The role of bile acids in the development of Barrett's Oesophagus and Oesophageal Adenocarcinoma: a systematic review

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The role of bile acids in the development of Barrett's Oesophagus and Oesophageal Adenocarcinoma: a systematic review

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Abstract:

Oesophageal adenocarcinoma (OAC) and its precursor, Barrett's oesophagus (BO), have overlapping risk factors, including gastro-oesophageal reflux disease. Refluxed contents contain bile acids (BAs) in an acidic environment. The aim of the current study was to investigate, in human subjects, tissues and cell-lines, potential associations of BAs with development or progression of BO to OAC, and to identify mechanisms underlying these effects. A systematic review of six computerised databases was conducted on original articles involving oesophageal tissue from human subjects or oesophageal cell-lines. All articles retrieved for inclusion examined effects of BAs, at neutral pH, on development or risk reduction of BO or OAC. Key findings from the 25 studies included were that deoxycholic acid exerted effects on BA-induced BO and OAC through several potentially co-operating mechanisms, including oxidative stress, DNA damage, inflammation, proliferation, apoptosis, enhanced clonogenicity and angiogenesis. In BO, taurodeoxycholic acid was associated with oxidative-stress, DNA damage and increased proliferation. Ursodeoxycholic acid prevented deoxycholic-acid-induced inflammation in non-malignant human oesophageal cells and BO. Lithocholic acid increased levels of SMAD4, promoting apoptosis in BO. In conclusion, BAs are associated with biological features linked to cancer development, which could be targeted therapeutically, through medication, bacterial supplementation, or lifestyle modifications.

Keywords: Oesophageal Adenocarcinoma, Barrett's Oesophagus, bile acids, deoxycholic acid, taurodeoxycholic acid, ursodeoxycholic acid, lithocholic acid

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Declarations:

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Conflict of Interest:

The authors have no conflicts of interest to declare.

Data Availability Statement:

All of the data procured during the production of this systematic review has been presented in tabular form in the present study. The keywords used in our database searches are available in Supplementary Material. All ethics declarations have been made in the individual studies.

Code Availability:

The production of code is irrelevant to this systematic review.

Author Contributions:

ALC designed the protocol for the present systematic review. She input the keywords into the six databases, checked reference lists of the papers generated, compiled the shortlisted papers in Rayyan, screened the shortlisted papers for eligibility and synthesised relevant information from the papers in six separate tables. She also wrote-up the current paper.

ARV input keywords into the six databases, compiled the shortlisted papers in Rayyan, screened the shortlisted papers for eligibility and synthesised relevant information from the papers in six separate tables: information which was checked against ALC's findings.

SLMCK reviewed drafts of this article and provided feedback.

FJD reviewed drafts of this article and provided feedback.

JJM provided the hypothesis for the study, provided input at all stages of the research and reviewed drafts of the article, providing feedback.

Introduction:

Oesophageal Cancer and Oesophageal Adenocarcinoma

Oesophageal malignancies are the sixth-leading cause of cancer-related mortality worldwide (1,2). Over 600,000 new cases of OCs occurred globally in 2020 (3). Northern European countries, including Ireland, rank among the highest incidences for oesophageal cancers (OCs) worldwide (4). There are two main subtypes of OCs: oesophageal squamous cell carcinoma and oesophageal adenocarcinoma (OAC) (1). The OAC subtype is the most common form of OC in Westernized regions such as Northern Europe, Northern America and Oceania (5). OAC is the most common form of OC in Ireland, where it accounts for 50.9% of all OCs diagnosed from 2010 to 2014 (6). European and North American data also indicate that OAC is nine times more common in men than in women (7).

OC survival rates vary from country to country (8,9). The age-standardised, five-year net survival for OCs (2010-2014) were 23.5%, 16.3%, 14.7%, 16.9%, 19.4% and 16.2% for Australia, Canada, Denmark, New Zealand, Norway and the UK, respectively (8). This compares to the age-standardised, five-year net survival for OCs (2014-2018) in Ireland of 24% (9). OAC survival rates also vary from country to country (10–12). In most populations, OAC has an overall 5-year survival of under 15% (11,13), with values as low as 11% for men, and 13% for women, being reported in the United Kingdom (14).

The incidence of OCs and OAC is on the rise (3,15). The incidence of OAC in England, for example, has increased more than six-fold in the last thirty years (16). This increase in OAC is noted in conjunction with a rise in abdominal obesity, gastro-oesophageal reflux disease (GORD) and Barrett's oesophagus (BO), with a concomitant decrease in *Helicobacter pylori*

infections (17). Nine hundred and fifty-seven thousand new cases of OCs are predicted to occur by 2040 worldwide (4). It is also predicted that new OAC cases will rise by 82% for the nineteen year period of 2021 to 2040 (18). In England, for example, the age-standardised incidence rate of OAC from between 1972 and 1992 increased from 4.8 to 12.3 per 100,000 in men and from 1.1 to 3 per 100,000 in women (16).

Barrett's Oesophagus and Gastro-Oesophageal Reflux Disease

BO is a risk factor for OAC (19). In BO, the normal squamous cells of the oesophagus are replaced by columnar cells (19). In a systematic review of 103 studies, the prevalence of BO in the general population was 3.89% and the prevalence of BO in patients with GORD was approximately double (7.8%) (20). A meta-analysis revealed that the incidence (1966 to 2011) of OAC in BO patients ranged from 1 in 500 to 1 in 300 (21). Patients with BO are 10-55% times more likely to develop OAC (22). BO and OAC have overlapping risk factors including male gender, ever smoking, obesity, prolonged GORD, hiatus hernia and an absence of an *Helicobacter pylori* infection (12). However, the primary stimuli promoting BO- and OAC development are unclear.

GORD is a condition where the contents of the stomach are regurgitated into the distal oesophagus (23). It has a prevalence of 10-20% in Western Europe and is a major risk factor for the development of both OAC and BO (24). Ten to fifteen percent of individuals with reflux-predominant symptoms may have BO (25). The refluxed contents contain stomach acid (hydrochloric acid, HCl), gastric secretions (pepsinogen, intrinsic factor, bicarbonate and mucous) and bile acids (BAs) (25). The contribution of BAs to the development of OAC and BO is currently unclear.

147

148 **Molecular processes and markers underpinning cancer development**

149 Several biological processes underlying cancer development have also been linked to OAC
150 (26–31). These include oxidative stress, DNA damage, inflammation, cell proliferation,
151 apoptosis, resistance to apoptosis, clonogenicity and angiogenesis (26–31). Each of these
152 cancer-related processes are associated with key, sometimes overlapping, molecular markers
153 (26–31).

154

155 *Oxidative stress* is typically measured by the production of reactive oxygen species (ROS) (32).
156 Another common marker is expression of Delta-like Protein 1 (DLL-1) (33). DLL-1 is a ligand of
157 the Neurogenic locus notch homolog protein (Notch) 1 and Notch 2 protein. These proteins
158 inhibit oxidative stress in non-malignant cells (34).

159

160 *DNA damage*, often observed in conjunction with oxidative stress, is associated with a range
161 of markers, including: Notch 1, K13, phospho-Histone 2A Family, Member X (p-H2AX), NADPH
162 Oxidase 5 (NOX5-S), Glutathione Peroxidase 1 (GPX1) and 8-Hydroxyguanosine (8OHdG) (26).

163

164 *Inflammatory markers* linked to cancer include: cyclooxygenase 2 (COX2), chemokine (C-X-C
165 motif) ligand (CXCL), interleukin (IL)-6, IL-8, IL-1β, Nuclear factor kappa-light-chain-enhancer
166 of activated B cells (NF-κB), nuclear factor of kappa light polypeptide gene enhancer in B-cells
167 inhibitor, alpha (IκBα), tumour necrosis factor alpha (TNF-α), Notch 1-4, interferon gamma
168 (IFN-γ), granulocyte-macrophage colony stimulating factor (GM-CSF), p-H2AX, phospho-p65,
169 GPX1, catalase, prostaglandin E2 (PGE2) and Mucin 2 (MUC2) (26,29).

