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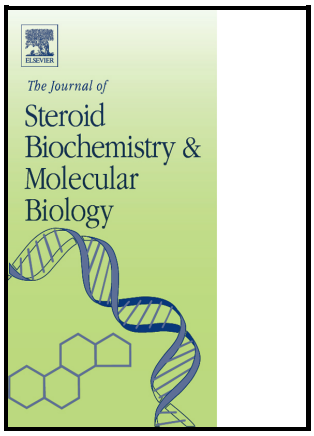

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Is cholesterol a risk factor for breast cancer incidence and outcome?

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Running title: Cholesterol and Breast Cancer

Abstract

Cholesterol plays important roles in many physiological processes, including cell membrane structure and function, hormone synthesis, and the regulation of cellular homeostasis. The role of cholesterol in breast cancer is complex, and some studies have suggested that elevated cholesterol levels may be associated with an increased risk of developing breast cancer, while others have found no significant association. On the other hand, other studies have shown that, for total cholesterol and plasma HDL-associated cholesterol levels, there was inverse association with breast cancer risk.

One possible mechanism by which cholesterol may contribute to breast cancer risk is as a key precursor of estrogen. Other potential mechanisms by which cholesterol may contribute to breast cancer risk include its role in inflammation and oxidative stress, which have been linked to cancer progression. Cholesterol has also been shown to play a role in signaling pathways regulating the growth and proliferation of cancer cells. In addition, recent studies have shown that cholesterol metabolism can generate tumor promoters such as cholesteryl esters, oncosterone, 27-hydroxycholesterol but also tumor suppressor metabolites such as dendrogenin A.

This review summarizes some of the most important clinical studies that have evaluated the role of cholesterol or its derivatives in breast cancer. It also addresses the role of cholesterol and its derivatives at the cellular level.

Abbreviations

5,6-EC, 5,6-epoxycholesterol; 27HC, 27-hydroxycholesterol; ABCA1, ATP-binding cassette sub-family A member 1; Akt, protein kinase B; ABCG5, ATP-binding cassette transporter G5 ; ABCG8, ATP-binding cassette transporter G8; ACAT, Acyl-coenzyme A:cholesterol acyltransferase; apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; BAX, Bcl-2–associated X; Bcl-xL; B-cell lymphoma-extra large; BMI, body mass index; ChEH, cholesterol-5,6-epoxide hydrolase; cholestane-3β,5α,6β-triol, CT; CYP27A1, cytochrome P450 family 27 subfamily A member 1; CYP7B1, cytochrome P450 family 7 subfamily B member 1; DDA, dendrogenin A; dendrogenin A, 5alpha-hydroxy-6beta-[2-(1H-imidazol-4-yl)-ethylamino]-cholestan-3beta-ol; EMT, epithelial-mesenchymal transition; EPIC, European Investigation into Cancer and Nutrition; ER, estrogen receptor alpha; ERK1/2, Extracellular signal-regulated kinases 1/2; FBW7, F-box protein W7; GNDF: glial cell line-derived neurotrophic factor; GDNF-RET, glial cell line-derived neurotrophic factor- Rearranged during transfection; GR, glucocorticoid receptor; HDL, high density lipoproteins ; HER2, human epidermal growth factor receptor 2; HMGCR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HR, hazard ratio; LDL, low density lipoprotein; LDLR, LDL receptor; Lp(a), lipoprotein A; LXR, liver-X-receptor; LXRα, liver-X-receptor alpha; LXRβ, liver-X-receptor beta; MDM2, mouse double minute 2; MMP9, matrix metalloproteinase 9; MPTP, Mitochondrial Permeability Transition Pore; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; NOR1, nuclear receptor 4A3; NPC1L1, Niemann-Pick C1-Like 1; Nur77, nuclear receptor 4A1; OCDO, 6-oxo-cholestan-3β,5α-diol; OR, odds ratio; PARP, poly-ADP ribose polymerase; PCSK9, Proprotein convertase subtilisin/kexin type 9; PDX, patient derived xenograft; PI3K, Phosphoinositide 3-kinase; PP2A, Protein phosphatase 2A; PPAR-α, peroxisome proliferator-activated receptors alpha; PR, progesterone receptor; RORγ, Retinoic acid receptor-related orphan receptor gamma; ROS, reactive oxygen species; RET, rearranged during transfection tyrosine kinase; RR, ratio risk/reward; SCP1, sterol carrier protein 1; SR-BI, Scavenger receptor class B, type I; SREBP1, sterol regulatory element-binding protein 1; SREBP2, sterol regulatory element-binding protein 2; StAR, Steroidogenic acute regulatory protein; StARD3, StAR Related Lipid Transfer Domain Containing 3; STAT3, Signal transducer and activator of transcription 3; TCGA, The Cancer Genome Atlas; TN, triple negative

Keywords: breast cancer, cholesterol, lipoproteins, metabolism, signaling pathway, clinical studies

1. Introduction

If the relationship between lipid metabolism dysregulation and the risk of cardiovascular disease is known and well established, the same relationship has not been clearly established for cancer and more specifically breast cancer. Research studies have been carried out to study the association between breast cancer risk and plasma cholesterol levels, or more specifically plasma HDL-cholesterol or LDL-cholesterol levels, or even levels of the corresponding associated proteins, which are apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB), respectively. These studies are all the more important since blood cholesterol levels are easily measurable in the clinical practice. If they prove to be true markers for the risk of breast cancer, their use could therefore be immediate and effective [1]. We will address this issue by successively discussing the contributions of epidemiological studies, in addition to targeted *in vivo* and *in vitro* studies on the subject.

2. Responses from epidemiological studies (Table 1)

2.1. Influence of Dietary Cholesterol

Dietary cholesterol may have an important role in breast cancer. Accordingly, the Kim et al [2] cohort study in Korea found that elevated cholesterol intake from the diet increased the risk of breast cancer for all women (HR=1.69 [1.01-2.82]). Hu et al [3] have also studied the influence of a diet rich in cholesterol. For this purpose, they collected data via food surveys from 24,771 individuals (men and women) between 1994 and 1997 in Canada. Among these individuals, there were 19,732 cases (different types of cancer) and 5,039 controls, and the questionnaires aimed at recording eating habits from the participants during the two years preceding the study. Results of this analysis showed that a diet rich in cholesterol increased the risk of cancer of the stomach, colon, rectum, pancreas, lungs, kidneys, bladder, non-Hodgkin lymphomas, and breast (HR=1.45 [1.14-1.85]). After analyzing the effect of menopause, it

appeared that only postmenopausal women were more at risk of developing breast cancer (HR=1.48 [1.07-2.07], against HR=1.10 [0.75-1.62] for premenopausal women). On the other hand, elevated dietary cholesterol decreased the risk of prostate cancer, and no association was demonstrated between the consumption of cholesterol and cancer of the ovary, testes, brain, and leukemias. A meta-analysis from Li et al. show the positive association between dietary cholesterol levels and breast cancer risk (HR=1.29[1.06-1.56]) [4].

2.2. Association with circulating cholesterol levels and cancer risk in general: Each cancer has specific requirements

Large cohorts conceptually constitute one of the richest and most reliable source of information. However, not all agree on the potential effect of cholesterol on the risk of cancer incidence. There are different assumptions underlying the idea that cholesterol is important for the initiation or development of breast cancer, and even cancer in general. One of them is based on the observation that, since cholesterol is a major element involved in the composition of various cellular membranes, it could therefore be essential for cancer cells, which may require rapid cholesterol acquisition to synthesize new membranes. However, it appears that cholesterol does not affect all types of cancer in the same manner. For example, Kitahara et al [5] prospectively studied data from 1,189,719 Koreans (men and women) and reported that high levels of total serum cholesterol (greater than 160 mg/dL) were positively associated with prostate cancer (HR=1.24 [1.07-1.44]) and colon cancer risk (HR=1.12 [1.00-1.25]) for men, and breast cancer risk (HR=1.17 [1.03-1.33]) for women. However, they were associated with a reduced risk of liver cancer (HR=0.42 [0.38-0.45] for men and HR=0.32 [0.27-0.39] for women), stomach cancer (HR=0.87 [0.82-0.93] for men and HR=0.86 [0.77-0.97] for women), and lung cancer for men (HR=0.89 [0.82-0.96]). Knekt et al. [6] studied the relationship between serum cholesterol levels and the risk of developing different types of cancer in a Finnish cohort of 39,268 subjects (men and women) aged 15 to 99 years. Cholesterol levels

were negatively associated with the risk of cancer (all cancers combined) for non-smoking men in particular, but also for women. However, the study's conclusions also differed depending on the type of cancer. Accordingly, men with elevated cholesterol levels were less at risk of developing prostate cancer, lymphoma or leukemia, but more at risk of developing colorectal cancer. On the other hand, women with elevated cholesterol levels were less at risk of developing colorectal cancer. Osaki et al [7] retrospectively examined the association in Japan between the incidence of liver or breast cancer and the metabolic syndrome, which plays a role in the metabolism serum cholesterol. Their study included 23,715 subjects (men and women) that were followed for 9.1 years on average, and it highlighted a significant relationship between the presence of the metabolic syndrome and the risk of liver cancer (HR=1.89 [1.11-3.22] for men and HR=3.67 [1.78-7.57] for women), and with the risk of breast cancer for women (HR=2.87 [1.67-4.94]). Importantly, the latter association increased considerably when only menopausal women aged 55 and over were taken into account (HR=6.73 [2.93-15.43]). The Metabolic syndrome and Cancer Project (Me-Can), which as its name indicates was set up to examine the relationship between cancer and the metabolic syndrome, combines seven cohorts from Norway, Sweden and Austria, and therefore brings together 289,273 men and 288,057 women that were on average followed for 11.7 years [8]. Analysis of the data from these cohorts shows that elevated total cholesterol levels are associated with a reduced risk of cancer in general for men (HR=0.94 [0.88-1.00] when comparing the fifth quantile with the first quantile). These findings more specifically hold true for the risk of liver cancer (HR=0.14 [0.07-0.29]), pancreatic cancer (HR=0.52 [0.33-0.81]), skin cancer excluding melanomas (HR=0.67 [0.46-0.95]), and lymphatic cancers (HR=0.68 [0.54-0.87]). For women, we observed a reduced risk of cancer in general (HR=0.86 [0.79-0.93]) and more specifically of gallbladder cancers (HR=0.23 [0.08-0.62]), melanoma (HR=0.61 [0.42-0.88]), lymphatic cancers (HR=0.61 [0.44-0.83), and breast cancer (HR=0.70 [0.61-0.81]). The nested case-

control study by Agnoli et al. [9] also aimed at measuring the impact of the metabolic syndrome (both as a whole and by considering each of its elements separately) on the incidence of post-menopausal breast cancer. According to their results, displaying the metabolic syndrome increased the risk of developing breast cancer (RR=1.58 [1.07-2.33]), an increase, which became all the more significant with the addition of each element of the metabolic syndrome (waist circumference over 40 inches (men) or 35 inches (women), blood pressure over 130/85 mm Hg, fasting triglyceride (TG) levels over 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol levels less than 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar over 100 mg/dl [10]). On the other hand, taken separately, only low levels of serum HDL-cholesterol and high levels of serum triglycerides were, according to this study, significantly and positively associated with the risk of breast cancer.

