Research article

The ketone body β-Hydroxybutyrate as a fuel source of chondrosarcoma cells

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ABSTRACT

Chondrosarcoma (CS) is a malignant bone tumor arising from cartilage-producing cells. The conventional subtype of CS typically develops within a dense cartilaginous matrix, creating an environment deficient in oxygen and nutrients, necessitating metabolic adaptation to ensure proliferation under stress conditions. Although ketone bodies (KBs) are oxidized by extrahepatic tissue cells such as the heart and brain, specific cancer cells, including CS cells, can undergo ketolysis. In this study, we found that KBs catabolism is activated in CS cells under nutrition-deprivation conditions. Interestingly, cytosolic β-hydroxybutyrate dehydrogenase 2 (BDH2), rather than mitochondrial BDH1, is expressed in these cells, indicating a specific metabolic adaptation for ketolysis in this bone tumor. The addition of the KB, β-Hydroxybutyrate (β-HB) in serum-starved CS cells re-induced the expression of BDH2, along with the key ketolytic enzyme 3-oxoacid CoA-transferase 1 (OXCT1) and monocarboxylate transporter-1 (MCT1). Additionally, internal β-HB production was quantified in supplied and starved cells, suggesting that CS cells are also capable of ketogenesis alongside ketolysis. These findings unveil a novel metabolic adaptation wherein nutrition-deprived CS cells utilize KBs for energy supply and proliferation.

1. Introduction

Chondrosarcoma (CS) is a cartilage-producing malignant tumor that occupies the second place of bone sarcomas, following osteosarcoma [1]. This neoplasm may appear *de novo* or derive from the malignant transformation of benign cartilage tumors such as osteochondroma and enchondroma. CS is classified based on its location as central or peripheral, depending on whether it occurs in the medullary cavity or the articular surface. Histologically, CS encompasses various types, including conventional (the most common), dedifferentiated, clear cell, and mesenchymal variants [2]. The prognosis and treatment of CS depend on histologic grade and location, making the curettage and resection the first choice. However, identifying molecular markers for pharmacological or prognostic applications is imperative, especially in cases where complete tumor resection is not feasible in certain anatomical sites [3].

Tumor progression involves the metabolic reprogramming of cancer cells, wherein various metabolic pathways collaborate to meet

the heightened bioenergetic and biosynthetic demand required for tumor cell survival [4]. Among the metabolic pathways studied in chondrosarcoma (CS), a prominent focus lies on the generation of the oncogenic metabolite δ-2-hydroxyglutarate (D2HG) from α-ketoglutarate in the Krebbs cycle given the gene mutations of isocitrate dehydrogenase −1 and −2 (IDH1, IDH2) [5]. Furthermore, D2HG contributes to changes in epigenetic pathways, such as cell growth and migration, frequently correlated to poor patient survival [6]. Consistent with IDH1 mutations, increased biosynthesis of cholesterol levels is also higher in CS, even though the role of this lipid in tumor development is not elucidated yet [7]. Additionally, heightened activity of lactate dehydrogenase A (LDHA) and glycolysis has been demonstrated in CS cells, prompting investigations into LDHA inhibitors such as oxamate and 1-phenylseleno-4-trifluouro-methyl benzene have been evaluated in CS mouse models [8,9]. While the metabolic pathways mentioned above have elucidated certain metabolic characteristics of chondrosarcoma, many other pathways remain unknown, such as the metabolism of ketone bodies (KBs). Notably, recent research from our group delineated the serum metabolic profile of cartilage tumors in patients diagnosed with enchondromas and chondrosarcomas, revealing dysregulation in the synthesis and degradation of KBs as a significant pathway [10]. KBs, including acetoacetate, acetone, and β-hydroxybutyrate (β-HB), are alternative energy sources in low glucose conditions. KBs are produced by the liver from β-oxidation of fatty acids during fasting or intense physical activity; after its secretion to the systemic circulation, they fuel target organs such as skeletal muscle, brain, and heart [11]. In cancer scenarios, it has been demonstrated that some neoplastic cells cannot use the KBs. Consequently, the ketogenic diet, characterized by high fat and low carbohydrate intake, has shown anti-tumour effects in various types of cancers, including brain, prostate, breast, lung, gastric, and colon [12,13]. However, owing to the heterogeneity of tumorigenic cells, some express ketolytic enzymes to degrade KBs. This metabolic adaptation has been highlighted in HeLa and hepatocarcinoma cells Hep3B, which, under nutrient deprivation, upregulate the expression of the ketolytic enzyme 3-hydroxybutyrate dehydrogenase 1 (BDH1) and (3-oxoacid-CoA transferase 1 (OXCT1) to support proliferation [14,15].