170

Proliferation markers include: Caudal Type Homeobox 1 (CDX-1), Caudal Type Homeobox 2 (CDX-2), phospho-p65, phospho-p38, Ki67, Krüppel-like Factor 4 (KLF 4), Octamer-binding transcription factor 4 (OCT 4), Sphingosine-1-phosphate receptor 2 (S1PR2) and the microRNAs, miR-221, miR-222, miR-143, miR-145 and miR-192 (28).

Apoptosis-related markers include B-cell lymphoma 2 (Bcl-2), Notch 1, hairy and enhancer of split-1 (Hes1), CC3 and SMAD family member 4 (SMAD4) (30). There can also be significant overlap between apoptosis markers and proliferation markers, for example, CDX-2, phospho-p38, miR-221, miR-222 and miR-143, or inflammation markers NF-κB and Notch 1 and 4 (26,28–30).

Clonogenicity markers include cMyc and Lin28 (31). cMyc also overlaps with many of the processes noted above, particularly apoptosis and proliferation (35).

Angiogenesis and related processes are often inferred through the detection of Vascular Endothelial Growth Factor (VEGF) and miR-145 (27).

There has been no recent systematic study on whether BAs have an effect (positive or negative) on these cancer-associated processes and their corresponding molecular markers.

Bile acids

BAs are cholesterol derivatives which exert both metabolic and hormonal effects on the human body (36). They are a component of bile whose functions include emulsification of lipids (to aid absorption in the small intestine), absorption of fat-soluble vitamins, metabolism

and excretion of cholesterol, excretion of bilirubin and the dissolution of gallstones (36). There are both primary (made in the liver) and secondary (made in the small intestine) BAs (36). Examples of primary BAs include cholic acid (CA) and chenodeoxycholic acid (CDCA), whereas examples of secondary BAs include deoxycholic acid (DCA), taurodeoxycholic acid (TDCA), ursodeoxycholic acid (UDCA) and lithocholic acid (LCA) (36). Secondary BAs are produced through the metabolism of primary BAs in the presence of intestinal bacteria such as *Bacteroides spp., Bifidobacteria, Lactobacilli, Clostridia, Enterococci and Listeria* (36). BAs exist in both unconjugated forms or conjugated to the amino acids, glycine or taurine (36). Examples of conjugated BAs include glycocholic acid (GCA), glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA) and taurochenodeoxycholic acid (TCDCA) (37,38). Conjugated BAs are the most predominant type in bile, accounting for greater than 90% of total BAs in the bile mixture (36,37). The disparity between concentrations of conjugated to unconjugated BAs in stomach juice have been highlighted in a study by Zhao *et al.* (37). Here, gastric juice samples (with or without BAs present) were taken from gastritis patients and healthy controls: the authors noted that, within the gastritis group with BAs present, the concentration of conjugated BAs was 100 times higher than the concentration of unconjugated BAs (37). This observation is in contrast to the approximately equal proportion of conjugated-to-unconjugated BA ratio detected in the gastric juice of healthy subjects (37). Healthy subjects have a wide spectrum of individual BAs (23 in total) whereas patients with BA reflux had predominantly the conjugated BAs, GDCA, TCDCA, TCA, GCDCA and GCA (all were present at concentrations higher than 50μM) (37). In a separate BO study by Nehra *et al*., DCA and TDCA were the most commonly featured BAs in BO (38). The profile of BAs in the OAC refluxate also contains a higher proportion of conjugated BAs (39). Specifically, a rat model of OAC showed that the refluxate contained

more TCA, TDCA, TCDCA and TUDCA than controls (39). BAs are either deposited into the duodenum (primary BAs) or recirculated back to the duodenum (secondary BAs); from here, they can enter the stomach and be refluxed into the distal oesophagus (36). Hydrophobic BAs, including GCA, LCA and DCA, are considered to be the most toxic of BAs, given that they are a major factor in inducing liver-cell death (40). The hydrophilic BAs include CA, CDCA and UDCA and are reported to be cancer-protective (40). The toxic effects of BAs vary depending on the pH of their environment (41).

The incidence of BO and OAC continues to rise, despite acid-neutralising and acid-suppressing treatments being prescribed to GORD and BO patients (16,23,42). Such an observation indicates that BAs alone may be exerting oncogenic effects, independent of acidity (23). The resting pH of the distal oesophagus in GORD patients is between 5 and 6 (43). The pH of the oesophagus of BO patients with GORD reaches between pH 2 and pH 4 more frequently than in oesophagitis patients with GORD (44–46). There is a lack of data on the pH of the oesophagus of OAC patients. The two most common treatments for GORD (proton pump inhibitors and H2 blockers) are both available over the counter. Up to 74% of patients with achalasia (a failure of the lower oesophageal sphincter to close, resulting in acid reflux) are treated with acid-suppressing medication (47). It is possible, given the correct dose of acid-neutralising or acid-suppressing medication, that the pH of the oesophagus of at least some OAC patients is neutral (pH 7) or close to neutral (pH 6.5 or over). Investigating the effects of neutral-pH, BAs on the development of BO and OAC is therefore physiologically relevant for at least some patients.

BAs themselves are acidic, with pKa values of unconjugated BAs being greater than 4, that of glycine conjugates being greater than 6 and that of taurine conjugates being greater than 2 (38). It is known that exposure to BAs, in combination with a low-pH environment often present in the oesophageal refluxate, is linked to the development of BO and OAC (48). However, little research has focused on the effects of BAs alone, independent of acidity, on the development of BO and OAC. It is thought that BAs upregulate the expression of metaplasia-promoting genes leading to the columnar-cell phenotype characteristic of BO (49). As GORD progresses to BO, and BO progresses to OAC, the proportion of HCl and secretions in the refluxate decreases dramatically, leaving predominantly BAs (38,50,51). This happens for a number of reasons (52–54). Firstly, BO and OAC patients are often obese and human obesity is associated with altered BA metabolism (52). BO and OAC patients often take acid-suppressing or acid-neutralising medication which reduces the proportion of acid in the distal oesophagus (53). Finally, acid suppression through medication can lead to bacterial overgrowth and increased secondary BAs (54). Unsurprisingly, BO patients have higher concentrations (greater than 200μM in 50% of cases) and different profiles of BAs in their refluxate, especially increased secondary BAs, compared to the refluxate of individuals without BO (50). Collating the evidence on the effects of BAs alone, or a mixture of BAs, in a neutral-pH environment would allow for a toxicity profile of individual BAs, relative to each other and independent of the influence of acidic pH. Such a profile may identify individuals most likely to progress onto BO or OAC and may present therapeutic avenues.

Two systematic reviews on BA-exposure in the oesophagus have been carried out: one in 2011 and the other, 2012 (19,55). They examined the effects of acidic environments (pH of under 7) alone, BAs at a neutral pH and a combination of BAs in acidic environments (19,55).

McQuaid *et al*. examined 83 original articles on human participants, human oesophageal tissue or human cell-lines (19). Their study outcomes included oesophagitis, BO and OAC and any underlying mechanisms (19). Bus *et al.* examined 6 cell-line studies with BO and OAC being primary outcomes (55). The authors of each review concluded, amidst variation in study designs, that BAs (including those independent of acidic environments) may contribute to the symptoms of oesophagitis, BO and OAC (19,55). There is thus a need for an update on the topic, with an expanded focus on the effects of BAs at a neutral pH and on OAC development.

In the current study, we carried out a systematic review of research papers covering the topic of BA-exposure on the development or risk of BO or OAC. Such a review was carried out on human-subject, human-tissue and human cell-line studies only.

Specifically, we aimed to:

i) Investigate, in human-subject, human-tissue and human cell-line studies, the potential association of neutral-pH, BAs with the development of BO or OAC.

ii) Identify any underlying neutral-pH, BA-related mechanisms leading to the development of BO or OAC.