2.3. Association between plasma cholesterol and breast cancer risk

Some studies tend to show that cholesterol is rather associated with a reduced risk of breast cancer. Accordingly, the cohort studied by Tulinius et al [11] included 11,580 Icelandic women and 11,366 Icelandic men and showed that serum cholesterol levels were negatively associated with breast cancer (and cancer in general, all sites combined) for women. In their study, Llanos et al [12] included 97 cases and 102 controls, all African-American women, and they found that elevated levels of total cholesterol and LDL-cholesterol reduced the risk of breast cancer (OR=0.46 [0.25-0.85] for total cholesterol and OR=0.41 [0.21-0.81] for LDL-cholesterol), while low levels of HDL-cholesterol increased this risk (OR=1.99 [1.06-3.74]). A prospective study of 7,557 subjects from the French cohort “Vitamin Supplement and Antioxidant Minerals Study” [13] allows to analyze the relationship between cancer -in general, then more specifically breast and prostate cancer - and triglyceride, total cholesterol and glucose levels. According to the results, none of the elements studied were significantly linked to the risk of prostate cancer, but total cholesterol levels were inversely associated with

the risk of cancer in general (HR=0.91 [0.82-1.00] for a 1mmol/L increase) and more specifically with the risk of breast cancer (HR=0.83 [0.69-0.99] for a 1 mmol/L increase). The observation was the same for HDL-cholesterol levels (HR=0.61 [0.46-0.82] for the risk of cancer in general; HR=0.48 [0.28-0.83] for the risk of breast cancer, considering an increase of 1 mmol/L), and for apoA-I levels (HR=0.56 [0.39-0.82] for the risk of cancer in general considering an increase of 1 g/L, and HR=0.36 [0.18-0.73] for the risk of breast cancer). These findings suggest that the type of lipoprotein to which cholesterol is associated with, may alter cancer risk. A more recent meta-analysis suggested a small inverse association between total cholesterol and HDL-cholesterol levels and the risk of breast cancer [1]. However, no association was found with LDL-cholesterol levels in this study.

2.4. BMI, triglycerides, cholesterol and breast cancer

Other studies have demonstrated an association with other metabolic factors and with the type of tumor. Kim et al. [2] conducted a case-control study in Korea between 2004 and 2005 including 690 cases and 1,380 controls. The results of their study showed that premenopausal women with high levels of HDL-cholesterol were less at risk of developing breast cancer (OR=0.49 [0.33-0.72] when comparing concentrations of HDL-cholesterol greater than or equal to 60 mg/dl to concentrations of less than 50 mg/dl). This finding was even more important in non-obese women (OR=0.34 [0.22-0.53]). Furthermore, having both low HDL-cholesterol levels (below 50 mg/dL) and high triglyceride levels (above or equal to 150 mg/dL) further increased the risk of ER- and PR-negative breast cancer (OR = 2.20 [1.32 - 3.67]). By analyzing data from two cohorts (a total of 38,823 Norwegian women), Furberg et al [14], also concluded that there was an inverse association between the risk of breast cancer and HDL-cholesterol levels (RR=0.75 [0.58-0.97] by comparing the highest quartile to the lowest quartile). This association was found to be stronger for women whose BMI was greater than 25 (RR=0.43 [0.28-0.67]). A year later, the same authors used data from 206 women in

the Norwegian Energy Balance and Breast Cancer Aspects (EBBA) cohort, ages 25 to 35, and noted that women with BMI over 23.6 had a salivary estradiol concentration that increased with low level of serum HDL-cholesterol, and that women with a BMI greater than 23.6 and an elevated LDL-cholesterol/HDL-cholesterol ratio (greater than 2.08, and in the 75th percentile) had salivary concentrations of estradiol more elevated than others [15]. Their results indicate that BMI is strongly associated with estradiol concentration, that HDL-cholesterol levels are inversely associated with serum leptin, insulin, and dehydroepiandrosterone sulfate concentrations, and that breast density is positively associated with a so-called "healthy" metabolic profile (low BMI and elevated HDL-cholesterol serum concentrations) and elevated salivary progesterone concentrations. These results tend to identify HDL-cholesterol as a biomarker for breast cancer risk: low levels of HDL-cholesterol would be associated with an "at risk" hormonal profile with particularly elevated levels of estrogens in the case of overweight or obese women. Lofterød et al. [16] analyzed the levels of triglycerides and HDL-cholesterol in 434 women with breast cancer, measured before their diagnosis: considering breast cancers as a whole, no significant relationship was found between the risk of mortality and the levels of triglycerides, HDL-cholesterol, or cholesterol. However, women with Triple-Negative breast cancer and elevated triglyceride levels were more at risk of dying (HR=2.99 [1.17-7.63] when comparing the highest tertile to the lowest), and those with elevated levels of HDL-cholesterol less at risk of dying (HR=0.33 [0.12-0.89]). On the other hand, no significant association were demonstrated for luminal A breast cancer (defined as follows: ER-positive, PR-positive, HER2-negative, and Ki-67 <20%) or B (ER-positive and / or PR-positive, HER2-positive, or HER2-negative and Ki-67 ≥ 20% or PR-negative) and the levels of triglycerides, total cholesterol, or HDL-cholesterol. Finally, patients carrying HER2-positive tumors (ER-negative, PR-negative, HER2-positive) also did not see their mortality associated in any way with total cholesterol or HDL-cholesterol levels. However, they were less at risk of dying if

they had elevated triglyceride levels (HR=0.06 [0.01-0.55]). A meta-analysis by Ni et al [17] also slightly nuances the effect of cholesterol: by combining the results of 15 prospective cohort studies (1,189,635 participants) on the association between the risk of breast cancer and the levels of cholesterol and triglycerides, they did not show any significant association, except in the case of postmenopausal women, for whom elevated concentrations of HDL-cholesterol were associated with a decreased risk of breast cancer (RR = 0.77 [0.64 - 0.93]). Borgquist et al. [18] used data from the Swedish cohort "Malmö Diet and Cancer Study", which included 17,035 women and 11,063 men between 1991 and 1996, and which measured the initial levels of apoA-I and apoB in the entire cohort and HDL-cholesterol and LDL-cholesterol levels for 5,281 of the participants. Their results did not show any association between these biomarkers and the overall risk of cancer in all of the cohort's data. On the other hand, apoB levels were positively associated with the risk of cancer in men (HR=1.06 [1.01-1.10]), inversely associated with the risk of breast cancer for women (HR= 0.92 [0.86-0.99]), and associated with an increased risk of colorectal cancer for both women and men (HR=1.08 [1.01-1.16]). ApoA-I, which is the main protein of HDL, was inversely associated with the risk of lung cancer for both women and men (HR=0.88 [0.80-0.97]). HDL-cholesterol levels were inversely associated with the overall risk of cancer (HR=0.89 [0.84-0.94]), and LDL-cholesterol levels were not significantly associated with the overall risk of cancer. No significant association was demonstrated between HDL-cholesterol or LDL-cholesterol levels and breast cancer.

On the contrary, other studies have indicated that elevated HDL-cholesterol levels are associated with an increased risk. For example, Martin et al. [19] carried out a nested case-control study including 4,690 women followed for 10 years on average, and they chose among them 279 cases and 558 controls. Their results indicate that HDL-cholesterol levels are positively associated with the risk of breast cancer (HR=1.23, p=0.05 when comparing the 75th with the 25th percentile) and similar results were observed for apoA-I levels (HR=1.28,

p=0.02). On the other hand, non-HDL cholesterol levels were negatively associated with the risk of breast cancer (HR=0.81, p=0.03), and the same was true for apoB levels (HR=0.78, p=0.01). Katzke et al [20] used a sub-cohort of the EPIC-Heidelberg cohort and found that women with elevated levels of circulating apoB and triglycerides were less likely to develop breast cancer (HR=0.71 [0.52-0.98] and HR=0.65 [0.46-0.92], respectively, comparing the highest quartile to the lowest quartile), while women with elevated levels of HDL-cholesterol were more at risk (HR=1.39 [1.01-1.93]). The same authors also reported that men with elevated levels of Lp(a) - a particularly atherogenic LDL particle - were more likely to develop prostate cancer (HR=1.43 [1.02-2.03]), and individuals with elevated levels of apo(a) were less likely to develop lung cancer (HR=0.52 [0.30-0.91]). Furthermore, elevated levels of triglycerides, HDL-cholesterol, apo(a), and Lp(a) were associated with a decrease in cancer mortality (all cancers combined, HR=0.71 [0.54-0, 94], HR=0.67 [0.50-0.91], HR=0.71 [0.54-0.93], and HR=0.74 [0.57-0.98], respectively). Similarly, a meta-analysis also showed that total cholesterol and HDL-cholesterol were correlated with improved survival of cancer patients [21].