Conversely, upregulation of enzymes involved in ketogenesis or KBs synthesis, such as HMG-CoA lyase (HMGCL), has been detected in melanoma cells [16] and pancreatic ductal adenocarcinoma in mice, promoting tumor growth [17]. Nevertheless, the effects of KBs on bone tumors such as CS have not been explored to date. Therefore, in this study, the results establish the metabolic reprogramming of CS cells by regulating ketolytic enzymes under nutritional starvation conditions, thereby promoting sarcoma cell proliferation.

2. Material and methods

2.1. Cell culture

The human chondrosarcoma cell line SW 1353 was acquired from the American Type Culture Collection (HTB-94, ATCC Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (12800-058 GIBCO, Auckland NZ) supplemented with 10 % fetal bovine serum (FBS) (S1560-500 Biowest, Riverside, MO, USA) and 1 % penicillin-streptomycin (15240-062 GIBCO, NY, USA) in a humidified incubator at 37 °C and 5 % CO_2. Once SW 1353 cells reached a confluency of about 80 %, they were trypsinized and cultured in flasks according to the experiment: 1×10^4 cells in 96-well culture plates for proliferation assays; 2.1×10^6 cells in T75 flasks for Western Blot (WB); 2.5×10^5 cells in 6-well culture plates for semi-quantitative RT-PCR. All experiments were performed at least in triplicate, culturing SW 1353 cells in three different conditions: in supplemented medium (supplied), without FBS (starvation), or without FBS adding L-β-hydroxybutyrate (β-HB) (Sigma-Aldrich, Toluca, Mexico) at concentrations of 10 or 25 mM, for 12, 24, or 48 h.

2.2. Proliferation assays

Cell proliferation assays were conducted by adding 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (M2128; Sigma-Aldrich, Toluca, Mexico) to the cells, followed by incubation in a humidified incubator for 5 h. After removal of the medium, formazan crystals were dissolved using 100 μl of DMSO (D8418, Sigma-Aldrich, Toluca, Mexico). The absorbance was read at 570 nm using 630 nm as reference wavelength on a multiwell plate reader Epoch Microplate Spectrophotometer (Biotek Instruments, Inc., Winooski, VT, USA). The proliferation index was calculated by dividing the number of viable cells in each group at 24 or 48 h by the number of viable cells at 0 h.

2.3. Western blot

The cultured cells were washed with PBS and lysed with RIPA buffer (89901, Thermo Scientific, Waltham, MA, USA). Thirty μg of protein, quantified using PierceTM 660 nm protein assay reagent (23275, Thermo Scientific), was fractionated in a 10 % SDS-PAGE and transferred to a nitrocellulose membrane of 0.45 μM pore–size (1620115, BioRad, Hercules, CA, USA) in a Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BioRad). The blots were blocked with 1 % non-fat dry milk for 30 min, followed by incubation with rabbit primary antibodies against OXCT1 1:1500 dilution (Cat. No. 203401/T46; Sino Biological, Wayne, Pennsylvania), BDH1 1:1500 (Cat. No. MBS1491516; MyBioSource, San Diego, CA, USA), ACAT1 1:500 (Cat. No. PAD664Hu01; MyBioSource), MCT1 1:1000 dilution (Cat. No. GTx35258; GeneTex, Irvine, CA, USA) or β-actin 1:5000 dilution (Cat No. FNab00869; Sigma Aldrich) with shaking for 1.5 h at room temperature. Subsequently, goat Anti-Rabbit IgG (Cat. No. A0545; Sigma-Aldrich) was incubated for 1 h, and the signal was developed using Western ECL Substrate (Bio-Rad). Densitometric analysis of the scanned images was performed using Image Studio™ Lite Software (LI-COR Biosciences, Lincoln, NE, USA).

