Role of 27-hydroxycholesterol and its metabolism in cancer progression: Human studies.

Role of 27-Hydroxycholesterol and its metabolism in cancer progression: human studies.

Fiorella Biasi[a], Valerio Leoni[b], Paola Gamba[a], Gérard Lizard[c], Giuseppe Poli[a,1]

[a] Department of Clinical and Biological Sciences, University of Turin, San Luigi Hospital, Orbassano (Turin), Italy.

[b] Laboratory of Clinical Chemistry, Hospital of Desio, and Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy.

[c] Université de Bourgogne Franche-Comté, Faculté des Sciences Gabriel, Dijon, France.

[1] Corresponding author: giuseppe.poli@unito.it

Short title: oxysterols and human cancer

Abstract

Direct translation of findings achieved in experimental cell or animal models to humans is always a quite difficult if not impossible task. As regards the role of 27-hydroxycholesterol and related metabolism in human cancer development we deemed of interest to focus only on the epidemiological and ex vivo human studies so far available in literature. There are some studies in humans that support an adverse effect of 27OHC in breast cancer, based upon the oxysterol's recognized ability to bind to and modulate esterogen receptors. The detrimental role of this side chain oxysterol would be evident in cancer progression, mainly in post-menopausal women and in an advanced stage of the disease. Other human studies, however, would rather correlate 27OHC intratumoral levels with a better prognosis. The analyses on human prostate cancer specimens performed to date are all against a detrimental contribution of 27OHC, rather suggesting interesting anti-prostate cancer effects exerted by this oxysterol. Finally, an increased 27OHC synthesis would favor cancer progression in colon, brain and thyroid tissues, as found for breast cancer.

Key words: 27-hydroxycholesterol, cytochrome P450 27A1, cytochrome P450 7B1, cancer progression, human studies.

Abbreviations:

27OHC: 27-hydroxycholesterol; CYP27A1: cholesterol 27-hydroxylase; CYP7B1: oxysterol 7-alpha-hydroxylase; ER: estrogen receptor; LXRs: Liver X Receptors; ROR: retinoic acid receptor related orphan receptor (ROR): PPARs: peroxisome proliferator-activated receptors; TLRs: toll like receptors; SREBP: sterol regulatory element binding protein; INSIG: insulin-induced gene 1 protein; LC-MS: liquid chromatography- mass spectrometry; MS-MS: tandem mass spectrometry; TCGA: the cancer genome atlas; EPIC: European

prospective investigation into cancer and nutrition; RFS: recurrence-free survival; OS: overall survival; TNM: tumor, node, metastasis (classification).

1. Introductory remarks

Cholesterol metabolism certainly plays a crucial role in human physiology, since leading to the synthesis of a number of important if not even vital molecules like estrogens, progestins, testosterone, vitamin D, biliary acids. In addition, the enzyme-driven oxidation of cholesterol generates a family of 27carbon-centered compounds termed oxysterols, being those containing an additional hydroxyl group on the sterol's side chain of high pathophysiological relevance [1,2 Schroepfer 2000; Sottero et al., 2019].

27-Hydroxycholesterol (27OHC) is by far the most abundant side chain oxysterol in human peripheral blood [3-5 Deleztovic 19..; Leoni and Caccia 2011; Pataj et al. 2016], in colostrum and milk [6 Civra et al. 2019]. It is generated from cholesterol by the ubiquitous mitochondrial enzyme cholesterol 27-hydroxylase (CYP27A1) and it is metabolized mainly in the liver by another cytochrome P450 enzyme, i.e. oxysterol 7-alpha-hydroxylase (CYP7B1), that conveys the oxysterol towards bile acid [7 Umetani et al., 2011] (Figure 1).

The serum/plasma 27OHC concentration approximately ranges between 0.3 and 0.8 µM, a quite relevant amount if compared for instance to that of different vitamins (Figure 2). In human colostrum, 27OHC content may be even higher than 1 µM [6 Civra et al. 2019]. These facts, in evolutionary terms, strongly sustain a physiological role of this oxysterols, most likely in multiple ways and biological systems. In this relation, increasing experimental evidence points to a broad spectrum antiviral activity of 27OHC [8 Lembo et al. MAM 2016] as well as some modulatory role of it in the innate immunity [9-11 Spann et al., 2013; Kim et al. 2014; Son et al., 2013].

However, if on one hand a complete understanding of the actual physiological role/s of 27OHC is/are yet to be achieved, on the other hand, excessive or inappropriate amounts of this side-chain oxysterols have been repeatedly associated with pathologic processes or diseases [for a review see 12 Sottero et al., 2019]. It should be noted that in most cases, data supporting the implication of 27OHC in the pathogenesis of given diseases have been

obtained in experimental in vitro and in vivo systems, suitable models to investigate possible molecular mechanisms underlying the oxysterol's effects but not sufficient to apply straight away the findings so obtained to the human setting.