LDL-cholesterol, in particular, could also be a risk factor for breast cancer. Studies by Rodrigues Dos Santos et al [22] prospectively analyzed the lipid profile of 244 women with breast cancer and found that patients with elevated LDL-cholesterol levels had larger tumors, at a more advanced stage of differentiation, with a faster proliferation rate using Ki67 as a marker. The latter tumors were more often HER2-positive, and were diagnosed at later stages. Elevated LDL-cholesterol levels were also associated with lower survival (after adjustment for the tumor stage and immunohistochemical subtype). However, it should also be noted that interactions between certain risk factors could occur. Accordingly, Ha et al. [23] included 170,374 pre-menopausal Korean women in their analysis, and by adjusting only for age, found a significant positive association between total serum cholesterol levels and breast cancer risk

(HR=1.11 [1.04-1.19] for an increase of 1 mmol/L). This association remained significant after also adjusting for known risk factors for breast cancer (age at the onset of the first period, age at the time of first delivery, having or not having children, taking hormone treatments, length of the breastfeeding period, smoking, alcohol consumption), but this significance disappeared as soon as the BMI was taken into account (HR=1.09 [1.01-1.18], against HR=1.06 [0.98-1.15]).

Finally, other studies did not permit to conclude as to the existence of a possible association between serum cholesterol and breast cancer, whether positive or negative. Accordingly, a prospective study by Gaard et al. [24] used data from 31,209 Norwegian women aged 20 to 54 to study the relationship between blood lipid levels and the risk of breast cancer. They found that women whose total cholesterol levels were in the lowest quartile appear to be less at risk of developing breast cancer (RR=0.87 [0.61-1.23]), but this result was not statistically significant. The same was true for HDL-cholesterol (RR=1.02 [0.73-1.42]), LDL-cholesterol (RR=0.93 [0.67-1.29]), and triglycerides levels (RR=0.82 [0.58-1.16]). The cohort study reported by Hiatt et al [25], involving 160,135 subjects (men and women) from California, also found no association between cholesterol levels and cancer risk, except for lymphomas and cervical cancers in men (with decreased risk when comparing the highest quintile to the lowest quantile). Steenland et al [26] studied 1,250 cases of cancer (657 men and 593 women) among 14,407 subjects from the "National Health and Nutrition Survey I" cohort (1970-1987) and concluded that there was a slight inverse association between cholesterol levels and cancer risk (in general) for men but not for women. Moorman et al [27], with their nested case-control study, tested the hypothesis that women with high levels of HDL-cholesterol were more at risk of developing breast cancer. This idea was based on the fact that many known risk factors for breast cancer are associated with elevated levels of HDL-cholesterol. These factors include estrogen levels, nulliparity or low parity, alcohol

consumption, especially in pre-menopausal women. The study population included 95,000 women. Among them, 200 cases were randomly chosen, and each of them was associated with a control. The results of this study did not show any significant difference between HDL-cholesterol levels from the cases and those of the controls. They nevertheless revealed a significant interaction between serum HDL-cholesterol levels and the menopausal status: postmenopausal women with breast cancer had reduced HDL-cholesterol levels (3.48 mg/dL, [-7.05-0.09]) compared to their controls. On the other hand, pre-menopausal women with breast cancer, had elevated HDL-cholesterol levels (2.05 mg/dl, [-0.94-5.03]). However, no significant association between HDL-cholesterol levels and the risk of breast cancer was demonstrated in either group. His et al. [28] analyzed 583 cases and 1,043 controls from the prospective "E3N" cohort and did not find any significant association between serum lipid concentrations and risk of breast cancer or mortality from breast cancer. On the other hand, Melvin et al [29], in Sweden, included 234,494 women, for whom triglycerides, total cholesterol, and glucose levels were measured. For 27,394 of these women, HDL, LDL, apoB and apoA-I levels were also available. Their study found a slight decrease in the risk of breast cancer when triglyceride levels increased (HR=1.01 [0.94-1.09], 0.93 [0.86-1.00] and 0.91 [0.84-0.99] by comparing the second, third and fourth quartile to the first quartile, respectively), but no other significant association between the risk of breast cancer and total cholesterol, HDL, LDL, apoB, or apoA-I levels emerges from their study. Kucharska-Newton et al [30] (case-control study nestled in the "Atherosclerosis Risk in Communities" cohort), were also unable to detect a significant association between HDL-cholesterol levels and the risk of breast cancer for all the combined menopausal statuses, but nevertheless concluded that there was a slightly significant association for premenopausal women (HR=1.67 [1.06-2.63]). Similarly, no association between circulating lipid levels and mammographic density or breast cancer risk was observed in the Nurses' Health Study (NHS) and Nurses' Health Study II

(NHSII) reported by Lucht et al. [31]. Finally, in a population of breast cancer patients, an important recent studies identified a positive association between Ki67 expression in tumor and Total and LDL-cholesterol and an inverse correlation between Ki67 expression in tumor and HDL-cholesterol and apoA-I serum levels [32].

3. Importance of other Factors regulating cholesterol metabolism in cancer

3.1. Role of oxysterols

Some cholesterol metabolites have also been proposed to play a role in breast cancer [33]. Among them, oxysterols represent an expanding family of bioactive compounds, which are produced by enzymatic and non-enzymatic oxygenation of cholesterol [34]. Interestingly, two categories of cholesterol metabolites with opposite properties regarding cancer development have been described: oxysterols with tumor promoter activities such as 27-hydroxycholesterol that can stimulate ER(+) breast cancer [35] and oncosterone that stimulates both ER(+) and TN breast cancer [36] on one hand, and tumor suppressor properties such as dendrogenin A [36-40] . According to their chemical structure, these molecules are capable of activating various nuclear receptors such as the ERα [41], LXRα and LXRβ [42], GR [36], RORγ [43], and the membrane receptor smoothened [44], thereby regulating tumor progression [45]. It was therefore proposed that cholesterol metabolites, rather than cholesterol itself, played a role in breast cancer. The nested case-control study by Lu et al. [46], in the "European Investigation into Cancer and Nutrition" (EPIC) cohort, included 530 cases and 1036 controls (up to two per case) and made it possible to analyze the relationship between serum concentrations of 27-hydroxycholesterol (27HC) and the risk of breast cancer. 27HC is probably the most studied oxysterol in breast cancer (Table 2). According to their results, 27HC levels were not globally associated with the risk of breast cancer (RR=0.9 [0.66-1.22] when comparing the fourth quartile to the first one), but postmenopausal women with elevated levels of 27HC were less at risk for breast cancer (RR=0.56 [0.36-0.87], while there was no significant

difference in the case of premenopausal women (RR=1.33 [0.75-2.38]). A more recent study has also examined the role of other types of oxysterols. In their work, Kloudova-Spalenkova et al. showed that plasma levels of 7α-hydroxycholesterol and 27-hydroxycholesterol were reduced in patients with small tumors compared with those of patients with larger tumors [47]. Furthermore, serum levels of 5,6β-epoxycholesterol (5,6β-EC) and cholestane-3β,5α,6β-triol (CT) were decreased in patients with stage IA disease compared with those of patients with larger tumors or more advanced tumor stage. Interestingly, patients with increased serum CT levels had reduced disease-free survival compared with patients with reduced levels of this oxysterol. Interestingly, plasma levels of 27HC have been positively correlated with physical activity and alcohol consumption, and women smoking at blood collection and are negatively correlated with full-term pregnancy [48]. Finally, a recent study has identified a strong correlation between free and total oxysterol/sterols in breast cancer patients [49]. Taken together, these studies suggest that serum oxysterols may also contribute to the disease aggressiveness.

3.2. Role of drugs targeting cholesterol metabolism

If cholesterol is an important player in cancer, it follows that targeting its metabolism should allow us to mitigate its effects on cancer. In that regard, Eliassen et al [50] have examined the role of lipid-lowering drugs in general and did not highlight any significant effect of drugs on breast cancer risk (RR=0.99 [0.86-1.13]). The same observation could be made with regard to statins (RR=0.91 [0.76-1.08]). The same authors also analyzed serum cholesterol levels and did not show any significant association (RR=1.04 [0.91-1.17] by comparing the highest levels to the lowest one). Other studies have obtained similar results [51, 52]. However, while breast cancer incidence does not appear to be affected by statins, studies have suggested that statin may reduce recurrence and mortality associated with breast cancer [53]. Consequently, Li et al. have shown in a retrospective study that long-term statin use was

associated with improved overall survival and disease-free survival [54]. Furthermore, studies from Kim et al. also showed that high statin usage was associated with reduced cancer incidence in men and women, when compared to hypercholesterolemic patients [55]. In a more recent study, Bjarnadottir et al. found that no association between cancer mortality and *HMGCR* expression or statin use [56]. However, they observed an association between *HMGCR* expression and unfavorable tumor characteristics. Importantly, dose and duration of statin treatment were not available for this study. A recent study has since confirmed the importance of statin in the reduction of breast cancer risk recurrence among postmenopausal patients [57]. Furthermore, a meta-analysis has also suggested that statin treatment is associated with reduction from all-cause mortality in breast cancer patients and appears to be associated with reduced mortality due to breast cancer [58]. The BIG 1-98 study was a randomized, phase III, double-blind trial that enrolled 8,010 postmenopausal women with early-stage, hormone receptor–positive invasive breast cancer from 1998 to 2003. In their study, Borgquist et al. showed that statin treatment in conjunction with adjuvant endocrine therapy (i.e, tamoxifen, letrozole) was associated with improved disease-free survival [59]. Fibrates, which are another class of hypolipidemic drugs, have also been examined. Fibrates are activator of peroxisome proliferator-activated receptors alpha (PPAR-α), and they regulate fatty acid and lipoprotein metabolism [60]. Limited data are available on this drug, but a meta-analysis has shown that fibrates did not have any effect on cancer risk or cancer mortality [61].