2.4. Semi-quantitative reverse transcription-polymerase chain reaction

SW 1353 cells were PBS washed, and the RNA was isolated using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). Total RNA concentration was evaluated using an Epoch Microplate Spectrophotometer at 260 nm. The cDNA was produced using two μg of total RNA and M-MLV reverse transcriptase (Invitrogen). PCR reactions (25 μL) were carried out using one μL of the cDNA reaction, 0.4 μL of MyTaq Polymerase (BIO-21106 Bioline Meridian Bioscience, OH, USA), five μL of MyTaq Reaction Buffer, and 0.2 μM each of OXCT1, ACAT1, BDH1, BDH2, MCT1 or the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers as housekeeping gene (Table 1). The optimized PCR conditions were as follows: 95 °C for 5 min and 35 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. The amplified DNA was analyzed on a 2 % agarose gel and stained using RedGel ®(Biotium Inc., Hayward, CA, USA). Gel images were acquired in a ChemiDoc-ItTM transilluminator (UVP, Upland, CA, USA), and the integrated pixel density (PD) of each band was calculated using Image J software [18]. The PD of each gene band was normalized by dividing the PD of the gene of interest by that of the housekeeping gene (GAPDH) band in each sample. The change in the expression of each gene was calculated by dividing the expression of the normalized gene in the specified group by that of the expression of the normalized gene in the control group. All gel images can be found in the supplementary.

2.5. β-HB measurements

SW 1353 cells were detached with trypsin 0.25 % (Sigma-Aldrich) and used to quantify intracellular β-HB production using the β-HB Assay Kit (MAK041 Sigma-Aldrich).

2.6. Statistical analysis

To analyze differences in the mRNA and protein expression levels, we conducted a two-way ANOVA and Bonferroni post hoc test comparing groups using GraphPad Prism version 9.0 (GraphPad, San Diego, CA, USA).

3. Results

3.1. β-hydroxybutyrate rescues chondrosarcoma cells from starvation

To assess the capability of CS cells to utilize KBs as an alternative energy source under nutritional stress, SW 1353 cells were cultured without FBS to starvation conditions, with the addition of 10 or 25 mM of β-HB as a KB source. Then, we evaluated its proliferation index at 24 and 48 h, as shown in Fig. 1. Starved cells showed an inhibited proliferation as early as 24 h compared to the supplied group. Nevertheless, adding 10 or 25 mM of β-HB to starved cells attenuated the proliferation inhibition at 24 h, and the cell population reached the supplied group with the two concentrations at 48 h. The addition of ten mM of β-HB to supplied cells induced proliferation similar to the only supplied group (the control group) at 48 h, while at 25 mM, increased proliferation was observed since the 24 h of β-HB treatment compared to the control group. It is important to mention that the induction of proliferation was β-HB concentration-dependent in SW 1353 cells under starvation conditions because, at 1 and 2.5 mM, the proliferation index did not differ significantly from the starvation group (Fig. S1). These findings indicate that CS cells can metabolize KBs to proliferate under nutritional stress and when the two energy sources, glucose and KBs, are available.

3.2. Effects of β-hydroxybutyrate supplementation on the expression of the monocarboxylate transporter MCT1 in chondrosarcoma cells under starvation

As we found that CS cells used β-HB to support proliferation under nutritional stress, we investigated the expression of critical genes involved in energy production under metabolic stress. Since the initial step in KBs metabolism depends on transportation to the cell's cytosol, we assessed the mRNA and protein expression of the monocarboxylate transporter MCT1 in SW 1353 cells under nutritional stress.

Table 1
RT-PCR primers sequences.