This is the case, for instance, of the yet undefined actual role, whether positive or negative, played by 27OHC in cancer, mainly breast carcinoma, with the large majority of preclinical studies suggesting an enhancing effect of the oxysterol on the latter disease progression, based on its recognized property of being a good extrogen receptors ligand [13,14 Umetani et al. 2007; Du Sell et al., 2008]. Direct translation of findings achieved in experimental cell or animal models to humans is always a quite difficult if not impossible task. Hence, we deemed useful to focus the present review analysis on the epidemiological and ex vivo human studies so far available in literature with regard to the role of 27OHC and related metabolism in human cancer development.

2. 27OHC and breast cancer: studies in humans.

First significant clues of a possible implication of 27OHC in the progression of estrogen receptor-positive breast carcinomas up to the metastatic step were provided by Nelson, McDonnell and colleagues, examining human cancer specimens. Out of 59 cancer samples collected, a relatively low level of CYP27A1, the enzyme generating 27OHC, was detected in 48 specimens. Of the remaining 11 cancer samples, all expressing high levels of CYP27A1 71% were ER positive and 29% ER negative, 45% of grade 2 and 55% of grade 3. Authors' conclusion was that CYP27A1 expression levels likely correlated with tumor grade [15 Nelson 2013 Science]. A claim actually based on very few cases, in need of being confirmed on a much wider casistics.

Of interest, in the same paper authors reported on their much wider analysis of CYP7B1 mRNA and CYP27A1 expression in various human breast cancer datasets, showing that a high expression of CYP7B1, the enzyme that metabolizes 27OHC, was associated with a

better survival rate in luminal A type carcinoma (n= 1170), while no significant correlation with survival outcome was observed for CYP27A1 in the same type of breast cancer (n= 482) [15 Nelson 2013 Science]. This CYP7B1-related observation would point to a faster removal of 27OHC by CYP7B1 as a positive prognostic index at least in human breast cancer of luminal A type, that accounts for 30-45% breast cancer cases.

In the same year, Wu and colleagues measured the peritumoral and intratumoral level of 27OHC in 66 women with ERα+ breast cancer, detecting a much higher oxysterol amount within the neoplastic tissue, well correlated with reduced expression of CYP7B1, but not correlated with serum 27OHC levels [16 Wu et al. Cell Rep 2013]. They further measured the expression of CYP7B1 but also that of CYP27A1 in 406 ER+ breast carcinoma samples and 63 normal breast tissue samples from The Cancer Genome Atlas (TCGA). While CYP27A1 expression did not show any significant difference between cancer and normal tissue specimens, CYP7B1 expression was found almost halved in cancer as to normal breast tissue [16 Wu et al. Cell Rep 2013]. Once again, the modulation of the 27OHC metabolizing enzyme was indicated as potentially crucial in regulating breast cancer progression.

Only very recently, a further human study supporting a negative role of 27OHC in breast cancer development became available. Kimbung and colleagues afforded a semiquantitative measurement of CYP27A1 enzyme level by immunohistochemistry in tumor specimens obtained from two separate prospective cohorts of invasive breast cancer bearing patients (n = 645 and n = 813, respectively) and correlation between the intratumoral enzyme level and prognosis assessed. Relatively high CYP27A1 was detected in 20-30% of the cases, especially in tumors of Nottingham histological grade III, big size and lack of hormone receptors. However, it should be noted that while in the first cohort of 645 women, whose 90.2% were ≥ 55 years of age, a high CYP27A1 intratumoral level appeared to be a consistent indicator of adverse prognosis, in the patients of the second cohort bearing ER+

cancer, with a much lower percent of postmenopausal women as to the first group, the inverse correlation between high CYP27A1 and survival resulted to be non statistically significant [17 Kimbung et al 2017].

To sum up, the human studies so far available that support an adverse effect of 27OHC in breast cancer, essentially focused on the oxysterol's synthesis by breast CYP27A1 and removal by breast CYP7B1, suggesting a detrimental role of this side chain cholesterol oxide 1) in cancer progression, 2) mainly in post-menopausal women and 3) in an advanced stage of the disease. The findings of these human studies are in line with the various pre-clinical ones pointing to an enhancing effect of 27OHC on breast cancer progression. They were all inspired by the demonstration that 27OHC acts as a valid selective estrogen receptor modulator (SERM) [14 DuSell et al.2008].

As a matter of fact, to opposite conclusions led some human studies on 27OHC and breast cancer, part of them of epidemiological type. For sake of clarity, it must be pointed out, as some authors did already [18 Le Cornet et al. 2020], that the main end point of studies on human cancer specimens is the impact of this oxysterol on ER+ breast carcinoma progression, while epidemiological studies evaluate a possible correlation between 27OHC blood levels and risk of breast cancer.