3.3. Why is it difficult to observe a clear effect of cholesterol on cancer in epidemiological studies

At first glance, differences in the results between the different studies may be disconcerting. Several hypotheses can come into play. On one hand, some of the risk factors for breast cancer are interconnected, and depending on the adjustments that are made, conclusions may change. For example, taking BMI into account in a regression model can mask

the effect of cholesterol. On the other hand, the impact of cholesterol ingested via the diet can be difficult to measure, since it is difficult to accurately transcribe the composition of a subject's diet. The effect can also depend on the type of cholesterol measured (HDL, LDL, or total). Importantly, the prevalence of certain types of nutrients may also varied according to the examined population. Finally, the impact of cholesterol can change depending on when it is measured (before or after the diagnosis). There is indeed a bias, because cancer itself can increase or decrease the levels of cholesterol in the blood [1, 62, 63]. As mentioned earlier, it is also important to note that some factors involved in the development of breast cancer can also affect plasma cholesterol levels and influence other pathologies such as atherosclerosis [64, 65]. In the case of estrogens, which play an important role in breast cancer, they also regulate plasma cholesterol levels [66] and may therefore regulate cancer risk directly and indirectly. Therefore, the effect of cholesterol depends on many variables including BMI, plasma triglycerides levels, estrogen levels, the type of tumor (luminal A, luminal B, triple negative, HER2+), dietary components, and possibly the environment. A recent studies also identified a positive non-linear and age-dependent association between HDL-cholesterol levels and breast cancer [67]. In this study, the authors observed that the overall association between total plasma cholesterol or LDL-cholesterol and breast cancer were both positive. As in the case of HDL, the association between BMI and breast cancer was confounded by age, and there was no association with plasma triglyceride. Finally, lipid treatments gave erroneous associations [67]. Other uncontrolled parameters (Lp(a), PCSK9, diabetes, kidney disease, bacterial or viral infection) can also alter plasma cholesterol levels. Interestingly, a dysregulation cholesterol regulatory gene has been identified in patients with breast cancer [68]. Consequently, in vitro studies or in vivo studies using animal models have been performed to more specifically address the role of cholesterol. It is also important to note that lipoproteins can carry cholesterol but can also carry other types of lipids or molecules

depending on the individual, their diet, or environment. For example, HDL have been shown to carry F_2-isoprostanes [69, 70], which are prostaglandin-like compounds produced by radical peroxidation of arachidonic acid. Interestingly, F_2-isoprostanes levels have been shown to be elevated in patients with atherosclerosis [71, 72] and cancer [73]. We have also recently shown that phytoprostanes, which are produced by the radical peroxidation of alpha linolenic acid, can control cancer cell proliferation and migration [74] and may therefore play a role in cancer progression [75].

Phytosterols are plant sterols that are not produced by animals. Their structure is similar to that of cholesterol, with differences in their side chain. Phytosterols can be sub-divided into sterol and stanols, with the latter ones being saturated sterols (e.g., campestanol). The most abundant phytosterols in the human diet are β-sitosterol and campesterol [76]. Phytosterols have also been shown to play an important role in the development of cancer. While some epidemiological studies have suggested a role for phytosterols in the inhibition of cancer growth, the impact of these analyses remains limited due to the lack of controlled studies [77]. However, animal experimentation started to provide additional insights into the role of phytosterols. In the case of breast cancer studies, feeding animals with a phytosterol-enriched diet was associated with reduced tumor formation in a xenograft model of breast cancer tumor cells [78, 79]. *In vitro* studies have obtained consistent results with phytosterols inhibiting cholesterol synthesis [80], reducing cellular proliferation via the regulation of cell cycle progression and apoptosis [80, 81]. Despite their similarity, cholesterol and phytosterols are not absorbed by the intestine at the same levels [82]. In fact, the intestinal absorption of plant sterols is discriminated against, and plasma and tissue concentrations of phytosterols are normally at least one hundred times lower than those of cholesterol [83]. However, once in the circulation, cholesterol and phytosterols are associated with HDL and their metabolisms are linked [84]. Several clinical studies have demonstrated the hypocholesterolemic role of a diet

enriched in phytosterols [85-87]. In most cases, reduced levels of plasma cholesterol have been observed with increased dietary uptake of phytosterols. Taken together, these data suggest that plasma phytosterol levels may also play a role in breast cancer risk, and phytosterol effect on plasma cholesterol in additional to its direct effect on breast cancer cell may explain some of the discrepancies observed in clinical studies.

Finally, a recent study has examined the consequence of genetic alterations associated with a modulation of circulating lipid levels [88]. In their approach, the authors specifically examined the effect of genetic variants associated with modulation in HDL-cholesterol or LDL-cholesterol. They found that genetically elevated LDL-cholesterol and HDL-cholesterol levels are associated with an increased breast cancer risk. However, they could not rule out that these genetic alterations could have other effects not involving plasma cholesterol levels.

Additional considerations

In general, small sample populations and the small number of cancer cases for some studies may also affect the results [15-17, 19]. Furthermore, in some cases, not sufficient details regarding the menopausal status [12], the presence of anti-cholesterol therapy [5, 8, 18, 23], family history for other cancer types or other comorbidities [16, 17, 29] were fully investigated.

Hormone replacement therapy may affect the metabolism of lipid and, therefore, the type of tested population (with or without hormone replacement therapy) may also explain the different results obtained in post-menopausal women [14, 19, 20].

In meta-analyses, it may be difficult to compare different studies because of clinical or methodological differences between studies. Important variations may include geographic region, menopausal status, number of cases, follow-up duration, and covariate analysis selection [17]. In some cases, serum or plasma samples were collected over a period of several

years and analyzed years later. Lipids may exhibit significant decreased in stability with over year of storage even at -80°C [89] . In some cases, non-fasting blood sample were used [19]. Additionally, alcohol intake may also lead to elevated HDL-C levels [90], and is associated with increased breast cancer risk. This aspect was not always specified in these studies. In diet studies [2], the effect of dietary cholesterol may also be confounded by the presence of nitrate and nitrite, vegetable, soy products, or even cholesterol auto-oxidation products. The metabolic syndrome also played a role in cholesterol metabolism and breast cancer [9, 15]. However, the metabolic syndrome also affects circulating levels of insulin, which plays essential roles in the regulation of cellular proliferation as well as circulating estrogen and testosterone levels. In particular, estrogen levels also play a role in the regulation of circulating cholesterol levels [91]. In particular, endogenous estrogen levels and hormone replacement therapy lead to decreases LDL-C and increases HDL-C levels. Importantly, cholesterol is a precursor of estrogen, and estrogen can be transported by lipoproteins containing cholesterol such as HDL [92]. Estrogen exposure has also been associated with increased risk of developing breast cancer [93]. Taken together, these data also suggest a complicate and potentially confounding role for estrogen in the regulation of cholesterol metabolism and breast cancer.

Taken together, these studies suggest the need for a significant project with a large population. This project would finely identify and analyze all the parameters that are known to play a role in the metabolism of cholesterol/sterol and test their effect on breast cancer risk.

4. Responses from in vivo preclinical studies

Epidemiological studies are not the only ones to provide answers to our initial question, namely, is cholesterol a risk factor for breast cancer or does cholesterol affect cancer progression? Important data have also been obtained with interventional studies with animal models or human clinical studies.

4.1. Dietary studies in animal models

Dos Santos et al [94] were interested on examining the impact of elevated cholesterol consumption on tumor growth with mouse models. Using three cancer cell lines (MDA-MB-231, HTB-20, and 4T1) and two different diets (a diet enriched in cholesterol diet and a control diet), they showed that an enriched-cholesterol diet could stimulate *in vivo* xenograft tumor growth by 20%. In one of their mouse models, enriching the diet with cholesterol also led to an increase in metastasis formation. Cleary et al [95] used a mouse model of post-menopausal breast cancer (transgenic MMTV-TGF mice) and tested two diets - a low and a high fat diet - and found that mice on a high-fat diet developed breast tumors faster than those on a low-fat diet. Furthermore, within the first group, obese mice developed tumors the fastest. With very similar methods, Dogan et al [96] observed an increased incidence of palpable mammary tumors following feeding with a diet enriched in fat and more particularly for mice that were prone to obesity. Their results also showed that serum leptin levels were more elevated in obesity-prone mice fed a high-fat diet. Dogan et al also noted that some pro-apoptotic proteins (PARP, caspase-3, Bcl-xL) levels were more elevated in tumors from mice on a low-fat diet than from mice on a high-fat diet. Pelton et al [97] also studied the impact of a high-fat diet on the development of mammary tumors in mice, but this time by injecting MDA-MB-231 breast cancer cells into immunodeficient mice. Mice fed a high-fat diet displayed increased serum cholesterol levels and developed mammary tumors faster. No difference was noted in terms of intra-tumor cholesterol levels, or in terms of plasma insulin levels. Mice on a high-fat diet but also taking ezetimibe - a treatment used to decrease intestinal cholesterol absorption - developed tumors slower than those without treatment. This finding highlights the role of dietary cholesterol on disease progression. On the other hand, after sorting all the mice (regardless of the diet or treatment followed) into two groups according to their serum cholesterol level, Pelton et al found that hypercholesterolemic mice developed larger tumors,

and identified a positive correlation between serum cholesterol levels and tumor size. Within these two groups (hypercholesterolemic mice versus all the others), no difference in insulin levels were highlighted, and considering all the mice as a whole, no association between tumor development and insulin levels was revealed.

