Crithmum maritimum restores the lipid and metabolic profiles of liver cancer cells to a normal phenotype

Davide Gnocchi
University of Bari Aldo Moro
Dragana Nikolic
University of Bari Aldo Moro
Rosa Rita Paparella
University of Bari Aldo Moro
Carlo Sabbà
University of Bari Aldo Moro
Antonio Mazzocca
antonio.mazzocca@uniba.it
University of Bari Aldo Moro

Research Article

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Abstract

Hepatocellular carcinoma (HCC) is becoming an alarming epidemiological clinical problem worldwide. Pharmacological approaches currently available do not provide adequate responses due to poor effectiveness, high toxicity, and serious side effects. We previously demonstrated that the wild edible plant *Crithmum maritimum* effectively slows the growth of liver cancer cells in vitro by reducing the bioenergetic and metabolic characteristics typical of transformed cells, particularly the fermentative phenotype (Warburg effect). Moreover, we found that *Crithmum maritimum* improves the expression of markers of differentiated hepatocytes. Here, we aimed to further characterize the effects of *Crithmum maritimum* on lipid accumulation and metabolism in HCC cells with different degrees of transformation. Additionally, we wanted to study markers of cellular metabolic health, such as AMP-activated protein kinase (AMPK), Sirtuin 1 (SIRT1), and Sirtuin 3 (SIRT3), as well as the insulin signaling pathway. To better model the biological spectrum of HCC, we employed HCC cell lines with varying degrees of transformation and invasiveness. Results indicate that *Crithmum maritimum* prevents lipid accumulation, downregulates lipid and cholesterol biosynthesis, and modulates markers of metabolic health, such as AMPK, SIRT1 and SIRT3. This effect is differentially modulated in different HCC cell lines, revealing an important functional versatility of *Crithmum maritimum*. These findings confirm the importance of *Crithmum maritimum* as a valuable nutraceutical, reinforcing its role in improving metabolic health.

Introduction

Today, metabolic diseases are growing at an alarming rate, driving the growth of many diseases, including cancer, cardiovascular disease, and neurodegenerative diseases [1–4]. Plant-derived products are regaining consideration in several fields of medicine, including oncology [5]. Moreover, the importance of plant-based diets and nutraceutical supplements is growing [6]. Hepatocellular carcinoma (HCC) is now an epidemiologic emergency and is the sixth leading cause of cancer-related death worldwide and is expected to become the third leading cause in Western countries by 2030 [7]. Metabolic dysregulation is now considered an important driver of HCC, as many HCC cases are the final stage of pathological progression from hepatic lipid accumulation (steatosis) [8] to inflammation, fibrosis and ultimately development of HCC [9]. Indeed, it has been estimated that about 35% of HCC cases can develop directly from simple steatosis [10]. The main treatment strategy for HCC is surgery, while drug treatment relies on a combination of tyrosine kinase inhibitors and immunotherapy [11]. However, these interventions have not lived up to expectations from an effectiveness and toxicity perspective. In this context, there is an urgent need to scientifically prove the efficacy of new, less toxic, and more efficacious specific opportunities for prevention and treatment of HCC. From this perspective, plant-based nutrition and nutritional supplements can provide valuable support [12]. We as first demonstrated the cytostatic effect of the ethyl acetate extract of *Crithmum maritimum* in HCC [13], linking it to a “rescheduling” of the tumour “metabolic signature”, with decrease of the levels of several metabolites, such as lactate, amino acids, cholesterol and polyunsaturated fatty acids (PUFA), as well as choline and phosphocholine, which fuel tumour growth. Additionally, we found increased levels of healthy monounsaturated fatty acids

(MUFA) [14]. We also previously demonstrated that *Crithmum maritimum* promotes oxidative phosphorylation (OXPHOS) [15], and can be used to reduce the dose and toxicity of tyrosine kinase inhibitors [16]. We later found that *Crithmum maritimum* sensitises HCC cells to tyrosine kinase inhibitors by inhibiting the (lactate) fermentation phenotype and improves the characteristic cancer metabolic profile of HCC, thereby promoting more closely related features of normal hepatocytes [17].

Here, we focused on further characterising the nutraceutical properties of *Crithmum maritimum* on the regulation of the metabolic phenotype of HCC, using four cell lines with different degree of aggressiveness and differentiation state, and therefore with a different metabolic phenotype, ranging from prevalent oxidative phosphorylation (OXPHOS) to prevalent lactic acid fermentation/Warburg effect [18]. This broad metabolic diversity was chosen to better characterise the effects of *Crithmum maritimum* on the biological and metabolic diversity found in liver metabolic diseases and HCC.

In this study, we found that *Crithmum maritimum* has potent homeostatic effects in HCC cells, reducing genes controlling lipogenesis and cholesterol production and activating metabolic health markers such as AMP-activated protein kinase (AMPK) [19], Sirtuin 1 (SIRT1) and Sirtuin 3 (SIRT3), and to the improvement of insulin response. All these metabolic markers are dysregulated in HCC, so *Crithmum maritimum* has a very promising potential as a therapeutic and preventive nutraceutical tool.