Kimbung and colleagues, in a study on 1,881 women with breast cancer, observed that an increased intratumoral concentration of CYP27A1 directly correlated, in a statistical marginal way, with both recurrence-free survival (RFS) and overall survival (OS). Such a correlation appeared much stronger when only ER+ tumors and patients ≤ 50 years of age were evaluated [17 Kimburg et al 2017]. These findings could be considered as not really conflicting whit those achieved by the same group later on, that on the contrary suggested an inverse correlation between CYP27A1 and RFS and OS parameters, but mainly in postmenopausal women bearing large size and high grade breast carcinomas [19 Kimbung et al 2020].

In a small prospective, non randomized trial involving patients with invasive breast cancer, both metastatic (n=19) and non metastatic (n=10) ones, the plasma levels of various oxysterols, before and after 1 month treatment with either aromatase inhibitor or tamoxifen, were measured by GC-MS. As far as 27OHC was concerned, its blood level at time 0, that is before the administration of anti-hormonal therapy, did not significantly differ between patients with metastatic or non metastatic breast.cancer. Author also observed a significant increase in 27OHC plasma level after hormonal therapy, but only in the group treated with the aromatase inhibitor [20 Dalenc et al., 2017].

As just mentioned, epidemiological analyses have quite different endpoints as to preclinical and clinical studies supported by histochemical feedbacks; epidemiology investigates a given disease from a wider and socially-oriented point of view, often providing useful information about the disease potential.

The first study ever on prediagnosis circulating 27HC and breast cancer risk in women was that provided by the group of Fortner in 2019. They carried out a nested case-control investigation within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg study. A total of 530 breast cancer cases matched to 1036 controls was analyzed. Indeed, a statistically significant correlation between high serum 27OHC concentration and reduced breast cancer risk was observed but only in postmenopausal women, whose percentage share in both patients and control groups was about 50% [21 Lu et al. 2019].

The same research group then analyzed whether the intratumoral expression of CYP27A1, CYP7B1, ERβ and Liver X receptorβ (LXRβ) was somehow associated with 27OHC serum level and breast cancer risk. Still within the frame of the EPIC-Heidelberg cohort, 287 breast cancer cases with tumor tissue available were examined, matched to 563 controls. No significant association was observed with all four molecules related to 27OHC metabolism and action [18 Le Cornet et al. 2020]. Of note, the lack of association between

circulating 27OHC and intratumoral 27OHC and CYP27A1 levels actually confirmed previous observations of Wu et al. [16 -2013] and Winburg et al. [17 -2017] respectively.

In Table 1, the presently available studies on human breast cancer with regard to pros and cons of an increased circulating and/or intratumoral level of 27OHC or CYP27A1 are summarized. Most likely, it will be difficult to achieve conclusive and unique proofs of the role of 27OHC in an extremely complex clinical setting such as breast carcinogenesis.

Indeed, it should be expected that an excessive accumulation of 27OHC within human breast cancer tissue might significantly contribute to further enhance neoplastic development, but several key points should be taken into account: 1) still missing is a solid proof of a direct correlation between increased intratumoral 27OHC content and neoplastic progression; in addition, increased CYP27A1 and/or decreased CYPB1 activities in cancer tissue are reliably but still indirect indices of 27OHC accumulation; 2) in human breast cancers (n=11 ER-positive and n=11 ER-negative carcinomas), large intra-tumor variations in 27OHC content have been observed [22 Solheim et al, SBMB 2019]; 3) 27OHC showed mixed agonist/antagonist effects with regard to estrogen receptors [14 Du Sell 2007]; 4) estrogen receptors are not the only receptors that 27OHC binds to. This oxysterol is indeed a good ligand of Liver X Receptors (LXRs), retinoic acid receptor related orphan receptor (ROR), peroxisome proliferator-activated receptors (PPARs), Toll Like Receptors (TLRs) and of the endoplasmic reticulum proteins: sterol regulatory element binding protein (SREBP) and Insulin-induced gene 1 protein (INSIG) [see for a review 23 Vurusaner et al. MAM 2016]; 5) a cysteine-guanine polymorphism in the CYP7B1 has been reported [24 Jakobsson et al. 2004], suggesting the opportunity to further investigate the impact of single nucleotide polymorphisms of CYP7B1 gene but also of CYP27A1 gene in cancer predisposiiton.

3. **27OHC and prostate cancer.**

With regard to a possible role of 27-hydroxycholesterol in modulating expression and progression of prostate adenocarcinoma, the large majority of experimental pre-clinical studies point to a 27OHC as able to exert significant promotion and exacerbation effects, as recently and comprehensively reviewed by the group of Ghribi [25 Marwarha et al. 2017].

However, it should be noted that in their careful analysis of the number of molecular targets of this oxysterol on prostate parechyma, authors clearly reported that both stimulatory and inhibitory signals were generated by 27OHC as far as cell proliferation, epithelial-mesenchymal transition, cell invasionm migration and metastasis are concerned [25 Marwarha et al. 2017]. Thus, a conclusive statement was yet to be achieved.

Interestingly, the studies on human prostate cancer specimens nowadays available in the literature are all against a detrimental contribution of 27OHC, rather suggesting anti-prostate cancer effects exerted by this side-chain oxysterol.