Gene		Sequence 5′–3′
OXCT1	Forward	CAC CAG TGC TCA TCG CCA TA
	Reverse	CAC ATA GCC CAA AAC CAC CAA
ACAT1	Forward	GGA GAG CAT GTC CAA TGT TCC
	Reverse	CGT CCT GTT CAT TTC GTG CAA
BDH1	Forward	TGG TTT TGG AAC CAC CGG GAG GA
	Reverse	GCT CCG CCG CAC TGG CAT AA
BDH2	Forward	GCT TCC AGC GTC AAA GGA GTT
	Reverse	CAG TTG CGA ATC TTC CCG TC
MCT1	Forward	GGT GGA GGT CCT ATC AGC AGT
	Reverse	CAG AAA GAA GCT GCA ATC AAG C
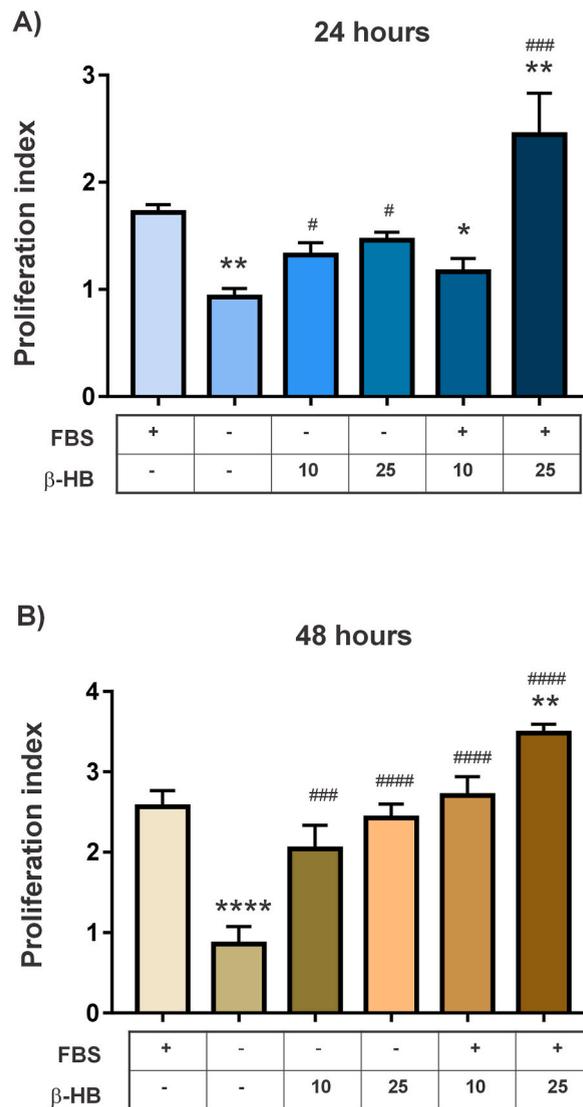
Fig. 1. Effect of β-HB at 10 and 25 mM in the proliferation of SW 1353 cells, A) 24 and B) 48 h. Cells supplied and supplied plus β-HB addition. The data are expressed as the mean ± standard deviation of three independent experiments. Statistical analysis was performed by one-way ANOVA multiple comparison and a post hoc Bonferroni multiple comparison test (n = 3 independent experiments). $*P < 0.05$, $**P < 0.01$, and $****P < 0.0001$ respect to the supplied condition; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.0001$ respect to the starvation condition.

As depicted in Fig. 2A, basal levels of MCT1 mRNA were detected in CS cells under supplied conditions. Interestingly, starvation did not affect MCT1 expression, at least for 24 h, but the addition of β-HB induced upregulation of mRNA expression, surpassing the supplied group at the same time. Regarding protein expression, MCT1 was detected in supplied SW 1353 cells under nutrition-depleted conditions; downregulation was observed at 24 h and partially restored at 48 h. The β-HB addition restored MCT1 protein expression comparable to the supplied cells (Fig. 2B).

3.3. Effects of β-hydroxybutyrate supplementation on expression of ketolytic enzymes BDH1, OXCT1, and ACAT1 in chondrosarcoma cells under starvation

Efficient ketolysis depends on the expression of the enzymes responsible for the breakdown of the KBs. Here, we examined the effect of KBs addition to starved cells on the gene expression and protein production of the ketolysis-related enzymes BDH1, OXCT1, and ACAT 1 in SW 1353 cells.

Basal mRNA expression of OXCT1 and ACAT1 was observed in supplied cells; however, BDH1 mRNA expression was unexpectedly undetectable (Fig. 3A and S2). Starvation downregulated OXCT1 mRNA levels after 24 h but did not significantly affect ACAT1 gene expression compared to the supplied cells. The addition of ten mM β-HB in starvation conditions promoted the expression of OXCT1

MCT1

A)