Methods:

Search Strategy:

We searched PubMed, EMBASE, CINAHL, Cochrane Library, Scopus and Web of Science for articles addressing our study aims. We limited our search to papers published from the 1st January 2013 to 31st December 2022. This was because two systematic reviews on the topic of bile acids and the development of BO were published in 2011 and 2012, respectively (55,56). A medical librarian assisted with the development of the search strategies. Details of keywords used have been included in Supplemental Table S1. In brief, we searched keywords for the following concepts: OAC, BO, BAs, cancer-biology outcomes and study design. Only original articles were included. We did not search for abstracts or unpublished data. There were no language restrictions. Any non-English papers were translated using Google Translate.

Initial screening for study eligibility was performed by two authors (AC and ARV) who independently screened all titles and abstracts on Rayyan (https://rayyan.ai/). Where there was disagreement, the reason for inclusion or exclusion was discussed. Full articles were retrieved for the abstracts meeting the inclusion criteria. A search of the bibliographies of included articles was also carried out to retrieve articles potentially missed by the initial search strategy.

Study Inclusion Criteria:

To determine eligibility for inclusion, the full text or abstract of all retrieved articles was reviewed by two authors (AC and ARV). Disagreement was resolved by discussion between the two reviewers.

For human studies, we included studies on male or female OAC patients, BO patients and studies on patients with both conditions. Patients were all 18 years old or over. The effect of BA-exposure (to either individual BAs or a mixture) was assessed. We included the following study types: randomized controlled trials, cohort studies (prospective or retrospective) and case-control studies. Studies involving *in vivo* experimentation on human oesophageal tissue were included. There were no restrictions on patient ethnicity, patient socio-economic class or geographical region in which the studies took place.

Human studies (or parts of studies) on oesophageal squamous cell carcinoma (OSCC) were excluded. OAC patients had to be without secondary cancers. We excluded studies on acid exposure alone and exposure to acid and BAs as a mixture. Studies which only measured surrogate markers of oesophageal bile reflux, such as alkaline pH or bilirubin, were not included. Studies focusing solely on cancer migration, invasion or metastasis-related outcomes were excluded. We also excluded patients receiving treatment with proton pump inhibitors, BA sequestrants, antacids, H-2 blockers, alginates and prokinetics. Studies on patients receiving chemotherapy or radiation were excluded. We did not include narrative reviews, murine studies or human cross-sectional studies.

For *in vitro* studies, we included OAC and BO cell-lines of both male and female origin, from subjects with or without BO. Such cell-lines included FLO-1, OE33, OE19 and BAR-T cells. Any studies on normal, human, oesophageal cells (EPC2-hTeRT or HET1A, for example) were also examined. The effect of BA-exposure (to either individual BAs or a mixture) was assessed.

Studies (or parts of studies) on OSCC cell-lines were excluded. Non-human cell-lines or human non-oesophageal cell-lines were not included. We excluded studies on exposure to acid alone or to mixtures of acid and BAs. We excluded studies that involved exposing OAC or BO cells to proton pump inhibitors, BA sequestrants, antacids, H-2 blockers, alginates and prokinetics. Studies focusing solely on cancer migration, invasion or metastasis-related outcomes were excluded. We excluded any study where cells were exposed to chemotherapy or radiation. We excluded narrative reviews and human cross-sectional studies.

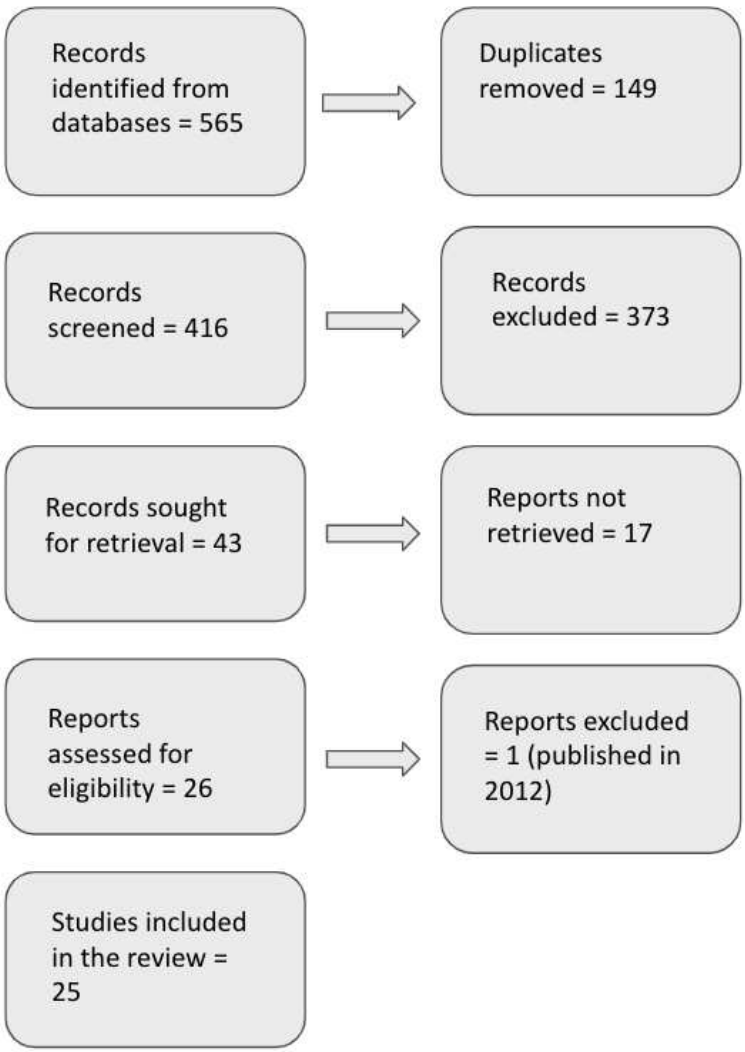
Data Abstraction and Validity Assessment:

A data abstraction form was developed prior to full article retrieval, tested by the authors on several known articles and revised to improve data recording. All articles meeting the inclusion criteria were reviewed independently by two authors and the data entered into the abstraction form. Any disagreements about the data were discussed between the two reviewers with consensus achieved in all cases. We used the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) to assess human study quality (57). We used the Quality Assessment Tool For In Vitro Studies (QUIN) to assess risk of bias in *in vitro* studies (58). We did not perform formal quality assessment of the *in vivo* studies.

A protocol for the present systematic review was registered on PROSPERO (ID: 398556)

Results:

Our initial search identified 565 unique titles. This number was reduced to 416 after duplicates were removed. Three hundred and seventy-three articles were excluded for the following reasons: not an original article (62 articles); murine or rat studies (19 articles); study on BAs and acid as a mixture (46 articles); no mention of BA alone (139 articles); no relevant outcomes provided (94 articles); and articles exclusively focusing on cell migration, invasion and metastasis (13 articles). After initial title and abstract review, by two authors, 43 complete articles were considered potentially relevant and retrieved for full review. Sixteen abstract-only articles were excluded (full texts not retrieved). The final number of articles included in the review was 25. All articles were published in English.



374 **Figure 1. PRISMA Flow Chart depicting the Process for Final Article Retrieval.**

Publications relating to Oxidative Stress:

Four studies reported observations relating to oxidative stress (32,33,48,59), see Table 1. All of the studies were on human cell-lines and all focused on BO (32,33,48,59). Three of the four studies examined the effect of DCA on oxidative stress (32,33,59) while the other investigated the effect of TDCA (48). Three studies used ROS production as a molecular marker (32,48,59) , whereas one examined DLL-1 (33). DCA increased ROS production in two studies (32,59). DCA increased DLL-1 in another study (33). The other BA examined, TDCA, also increased ROS production (48).

Publications relating to DNA Damage:

Seven studies reported observations relating to DNA damage (48,51,60–63), see Table 2. Three studies were carried out in human tissue (51,60,61), two on human cell-lines (48,63) and one examined the effects of BAs on both human tissue and cell-lines (62). Two of these studies focused on non-malignant oesophageal cells (61,62) while four focused on BO (48,51,60,63). Three studies examined the effects of DCA on DNA damage (62,63) whereas the effects of UDCA, TDCA, TCA + GCA mixture, UDCA and DCA (separate experiments) were examined in one study each (48,51,60,61).