Materials and Methods

Preparation and characterisation of Crithmum maritimum ethyl acetate extract

Crithmum maritimum ethyl acetate extract was prepared and characterised as we previously reported [13].

Cell lines and culturing

A detailed description can be found in Supplementary Information.

Oil Red O (ORO) staining for evaluation of lipid accumulation

A detailed description can be found in Supplementary Information.

Quantitative Real-Time PCR (q-RT-PCR)

A detailed description can be found in Supplementary Information.

Immunoblotting analyses

A detailed description can be found in Supplementary Information.

Statistical analyses

A detailed description can be found in Supplementary Information.

Results and Discussion

First, we explored the role of *Crithmum maritimum* in regulating lipid and metabolic health homeostasis in HCC. Figure 1 shows that *Crithmum maritimum* 0.5 mM ethyl acetate extract prevents lipid accumulation in two HCC cell lines, HepG2 and HepaRG. These two cell lines were chosen because they have been shown to be good models of lipid accumulation *in vitro* [20]. To evaluate lipid accumulation, we used Oil Red O staining, which stains neutral lipids [9].

******* $p < 0.001$ compared to vehicle; ****** $p < 0.01$ compared to Vehicle ### $p < 0.001$ compared to OA

Next, we analysed the expression of two major genes involved in the lipid biosynthetic pathway, namely fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC). Overexpression of these two genes is associated with hepatocarcinogenesis [21, 22]. Additionally, we considered the expression of HMG-CoA reductase (*HMG-CoA Red*), a key gene involved in cholesterol biosynthesis, whose overexpression is associated with the development of cancer [23]. Finally, we also evaluated the expression of CD36, a fatty acid transporter whose overexpression has been shown to be associated with the development of hepatic steatosis and HCC [24, 25]. Our results show that *Crithmum maritimum* is effective in reducing lipid dysregulation (Fig. 2), which promotes the development of HCC. Since these genes are overexpressed in HCC, *Crithmum maritimum* can be used to normalise the transformed lipid phenotype in well-established HCC.

As a successive step, we evaluated the nutraceutical potential of *Crithmum maritimum* in the regulation of genes that are considered reliable indicators of the metabolic status of cells. In particular, we analysed the expression of Lactate Dehydrogenase A (*LDHA*), Lactate Dehydrogenase B (*LDHB*), AMP-activated protein kinase (*AMPK*), Sirtuin 1 (*SIRT1*), Sirtuin 3 (*SIRT3*). Each of these genes controls key steps in the regulation of metabolic homeostasis, and their expression is dysregulated in tumour cells and also in HCC. In particular, *LDHA* is usually upregulated [26], whereas *LDHB* is downregulated [27]. All the other genes are downregulated [28]. In particular, the activation of *AMPK, SIRT1* and *SIRT3* has been suggested as a preventative and therapeutic opportunity for HCC [29–31]. The data reported in Fig. 3 demonstrate that *Crithmum maritimum* effectively reverses the pathogenic expression pattern of these genes (Fig. 3a-d). Quantitative differences can be observed in the different cell lines used, which model different degrees of transformation, differentiation, and invasiveness of HCC. For example, HLE are less differentiated and relay more on lactic acid fermentation compared with Huh7 [18], and Huh7 are in turn more aggressive and more fermentative compared to HepG2 and HepaRG, which are the least aggressive and less tumorigenic. In particular, HepaRGs are considered a good model for non-transformed hepatocytes, even though they cannot be compared with primary hepatocytes. Notably, the effect of *Crithmum maritimum* was evident despite the different characteristics of the cell lines. The effect on gene expression has been substantiated by Western Blot analyses, by the evaluation of the activation by phosphorylation at Thr172

of AMPK. The results demonstrated that the AMPK signalling pathway was activated in HepG2 and Huh7 (Fig. 3e). Figure 3f summarises the main concepts expressed in the reported results.

Finally, we investigated the role of *Crithmum maritimum* in the regulation of the insulin signaling pathway, whose dysregulation has been implicated in the development and progression of HCC. In particular, we measured the expression of the three main genes involved in the control of the pathway, namely Insulin Receptor (*IR*), Insulin Receptor Substrate 1 (*IRS-1*) and Insulin Receptor Substrate 2 (*IRS-2*). Moreover, we evaluated the activation by phosphorylation of Akt at Ser473, which is a well-recognised marker of activation of the insulin signalling pathway. The results reported in Fig. 4 depicts a very interesting scientific picture, as *Crithmum maritimum* shows a differential effect that varies according to the degree of aggressiveness and Warburg phenotype of the cell line used. In fact, in HepG2, which display a “low tumorigenic” phenotype, both gene expression and Western blot analyses show a moderate activation of insulin signalling (Fig. 4a, e), while in HepaRG no significative effect was observed (Fig. 4b). In contrast, in the more tumorigenic and invasive Huh7 and HLE, *Crithmum maritimum* inhibited insulin signalling (Fig. 4c-e). These results are graphically summarised in Fig. 4f. Of note, it has been reported that both *IRS-1* and *IRS-2* are upregulated in HCC [32].