B)
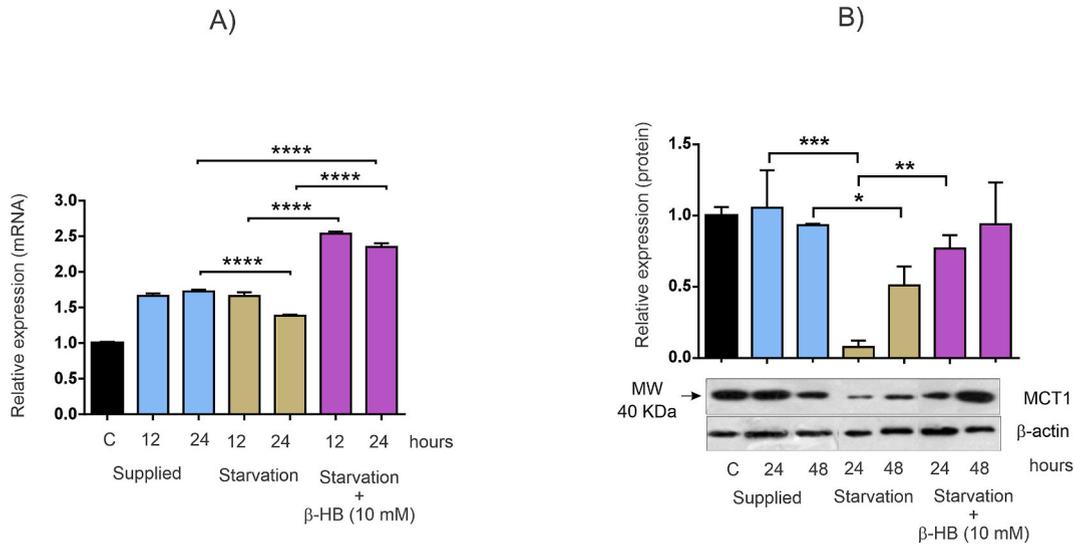
Fig. 2. Effects of β-HB supplementation on the expression of monocarboxylate transporter MCT1 in SW 1353 cells under starvation. A) Relative gene expression (fold change) of MCT1 determined by RT-PCR and normalized to levels of GAPDH. B) Relative protein expression (fold change) of MCT1 detected by SDS-PAGE and Western blot using β-actin as the loading control. The data are expressed as the mean ± standard deviation, n = 3 independent experiments. Statistical analysis was performed by one-way ANOVA multiple comparison and a post hoc Bonferroni multiple comparison test, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$. C = control supplied 0 h.

and ACAT1 mRNA levels at 24 h in comparison with the nutritional stress group (Fig. 3A).

Consistent with mRNA results, BDH1 protein production was not detected in supplied cells (Fig. S3), while basal levels of OXCT1 and ACAT1 protein were confirmed. For 24 and 48 h, starvation significantly reduced OXCT1 protein production, partially recovered with β-HB addition (Fig. 3B). Conversely, starvation enhanced twice ACAT1 protein production compared to the supplied group, and
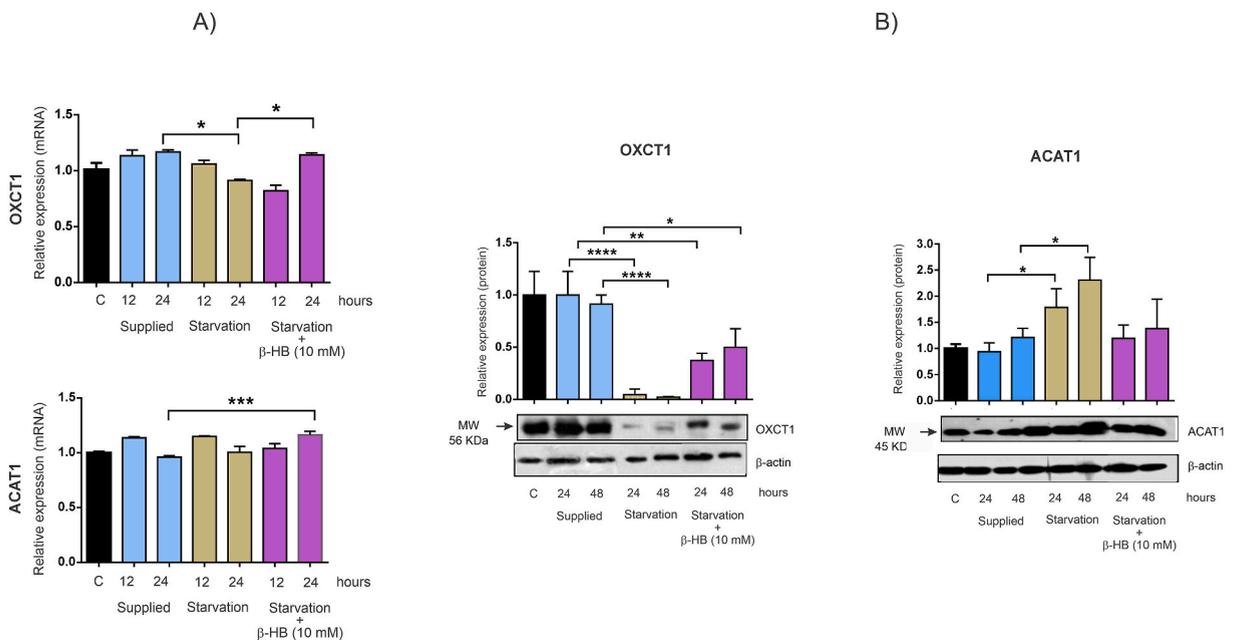
A)

