td>Fatty acids</td>
<td>Oleic acid</td>
<td>54,235 ± 38</td>
<td>Suppresses Her2/neu expression in cancer cells</td>
<td>[50]</td>
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<td>α-Linolenic acid</td>
<td>36,341 ± 42</td>
<td>Suppresses Her2/neu expression in BC cells</td>
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<td>Arachidonic acid</td>
<td>24,094 ± 18</td>
<td>Prevention against coronary heart disease</td>
<td>[52]</td>
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<tr>
<td></td>
<td>Docosahexaenoic acid (DHA)</td>
<td>12,111 ± 16</td>
<td>Substrate of specific lipid oxygenases to form bioactive inflammatory mediators Increased survival of metastatic BC patients treated with chemotherapy Prevention against coronary heart disease</td>
<td>[16]</td>
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### Endocrine and metabolic diseases

#### Table 2

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<td>Docosahexaenoic acid (DHA)</td>
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(G Segal, P de Medina, MR Paillas, B Payre, F Dalenc, VC Jordan, S Silvente-Poirot, M Poirot, unpublished data). Again, these data are inconsistent with deleterious effects of 5,6-EC since these anti-cancer and chemopreventive molecules that are used in long-term treatment are improving overall survival in a phase III randomized trial for metastatic BC when associated with doxorubicin [20]. This result may be due to the impact on tumor initiating cells (TIC) since therapeutic doses of tamoxifen were reported to directly target and kill breast TIC in four different models of BC [21]. Since the exact mechanism of action of tamoxifen (DPPE) is not known, the inhibition of ChEH and the impact of 5,6-ECs in these effects deserve further studies. Consistent with this hypothesis, high concentrations of 4-hydroxy-Tam also attenuated tumor sphere formation in MCF7 cells independently of the presence of the ER [22].

Moreover, clomiphene, which induced apoptosis independently of the ER in leukemia cell lines and patient samples [23], appeared to stabilize disease and to prolong survival in a subset of patients with recurrent or chemotherapy resistant acute myeloid leukemia in a pilot phase study [24]. Together, these results shed light on a
6 Endocrine and metabolic diseases

mechanism independent of the ER and the rationale to study the involvement of ChEH in the effects of these therapeutic molecules.

**Molecular mechanisms involved in the anti-cancer effects of 5,6-EC**

An important question is the characterization of the molecular mechanisms by which 5,6-EC accumulation may contribute to the therapeutic effects of anti-cancer agents [8**]. 5,6-EC may act on mitogenic signal transduction by modulating the biophysical properties of membranes and by interacting with phospholipids [25]. 5,6α-EC was also reported to inhibit topoisomerase II concomitantly with the inhibition of tumor cell growth, their arrest in the G2/M phase of the cycle and the increase in sub G1 phase [26]. Importantly, 5,6α-EC is a ligand of the Liver X Receptor (LXR) α and β. LXRα is nuclear receptors and transcriptional factors involved in cholesterol transport and metabolism, lipid synthesis, innate and adaptive immune system modulation and in the regulation of normal and tumor cell growth [27–29]. It was reported that 5,6α-EC is a modulator of LXRs with antagonist, agonist and inverse agonist activity depending on the cells and genes studied [30**]. 5,6α-EC-3β-sulfate (5,6α-ECS), a sulfated metabolite of 5,6α-EC, is an antagonist of LXRs [31]. 5,6α-ECS and 5,6α-EC were both shown to contribute to the induction of cell differentiation and death by Tam through an LXR-dependent mechanism in ER-positive and ER-negative BC (G Segala, P de Medina, MR Paillasse, B Payre, F Dalenc, VC Jordan, S Silvente-Poirot, M Poirot, unpublished data).

**ChEH activation and cancer development and resistance**

Pitroda et al. have established that mucin 1, a glycoprotein aberrantly overexpressed in numerous cancers, induces a lipid and sterol metabolism transcriptional signature in which a set of 38 enzymes and transporters are upregulated in ER-positive BC and they established that this signature is predictive of resistance to Tam treatment [32]. LXRs are in the heart of this signature that includes the gene coding DHCR7, one of the subunits of ChEH/AEBS [8**,9]. These results suggest that the upregulation of ChEH expression and activity may lead to cancer resistance and is consistent with the fact that the inhibition of the ChEH and 5,6-EC accumulation may contribute to the anti-tumor and chemopreventive action of Tam and other AEBS/ChEH inhibitors.

On the basis of these results, if we reconsider the previous publication of Chen and Black [3] that reported that ChEH activity gradually increased 8 weeks after exposure of mouse skin to UV radiation and reached a maximum activity at the time of the appearance of skin tumors at 15 weeks and then remained above the basal level, it can be postulated that the increase in ChEH activity may be linked to skin carcinogenesis and thus to the metabolism of 5,6-EC into CT and CT metabolites.

An increased activity of ChEH could also be suspected in patients with precancerous colon lesions or with colon cancer since they were found to excrete higher levels of CT, the product of ChEH activity, in their feces than normal controls [33]. Patients with chronic ulcerative colitis, a high-risk group for the development of colon cancer, were also found to have increased fecal excretion of CT and cholesterol [34].

Interestingly, Cheng et al. found that CT was weakly mutagenic through reactive oxygen species production suggesting that one or more oxidation products of CT may be involved in this effect [35]. It was also reported that an oxidative metabolite of CT [36] is a promoter of tumors and stimulates tumor invasiveness in vitro and in vivo [37**].

**Conclusion**

The recent identification of ChEH and the discovery that several anti-cancer and chemopreventive molecules used in the clinic are inhibitors of ChEH at therapeutic doses associated with the demonstration that ChEH substrates are stable and un-reactive epoxides under non-catalytic conditions sheds light on the potential to inhibit ChEH for cancer therapy and prevention. Moreover, the fact that increased activity of ChEH may lead to cancer development and resistance opens new avenues of research that deserve further investigations. The molecular identification of ChEH and the discovery of new synthetic and natural ChEH inhibitors have certainly removed a lock that will enhance studies on the physiological role of this enzyme, its implication in cancer and other pathologies and enable the development of new pharmacological tools to conduct these studies. In addition, the search for more potent endogenous inhibitors of ChEH deserves further investigations.

**Acknowledgements**

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This study characterizes the substrates specificity of ChEH and identifies 7-ketocholesterol, 6-ketocholestanol, and 7-ketocholanol as specific inhibitors of this enzyme while none of the xenobiotic epoxide hydrolase inhibitors or activators affects ChEH activity, suggesting that the ChEH is distinct from the mEH.


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8 Endocrine and metabolic diseases


37. de Medina P, Silvente-Poirot S, Poirot M: Methods for determining the oncogenic condition of cell, use thereof, and methods for treating cancer. Word Patent, 2010/149941; 2010 This patent shows that an oxidative metabolite of CT is a promoter of tumors in vitro and in vivo and that its synthesis is inhibited by ChEH inhibitors.