We previously reported that *Crithmum maritimum* inhibits HCC cell growth [13] and modulates metabolic [14] and bioenergetic characteristics by activating OXPHOS [15]. We also demonstrated that *Crithmum maritimum* reduced the expression of HCC markers *α-fetoprotein* (*α-FP*) and *α1-antitrypsin* (*α1-AT*) and induced a shift in the HCC bioenergetic profile from lactic acid fermentation to OXPHOS [33, 34]. Indeed, we recently showed that OXPHOS inhibition is associated with drug resistance in HCC cell lines [35]. In our future studies, we will continue to explore OXPHOS-mediated drug sensitivity promotion and explore the mechanisms behind the drug sensitization effects of *Crithmum maritimum*. This may provide additional translational applications for the nutritional properties of *Crithmum maritimum*.

Overall, *Crithmum maritimum* shows a strong multifaceted bioactive action, which represents a very important resource in the management of HCC and possibly of other types of tumours. It is worth to underline here the systemic and multi-targeted action exerted by the blend of compounds found in *Crithmum maritimum* (Fig. S1), which guarantees a full-spectrum action on several of the central bioenergetic traits characteristic of hepatocytes. Finally, it is important to emphasize that the effects of *Crithmum maritimum* appear to be modulated according to the metabolic phenotype of the interacting cells, as shown in Fig. 5 below.

Conclusions

Our current findings provide evidence that *Crithmum maritimum* actively interacts with HCC cells to improve their lipid and metabolic profiles and orient them towards the phenotype of non-transformed cells. Moreover, the normalization of well-known “metabolic health promoters”, such as AMPK, SIRT1 and SIRT3, as well as the promotion of insulin sensitivity, strengthens the importance of Crithmum *maritimum* as a valuable and concrete nutraceutical option to support lifestyle protocols aimed to prevent and cure

dysmetabolic conditions, which are becoming a serious health concern worldwide but especially in Western countries [1–4].

Based on our previous findings and what we present here, we propose *Crithmum maritimum* as a nutraceutical tool for developing strategies aimed at supporting conventional HCC pharmacotherapy and reducing side effects. In addition, supplementation with *Crithmum maritimum* may be used to prevent metabolic diseases of the liver and possibly other types of cancer. Future research will be directed to the preparation of formulations for human clinical trials to conduct case-control studies on HCC patients, combining appropriate extract preparations with standard therapies. In line with this, we are about to start a clinical study to test the effect of a formulation of *Crithmum maritimum* as a nutraceutical supplement for the improvement of metabolic health, with particular focus on triglycerides and cholesterol levels, and to insulin sensitivity.

Declarations

Author contributions:D.G. conceived the article, performed the experiments and wrote the manuscript, D.N. performed the experiments, R.R.P. performed the experiments, C.S. revised the manuscript, and A.M. conceived the article, wrote and revised the manuscript.

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Ethical Approval: “not applicable”

Consent to Participate: This article has been approved by all authors.

Consent for Publication: This manuscript has not been copyrighted or published elsewhere.

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Availability of data and materials: All data presented in this study are available upon request.

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

References

1. Zhang Y, Ding Y, Zhu N, Mi M, Lu Y, Zheng J, Weng S, Yuan Y (2023) Emerging patterns and trends in global cancer burden attributable to metabolic factors, based on the Global Burden of Disease Study 2019. Front Oncol 13:1032749. 10.3389/fonc.2023.1032749
2. Hu W, Zhai C, Sun H, Gong X, Cui L, Cai L, Zong Q, Yu G, Wang F, Zou Y (2023) The global burden of disease attributable to metabolic risks in 204 countries and territories from 1990 to 2019. Diabetes Res Clin Pract 196:110260. 10.1016/j.diabres.2023.110260