Years ago, a small histochemical study on 12 specimens from human localized prostate cancers showed a significant decrease expression of CYP7B1 gene in the tumor tissue, likely dependent on hypomethylation of its promoter region [26 Ollson et al., 2007]. This finding should indirectly suggest a reduced rate of metabolic disposition of 27OHC.

In the same year, an independent investigation consisting in a RNA microarray analysis on specimens from 18 patients with hormone-refractory prostate carcinoma, 12 of them at metastatic stage, observed a net down-regulation of CYP27A1 expression. Such finding was interpreted by the authors as an advantage exploited by cancer cells for survival and further growth [27 Tamura et al. 2007].

More recently, coupled histochemical-bioinformatic analyses on a cohort of 329 patients with prostate cancer were able to detect a net drop of CYP27A1 expression in the cases with higher tumor grade and reduced disease-free survival [28 Alfaqih et al., 2017]. Hence, further data were obtained in the clinical setting indirectly indicating a decreased

concentration of 27OHC in advanced prostate cancer, due to a diminished oxysterol's synthesis.

Identical conclusion was provided by a longitudinal study carried out on two cancer cohorts of 404 and 254 prostate cancer patients, aiming at evaluating also metastasis development and death. Expression of CYP27A1 was again found lower in advanced stage carcinomas, with high Gleason score. Importantly, the clinical cases from both cohorts with higher risk of developing metastasis and eventually death were consistently showing a relatively low expression of the 27OHC synthetic enzyme in the neoplastic tissue [29 Khan et al. 2019].

Finally, a careful CYP27A1 oriented transcriptome profiling performed on a cohort of 794 prostate adenomas and 1,321 prostate carcinomas virtually achieved in the Prostate Cancer Transcriptome Atlas, allowed to demonstrate a gradual decrease of this enzyme expression from the benign neoplasias down to the hormone-resistant metastatic carcinomas [30 Dambal et al., 2020]. These authors completed their study with in vitro and in vivo experimental analyses all consistently showing a net inhibitory effect exerted by 27OHC on the IL6-JAK-STAT3 signaling pathway, that is well know to enhance prostate cancer cell growth [30 Dambal et al., 2020].

Actually, presently available data on intratumoral CYP27A1 concentration and its correlation with the disease progression in breast and prostate cancers appear to provide opposite evidence, being an increased tumor tissue level of this enzyme detrimental in breast carcinoma while beneficial in the prostatic one. Only solid data stemming in the near future from a direct measurement of the oxysterol 27OHC should allow to properly integrate and possibly confirm the CYP27A1 and also the CYP7B1 cancer-related findings.

4. 27OHC and other human cancers.

Indeed, the investigation of a potential implication of 27OHC in human carcinogenesis has so far been essentially limited to two hormone sensitive type of cancers, namely those developing in breast and prostate. Just few data are available in this relation in other tumor types.

With regard to the colon, while a supraphysiological concentration of 27OHC (10μM) was shown to significantly decrease the proliferation rate of Caco2 and SW620 intestinal cell lines, through the induction of apoptosis [31 Warns et al., 2018], in the only randomized clinical trial to date available, this oxysterol's plasma concentration rather appeared to be directly associated with the risk of developing colorectal adenoma [32 Passarelli et al., 2021]. Namely, by correlating 27OHC plasma levels in 1,246 individuals with their chance to develop colon preneoplastic neoplasias, an oxysterol concentration in the highest physiological range was combined to 80% increased possibility to have an advanced colorectal adenoma. [32 Passarelli et al., 2021].

To our knowledge, the only 27OHC-related study performed on human colorectal cancer specimens so far available is that performed by Rossin and colleagues [33 Rossin et al. 2019]. Surgical samples of tumor tissue as well as tumor adjacent tissue were obtained from 26 patients operated for colorectal cancer at different steps of progression. Tumor staging was made according to TNM and Dukes modified Aster-Coller classification (3 cases at stage I, 8 at stage II, 12 at stage III and 3 at stage IV). A statistically significant increase of 27OHC intratumoral concentration was detected by GC-MS only in the stage III specimens, that is the advanced stage colorectal carcinomas. Stage IV cancer specimens, actually very few, did not show any increased concentration of this oxysterol, presumably because of the extreme dedifferentiation of the tumor tissue itself. None of the four cancer stages showed increased 27OHC levels in the adjacent tissue [33 Rossin et al., 2019]. Because of the very small casistics, conclusive comments cannot be drawn about 27OHC

in human colon cancer, even if the impression of a rise of its intratumoral concentration at an advanced stage remains.

As far as brain cancers are concerned, LC-MS measurement of 27OHC was carried out in gliobastoma tissue specimens from 37 patients, further divided in two groups, on the basis of relatively low (n=21) and high (n=16) cancer content of this oxysterol. The overall survival of gliobastoma patients showing a higher 27OHC tumor tissue content resulted to be much lower than that of the other group [34 Liu et al., 2019].