4.2. Role of protein regulating cholesterol metabolism in animal models

Alikhani et al [98] used a different approach to induce dyslipidemia. They injected metastatic (Mvt-1) and non-metastatic (Met-1) breast cancer cells derived from the transgenic MMTV-PyMT / FVB-N mouse model in *Apoe*$^{-/-}$ mice (mouse model in which serum cholesterol levels are elevated) or in control wild-type mice. Both groups of mice were on a high cholesterol/high fat diet. During this experiment, *Apoe*$^{-/-}$ mice displayed accelerated tumor growth and increased lung metastasis. Injection of Mvt-1 cells also led to increased metastases in *Apoe*$^{-/-}$ mice compared to control mice. Administering a PI3K inhibitor (BKM120) to the mice slowed down tumor growth and metastasis formation in *Apoe*$^{-/-}$ mice, suggesting that this signaling pathway is implicated in this effect. Gallagher et al [99] also used two immunodeficient mouse models (*Rag1*$^{-/-}$/*Ldlr*$^{-/-}$ and *Rag1*$^{-/-}$/*ApoE*$^{-/-}$) and two non-immunodeficient mouse models (*Ldlr*$^{-/-}$ and *ApoE*$^{-/-}$) to study the impact of elevated LDL-cholesterol levels on breast cancer derived from MDA-MB-231 cells or from mice overexpressing Her2/Neu, respectively. The tumors generated by either of these models strongly expressed LDLR and their size increased in mice with elevated serum LDL-cholesterol levels. By inhibiting LDLR expression, tumors of mice overexpressing Her2/Neu displayed reduced growth in *Ldlr*$^{-/-}$ and *ApoE*$^{-/-}$ mice. Gallagher et al also reported, after analysis of a public database, that elevated *LDLR* expression in human breast cancer tissues was associated with decreased overall and relapse-free survival in women treated with systemic hormonal therapy. These data are consistent with our studies using PyMTTg mice in which we have showed that a diet enriched in cholesterol is associated with accelerated tumor formation

and increased tumor aggressiveness [100]. We have also shown that reduced SR-BI-mediated cholesterol entry into tumor is associated with reduced tumor formation in a mouse model of xenograft tumors [101]. However, our more recent studies suggest that the promoting the elimination of cholesterol via apoA-I or apoE expression in cancer cells has different effects depending on the type of breast cancer cells. In MCF-7 cells, it appears that a dysregulation of cholesterol metabolism can also modulate activation of the epithelial–mesenchymal transition (EMT) pathway [102]. Finally, consistent with a role for cholesterol in breast cancer, Ehmsen et al have shown that triple negative patient tumors and their corresponding PDXs as well mammospheres displayed an increased expression of proteins involved in the endogenous synthesis of cholesterol [103]. Taken together, these data suggest that efficient use of cholesterol by cancer cells can facilitate tumor growth, and that removal or reduced cholesterol acquisition by cancer cells can limit tumor progression.

4.3. Impact of cholesterol metabolites in animal models

Finally, there are also several important studies that have implicated various metabolites of cholesterol. These studies are important as they may explain some of the discrepancies that have been observed with epidemiological studies: Different cholesterol metabolites appear to have very different roles in the regulation of breast cancer risk and development. For example, the role of 27HC, which is both a ligand of the ERα as well as a ligand of the LXR, has been examined. Nelson et al [35] have shown that it stimulates *in vivo* tumor growth (ER-dependent and LXR-dependent) in a mouse model. Inhibiting the conversion of cholesterol to 27HC by an inhibitor of the cytochrome P450 oxidase CYP27A1, which is responsible for the synthesis of 27HC, allows to reduce the pro-proliferative effects of 27HC. The same authors also investigated what occurs in humans with an immunohistochemical analysis of human tumors, and they found a positive association between CYP27A1expression levels and tumor grade: the more elevated the expression was in both tumors and macrophages, the more advanced the

tumor grade was. Wu et al [104] have also shown that 27HC stimulates the growth of tumors originating from xenografts of MCF-7 cells in immunodeficient mice. These authors also reported that patients with ER-positive breast cancer present with more elevated levels of 27HC in the normal breast tissue than those found in healthy women. An increase in 27HC in tumors was also associated with a decrease in the expression of the enzyme CYP7B1, which catabolizes 27HC. This decreased expression was correlated with a decrease in patient survival. Other metabolites of cholesterol have been shown to play a role in breast cancer. Other studies have even proposed a role for 27HC in the regulation of the number of cytotoxic CD8+T lymphocyte and the development of metastasis [105].

Recent studies have also identified other cholesterol derivatives that may play an important role in the breast cancer. 5,6-epoxycholesterol is another oxysterol that may play a critical role [106]. 5,6-epoxycholesterol (5,6-EC) exists as two diastereoisomers: 5,6α-EC, 5,6β-EC. They are auto- and photo-oxidation products derived from cholesterol and can be found in human biological fluids and tissues [107]. Importantly, 5,6α-EC can control the activity of the nuclear receptors LXRα and LXRβ [108]. 5,6-EC is transformed into CT by the cholesterol-5,6-epoxide hydrolase (ChEH) in mammals [109]. This compound can in turn be transformed into oncosterone (6-oxo-cholestan-3β,5α-diol, OCDO), which has been shown to play an important role in cancer progression as it can promote cellular proliferation of ER+ and triple negative breast cancer cells [36]. Importantly, it was also demonstrated that the enzymes involved in its synthesis were overexpressed in tumor obtained from breast cancer patients compared to adjacent normal tissues, and their levels correlated with patient survival. Oncosterone levels were also increased in breast cancer tissues compared with normal tissues. Mechanistically, it was shown that oncosterone can act via the glucocorticoid receptor and activate cell cycle progression [36]. On the other side, 5,6-EC can also be transformed into dendrogenin A via the enzyme DDA synthase [37]. Dendrogenin A is synthesized in healthy

mammalian tissues, and its metabolism is deregulated in cancer cells. It is not found, for example, in tumors of breast or skin cancer, which could be due to a deficit of the enzyme DDA synthase [37, 110]. Studies suggest that it may play a role as a tumor suppressor metabolite since it promotes cellular differentiation, cancer cell death [111] and modified the tumor microenvironement through the stimulation of the secretion by tumors of immunogenic antitumor exosomes [40, 112]. Tumor studies have also shown that treatment with dendrogenin A of mouse bearing tumors reduces tumor growth [37, 38]. It also appears that DDA is produced by differentiated cells but not by cancer cells [37]. Similarly, breast tumors display reduced levels of this compound compared to normal tissues [37]. Mechanistically, it was further shown that DDA can modulate the activity of LXRβ, promote lethal autophagy in cancer cells and stimulate immunity against tumors [38]. Not only is it capable of transforming the phenotype of cancer cells into a phenotype of normal cells, both *in vitro* and *in vivo*, but used at high doses, it appears to be toxic to cancer cells [37, 38, 111]. It can induce cellular differentiation of melanoma cells into melanocytes, or differentiation of pluripotent carcinoembryonic cells into neurons [37]. It also induces polarization and milk production by breast cancer cells [111]. *In vivo*, it slows down the development of breast cancer or melanoma tumors in immunocompetent mouse models, in particular by inducing cell differentiation of cancer cells [37, 110]. Cell death mediated by dendrogenin A does not really go through a classical apoptosis pathway since caspase inhibitors cannot counteract the effects of dendrogenin A. Cytotoxicity mechanisms actually involve the transcription factors Nur77 and NOR1 and are associated with the induction of a lethal autophagy [38]. Dendrogenin A is a ligand for LXRβ. Its association can modulate LXRβ activity and therefore act on the transcription of a certain number of genes regulating cholesterol metabolism. DDA inhibits the expression of ABCA1 but stimulates the expression of the LDLR receptor [38].

Taken together, these studies suggest that cholesterol and its derivatives can directly interfere with tumor progression. Furthermore, depending on the cholesterol metabolites examined, different signaling pathways can be activated with different outcome.

5. Responses from in vitro studies: signaling pathways regulated by cholesterol

In vitro studies have also provided a number of responses, in particular by highlighting certain molecular mechanisms potentially involved in the influence of cholesterol on breast cancer. Cellular cholesterol homeostasis is remarkably affected in cancer cells (**Figure 1**) and Studies have now shown that cholesterol and its metabolites can modulate various signaling pathways involved in the regulation cancer progression (proliferation, survival, migration , and invasion of cancer cells, **Figure 2**).

5.1. Cholesterol in cancer progression

Alikhani et al [98] studied the response of the metastatic breast cancer cells Mvt-1 to cholesterol and concluded that the latter stimulates phosphorylation of Akt and proliferation. They further confirm that removing cholesterol from the medium eliminated the effect on Akt phosphorylation. Dos Santos et al [94] incubated MDA-MB-231, HTB-20, and HTB-126 cells with LDL and showed that the latter stimulates the proliferation of these three cell lines, stimulated wound-healing mediated migration, and caused a loss of adhesion of MDA-MB-231 cells. An additional analysis on microchips also allowed them to demonstrate that in the presence of LDL, certain genes encoding adhesion proteins were under-transcribed, in particular that of cadherin-related family member 3, CD226, claudin 7, occludin, integrin β8. In addition, certain pro-proliferative proteins were on the contrary overactivated (Akt, ERK, Jnk, all three dependent on the ErbB2 pathway, **Figure 2**). Furthermore, Gallagher et al [99] have shown that inhibiting LDLR decreases cellular survival of MDA-MB-231 cells. This finding again suggests the involvement of LDL in cellular survival and proliferation.

Cancer cells also often contain significant amount of cholesteryl ester [113], which may play a role in cancer. In that case, it appears that an accumulation of cholesteryl esters within the breast tumor contributes to its aggressive nature, in particular by stimulating cellular proliferation. The first evidence that cholesteryl esters presents tumor promoter activities came from Paillasse and collaborators [114], which led to the proof of concept that inhibition of cholesterol esterification may represent a new strategy to fight cancer [115]. The accumulation of cholesteryl esters in breast cancer was observed by Gonzalo-Calvo et al [116], who analyzed by thin layer chromatography the tumor composition in cholesteryl esters, free cholesterol and triglycerides from 30 breast tumor samples. By modeling the relationship between these levels and different markers of the cancer phenotype, they found that there was a strong association between elevated levels of cholesteryl esters and the histological stage of the tumor (the higher the concentration in cholesteryl esters and the higher the tumor grade), Ki-67, and tumor necrosis. Gonzalo-Calvo et al also found that tumors enriched in cholesteryl esters had elevated quantities of LDLR and SRB1 at the mRNA and protein levels. Antalis et al [117] also studied certain markers of lipid storage in the MDA-MB-231 and MDA-MB-436 cell lines (both ER-negative) and in the MCF-7 cell line (ER–positive) and found that concentrations of triacylglycerols and cholesteryl esters, and cholesteryl ester synthesis were more elevated in the ER-negative cell lines than in the ER-positive cell line. ER-negative cells had also more lipid droplets, and displayed increased levels of Acyl-coenzyme A:cholesterol acyltransferase (ACAT) (mRNA and protein, as well as increased activity), caveolin-1, and incorporated more LDL. Proliferation of MDA-MB-231 cells could be stimulated by LDL, but that was not the case for MCF-7 cells, and the proliferation of ER-negative cells (and more specifically, the proliferation induced by LDL) could be reduced by inhibiting ACAT with the CP-113,818 inhibitor. These differences between ER-negative cells and ER-positive cells remarkably affect their capacities to store and internalize lipids. The same team subsequently demonstrated that

if these cells were cultured in a medium devoid of lipoproteins, MDA-MB-231 cells had fewer lipid droplets and migrated less (loss of 85%) [118]. By again supplementing the medium with LDL, cells regained their capacity to migrate in an ACAT-dependent manner. Furthermore, LDLR mRNA levels were 12 times more elevated in MDA-MB-231 cells than in the non-cancerous MCF-10A cells. Adding LDL to the lipoprotein-free environment decreased LDLR mRNA levels in MCF-10A cells but not in MDA-MB-231 cells. By inhibiting ACAT1, LDLR mRNA levels in MDA-MB-231 were reduced, but not comparable to those observed in MCF-10A. These findings may suggest that the activity of ACAT may be associated with increased expression of LDLR.