3. Collaborators GBDD (2023) Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet 402(10397):203–234. 10.1016/S0140-6736(23)01301-6
4. Chew NWS, Ng CH, Tan DJH, Kong G, Lin C, Chin YH, Lim WH, Huang DQ, Quek J, Fu CE, Xiao J, Syn N, Foo R, Khoo CM, Wang JW, Dimitriadis GK, Young DY, Siddiqui MS, Lam CSP, Wang Y, Figtree GA, Chan MY, Cummings DE, Noureddin M, Wong VW, Ma RCW, Mantzoros CS, Sanyal A, Muthiah MD (2023) The global burden of metabolic disease: Data from 2000 to 2019. Cell Metab 35(3):414–428e413. 10.1016/j.cmet.2023.02.003
5. Newman DJ, Cragg GM (2020) Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. J Nat Prod 83(3):770–803. 10.1021/acs.jnatprod.9b01285
6. Pena-Jorquera H, Cid-Jofre V, Landaeta-Diaz L, Petermann-Rocha F, Martorell M, Zbinden-Foncea H, Ferrari G, Jorquera-Aguilera C, Cristi-Montero C (2023) Plant-Based Nutrition: Exploring Health Benefits for Atherosclerosis, Chronic Diseases, and Metabolic Syndrome-A Comprehensive Review. Nutrients 15(14). 10.3390/nu15143244
7. de Lope CR, Tremosini S, Forner A, Reig M, Bruix J (2012) Management of HCC. J Hepatol 56(Suppl 1):S75–87. 10.1016/S0168-8278(12)60009-9
8. Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P (2018) Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. Cell Mol Life Sci 75(18):3313–3327. 10.1007/s00018-018-2860-6
9. Gnocchi D, Afonso MB, Cavalluzzi MM, Lentini G, Ingravallo G, Sabba C, Rodrigues CMP, Mazzocca A (2023) Inhibition of lysophosphatidic acid receptor 6 upregulated by the choline-deficient l-amino acid-defined diet prevents hepatocarcinogenesis in mice. Mol Carcinog 62(5):577–582. 10.1002/mc.23516
10. Grgurevic I, Bozin T, Mikus M, Kukla M, O'Beirne J (2021) Hepatocellular Carcinoma in Non-Alcoholic Fatty Liver Disease: From Epidemiology to Diagnostic Approach. Cancers (Basel) 13(22). 10.3390/cancers13225844
11. Forner A, Reig M, Bruix J (2018) Hepatocellular carcinoma. Lancet. 10.1016/S0140-6736(18)30010-2
12. Tran Q, Lee H, Kim C, Kong G, Gong N, Kwon SH, Park J, Kim SH, Park J (2020) Revisiting the Warburg Effect: Diet-Based Strategies for Cancer Prevention. Biomed Res Int 2020:8105735. 10.1155/2020/8105735
13. Gnocchi D, Cesari G, Calabrese GJ, Capone R, Sabba C, Mazzocca A (2020) Inhibition of Hepatocellular Carcinoma Growth by Ethyl Acetate Extracts of Apulian Brassica oleracea L. and Crithmum maritimum L. Plant Foods Hum Nutr 75(1):33–40. 10.1007/s11130-019-00781-3
14. Gnocchi D, Del Coco L, Girelli CR, Castellaneta F, Cesari G, Sabba C, Fanizzi FP, Mazzocca A (2021) (1)H-NMR metabolomics reveals a multitarget action of Crithmum maritimum ethyl acetate extract in inhibiting hepatocellular carcinoma cell growth. Sci Rep 11(1):1259. 10.1038/s41598-020-78867-1
15. Gnocchi D, Sabba C, Mazzocca A (2022) The Edible Plant Crithmum maritimum Shows Nutraceutical Properties by Targeting Energy Metabolism in Hepatic Cancer. Plant Foods Hum Nutr 77(3):481–483.

10.1007/s11130-022-00986-z

16. Gnocchi D, Castellaneta F, Cesari G, Fiore G, Sabba C, Mazzocca A (2021) Treatment of liver cancer cells with ethyl acetate extract of Crithmum maritimum permits reducing sorafenib dose and toxicity maintaining its efficacy. J Pharm Pharmacol. 10.1093/jpp/rgab070
17. Gnocchi D, Sabba C, Mazzocca A (2023) Crithmum maritimum Improves Sorafenib Sensitivity by Decreasing Lactic Acid Fermentation and Inducing a Pro-Hepatocyte Marker Profile in Hepatocellular Carcinoma. Plant Foods Hum Nutr 78(1):230–232. 10.1007/s11130-022-01037-3
18. Nwosu ZC, Battello N, Rothley M, Pioronska W, Sitek B, Ebert MP, Hofmann U, Sleeman J, Wolfl S, Meyer C, Megger DA, Dooley S (2018) Liver cancer cell lines distinctly mimic the metabolic gene expression pattern of the corresponding human tumours. J Exp Clin Cancer Res 37(1):211. 10.1186/s13046-018-0872-6
19. Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD + metabolism and SIRT1 activity. Nature 458(7241):1056–1060. 10.1038/nature07813
20. Ramos MJ, Bandiera L, Menolascina F, Fallowfield JA (2022) In vitro models for non-alcoholic fatty liver disease: Emerging platforms and their applications. iScience 25(1):103549. 10.1016/j.isci.2021.103549
21. Shen Y, Wang X, Ni Z, Xu S, Qiu S, Zheng W, Zhang J (2023) Identification of acetyl-CoA carboxylase alpha as a prognostic and targeted candidate for hepatocellular carcinoma. Clin Transl Oncol 25(8):2499–2513. 10.1007/s12094-023-03137-1
22. Hao Q, Li T, Zhang X, Gao P, Qiao P, Li S, Geng Z (2014) Expression and roles of fatty acid synthase in hepatocellular carcinoma. Oncol Rep 32(6):2471–2476. 10.3892/or.2014.3484
23. Murai T (2015) Cholesterol lowering: role in cancer prevention and treatment. Biol Chem 396(1):1–11. 10.1515/hsz-2014-0194
24. Zeng H, Qin H, Liao M, Zheng E, Luo X, Xiao A, Li Y, Chen L, Wei L, Zhao L, Ruan XZ, Yang P, Chen Y (2022) CD36 promotes de novo lipogenesis in hepatocytes through INSIG2-dependent SREBP1 processing. Mol Metab 57:101428. 10.1016/j.molmet.2021.101428
25. Luo X, Zheng E, Wei L, Zeng H, Qin H, Zhang X, Liao M, Chen L, Zhao L, Ruan XZ, Yang P, Chen Y (2021) The fatty acid receptor CD36 promotes HCC progression through activating Src/PI3K/AKT axis-dependent aerobic glycolysis. Cell Death Dis 12(4):328. 10.1038/s41419-021-03596-w
26. Sheng SL, Liu JJ, Dai YH, Sun XG, Xiong XP, Huang G (2012) Knockdown of lactate dehydrogenase A suppresses tumor growth and metastasis of human hepatocellular carcinoma. FEBS J 279(20):3898–3910. 10.1111/j.1742-4658.2012.08748.x
27. Chen R, Zhou X, Yu Z, Liu J, Huang G (2015) Low Expression of LDHB Correlates With Unfavorable Survival in Hepatocellular Carcinoma: Strobe-Compliant Article. Med (Baltim) 94(39):e1583. 10.1097/MD.0000000000001583
28. Gnocchi D, Sabba C, Massimi M, Mazzocca A (2023) Metabolism as a New Avenue for Hepatocellular Carcinoma Therapy. Int J Mol Sci 24(4). 10.3390/ijms24043710