In non-malignant oesophageal cells, a mixture of TCA + GCA upregulated p-H2AX (61). DCA suppressed Notch 1 and K13 gene expression in non-malignant oesophageal cells; such inhibition normally results in DNA damage from external factors (62). Minority MOMP (Mitochondrial Outer Membrane Permeabilization) is a process that can cause chronic cellular damage and slow carcinogenesis (63). Minority MOMP presumably leads to ROS production and oxidative stress and would include DNA damage (63). In BO studies, DCA promoted

Minority MOMP, which consequently led to DNA damage (63). Li *et al*. demonstrated, in BO, that TDCA exposure induced DNA damage (48). Oral UDCA increased GPX1 levels and prevented DNA-induced DNA damage in BO (51). 8-Hydroxyguanosine (8OHdG) is an RNA nucleoside which is an oxidative derivative of guanosine. Levels of 8OHdG is used as a biomarker of oxidative stress causing RNA damage (64). Exposure to UDCA did not change 8OHdG levels in BO (60).

Publications relating to Inflammation:

Ten studies focused on the effects of BAs on inflammation (32,51,62,65–71), see Table 3. Seven studies were on human cell-lines (32,51,65,68–71) and the remaining three were carried out on human tissue (62,66,67). Four studies were carried out on non-malignant oesophageal cells (65,66,70,71), while others focused on BO (n=5) (32,51,62,67,69) and OAC (n=1) (68). Most of the studies investigated the effects of DCA alone (n=5) (32,62,67,68,70). The effects of UDCA and DCA (separate experiments) were examined in two studies (51,71). The effects of TCA, GCA, TDCA, TCDCA (all separate experiments) and TCDCA were investigated in one study each (65,66).

In non-malignant cell-lines, DCA increased lipid droplets, COX-2 and CXCL-8 expression and IL-8 secretion (70). Another study in non-malignant oesophageal cells noted that DCA-induced production of IL-6 and IL-8 was attenuated by UDCA (71). TCDCA treatment identified multiple gene sets relating to inflammation in non-malignant cell-lines (66). Finally, in a study by Shan *et al*., none of the conjugated BAs examined (TCA, GCA, TDCA and TCDCA, all separate experiments) induced IL-8 production in non-malignant oesophageal cells (65).

A substantial number of studies have been carried out on the effect of DCA on inflammatory markers in BO and OAC (32,62,67,68,70). One BO study noted that DCA increased TNF-α, IL-8, IL-6, IL-1β (32); this study and another report on BO noted that DCA activated NF-κB (32,51). DCA also: induced expression of COX-2 and PGE2 (BO) (67); increased MUC2 expression (BO) (62); decreased Notch 1-4 (BO) (32); and activated COX-2 and IκBα expression (OAC) (68). Peng *et al*. demonstrated that, in BO, DCA activated the inflammatory markers, p-H2AX, phospho-p65 and NF-κB (51); by contrast, UDCA upregulated levels of the anti-inflammatory GPX1 and catalase enzymes in BO (51). BA exposure (specific BA not detailed) increased expression of macrophage-recruiting cytokines, IFN-γ, TNFα and GM-CSF in BO (69).

Publications relating to Cell Proliferation:

Eleven studies examined the effect of BAs on proliferation (51,60,62,66–68,72–78), see Table 4. Four were human studies (60,62,66,77) and seven were carried out in human cell-lines (68,72–76,78). Two studies were carried out on non-malignant oesophageal cells (66,72), four were carried out on BO (60,62,73,77) and five on OAC (68,74–76,78). The effects of DCA were examined in four studies (62,68,76,77). Two studies each were carried out on the effect of TDCA and TCA, respectively (66,73–75). One study each examined the role of UDCA, CA and CDCA (separate experiments) and a mixture of DCA + GCA + TCDCA (60,72,78).

In non-malignant oesophageal cells, TDCA did not enrich proliferation markers (66). In a micro-RNA study on non-malignant oesophageal cells, exposure to a mixture of BAs (DCA + GCA + TCDCA) did not significantly change levels of miR-143, -145 and -192 (72). In one BO study, DCA increased phospho-38 (67). DCA suppressed Notch 1 activity in BO (62), whereas TDCA exposure significantly increased Notch 4 in BO (73). UDCA did not change Ki67 levels in

BO (60). In OAC studies, DCA activated CDX2, KLF4 and OCT4 (68,76). In other OAC studies, TCA promoted cell proliferation markers, including S1PR2 (74,75). CA or CDCA exposure increased levels of miR-221 and -222 in OAC (78). CA and CDCA exposure degraded CDX2 in OAC (78).

Publications relating to Apoptosis:

Nine studies investigated the effects of BAs on apoptosis (32,59,60,62,67,72,77–79), see Table 5. Five studies were carried out on human cell-lines (32,59,72,78,79) and four were based on human tissue (60,62,67,77). One of these studies was carried out on non-malignant oesophageal cells (72), seven on BO (32,59,60,62,67,77,79) and one on OAC (78). The effects of DCA on apoptosis were evaluated in five studies (32,59,62,67,77). One study each focused on the following BAs: UDCA, LCA, CA and CDCA (separate experiments) and a mixture of DCA + GCA + TCDCA (60,72,78,79).

In non-malignant cell-lines, the mixture of BAs (DCA + GCA + TCDCA) did not significantly change miR-143 levels (72). In BO, DCA induced apoptosis of non-malignant cell-lines through increasing the expression of molecules such as VEGF, Bcl-2, phospho-p38, Notch 1 and Hes 1 (32,62,63,67,77). DCA induced BO-related, apoptotic resistance through the action of NF-κB and Bcl-2 (59). By contrast, DCA suppressed Notch 1 and Hes1, preventing apoptosis of BO cells (62). A study on UDCA did not note any change in CC3 levels in BO (60). Finally, LCA induced apoptosis of non-malignant cells through the upregulation of the SMAD4 gene in BO (79). In OAC, neither CA nor CDCA increased levels of miR-221 and -222 (78). By contrast, CA and CDCA exposure degraded the apoptosis-promoting CDX2 (78).

Publications relating to Clonogenicity and Angiogenesis:

One cell study focused on clonogenicity (63), see Table 6. It was carried out on OAC cells (63). DCA increased clonogenicity in these cells (63).

Two human studies focused on angiogenesis (67,72), see Table 6. One examined effects on non-malignant oesophageal cells (72) and the other on BO (67). The first study examined the effects of DCA (67), while the other examined a mixture of DCA + GCA + TCDCA (72).

DCA induced VEGF expression (67), leading to angiogenesis, while exposure to the BA mixture did not significantly change angiogenic miR-145 levels (72).

DISCUSSION:

The goal of the present study was to examine the effects that BAs (individually or as a mixture), at neutral pH, might have on the development or risk of BO and OAC. Two systematic reviews on the topic (carried out in 2011 and 2012, respectively) indicated that BAs in media of various acidic pH conditions may play a role in the aetiology of these conditions (55,56).

In the present systematic review, we examined 25 recent articles identified by a keyword search of six databases. Our key findings were as follows: DCA was reported to affect the biology of BO and OAC through a wide range of potentially co-operating mechanisms (32,33,51,59,62,63,67,68,70,76,77); DCA suppressed Notch 1-4 genes in non-malignant oesophageal cells (Notch 1 only) and BO, leading to oxidative-stress generation, inflammation (32), DNA damage, proliferation and apoptosis (62); TDCA was associated with oxidative-stress generation, DNA damage and increased cell proliferation in BO (65,66,73); UDCA prevented DCA-induced inflammation in non-malignant oesophageal cells and BO (51,71); and LCA increased levels of SMAD4, which can promote apoptosis in BO cells (79).