In the context of breast cancer, the role of 27HC has also been studied in vitro. 27HC is in fact capable of stimulating growth of some breast cancer cell lines. Produced by MCF-7 cells, it stimulates cellular proliferation in an ER-, and GDNF-RET-dependent manner [104]. Shen et al. [119] have also shown that it stimulates migration, invasion, and EMT of MCF7 and T47D cells as well as the expression of the matrix metalloproteinase 9 (MMP9) in these cell lines. They also found that 27HC activates STAT3 in ER-positive cells, and that inhibiting STAT3 reduces the effect of 27HC on MMP9-mediated migration and invasion. Furthermore, according to their work, 27HC also increased the expression of MMP9, vimentin and STAT3 in the ER-negative MDA-MB-231 cell line. The results of Torres et al [120] are consistent with these observations, and they show that exposure of MCF-7 cells to 27HC stimulates the EMT process by decreasing the expression of E-cadherin and β-catenin and causing a loss of adherent junctions. Conversely, when 27HC was removed from the medium, MCF-7 cells started to re-express E-cadherin and β-catenin, but without being able to adhere to each other to reconstitute an epithelium. They also expressed plasma membrane EGFR2/neu for a prolonged period, which suggests a mechanism of action regulated by EGFR2. Ma et al [121] suggested another mechanism of action, since they showed that 27HC could activate the oncoprotein Myc in

MCF-7 cells by increasing its stability at the transcriptional level and by inhibiting three of its negative modulators: PP2A, SCP1 and FBW7. It appears that 27HC can also act on the protein p53. Raza et al [122] have indeed demonstrated that exposing MCF-7 cells to 27HC reduces the activity of p53 at the transcriptional level, but does not cause any change in MDA-MB-231 cells. In addition, in this study, exposing MCF-7 cells to 27HC increased the expression of the E3 ubiquitin protein ligase MDM2 at the protein level, decreased the expression of p53, and stimulated an interaction between p53 and MDM2. By reactivating p53 (with the activator Tenovin-1) or by inhibiting MDM2 (with the inhibitor Nutlin-3 or by using an siRNA), the effect of 27HC on cellular proliferation was reduced. It is possible that there is a link between p53, which gene is frequently mutated in breast cancer, and the synthesis of cholesterol as previously suggested [123]. In addition, if p53 is no longer functional, the cholesterol synthesis pathway is over-activated, and this activation is sufficient to modify the normal breast structure [124].

5.2. Cholesterol and mitochondrial function

As the mitochondria is a major site implicated in the regulation cancer cell behavior, an imbalance in mitochondrial cholesterol levels may also contribute to the development of breast cancer [125]. In that case, expression of the protein StARD3, which, with StAR, is one of the two main proteins that regulates the entry of cholesterol into the mitochondria, is associated with a poor survival of cancer cells [126]. Alpy et al [127] have shown that when levels of this protein decrease, proliferation decreases and cell death increases in Her2-negative breast cancer cells. On the other hand, Vassilev et al [128] have shown that, when this protein is overexpressed, it increases cellular cholesterol levels, and consequently, decreases adhesion capacities and metastatic properties of MCF7 cells. After analyzing data from two Finnish cohorts (2,220 patients), Vassilev et al also reported, that the StARD3 protein was overexpressed in approximately 10% of breast cancer cases, and that this overexpression was

associated with an amplification of HER2, elevated proliferation rates, larger tumors, the presence of lymph node metastases at the time of diagnosis, ER- and PR-negative breast cancers, and expression of the p53 protein, and generally poor prognostic factors. With data from The Cancer Genome Atlas (TCGA) cohort [129], it was also shown that the genes encoding the proteins StAR and StARD3 were overexpressed in 30% of breast cancer cases, but without evidence of association between these genes and survival [130]. Another finding that allows to associate cancer and mitochondrial cholesterol homeostasis is through the expression of the ABCA1, which expression is modified in certain cases of cancer [131, 132]. In particular, a drop in the activity of this protein has been associated with the stimulation of colon cancer cell survival via an increase in mitochondrial cholesterol levels [133]. Increases mitochondrial cholesterol content may contribute to increased mitochondrial ROS generation, which may promote HIF-1α stabilization and promote cell survival and tumor progression [125]. Furthermore, its role in the regulation of cellular survival has also been demonstrated by its ability to inhibit the membrane-permeabilizing activity of BAX, and may therefore decrease the pro-apoptotic activity of BAX [134, 135].

5.3. Cholesterol and the regulation of signaling pathways

Implication of one of the suspected mechanisms of action by which cholesterol plays a role in cancer involves its central position in the regulation of different signaling pathways (**Figures 1** and **2**). Cholesterol can activate the Hedgehog pathway, for example, by covalently binding to the Smoothened receptor, which is a G protein-coupled receptor [136, 137]. While the Hedgehog pathway stimulates cell differentiation, polarization, and proliferation, it is also involved in different types of cancer such as skin, lung, brain, gastrointestinal, or leukemia [138] and breast cancer [139]. Cholesterol also binds to the PDZ domain of certain scaffolding proteins such as NHERF1/EBP50, which regulates several oncogenic proliferative pathways (e.g., PI3K/PTEN/Akt, Wnt/β-catenin, or even the EGFR receptor) [140]. Cholesterol is also

one of the main building blocks of lipid rafts, which play a key role in the regulation of various signaling pathways [141]. When cholesterol levels are modified, changes in the structure of lipid rafts alter their function in the cell. The roles of these membrane nano-domains are multiple. They play a major role in the regulation of cellular survival since they can regulate apoptosis triggering [142]. In addition, phosphorylation or dephosphorylation of kinases, cell death receptors, and calcium channels occurs in lipid rafts [143]. In that context, the protein Akt is more easily activated when it is translocated to lipid rafts [144]. Consistent with a role for membrane cholesterol, the use methyl-β-cyclodextrin has allowed investigators to modify the structure of lipid rafts by extracting cholesterol, in a process that inhibits Akt phosphorylation and triggers apoptosis of cancer cells [145]. Use of the same technique can also lead to the loss of CD44, which is a glycoprotein involved in migration, adhesion and metastasis [146]. The structure of rafts can also be impacted by statins, which hinder EGF-induced phosphorylation of the Src, Akt, and p42/44, and therefore, prevent cellular migration of cancer cells [147]. Caveolae, which are a subset of lipid raft containing the protein caveolin-1, also participate in the formation of invadopods and in the degradation of the extracellular matrix in breast cancer [148]. Disrupting lipid rafts with methyl-β-cyclodextrin can reduce the invasion of breast cancer cells, in a process associated with a drop in the metalloproteinase MMP9 levels [149]. Precursors or derivatives of cholesterol can also directly act on certain signaling pathways. Consequently, mevalonic acid can activate PI3K and the mTOR signaling cascade [150]. Certain isoprenoids, such as isopentenyl diphosphate, farnesyl pyrophosphate, or geranylgeranyl pyrophosphate, can modify Ras and Rho GTPases via their prenylation and therefore promote their translocation to the plasma membrane. However, once prenylated, they can also play a role in the activation of carcinogenic signaling pathways [151]. Other types of cholesterol metabolites such as oxysterols, can act via their association to LXR, which once

activated, has anti-tumor properties and can decrease mammary cancer cell proliferation and reduce *in vivo* tumor growth in mouse models [152, 153].

5.4. Regulation of Cholesterol Synthesis and Cancer

In addition to its involvement in signaling pathways, cholesterol plays a role as an essential component of the plasma membrane, and it is essential for cancer cells, which proliferate endlessly. Synthesis of cholesterol is therefore often over-activated when cancer develops and takes hold [154]. Artificially stimulating the synthesis of cholesterol also leads to the worsening of a cancer phenotype. This activation allows the stimulation breast cancer cell proliferation via a p53-dependent mechanism [155]. HMGCR, a key enzyme in cholesterol synthesis, can stimulate the migration of colon cancer cells by inhibiting geranyl-geranylation of GTPase RhoA, which participates in cell development and in the regulation of cell cycle [156]. Squalene epoxidase, which is a rate-limiting enzymes involved in the cholesterol synthesis pathway and which intervenes downstream of HMGCR, is overexpressed in HER2-positive and high-grade breast cancers, and its inhibition reduces cellular viability of breast cancer cells [157]. The PI3K/Akt/mTOR pathway is also associated with the synthesis of cholesterol [158]. In particular, inhibiting the mTORC1 complex prevents the nuclear accumulation of the Sterol Regulatory Element-Binding Protein 1 (SREBP1) and therefore prevents the activation of SREBP1 target genes. The reverse is also true: preventing the expression of SREBP1 inhibits Akt-mediated lipogenesis, decreases the effect of Akt on increasing cell size, and that of PI3K on stimulating cellular proliferation [158, 159]. Transcription of *SREBF2* (encoding SREBP2) has also been shown to be regulated during cancer progression [160]. While SREBP1 plays a critical role in fatty acid metabolism, SREBP2 preferentially regulates the transcription of gene involved in cholesterol metabolism. Its regulation also appears to be affected by rapamycin and

mTORC1 [161]. Recent studies have even suggested that SREBP2 may participate in the regulation of breast cancer bone metastasis in a mouse model [160].