29. Zeng X, Wang N, Zhai H, Wang R, Wu J, Pu W (2017) SIRT3 functions as a tumor suppressor in hepatocellular carcinoma. Tumour Biol 39(3):1010428317691178. 10.1177/1010428317691178
30. Wang RH, Sengupta K, Li C, Kim HS, Cao L, Xiao C, Kim S, Xu X, Zheng Y, Chilton B, Jia R, Zheng ZM, Appella E, Wang XW, Ried T, Deng CX (2008) Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. Cancer Cell 14(4):312–323. 10.1016/j.ccr.2008.09.001
31. Tseng HI, Zeng YS, Lin YJ, Huang JW, Lin CL, Lee MH, Yang FW, Fang TP, Mar AC, Su JC (2022) A novel AMPK activator shows therapeutic potential in hepatocellular carcinoma by suppressing HIF1alpha-mediated aerobic glycolysis. Mol Oncol 16(11):2274–2294. 10.1002/1878-0261.13211
32. Sakurai Y, Kubota N, Takamoto I, Obata A, Iwamoto M, Hayashi T, Aihara M, Kubota T, Nishihara H, Kadowaki T (2017) Role of insulin receptor substrates in the progression of hepatocellular carcinoma. Sci Rep 7(1):5387. 10.1038/s41598-017-03299-3
33. Tzonou A, Sparos L, Kalapothaki V, Zavitsanos X, Rebelakos A, Trichopoulos D (1990) Alpha 1-antitrypsin and survival in hepatocellular carcinoma. Br J Cancer 61(1):72–73. 10.1038/bjc.1990.16
34. Ohguchi S, Nakatsukasa H, Higashi T, Ashida K, Nouso K, Ishizaki M, Hino N, Kobayashi Y, Uematsu S, Tsuji T (1998) Expression of alpha-fetoprotein and albumin genes in human hepatocellular carcinomas: limitations in the application of the genes for targeting human hepatocellular carcinoma in gene therapy. Hepatology 27(2):599–607. 10.1002/hep.510270239
35. Gnocchi D, Kurzyk A, Mintrone A, Lentini G, Sabba C, Mazzocca A (2022) Inhibition of LPAR6 overcomes sorafenib resistance by switching glycolysis into oxidative phosphorylation in hepatocellular carcinoma. Biochimie. 10.1016/j.biochi.2022.07.016