To add more context to our observations, the present systematic review reports BA-related findings in the literature in diseases other than OAC. Blood and stool levels of BAs of healthy individuals are generally tightly regulated (80); despite this, blood and stool samples of diseased patients (specifically: colon cancer, colitis, gastric cancer, hepatocellular carcinoma, primary biliary cholangitis, melanoma and hepatocellular carcinoma) show increased levels of BAs compared to those of control subjects (80–84). It is currently unknown whether high bodily levels of BAs are a cause or consequence of the diseases in

question. By contrast, breast cancer patients have decreased gut concentrations of BAs (85) and the development of glioblastoma and nephroblastoma can be inhibited by UDCA and LCA, respectively (86,87). The digestive cancer observations are particularly pertinent to our study of BAs and OAC.

The Role of DCA in BO and OAC Tumour Biology:

DCA and Oxidative Stress:

DCA was associated with oxidative-stress in the findings from the present study (32,33,48,59). Many authors cited potential mechanisms underlying the DCA-related associations (62,88–112). In colon epithelial cells, the authors noted that DCA induced mitochondrial oxidative stress through the activation of NF-κB in these cells (88). It is thought that DLL-1 interacts with Notch proteins in BO and results in oxidative stress (33). DCA also induced oxidative stress in human colon adenocarcinoma cells, via the activation of NADPH oxidases (99).

DCA and DNA Damage:

Five studies in the literature examined the effect of DCA on DNA damage (92,98,100,102,103,113); these studies were carried out on non-malignant cells, BO and OAC. In non-malignant oesophageal cells, DCA had a non-linear concentration response for DNA damage (92); such a relationship provides researchers with the knowledge of the extent of DNA damage at a given concentration of DCA. DCA induced DNA damage in human colon epithelial cells in two studies (98,100). A study on benign Barrett's epithelial cells observed that DCA caused DNA damage in this condition (113). One study focused on the effect of DCA on DNA damage and OAC development (102). The DCA-related bile salt,

sodium deoxycholate, was associated with increased DNA damage in both non-malignant and OAC cell-lines (102). The same study revealed that a mixture of sodium glycocholate, glycocholic acid, sodium taurocholate and taurochenodeoxycholate led to DNA damage in OAC cells (102).

DCA and Inflammation:

DCA induces inflammation through a variety of mechanisms (100,104–106). In the DNA-damage-related study by Glinghammar et al., there was subsequent induction of inflammatory markers such as caspases, COX-2 promoter activity, NF-κB and AP-1 (100). DCA was found to induce gut dysbiosis and inflammation in the intestine (106). DCA has also been reported to activate the NLRP3 inflammasome and aggravated colitis in mice (105). In another murine study, intestinal inflammation was attenuated through the modulation of the gut-microbiota-farnesoid-X-receptor axis (104).

DCA and Cell Proliferation:

DCA affected cell proliferation in four studies (two on BO and two on OAC) in the present study (62,68,76,77). DCA increased cell proliferation in three out of these four studies (68,76,77): the remaining study decreased non-cancerous proliferation in BO through the suppression of Notch 1 function (62). Observations from the literature support the increase of proliferation in response to DCA exposure. Ochsenkühn *et al.* noted that serum DCA promoted hyperproliferation of the colonic mucosa, a key precursor to the development of colon cancer (107). Two studies stated the DCA doses at which proliferation of cancer of the colon occurred: 20µM and 5µM and 50µM, respectively (108,109).

DCA and Apoptosis:

Several studies in the literature state that DCA induced apoptosis, be it cancer-promoting or cancer-preventing (89–91,93,110–113); several molecular mechanisms have also been cited (89–91,93,110–113). DCA induced rat hepatocellular apoptosis through the inhibition of NF-κB production (89); such apoptosis can lead to fibrosis (89). Bcl-2 like protein 4 (Bax) is a key regulator of apoptosis (114). DCA can induce apoptosis in the human, colon-cancer cell-line, in the absence of Bax (91); such apoptosis disrupts the fine balance among proliferation, differentiation and apoptosis and is thought to be tumour-promoting (91). By contrast, DCA induced apoptosis in gastric-carcinoma cells through activation of an intrinsic mitochondrial-dependent pathway (93).

DCA and Apoptotic Resistance:

In line with an observation in the present study (59), some studies noted that DCA induced apoptotic resistance in BO (90,113). In Barrett's epithelial cells, DCA induced apoptotic resistance in cells with DNA damage; such resistance led to increased likelihood of worsening BO and was brought about by the activation of the same transcription factor, NF-κB (113). Apoptotic resistance was also a feature of progression to colon cancer in a study by Bernstein *et al.* (90).

DCA and Clonogenicity and Angiogenesis

In the current study, DCA increased clonogenicity of OAC cells through the activation of cMyc and Lin28 (115).

Angiogenesis and related processes have been noted in cancer cells (95–97). Song *et al*. noted that DCA promoted vasculogenic mimicry (a tumour blood supply which takes place independent of angiogenesis) through VEGF Receptor 2 activation; such activation further exacerbated intestinal carcinogenesis (95). Such an observation contrasts with that of two studies focusing on how a heparin-DCA conjugate can suppress angiogenesis and subsequent tumour growth (96,97).

DCA and Notch Proteins:

In contrast to the upregulation of several biological processes, DCA suppressed Notch 1-4 protein production in studies focusing on DNA damage (non-malignant oesophageal cells), inflammation (BO), proliferation (BO) and apoptosis (BO) (32,62). Notch proteins act as tumour suppressors in their native state but become oncogenic if mutated (62). Wang *et al*. showed that DCA suppressed Notch 1 activity in non-malignant oesophageal cells, leading to DNA damage and inflammation (62). Wang *et al*. also illustrated that Notch 1 gene suppression may lead to the development of BO, through the suppression of certain proliferation markers, apoptosis markers and a DNA-damaging marker (62). Feng *et al*. demonstrated that, in BO, DCA-exposure resulted in inflammation, which was partially induced by the suppression of Notch genes 1-4 (32). Xiao *et al*. indicated, in porcine enterocytes, that the inhibition of the Notch 1 protein increased oxidative stress, caused cell apoptosis, reduced autophagy and aggravated cell inflammation after exposure to the mycotoxin, deoxynivalenol (116). DCA appears to be toxic to the oesophagus and exerts its toxic effects partially through the deactivation of Notch signalling (62).

TDCA in BO and OAC Tumour Biology:

TDCA, a conjugated form of DCA, was associated with oxidative-stress generation (BO), DNA damage (BO) and increased proliferation (BO) in the current study (48,66,73). Contrary to the DCA-related results, TDCA exposure significantly increased Notch-4 gene expression in BO (73). This increased expression appears to increase BO cell proliferation (73). Although we noted the generation of oxidative stress in the present study, there is a paucity of studies examining the relationship between TDCA exposure and oxidative stress in the literature. The data from the present study indicate that, in BO, TDCA induced DNA damage through the activation of the NOX5-S protein (48).

TDCA-related Biology in Other Malignancies:

The results from some studies indicate that TDCA may be cancer protective. TDCA supplementation alleviated mucosal damage and improved cell survival after inflammation-induced intestinal injury (117). TDCA also increased intestinal epithelial cell proliferation through c-myc expression (118): this increased proliferation of normal cells is thought to be cancer protective. More research is needed to decipher the mixed results of TDCA cancer-related studies.

UDCA in BO and OAC Tumour Biology

Evidence presented in the current review suggests that UDCA can attenuate DCA-induced inflammation, as indicated by reduced IL-6 and IL-8 expression (71) and increased GPX1 and catalase levels (51). By contrast, it had no effect on markers of DNA damage, proliferation or apoptosis (60).