Intratumoral 27-HC levels was also measured in thyroid tissue specimens from adenomas (n=40), low/intermediate risk (n=30) and high risk carcinomas (n=18), poorly differentiated/anaplastic cancers (n=9). Hence, in thyroid cancer as in the case of colorectal cancer, some increase of 27OHC was essentially observed only in the most advanced stage of progression and dedifferentiation. Further, such an increase of 27OHC content significantly correlated to a reduced expression of CYP7B1 in the same small group of late stage thyroid cancers, pointing to a reduced metabolic disposition of the oxysterol, while CYP27A1 expression did not show any significant variations among the different groups of tumors analyzed [35 Revilla et al., 2019].

The few data available for 27OHC and human cancer of brain, thyroid and colon origin, all would indicate a direct correlation between 27OHC intratumoral concentration and disease progression, especially in its advanced steps. Certainly, more clinical studies are needed to elucidate this matter, complicated by the possible heterogeneity of the oxysterol's distribution within the cancer tissue.

5. Conclusions

Even if the actual role of oxysterols like 27OHC in cancer promotion and progression remains debatable, the studies performed on human cancer specimens and the epidemiological surveys provide some interesting deduction.

First, a decidedly harmful role of 27OHC in the development of all malignat neoplasias in humans cannot be certainly claimed. In fact, if an enhancing effect of 27OHC on primary breast cancer is probably dependent upon its binding to estrogen receptors, the oxysterol exerted promotion of lung metastases already seems not to involve ER [Nelson 2013]. Besides the to date available epidemiological studies suggesting a significant correlation between high 27OHC levels and reduced risk of breast cancer [18 Le Cornet; 21 Lu et al], one should consider that all reports related to human prostate cancer consistently indicate a protective and not a detrimental role of 27OHC as to tumor progression and growth [26 Olsson; 27 Tamura; 28 Alfaqih; 29 Khan; Dambal].

Second, a disproportionate 27OHC concentration is probably unfavorable in advanced stage breast cancers, but also in those affecting organs like colorectum, thyroid and brain. The underlying mechanism of a possible further development of advanced cancers by 27OHC, clearly estrogen-independent, could be the recognized pro-inflammatory and procell survival properties of supraphysiological amounts of this oxysterol [23 Vurusaner]. Indeed, advanced malignant tumors are often accompanied by extensive necrotic areas and surrounding inflammatory cells, by the way cells with high expression of CYP27A1, the 27OHC generating enzyme [37 Bjorkhem et al., 1994].

Finally, distribution and activity of the two enzymes regulating 27OHC metabolism, i.e. CYP27A1 and CYP7B1, do not seem homogeneous within the cancer tissue, already an extremely heterogeneous entity per se. The likely existing polymorphisms of these two enzymes, definitely worth to be deeper analyzed in the near future, further complicates the overall picture.

Much better to keep a polytheistic behavior in Medicine.

6. Acknowledgements

The authors gratefully acknowledge Dr. Rosanna Falbo, Laboratory of Clinical Chemistry, Hospital of Desio, ASST-Brianza, for linguistic revision of the manuscript. This work was supported in part by the University of Turin, Italy (grants BIAF_RILO_18_01 and BIA-F_RILO_19_01 to F.B.; grant GAMP_RILO_20_01 to P.G.) and the Uni-versity of Milano Bicocca, Milan, Italy (grants 2019-ATE-0481 and 2020-ATE-0433 to V.L.).

7. References

[1] G.J. Schroepfer, Oxysterols: modulators of cholesterol metabolism and other processes, Physiol. Rev. 80 (2017) 361–554.

[2] B. Sottero, G. Leonarduzzi, G. Testa, S. Gargiulo, G. Poli, F. Biasi, Lipid oxidation redived aldehydes and oxysterols between health and disease, Eur J Lipid Sci Technol 121 (1) (2019) 1700047. doi: 10.1002/ejlt.201700047.

[3] S. Dzeletovic, O. Breuer, E. Lund, U. Diczfalusy, Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry, Anal Biochem 225 (1) (1995) 73-80. doi: 10.1006/abio.1995.1110.

[4] V. Leoni, C. Caccia, Oxysterols as biomarkers in neurodegenerative diseases, Chem Phys Lipids 164 (2011) 515-524. doi: 10.1016/j.chemphyslip.2011.04.002.

[5] Z. Pataj, G. Liebisch, G. Schmitz, S. Matysik, Quantification of oxysterols in human plasma and red blood cells by liquid chromatography high-resolution tandem mass spectrometry J Chromatogr A 1439 (2016) 82–8.

[6] A. Civra, V. Leoni, C. Caccia, S. Sottemano, P. Tonetto, A. Coscia, C. Peila, G.E. Moro, P. Gaglioti, E. Bertino, G. Poli, D. Lembo, Antiviral oxysterols are present in human milk at diverse stages of lactation, J Steroid Biochem Mol Biol 193 (2019) 105424. doi: 10.1016/j.jsbmb.2019.105424.