Figures

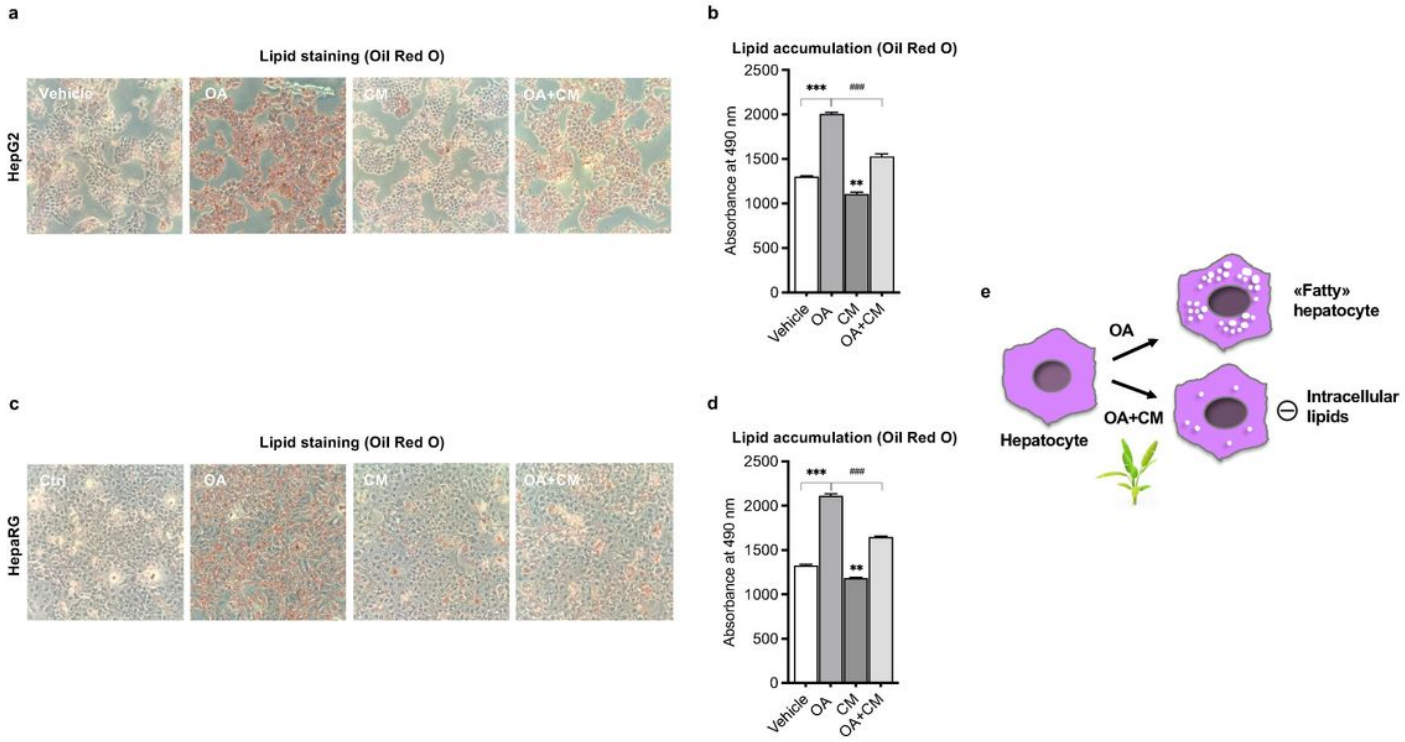

Figure 1

Crithmum maritimum prevents lipid accumulation in HCC cell lines. Lipid accumulation was evaluated by Oil Red O (ORO) staining. ORO pictures and quantification of HepG2 cells (a-b) and HepaRG cells (c-d). (e) Graphical summary of the key message described in the Figure.