UDCA-related Biology in Other Malignancies:

624 The literature supporting a link between UDCA and inflammation is the most
625 comprehensive of all BAs studied, but no study has been carried out on the effects of UDCA
626 on OAC. In macrophages, UDCA inhibited the pro-inflammatory responses induced by
627 lipopolysaccharide (119). The immunosuppressive action of UDCA has also been noted in
628 dendritic cells (120). Indeed, inhibiting the function of dendritic cells, through the BA-
629 sensitive Farnesoid X Receptor, allows UDCA to suppress eosinophilic airway inflammation
630 (121). UDCA administered to rats with spinal-cord injury not only dampened inflammatory
631 responses but also promoted functional recovery (122). A mechanism underlying these
632 UDCA-related associations has also been illustrated (103). UDCA pre-treatment of cells
633 inhibited COX-2 upregulation, DCA-induced activation of NF-κB and Activator Protein 1 and
634 translocation of NF-κB (103).
635
636 *UDCA and Cell Proliferation:*
637 The lack of an effect of UDCA on proliferation markers contradicts findings reported in the
638 literature. Martínez *et al*. and Serfaty *et al.* both noted the inhibition of cell proliferation by
639 UDCA: inhibition which prevented the development of colon cancer (110,123). The same
640 reduction in proliferation was observed in primary biliary cholangitis (124); this study used
641 the same molecular marker, Ki67, as the report included in the present systematic review
642 (60,124). Ki67 was also used to measure proliferation in a colorectal cancer model: UDCA
643 inhibited tumour growth, as indicated by Ki67 levels, in a concentration-dependent manner
644 (125).
645
646 *UDCA and Apoptosis:*

The literature also points to UDCA improving health through apoptotic mechanisms (86,126,127). Such observations were noted in human melanoma (126), glioblastoma (86) and hepatocellular carcinoma (127). Several molecular mechanisms have been cited (86,111,126–131). UDCA either inhibits cancer-promoting apoptosis or promotes the programmed cell death of cancer cells (86,111,126–131). Cancer-promoting apoptosis can occur at the level of the endoplasmic reticulum (ER) and in the mitochondrion (131). UDCA inhibited glioblastoma progression via ER-stress-related apoptosis (86). Mitochondrial UDCA effects were noted in human melanoma cells in two studies (111,126). A potential mechanism postulated is that UDCA inhibits DCA-induced harmful apoptosis through the modulation of mitochondrial transmembrane potential (as was observed in experiments on otherwise healthy rats) (111). Apoptosis is also activated in order to kill cancer cells (127–129). UDCA induced apoptosis of hepatocellular carcinoma (127,128). One study demonstrated that this programmed cell death was due to the activation, by UDCA, of the p53-caspase 8 pathway (128). DCA-induced apoptosis can also be attenuated by the UDCA-related stimulation of Akt-dependent survival signalling (129).

UDCA as a Therapeutic Target:

Given its tumour protective characteristics, UDCA may be an appropriate therapeutic target; the BA could, for example, be administered orally to potentially change the profile of BAs in the stomach of BO and OAC patients. A human trial by Banerjee *et al*., however, demonstrated that high-dose UDCA supplementation for six months increased UDCA blood levels but did not modulate selected markers of oxidative stress, DNA damage, cell proliferation, and apoptosis in BO (60). This lack of change may be due to the small sample size (29 patients), but a power calculation was not carried out (60). It could also be that

levels of these markers were not as elevated as they would be in OAC and, as such, no significant change between normal oesophageal cells and BO was observed.

LCA in BO and OAC Tumour Biology:

LCA is a metabolite of UDCA (79). It is a hydrophobic BA and is often considered to be toxic to human tissue (87). In data from the present systematic review, LCA induced SMAD4 expression and this in turn promoted apoptosis of BO cells (79). Furthermore, this apoptotic effect was independent of the tumour suppressor, p53 (encoded by the TP53 gene) (79). The LCA-stimulated apoptosis in BO is reinforced by the observation that LCA-associated apoptosis does not occur when SMAD4 is deleted (79). A SMAD4-related result is pertinent given that it is one of the three "driver genes" (along with TP53 and Mucin 5AC) for the development of OAC: it is responsible for the worsening of OAC, whilst TP53 promotes the progression of BO to OAC (132,133). The LCA-associated induction of SMAD4 in BO, rather than in OAC, might mean that apoptosis by SMAD4 in BO prevents the progression of BO to OAC; if so, LCA could prove to reduce the risk of BO to OAC progression. Since this beneficial SMAD4-associated apoptosis occurs independent of TP53 mutations, SMAD4 could be upregulated by LCA to prevent BO to OAC progression in patients with or without TP53 mutations. There is no evidence suggesting that LCA influences p53 function.

LCA and Apoptosis:

Despite the association presented in the present systematic review, no similar SMAD4 and LCA association could be identified in the literature. Nevertheless, there are studies linking LCA exposure and the activation or suppression of beneficial apoptosis (87,134–137). LCA induced apoptosis of breast cancer cells and human nephroblastoma cells (87,134,135). In

colorectal carcinoma, LCA inhibited the cancer-promoting MDM2 and MDM4, which in turn allowed for p53 to remain upregulated and p53-dependent apoptosis to occur (136). A differential apoptotic effect was noted in normal colonic cells (stimulation of apoptosis with LCA exposure) and in premalignant colon cells (nearly complete suppression of apoptosis with LCA exposure) (137); this almost complete suppression of apoptosis in the latter condition is likely a consequence of the disease.

Limitations of the Present Study:

The present study is not without its limitations. Most (44%) of the studies included examined the potential effects of DCA on non-malignant oesophageal cells, BO and OAC (32,33,51,59,62,63,67,68,70,76,77,115). More study on non-DCA BAs needs to be carried out in order to compare BA-specific results in a more balanced way. In contrast to DCA studies, relatively few studies (n=5) have examined the role of TDCA (48,66,73) and only one study examined the effects of LCA (79). Despite their predominant existence in normal oesophageal tissue, only nine studies (33%) in the current review focused on conjugated BAs (48,61,65,66,72–75,78); three of these studies revealed no change in outcomes measured (65,66,72). Only six (23%) of the studies assessed associations of BAs with OAC: a value disproportionate to BO-related studies (50%). There is a lack of human-subject and human-tissue studies (20%) compared to human *in vitro* studies (73.33%) or a combination of these study types (6.67%). There is also a complete absence of randomised controlled trials. There is thus a need for a human-subject study focused on BA profiles in OAC. Despite the clear gender disparity associated with OAC in the literature, and the sexual dimorphisms in cholesterol to BA conversion (138), no gender-related observations were made in the included studies of the present systematic review.

Potential for Intervention:

The findings from the present systematic review lay the groundwork for further hypotheses, relating in particular to lifestyle intervention. Blood levels of BAs increase with increasing body mass index (52,139): as such, slow and steady weight loss may result in lower concentrations of toxic BAs and would reduce the risk of gallstones (140). Additionally, prophylactic UDCA given during a period of weight loss aids in reducing gallstone risk (140). UDCA lowers total cholesterol: the precursor for BA synthesis (141). Similarly, beta glucan (found in oats) binds to BAs and cholesterol for excretion (142). Plant sterols also inhibit intestinal absorption of cholesterol (143). High-fat diets increase levels of DCA, raising the possibility that low-fat diets may reduce the levels of these BAs or result in a more health-promoting profile (144). The spice, turmeric (active compound: curcumin), reduces DCA levels in those consuming a high-fat diet. Since DCA consistently upregulates the inflammatory transcription factor, NF-κB, high polyunsaturated-fat diets or omega-3 supplementation may be possible therapeutic avenues (145). Finally, diallyl disulfide, a compound found in garlic, attenuated DCA-induced inflammation and apoptotic resistance in BO (59).