While blocking cholesterol influx can have anti-oncogenic properties, blocking cholesterol efflux may stimulate cancer progression. Accordingly, inhibiting NPC1L1 (influx) decreases plasma cholesterol levels, β-catenin levels, and phosphorylated ERK1/2 levels, and reduces the risk of colorectal tumorigenesis under specific conditions [162]. On the other hand, inhibiting ABCA1 (efflux) may increase intracellular cholesterol levels, which promote cancer progression and can render tumors more aggressive. In agreement with this hypothesis, it was shown that the *ABCA1* promoter is often hypermethylated in prostate cancer, but not in benign prostate tumors [163], and ABCA1 protein levels are reduced in invasive breast cancer [132]. Furthermore, when TP53 and Ras mutants are overexpressed, they inhibit ABCA1 and increase mitochondrial cholesterol levels, which in turn stimulates tumor growth *in vivo* [164]. The anti-oncogenic action of the protein ABCA1 may therefore be due, at least in part, to its ability to reduce mitochondrial cholesterol levels [164]. In fact, it has often been reported that mitochondria membranes of cancer cells contain more cholesterol than those of normal cells [135, 165]. Increased mitochondrial cholesterol content decreases the fluidity of these membranes and reduces the opening of the Mitochondrial Permeability Transition Pore (MPTP), and consequently prevents the release of pro-apoptotic molecules (e.g., cytochrome C) [133, 166, 167]. Inhibition of ABCA1 mediated by miR-33 microRNAs is also at the origin of pro-tumorigenic effects [168]. Certain carcinogenic mechanisms also involve other transporters, such as ABCG5 (“ATP-binding cassette transporter G5”) and ABCG8 (“ATP-binding cassette transporter G8”), in particular with regard to gallbladder cancer [169].

6. Conclusions

Data obtained from in vivo and in vitro studies have confirmed the important role of cholesterol and its metabolites in breast cancer. However, epidemiological studies are more difficult to analyze with confounding factors. They also suggest that other factors can either limit or aggravate the role of cholesterol. Among these factors, cholesterol metabolites may also have specific pro-or anti-tumorigenic activities. However, further studies specifically addressing the role of this components will have to be performed. Finally, as shown by several studies [32, 107, 170, 171], cholesterol metabolism and its regulation in the tumor and its microenvironment may be an important target by therapeutic treatment of patients.

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Tables

Table 1: **Overview of the responses from epidemiological studies to the question "Is cholesterol a risk factor for breast cancer?" "**

Positive Association					
Study	Association	Type of cholesterol	Risk type	Population	Additional information
Kitahara et al. [5]	HR = 1.17 [1.03–1.33]	Elevated total serum cholesterol (>160mg/dL)	Incidence		
Ha et al. [23]	HR = 1.11 [1.04–1.19]	Total serum cholesterol (Increase of 1mmol/L)	Incidence		Age adjustment only
	HR = 1.09 [1.01–1.18]	Total serum cholesterol (increase of 1mmol/L)	Incidence		Adjustment for breast cancer risk factors except BMI
Katzke et al. [20]	HR = 1.39 [1.01–1.93]	Elevated HDL-cholesterol (≥1.70 mmol/L)	Incidence		
Martin et al. [19]	HR = 1.23 [1.00–1.51]	Elevated HDL-cholesterol (≥1.72 mmol/L)	Incidence		
Hu et al. [172]	HR = 1.45 [1.14–1.85]	High dietary cholesterol intake (≥1880,266mg/week)	Incidence		

	HR = 1.48 [1.07–2.07]	High dietary cholesterol intake (≥1880,266mg/week)	Incidence	Postmenopausal women	
Kim et al. [2]	HR = 1.69 [1.01–2.82]	High dietary cholesterol intake (consumption of food enriched in cholesterol ≥ 2–3 times/month)	Incidence		
Osaki et al. [7]	HR = 2.87 [1.67–4.94]	The Metabolic Syndrome	Incidence		
	HR = 6.73 [2.93–15.43]	The Metabolic Syndrome	Incidence	Postmenopausal women > 55 yo	
Agnoli et al. [9]	RR = 1,58 [1.07–2.33]	The Metabolic Syndrome	Incidence		
			Negative Association		
Tulinius et al. [11]	RR = 0.9 (p=0.07)	Elevated serum cholesterol (increase of 1 mmol/L) measured more than 10 years before diagnosis	Incidence		
	RR = 0,930 (p=0.23)	Elevated serum cholesterol (increase of 1 mmol/L) measured more than 10 years before diagnosis	Incidence		
Llanos et al. [12]	OR = 0.46 [0.25–0.85]	Elevated total plasma cholesterol (≥200 mg/dL)	Incidence		
« SU.VI.MAX Study » [13]	HR = 0.83 [0.69 – 0.99]	Elevated total plasma cholesterol (increase of 1 mmol/L)	Incidence		
Llanos et al. [12]	OR = 1.99 [1.06–3.74]	Low HDL-cholesterol (<60 mg/dL)	Incidence		
« SU.VI.MAX Study » [13]	HR = 0,48 [0,28–0,83]	Elevated HDL-cholesterol (increase of 1 mmol/L)	Incidence		
Ni et al. [17]	RR = 0,77 [0,64–0,93]	Elevated HDL-cholesterol (≥ 0.35 mmol/L)	Incidence	Postmenopausal women	
Kucharska-Newton et al. [30]	HR = 1.67 [1.06–2.63]	Low serum HDL-cholesterol (<50 mg/dL)	Incidence	Postmenopausal women	
Ni et al. [17]	RR = 0.77 [0.64–0.93]	Elevated HDL-cholesterol (≥ 0.35 mmol/L)	Incidence	Postmenopausal women	
Lofterød et al. [16]	HR = 0.33 [0.12–0.89]	Elevated HDL-cholesterol (≥ 0.35 mmol/L)	Mortality		Triple-negative Cancer
Kim et al. [173]	OR = 0.49 [0.33–0.72]	Elevated HDL-cholesterol (≥60mg/dl)	Incidence	Pre-menopausal women	
			Incidence		

	OR = 0.34 [0.22–0.53]	Elevated HDL-cholesterol (≥60mg/dl)		Non obese pre-menopausal women	
	OR = 2.20 [1.32–3.67]	Low HDL-cholesterol (< 50mg/dl) et elevated triglycerides (≥ 150mg/dL)	Incidence		ER- and PR-negative cancer
Agnoli et al. [9]	RR = 1.60 [1.10–2.33]	Low HDL-cholesterol (≤55 mg/dL)	Incidence		
Furberg et al. [14]	RR = 0.75 [0.58–0.97]	Elevated HDL-cholesterol (>1.64 mmol/L)	Incidence	Postmenopausal women	
	RR = 0.43 [0.28–0.67]	Elevated HDL-cholesterol (>1.64 mmol/L)	Incidence	Obese postmenopausal women	
	RR = 1.44 [0.91–2.30]	Elevated HDL-cholesterol (>1.64 mmol/L)	Incidence	Pre-menopausal women	
Martin et al. [19]	HR = 0.81 [0.66–098], p=0.03	Elevated non-HDL cholesterol (≥4,29 mmol/L)	Incidence		
Rodrigues dos Santos et al. [22]	HR = 0.129 [0.017 – 0.978]	Elevated LDL-cholesterol (>117 mg/dL)	Relapse-free survival		
Llanos et al. [12]	OR = 0.41 [0.21 – 0.81]	Elevated LDL-cholesterol (≥130 mg/dL)	Incidence		
Lu et al. [46]	RR = 0.56 [0.36–0.87]	Elevated serum 27-hydroxycholesterol (≥221 nmol/L)	Incidence	Postmenopausal women	
« Metabolic syndrome and Cancer Project » [8]	HR = 0.70 [0.61–0.81]	The Metabolic Syndrome	Incidence		

No Association					
Ni et al. [17]	RR = 0.96 [0.86–107]	Elevated serum cholesterol	Incidence		
Lofterød et al. [16]	HR = 1.46 [0.87–2.44]	Elevated serum cholesterol (≥ 6.26 mmol/L)	Mortality		
Ha et al. [23]	HR = 1.06 [0.98–1.15]	Total serum cholesterol (increase of 1mmol/L)	Incidence		Adjustment for breast cancer risk factors including BMI
Gaard et al. [24]	RR = 0.87 [0.61–1.23]	Serum cholesterol (≥6.86 mmol/L)	Incidence		
Hiatt et al. [25]	No significant effect	Elevated serum cholesterol	Incidence		
His et al. [28]	OR = 0.98 [0.74–1.30]	Elevated serum cholesterol (≥5.56 mmol/L)	Incidence		

	OR = 1.56 [0.94–2.58]	Elevated serum cholesterol (≥5.56 mmol/L)	Relapse-free survival		
Melvin et al. [29]	HR = 0.97 [0.89–1.05]	Elevated serum cholesterol (≥6.30 mmol/L)	Incidence		
Hu et al. [172]	HR = 1.10 [0.75–1.62]	High dietary cholesterol intake (≥1880.266mg/week)	Incidence	Pre-menopausal women	
Eliassen et al. [50]	RR = 0.99 [0.86–1.13]	Treatments that Lower Cholesterol Levels	Incidence		
Furberg et al. [14]	RR = 1.44 [0.91–2.30]	Elevated HDL-cholesterol (>1.64 mmol/L)	Incidence	Pre-menopausal women	
Lofterød et al. [16]	HR = 0.82 [0.1–1.33]	Elevated HDL-cholesterol (≥ 0.35 mmol/L)	Mortality		
Borgquist et al. [18]	No association	HDL-cholesterol	Incidence		
Gaard et al. [24]	RR = 1.02 [0.73–1.42]	Elevated HDL-cholesterol (≥1.66 mmol/L)	Incidence		
Moorman et al. [27]	OR = 0.99 [0.97–1.01]	Elevated HDL-cholesterol (augmentation de 1 mg/dl)	Incidence		
	OR = 0.53 [0.20–1.39]	Elevated HDL-cholesterol (>37 mg/dl)	Incidence	Pre-menopausal women	
	OR = 0.70 [0.67–4.33]	Elevated HDL-cholesterol (>37 mg/dl)		Postmenopausal women	
His et al. [28]	OR = 0.75 [0.49–1.15]	Elevated HDL-cholesterol (≥2.63 mmol/L)	Incidence		
	OR = 1.45 [0.87–2.44]	Elevated HDL-cholesterol (≥2.63 mmol/L)	Relapse-free survival		
	OR = 0.95 [0.76–1.18]	Elevated HDL-cholesterol	Incidence		ER-positive breast cancers
	OR = 0.79 [0.38–1.66]	Elevated HDL-cholesterol	Incidence		ER-negative breast cancers
Melvin et al. [29]	HR = 1.05 [0.86–1.29]	Elevated HDL-cholesterol (≥1.98 mmol/L)	Incidence		
Kucharska-Newton et al. [30]	HR = 1.08 [0.84–1.40]	Low HDL-cholesterol (<50 mg/dL)	Incidence		
	HR = 0.93 [0.66–1.30]	Low HDL-cholesterol (<50 mg/dL)	Incidence	Postmenopausal women	
Gaard et al. [24]	RR = 0.93 [0.67–1.29]	Low LDL-cholesterol (≥4.72 mmol/L)	Incidence		
Borgquist et al. [18]	No association	LDL-cholesterol	Incidence		
His et al. [28]	OR = 0.91 [0.60 –1.38]	Elevated LDL-cholesterol (≥2.82 mmol/L)	Incidence		