******* p<0.001 compared to vehicle; ****** p<0.01 compared to Vehicle ### p<0.001 compared to OA

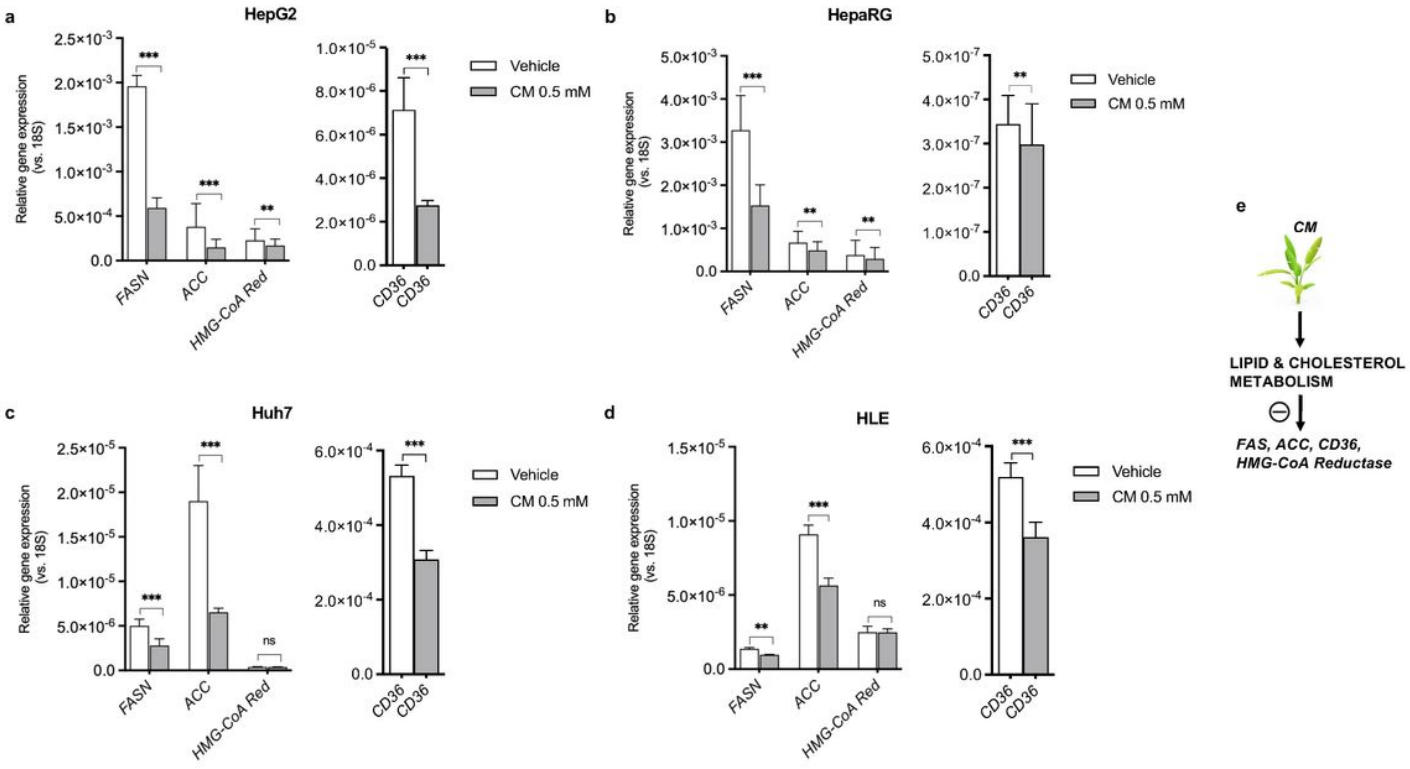

Figure 2

Crithmum maritimum restores the expression of lipid metabolism genes in HCC cell lines. The expression of four key genes for lipid and cholesterol metabolism in four HCC cell lines HepG2 (a), HepaRG (b), Huh7 (c), HLE (d) was detected by real-time q-PCR. *** p<0.001, ** p<0.01, ns not significant compared to vehicle

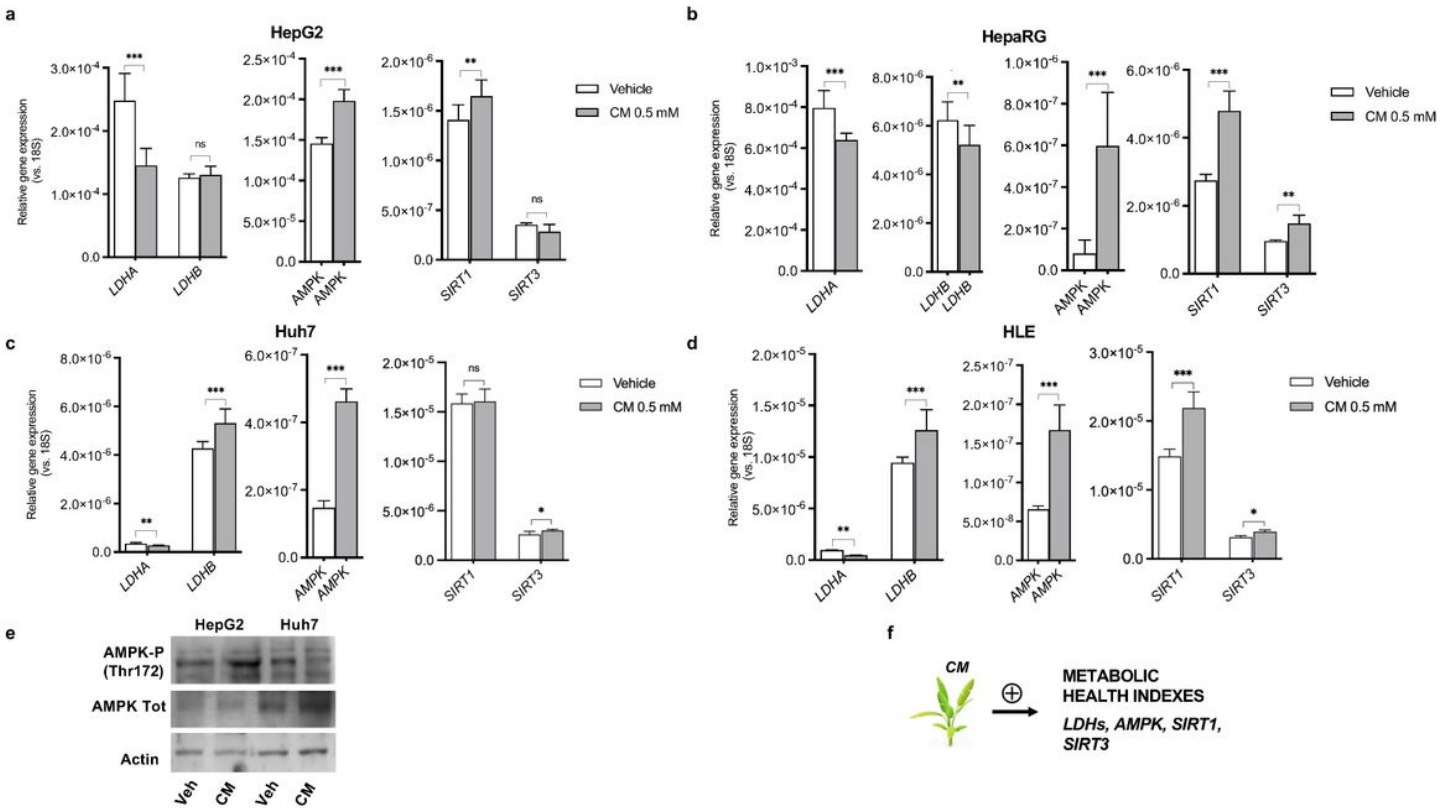

Figure 3

Crithmum maritimum improves the expression of metabolic health marker genes in HCC cell lines. The expression of key genes involved in the control of metabolic health status was evaluated by real-time q-PCR in four HCC cell lines HepG2 (a), HepaRG (b), Huh7 (c), HLE (d). The activation of AMPK has been analysed by Western Blot in HepG2 and Huh7 cells (e). (f) Graphical summary of the key message described in the Figure