Conclusions:

In conclusion, the current systematic review provides an update on the more recent evidence linking exposure to BAs (independent of acidity) and the development of BO and OAC; it also cites potential underlying mechanisms for the observed associations. Our analysis highlights roles for DCA, TDCA, UDCA and LCA in particular. DCA exerted pro-oncogenic effects on non-malignant oesophageal tissue, BO and OAC through a wide range

743 of cellular processes (32,33,51,59,62,63,67,68,70,76,77,115). DCA also suppressed Notch
744 signalling (32,62). Notch signalling is presumed to be tumour suppressive in the studies
745 examined, except when it interacts with DLL-1 (32,62). TDCA was associated with the
746 generation of oxidative stress in one BO study, the damaging of DNA in another BO study
747 and an increase in proliferation of BO cells in another (48,66,73). Less information is
748 available on the effects of TDCA on non-malignant oesophageal tissue, BO and OAC,
749 particularly in oxidative-stress generation. UDCA-exposure confers risk-reducing and
750 protective effects on non-malignant oesophageal cells, BO and OAC (51,60,71) and may be a
751 potential therapeutic target. Finally, the SMAD-4-related apoptosis results of the one study
752 of LCA action (79), while pertinent, need to be reinforced by further investigation. Further
753 research is needed to discern the tumour-promoting and tumour-suppressive functions of
754 Notch proteins in different contexts. The research on BAs and the development of BO and
755 OAC could be strengthened by conducting randomized controlled trials. A sample
756 randomized controlled trial would involve recruiting OAC patients and non-cancer controls.
757 Each group (OAC patients being Group A and non-cancer controls being Group B) would
758 either consume UDCA or plant sterols for a designated amount of time. Four outcomes
759 would be studied: changes (if any) in total BA concentration; improvement in BA profile;
760 reduction in oxidative stress and inflammation; and changes in OAC tissue morphology. To
761 our knowledge, the present systematic review is the most comprehensive study on the
762 effects of BA-exposure, independent of acidic pH environments, and BO and OAC
763 development to date. We show that BAs can act independently of acidic pH environments.
764 Taken together, BAs play a role in the development of BO and OAC, independent of acidic
765 environments, and could be targeted therapeutically, through medication, bacterial
766 supplementation, weight loss or dietary modification.

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773

Data Availability Statement:

All of the data procured during the production of this systematic review has been presented in tabular form in the present study. The keywords used in our database searches are available in Supplementary Material. All ethics declarations have been made in the individual studies.

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Table 1. Bile-Acid Exposure and Oxidative Stress in Barrett's Oesophagus Studies							
Authors	**Year**	**Study Type**	**Condition**	**Bile Acid(s)**	**Outcome**	**Molecule(s)**	**Result(s)**
Feng et al.	2016	Cell	BO	DCA	Oxidative Stress	ROS	DCA increased intracellular ROS
Feng et al.	2017	Cell	BO	DCA	Oxidative stress	ROS	DCA induced ROS production in a dose-dependent manner
Tamagawa et al.	2016	Cell	BO	DCA	Oxidative stress	DLL-1	DCA exposure increased DLL-1 production
Li et al.	2016	Cell	BO	TDCA	Oxidative stress	ROS	TDCA induced oxidative stress

Table 1. Bile-Acid Exposure and Oxidative Stress in Barrett's Oesophagus Studies

BO = Barrett's Oesophagus; DCA = deoxycholic acid; TDCA = taurodeoxycholic acid; ROS = reactive oxygen species; DLL-1 = delta-like protein 1

Table 2. Bile-Acid Exposure and DNA Damage in Experiments on Non-Malignant Oesophageal Cells or Tissue and Barrett's Oesophagus

NON-MALGINANT OESOPHAGEAL CELLS OR TISSUE							
Authors	**Year**	**Study Type**	**Bile Acid(s)**	**Condition**	**Outcome**	**Molecule(s) Examined**	**Result(s)**
Wang et al.	2018	Human and cell	DCA	Non-malignant oesophageal cells	DNA damage	Notch 1 and K13	DCA suppressed Notch 1 and K13
Jiang et al.	2016	Human	TCA + GCA mixture	Non-malignant oesophageal tissue	DNA damage	p-H2AX	TCA + GCA mixture upregulated p-H2AX
BARRETT'S OESOPHAGUS							
Xu et al.	2020	Cell	DCA	BO	DNA damage	Cytochrome C and caspase 3	DCA promoted minority MOMP. This resulted in DNA damage
Peng et al.	2014	Human	DCA	BO	DNA damage	p-H2AX and phospho-p65	Oesophageal perfusion with DCA increased p-H2AX and phospho-p65
Li et al.	2016	Cell	TDCA	BO	DNA damage	NOX5-S	TDCA induced DNA damage

Peng et al.	2014	Human	UDCA and DCA (separate experiments)	BO	DNA damage	GPX1	Oral UDCA increased GPX1 levels. Oral UDCA prevented DCA-induced DNA damage
Banerjee et al.	2016	Human	UDCA	BO	DNA damage	8OHdG	Exposure to UDCA did not change 8OHdG levels

Table 2. Bile-Acid Exposure and DNA Damage in Experiments on Non-Malignant Oesophageal Cells or Tissue and Barrett's Oesophagus

DCA = deoxycholic acid; TCA = taurocholic acid; GCA = glycocholic acid; p-H2AX = phospho Histone Family, Member X; BO = Barrett's Oesophagus; Minority MOMP = Minority Mitochondrial Outer Membrane Permeabilization; TDCA = taurodeoxycholic acid; NOX5-S = NADPH Oxidase 5; UDCA = Ursodeoxycholic acid; GPX1 = Glutathione Peroxidase 1; 8OHdG = 8-Hydroxyguanosine.

Table 3. Bile-Acid Exposure and Inflammation in Experiments on Non-Malignant Oesophageal Cells or Tissue, Barrett's Oesophagus and Oesophageal Adenocarcinoma

NON-MALIGNANT OESOPHAGEAL CELLS OR TISSUE							
Authors	**Year**	**Study Type**	**Bile Acid(s)**	**Condition**	**Outcome**	**Molecule(s)**	**Result(s)**
Carrossini et al.	2021	Cell	DCA	Non-malignant oesophageal cells	Inflammation	COX2, CXCL and IL-8	DCA increased LD, COX-2 and CXCL-8 expression and IL-8 secretion
Quilty et al.	2021	Cell	DCA and UDCA (separate experiments)	Non-malignant oesophageal cells	Inflammation	IL-6 and IL-8	DCA-induced production of IL-6 and IL-8 was attenuated by UDCA
Green et al.	2014	Human	TCDCA	Non-malignant oesophageal cells	Inflammation	NF-κB	TCDCA treatment identified multiple gene sets related to inflammation
Shan et al.	2013	Cell	TCA, GCA, TDCA, TCDCA (all separate experiments)	Non-malignant oesophageal cells	Inflammation	IL-8	None of the conjugated BAs, under a neutral condition, induced IL-8 production
BARRETT'S OESOPHAGUS							

Feng et al.	2016	Cell	DCA	BO	Inflammation	TNF-α, IL-8, IL-6, IL-1β, NF-κB, Notch 1-4	DCA increased TNF-α, IL-8, IL-6, IL-1β, NF- κB. DCA decreased Notch 1-4
Fedder et al.	2020	Cell	Bile acid	BO	Inflammation	IFN γ, TNFα and GM-CSF	Increased expression of macrophage-recruiting cytokines IFN γ, TNFα and GM-CSF
Peng et al.	2014	Cell	UDCA and DCA (separate experiments)	BO	Inflammation	NF-κB, p-H2AX, phospho-p65, GPX1, catalase.	DCA increased p-H2AX and phospho-p65. DCA activated NF-κB. Oral UDCA increased GPX1 and catalase levels
Taddei et al.	2014	Human	DCA	BO	Inflammation	COX-2, PGE2	DCA induced expression of COX-2 and PGE2
Wang et al.	2018	Human	DCA	BO	Inflammation	MUC2	DCA increased MUC2 expression
OESOPHAGEAL ADENOCARCINOMA							
Yamada et al.	2014	Cell	DCA	OAC	Inflammation	COX-2 and IκBα	DCA activated COX-2 and IκBα expression

Table 3. Bile-Acid Exposure and Inflammation in Experiments on Non-Malignant Oesophageal Cells or Tissue, Barrett's Oesophagus and Oesophageal Adenocarcinoma