	OR = 1.01 [0.61–1.69]	Elevated LDL-cholesterol (≥2.82 mmol/L)	Relapse-free survival		
	OR = 1.10 [0.89–1.36]	Elevated LDL-cholesterol	Incidence		ER-positive breast cancers
	OR = 0.99 [0.55–1.79]	Elevated LDL-cholesterol	Incidence		ER-negative breast cancers
Melvin et al. [29]	HR = 0.92 [0.75–1.13]	Elevated LDL-cholesterol (≥4.14 mmol/L)	Incidence		
Lu et al. [46]	RR = 0.9 [0.66–1.22]	Elevated 27-hydroxycholesterol (≥221 nmol/L)	Incidence		
	RR = 1.33 [0.75–2.38]	Elevated 27-hydroxycholesterol (≥221 nmol/L)		Pre-menopausal women	

Data presented in this Table originate from a review of the literature carried out using existing data until December 2019 and may not be exhaustive. They nevertheless demonstrate the complexity of the issue, and the differences in term of results that exist in the literature depending on the population concerned, the type of cholesterol measured, or the type of breast cancer considered. If the population is not specified, all women are examined.

Table 2: **Overview of the evidence associating 27-hydroxycholesterol with an increased or decreased breast cancer incidence and outcome**

Positive Association				
Study	Association	Observation	Population	Additional information
Nelson et al [35]	Increased expression of CYP27A1	Positive association with tumor grade	Patients with ER-positive tumor	
Wu et al [99]	Reduced expression of CYP7B1	Reduced levels in tumors vs normal tissue	Patients with ER-positive tumor	
Kimbung et al [174]	Increased intratumoral levels of CYP27A1	CYP27A1 as an indicator of adverse prognosis	invasive breast cancer bearing patients	

Negative Association			
Nelson et al [35]	Increased expression of CYP7B1	Improve survival	Patient with luminal A tumors

No Association			
Le Cornet et al. [175]	Circulating levels of	Limited association	Breast cancer patients

27HC and CYP7B1 and breast cancer	

Figure Legends

Figure 1: Main signaling pathways involved in breast cancer and the role of cholesterol. The PI3K / Akt / mTOR and RAS / RAF / MAPK pathways, are involved in cell proliferation, migration and invasion and can be activated by the HER2 or EGFR receptors. The latter can be activated by scaffolding proteins such as NHERF1 or EBP50, under the control of cholesterol. Associated with cholesterol, NHERF1 or EBP50 can also stimulate the Wnt/β-catenin pathway, which activates transcription of the Myc gene and stimulates proliferation. Cholesterol in lipid rafts can contribute to the activation of the PI3K and MAPK pathways. Furthermore, by binding to the Smoothened receptor, cholesterol can activate the Hedgehog pathway, which participates in the differentiation and polarization of breast cells, and stimulates proliferation and invasion. Finally, 27-hydroxycholesterol can serve as a ligand for the ERα receptor and contribute to increase proliferation, migration, invasion and stimulation of the Epithelio-Mesenchymal Transition.

Figure 2: Metabolism of Cholesterol and its Metabolites in Breast Cancer. In the present figure, we show that the metabolism of cholesterol and its metabolites can directly alter cellular function in cancer cells. Of note, 27HC, OCDO, and esterified cholesterol have been implicated in the regulation of various signaling pathways leading to tumors that are more aggressive. On the other hand, recent studies with dendrogenin A have demonstrated its beneficial effects against cancer. Importantly, accumulation of free cholesterol may be a consequence of a dysfunctional cholesterol esterification coupled to a dysregulated transport of free cholesterol.

Fig 1

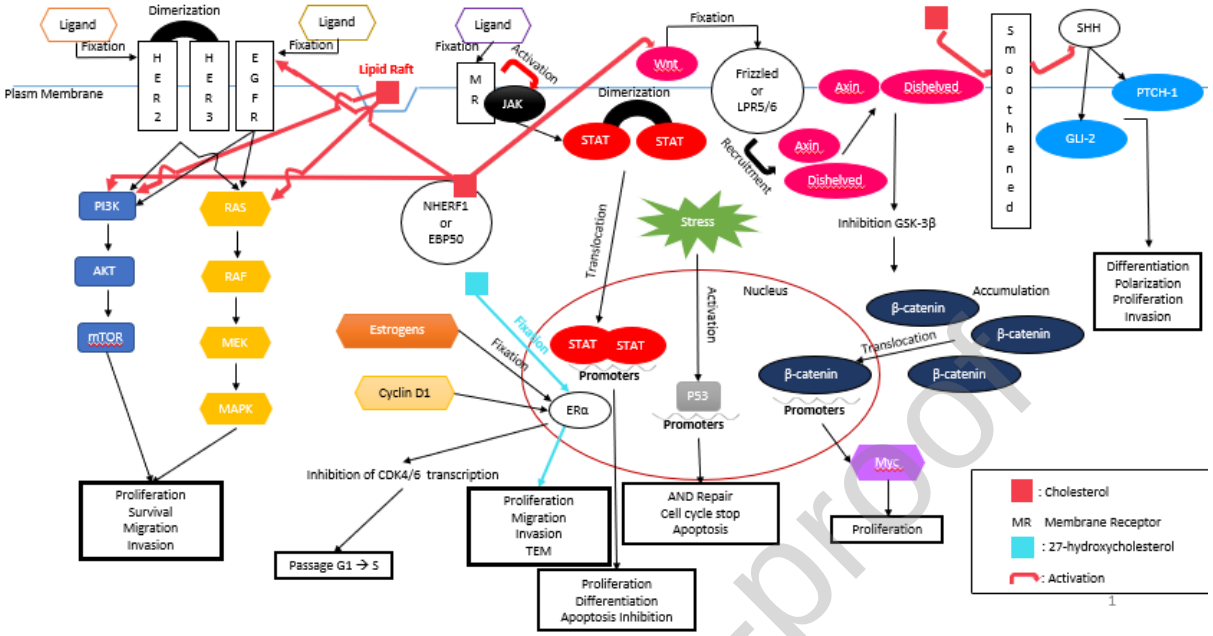
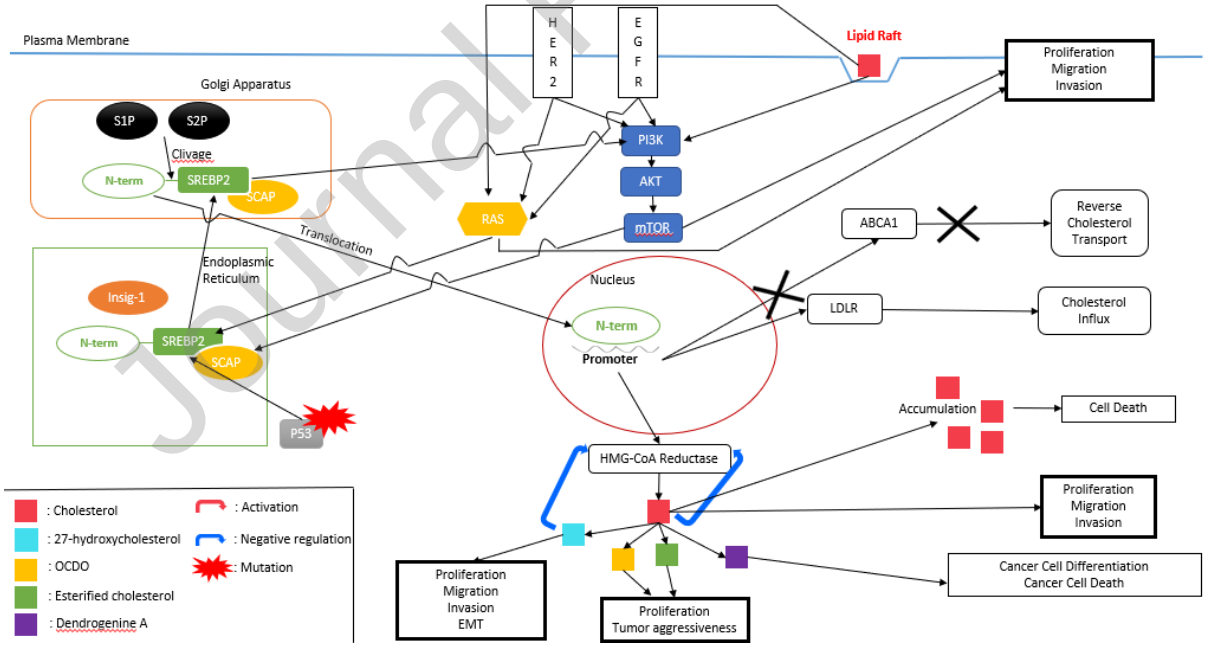


Fig 2



Conflicts of Interest: The authors declare no conflict of interest.

Highlights

- The role of cholesterol in breast cancer is complex.
- Clinical studies have revealed contradictory results.
- Modulation of cholesterol metabolism can stimulate inflammation and oxidative stress.
- Cholesterol can be metabolized into steroids that are tumor promoter.
- Cholesterol can be metabolized into tumor suppressors.