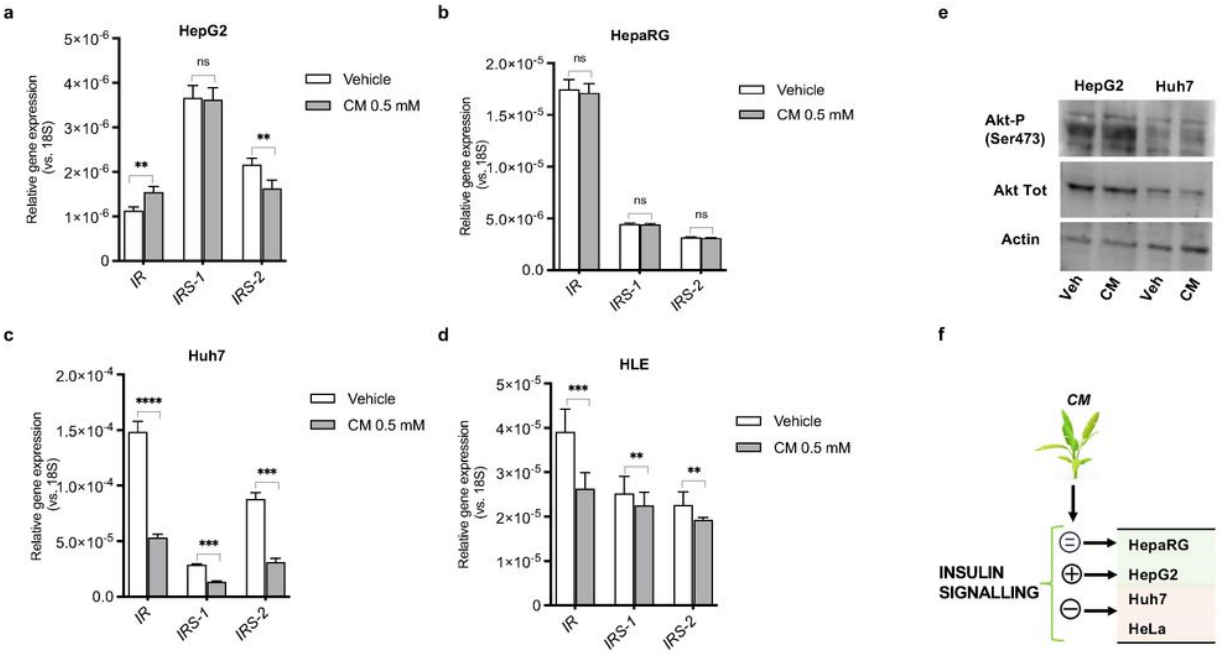

Figure 4

Crithmum maritimum differentially regulates insulin signalling depending on the degree of transformation of HCC cells. The expression of three genes involved in the control of insulin signaling was evaluated by real-time q-PCR in four HCC cell lines HepG2 (a), HepaRG (b), Huh7 (c), HLE (d). The activation of insulin signalling has been verified by Western Blot by the evaluation of Akt phosphorylation at Ser473 in HepG2 and Huh7 cells (e). (f) Graphical summary of the information presented in the Figure

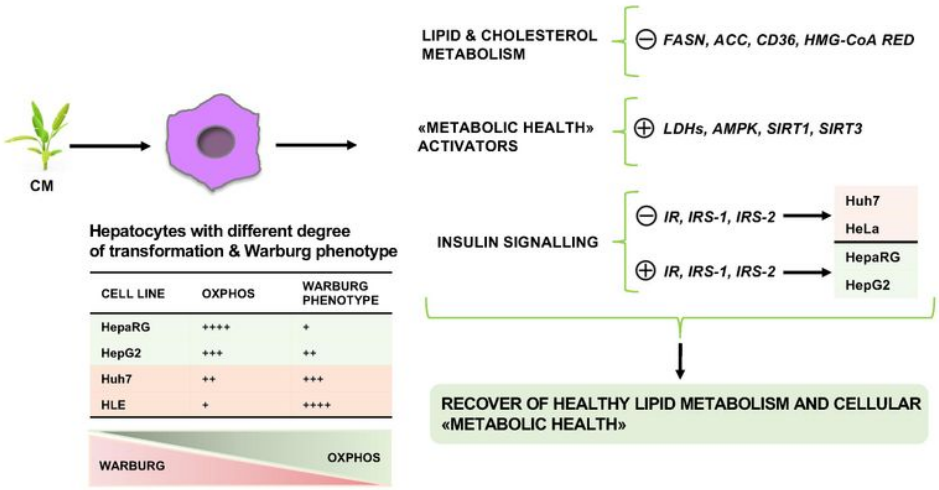

Figure 5

Schematic representation of the effects of *Crithmum maritimum* on HCC cell lines with varying degrees of transformation and Warburg phenotype. As described in the article, *Crithmum maritimum* prevents lipid accumulation, reduces the expression of key genes that control lipogenesis, and promotes metabolic markers of health

Supplementary Files

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