DCA = deoxycholic acid; COX-2 = cyclooxygenase 2; CXCL1 = chemokine (C-X-C motif) ligand 1; IL-8 = interleukin 8; LD = lipid droplets; UDCA = ursodeoxycholic acid; IL-6 = interleukin 6; TDCA = taurodeoxycholic acid; NF- κB = Nuclear factor kappa-light-chain-enhancer of activated B cells; TCA = taurocholic acid; GCA = glycocholic acid; TCDCA = taurochenodeoxycholic acid; BO = Barrett's Oesophagus; IκBα = nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; TNFα = tumour necrosis factor alpha; IL-1β = Interleukin 1 beta; IFN γ = Interferon gamma; GM-CSF= granulocyte-macrophage colony stimulating factor; p-H2AX = phospho Histone 2A Family Member X; GPX1 = glutathione peroxidase 1; PGE2 = prostaglandin E2; MUC2 = mucin 2; OAC = oesophageal adenocarcinoma

Table 4. Bile-Acid Exposure and Proliferation Experiments on Non-Malignant Oesophageal Cells or Tissue, Barrett's Oesophagus and Oesophageal Adenocarcinoma

NON-MALIGNANT OESOPHAGEAL CELLS OR TISSUE							
Authors	**Year**	**Study Type**	**Bile Acid(s)**	**Condition**	**Outcome**	**Molecule(s)**	**Result(s)**
Green et al.	2014	Human	TDCA	Non-malignant oesophageal cells	Proliferation	CDX1 and CDX2	TDCA did not enrich proliferation markers
Bus et al.	2014	Cell	DCA + GCA + TCDCA (mixture)	Non-malignant oesophageal cells	Proliferation	miR-143, miR-145 and miR-192	Mixture of BAs did not significantly change levels of miR-143, -145 and -192
BARRETT'S OESOPHAGUS							
Huo et al.	2020	Human	DCA	BO	Proliferation	phospho-p38	DCA increased phospho-38
Wang et al.	2018	Human	DCA	BO	Proliferation	Notch 1	DCA suppressed Notch 1
Cao et al.	2016	Cell	TDCA	BO	Proliferation	Notch 4	TDCA exposure significantly increased Notch 4

Banerjee et al.	2016	Human	UDCA	BO	Proliferation	Ki67	UDCA did not change Ki67 levels
OESOPHAGEAL ADENOCARCINOMA							
Yamada et al.	2014	Cell	DCA	OAC	Proliferation	CDX2	DCA activated CDX2
Chen et al.	2020	Cell	DCA	OAC	Proliferation	KLF 4 and OCT4	DCA promoted the expression of reprogramming factors KLF 4 and OCT4
Kanai et al.	2019	Cell	TCA	OAC	Proliferation	Calcein fluorescence ratio	TCA promoted cell proliferation in a dose-dependent manner
Liu et al.	2018	Cell	TCA	OAC	Proliferation	S1PR2	TCA promoted cell proliferation. TCA also activated S1PR2
Matsuzaki et al.	2013	Cell	CA and CDCA (separate experiments)	OAC	Proliferation	MiR-221 and MiR-222	CA or CDCA exposure increased levels of miR-221 and -222. CA and CDCA exposure degraded CDX2

Table 4. Bile-Acid Exposure and Proliferation in Experiments on Non-Malignant Oesophageal Cells or Tissue, Barrett's Oesophagus and Oesophageal Adenocarcinoma

TDCA = taurodeoxycholic acid; CDX1 / 2 = Caudal Type Homeobox 1 / 2; DCA = deoxycholic acid; GCA = glycocholic acid; TCDCA = taurochenodeoxycholic acid; miR-143 / 145 / 192 = micro-RNA 143 / 145 / 192; BO = Barrett's Oesophagus; UDCA = ursodeoxycholic acid; OAC = oesophageal adenocarcinoma; KLF4 = Krüppel-like Factor 4; OCT 4 = Octamer-binding transcription factor 4; TCA = taurocholic acid; S1PR2 = Sphingosine-1-phosphate receptor 2; CA = cholic acid; CDCA = chenodeoxycholic acid; miR-221 / 222 = micro-RNA 221 / 222.

Table 5. Bile-Acid Exposure and Apoptosis in Experiments on Non-Malignant Oesophageal Cells or Tissue, Barrett's Oesophagus and Oesophageal Adenocarcinoma

NON-MALIGNANT OESOPHAGEAL CELLS OR TISSUE							
Authors	**Year**	**Study Type**	**Bile Acid(s)**	**Condition**	**Outcome**	**Molecule(s)**	**Result(s)**
Bus et al.	2014	Cell	DCA + GCA + TCDCA (mixture)	Normal oesophageal cells	Apoptosis	miR-143	Mixture of BAs did not significantly change miR-143 levels
BARRETT'S OESOPHAGUS							
Taddei et al.	2014	Human	DCA	BO	Apoptosis	VEGF	DCA induced apoptosis
Feng et al.	2016	Cell	DCA	BO	Apoptosis	Bcl-2	DCA increased Bcl-2
Huo et al.	2020	Human	DCA	BO	Apoptosis	phospho-p38	DCA increased phospho-p38
Wang et al.	2018	Human	DCA	BO	Apoptosis	Notch 1 and Hes-1	DCA suppressed Notch 1 and Hes1
Feng et al.	2017	Cell	DCA	BO	Apoptosis	NF-κB and Bcl-2	DCA induced apoptotic resistance

Banerjee et al.	2016	Human	UDCA	BO	Apoptosis	CC3	UDCA did not change CC3 levels
Singh et al.	2018	Cell	LCA	BO	Apoptosis	SMAD4	LCA induced SMAD4, which in turn promoted apoptosis
OESOPHAGEAL ADENOCARCINOMA							
Matsuzaki et al.	2013	Cell	CA and CDCA (separate experiments)	OAC	Apoptosis	miR-221, miR-222 and CDX2	Neither CA nor CDCA increased levels of miR-221 and -222. CA and CDCA exposure degraded CDX2.

Table 5. Bile-Acid Exposure and Apoptosis in Experiments on Non-Malignant Oesophageal Cells or Tissue, Barrett's Oesophagus and Oesophageal Adenocarcinoma

DCA = deoxycholic acid; GCA = glycocholic acid; TCDCA = taurochenodeoxycholic acid; miR-143 = micro-RNA 143; BO = Barrett's Oesophagus; VEGF = vascular endothelial growth factor; Bcl-2 = B-cell lymphoma 2; Hes 1 = hairy and enhancer of split-1; NF-κB = Nuclear factor kappa-light-chain-enhancer of activated B cells; UDCA = ursodeoxycholic acid; LCA = lithocholic acid; SMAD4 = SMAD family member 4; CA = cholic acid; CDCA = chenodeoxycholic acid; OAC = oesophageal adenocarcinoma; miR-221 / 222 = micro-RNA 221 / 222; CDX2 = caudal type homeobox 2.

Table 6. Bile-Acid Exposure and Clonogenicity and Angiogenesis in Non-Malignant Oesophageal Cells or Tissue, Barrett's Oesophagus and Oesophageal Adenocarcinoma							
CLONOGENICITY							
Authors	**Year**	**Study Type**	**Bile Acid(s)**	**Condition**	**Outcome**	**Molecule(s)**	**Results**
Xu et al.	2017	Cell	DCA	OAC	Clonogenicity	cMyc and Lin 28	DCA increased clonogenicity in OAC cells
ANGIOGENESIS							
Bus et al.	2014	Human	DCA + GCA + TCDCA (mixture)	Non-malignant oesophageal cells	Angiogenesis	miR-145	BA exposure did not significantly change mi-145
Taddei et al.	2014	Human	DCA	BO	Angiogenesis	VEGF	DCA induced VEGF expression

Table 6. Bile-Acid Exposure and Clonogenicity and Angiogenesis in Non-Malignant Oesophageal Cells or Tissue, Barrett's Oesophagus and Oesophageal Adenocarcinoma

DCA = deoxycholic acid; OAC = oesophageal adenocarcinoma; GCA = glycocholic acid; TCDCA = taurochenodeoxycholic acid; miR-145 = microRNA 145; BO = Barrett's Oesophagus; VEGF = vascular endothelial growth factor

Supplementary Files

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- SupplementaryTableS1DiscoverOncology.pdf
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