[7] M. Umetani, P.W. Shaul, 27-Hydroxycholesterol: the first identified endogenous SERM, Trends Endocrinol Metab 22(4) (2011)130–135. doi: 10.1016/j.tem.2011.01.003.

[8] D. Lembo, V. Cagno, A. Civra, G. Poli, Oxysterols: An emerging class of broad spectrum antiviral effectors, Mol. Aspects Med. 49 (2016) 23-30. https://doi: 10.1016/j.mam.2016.04.003.

[9] N.J. Spann, C.K. Glass, Sterols and oxysterols in immune cell function, Nat Immunol 14 (2013) 893-900. doi: 10.1038/ni.2681.

[10] S-M. Kim, B-Y. Kim, S-A. Lee, S-K. Eo, Y. Yun, C-D. Kim, K. Kim, 27-Hydroxycholesterol and 7alpha-hydroxycholesterol trigger a sequence of events leading to migration of CCR5-expressing Th1 lymphocytes, Toxicol Appl Pharmacol 274 (2014) 462-470. doi: 10.1016/j.taap.2013.12.007.

[11] Y. Son, S-M. Kim, S-A. Lee, S-K. Eo, K. Kim, Oxysterols induce transition of monocytic cells to phenotypically mature dendritic cell-like cells, Biochem Biophys Res Communs 438 (2013) 161–168. doi:10.1016/j.bbrc.2013.07.046.

[12] B. Sottero, D. Rossin, E. Staurenghi, P. Gamba, G. Poli, G. Testa, Omics analysis of oxysterols to better understand their pathophysiological role, Free Radic Biol Med 144 (2019) 55-71. doi: 10.1016/j.freeradbiomed.2019.05.026.

[13] M. Umetani, H. Domoto, A.K. Gormley, I.S. Yuhanna, C.L. Cummins, N.B. Javitt, K.S. Korach, P.W. Shaul, D.J. Mangelsdorf, 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen, Nat Med 13(10) (2007) 1185–92.

[14] C.D. DuSell, M Umetani, P.W. Shaul, D.J. Mangelsdorf, D.P. McDonnell, 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator, Mol Endocrinol 22(1) (2008) 65–77.

[15] E.R. Nelson, S.E. Wardell, J.S. Jasper, S. Park, S. Suchindran, M.K. Howe, N.J. Carver, R.V. Pillai, P.M. Sullivan, V. Sondhi, M. Umetani, J. Geradts, D.P. McDonnell, 27-

Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology, Science 342(6162) (2013) 1094-1098. doi:10.1126/science.1241908.

[16] Q. Wu, T. Ishikawa, R. Sirianni, H. Tang, J.G. McDonald, I.S. Yuhanna, B. Thompson, L. Girard, C. Mineo, R.A. Brekken, M. Umetani, D.M. Euhus, Y. Xie, P.W. Shaul, 27-Hydroxycholesterol promotes cell-autonomous er-positive
breast cancer growth, Cell Rep 5(3) (2013) 637–645. doi:10.1016/j.celrep.2013.10.006.

[17] S. Kimbung, C-Y. Chang, P-O. Bendahl, L. Dubois, J.W. Thompson, D.P. McDonnell, S. Borgquist, Impact of 27-hydroxylase (CYP27A1) and 27-hydroxycholesterol in breast cancer, Endocr Relat Cancer 24(7) (2017) 339-349. doi: 10.1530/ERC-16-0533.

[18] C. Le Cornet, B. Walter, D. Sookthai, T.S. Johnson, T. Kühn, E. Herpel, R. Kaaks, R.T. Fortner, Circulating 27-hydroxycholesterol and breast cancer tissue expression of CYP27A1, CYP7B1, LXR-β, and ERβ: results from the EPIC-Heidelberg cohort, Breast Cancer Res 22 (2020) 23. doi.org/10.1186/s13058-020-1253-6.

[19] S. Kimbung, M. Inasu, T. Stålhammar, B. Nodin, K. Elebro, H. Tryggvadottir, M. Ygland Rödström, K. Jirström, K. Isaksson, H. Jernström, S. Borgquist, CYP27A1 expression is associated with risk of late lethal estrogen receptor-positive breast cancer in postmenopausal patients, Breast Cancer Res 22 (2020) 123, doi.org/10.1186/s13058-020-01347-x.

[20] F. Dalenc, L. Iuliano, T. Filleron, C. Zerbinati, M. Voisin, C. Arellano, E. Chatelut, P. Marquet, M. Samadi, H. Roché, M. Poirot, S. Silvente-Poirot, Circulating oxysterol metabolites as potential new surrogate markers in patients with hormone receptor-positive breast cancer: Results of the OXYTAM study, J Steroid Biochem Mol Biol 169 (2017) 210–218. doi.org/10.1016/j.jsbmb.2016.06.010.

[21] D.L. Lu, C. Le Cornet, D. Sookthai, T.S. Johnson, R. Kaaks, R.T. Fortner, Circulating 27-hydroxycholesterol and breast cancer risk: results from the EPIC-Heidelberg cohort, J Natl Cancer Inst 111(4) (2019) djy115. doi: 10.1093/jnci/djy115.

[22] S. Solheim, S.A. Hutchinson, E. Lundanes, S.R. Wilson, J.L. Thorne, H. Roberg-Larsen, Fast liquid chromatography-mass spectrometry reveals side chain oxysterol heterogeneity in breast cancer tumour samples, J Steroid Biochem Mol Biol 192 (2019) 105309. doi.org/10.1016/j.jsbmb.2019.02.004.

[23] B. Vurusaner, G. Leonarduzzi, P. Gamba, G. Poli, H. Basaga, Oxysterols and mechanisms of survival signaling, Mol. Aspects Med 49 (2016) 8-22. doi.org/10.1016/j.mam.2016.02.004.

[24] J. Jakobsson, H. Karypidis, J.E. Johansson, H.K. Roh, A. Rane, L. Ekstrom, A functional C-G polymorphism in the CYP7B1 promoter region and its different distribution in Orientals and Caucasians, Pharmacogenomics J 4(4) (2004) 245–250.

[25] G. Marwaha, S. Raza, K. Hammer, O. Ghribi, 27-Hydroxycholesterol: A novel player in molecular carcinogenesis of breast and prostate cancer, Chem Phys Lipids 207 (2017) 108-126. doi.org/10.1016/j.chemphyslip.2017.05.012.

[26] M. Olsson, O. Gustafsson, C. Skogastierna, A. Tolf, B.D. Rietz, R. Morfin, A. Rane, L. Ekström, Regulation and expression of human CYP7B1 in prostate: overexpression of CYP7B1 during progression of prostatic adenocarcinoma, Prostate 67(13) (2007) 39-46. doi: 10.1002/pros.20630.

[27] K. Tamura, M. Furihata, T. Tsunoda, S. Ashida, R. Takata, W. Obara, H. Yoshioka, Y. Daigo, Y. Nasu, H. Kumon, H. Konaka, M. Namiki, K. Tozawa, K. Kohri, N. Tanji, M. Yokoyama, T. Shimazui, H. Akaza, Y. Mizutani, T. Miki, T. Fujioka, T. Shuin, Y. Nakamura, H. Nakagawa, Molecular features of hormone-refractory prostate cancer cells by genome-wide gene expression profiles. Cancer Res 67 (2007) 117–25. doi:10.1158/0008-5472.CAN-06-4040.

[28] M.A. Alfaqih1,2,4, E.R. Nelson, W. Liu, R. Safi, J.S. Jasper, E. Macias, J. Geradts, J. W. Thompson, L.G. Dubois, M.R. Freeman, C-y. Chang, J-T. Chi, D.P. McDonnell, S.J. Freedland, CYP27A1 loss dysregulates cholesterol homeostasis in prostate cancer, Cancer Res 77(7) (2017) 1662–1673. doi:10.1158/0008-5472.CAN-16-2738.

[29] N.A. Khan, K.H. Stopsack, E.H. Allott, T. Gerke, E. L. Giovannucci, L.A. Mucci, P.W. Kantoff, Intratumoral sterol-27-hydroxylase (CYP27A1) expression in relation to cholesterol synthesis and vitamin D signaling and its association with lethal prostate cancer, Cancer Epidemiol Biomarkers Prev 28(6) (2019) 1052-1058. doi:10.1158/1055-9965.EPI-18-1083.

[30] S. Dambal, M. Alfaqih, S. Sanders, E. Maravilla, A. Ramirez-Torres, G.C. Galvan, M. Reis-Sobreiro, M. Rotinen, L.M. Driver, M.S. Brehove, T. Jovanovic Talisman, J. Yoon, S. You, J. Turkson, J-T. Chi, M.R. Freeman, E. Macias, S.J. Freedland, 27-Hydroxycholesterol impairs plasma membrane lipid raft signaling as evidenced by inhibition of IL6-JAK-STAT3 signaling in prostate cancer cells, Mol Cancer Res 18(5) (2020) 671-684. doi:10.1158/1541-7786.MCR-19-0974.

[31] J. Warns, G. Marwarha, N. Freking, Othman Ghribi, 27-Hydroxycholesterol decreases cell proliferation in colon cancer cell lines, Biochimie 153 (2018) 171-180. doi:10.1016/j.biochi.2018.07.006.

[32] M.N. Passarelli, B.M. Thompson, J.G. McDonald, D.C. Snover , T.J. Palys, J.R. Rees, E.L. Barry, J.A. Baron, Circulating 27-hydroxycholesterol and risk of colorectal adenomas and serrated polyps, Cancer Prev Res (Phila) (2021) Jan 6. doi: 10.1158/1940-6207.CAPR-20-0414. Online ahead of print.

[33] D. Rossin, I.H.K. Dias, M. Solej, I. Milic, A.R. Pitt, N. Iaia, L. Scoppapietra, A. Devitt, M. Nano, M. Degiuli, M. Volante, C. Caccia, V. Leoni, H.R. Griffiths, C.M. Spickett, G. Poli, F. Biasi, Increased production of 27-hydroxycholesterol in human colorectal cancer advanced stage: Possible contribution to cancer cell survival and infiltration, Free Radic Biol Med 136 (2019) 35-44. doi.org/10.1016/j.freeradbiomed.2019.03.020.

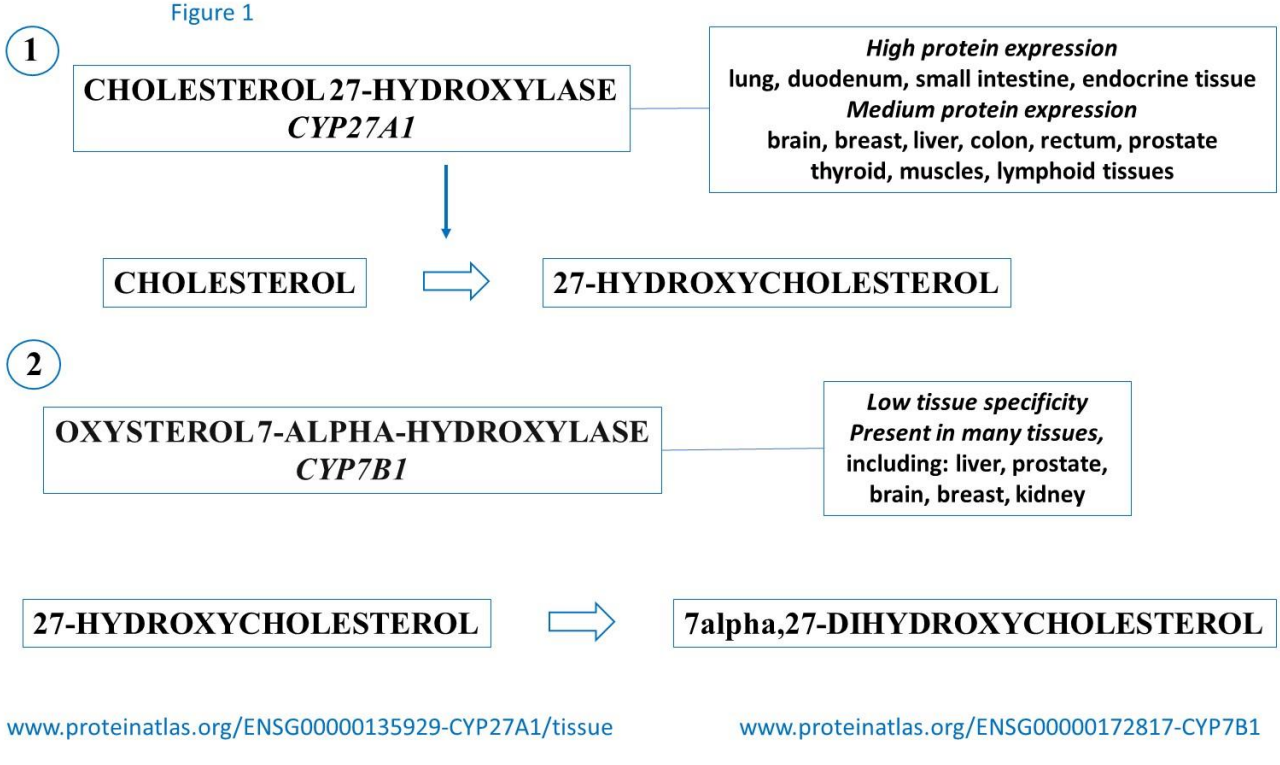
[34] L. Liu, M‐Y. Li, Y. Xing, X‐Y. Wang, Y. Wang, The oncogenic roles of 27‐hydroxycholesterol in glioblastoma, Oncol Lett 18(4) (2019) 3623-3629. doi: 10.3892/ol.2019.10690.

[35] G. Revilla, M. de Pablo Pons, L. Baila-Rueda, A. García-León, D. Santos, Ana Cenarro, M. Magalhaes, R.M. Blanco, A. Moral, J.I. Pérez, G. Sabé, C. González, V. Fuste, E. Lerma,

M. dos Santos Faria, A. de Leiva, R. Corcoy, J. C. Escolà-Gil, E. Mato, Cholesterol and 27-hydroxycholesterol promote thyroid carcinoma aggressiveness, Sci Rep 9 (2019) 10260. doi.org/10.1038/s41598-019-46727-2.

[36] G. Poli, F. Biasi, Potential modulation of cancer progression by oxysterols, Mol Aspects Med 49 (2016) 47-48. doi.org/10.1016/j.mam.2016.04.002

Figure 1



www.proteinatlas.org/ENSG00000135929-CYP27A1/tissue

www.proteinatlas.org/ENSG00000172817-CYP7B1

Figure 2