B)

Fig. 3. Effects of β-HB supplementation on expression of ketolytic enzymes OXCT1 and ACAT1 in SW 1353 cells under starvation. A) Relative gene expression (fold change) of OXCT1 and ACAT1 determined by RT-PCR, normalized to levels of GAPDH. B) Relative protein expression (fold change) of OXCT1 and ACAT 1 detected by SDS-PAGE and Western blot using ®-actin as a loading control. The data are expressed as the mean ± standard deviation, n = 3 independent experiments. Statistical analysis was performed by one-way ANOVA multiple comparison and a post hoc Bonferroni multiple comparison test, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, ; $****p < 0.0001$. C = control supplied 0 h.

notably the addition of β-HB restores ACAT1 expression values as in the control group at both culture times.

The presence of OXCT1 and ACAT1 enzymes in SW 1353 cells confirms their ketolytic capacity, while the upregulation or maintenance induced by β-HB under nutritional stress suggests that KBs can be used as a source of energy recovery. However, the lack of BDH1 expression is inconsistent with the complete enzymatic machinery required for ketolysis. Therefore, we further evaluated BDH2 expression, which has been considered an alternative way to complete the ketolytic process [19]. In contrast to the BDH1 expression, BDH2 was detected in SW 1353 cells (Fig. 4). Starvation inhibited its expression compared with supplied cells, and notably, supplementation with β-HB showed a trend toward restoring its expression to levels comparable to supplied cells.

3.4. β-hydroxybutyrate is produced by chondrosarcoma cells

Having established that CS cells engage in ketolysis, the subsequent inquiry was whether they could synthesize KBs. Therefore, β-HB production was measured in SW 1353 under supplied and starvation conditions.

Fig. 5 shows that when the cells were provided with adequate nutritional supply, β-HB production was approximately 0.1 mM within the first 76 h and doubled at 96 h. In contrast, β-HB levels slightly increased compared to 0 h for starvation condition but remain below 0.1 mM even at 96 h. This finding strongly suggests that CS cells are capable of ketogenesis, underscoring the notion that β-HB may play a crucial role as an energy source during nutritional stress and in supplemented conditions.

4. Discussion

Oncogenesis is a multifactorial process in which several biochemical pathways control cell proliferation and differentiation. Cancer cells exhibit several metabolic adaptations, the Warburg effect being the most well-known [20,21]. For instance, the metabolism of KBs in cancer cells is still unclear and contradictory [11,22]; while some studies propose ketogenic diets (KDs), low in carbohydrates and rich in fats, as favorable to combat a wide range of malignant tumors [12,13,23,24], in addition to being effective as adjuvants with radio or chemotherapy [25], others suggest that KBs may promote tumor growth and metastasis [26,27]. Given these discrepancies and our previous findings indicating dysregulated KBs metabolism in CS patients, this study aimed to investigate the effect of the KB β-HB on CS cell proliferation under metabolic stress and analyze their ketolytic machinery [10].

We reveal the metabolic plasticity of CS cells by showing their ability to utilize β-HB to induce cell proliferation under starvation conditions. Similar findings have been reported in other cancer cells, such as HepG2 and HeLa cells, and even in normal rabbit chondrocytes [28], the non-malignant counterpart of CS cells.

Since KBs such as β-HB, pyruvate, and lactate are incorporated into the cells via MCT1 [29], studying their expression in CS was necessary. We found that MCT1 expression decreases in the absence of FBS and is recovered with β-HB addition. This result confirms that CS cells can take up KBs, similar to what has been reported in osteosarcoma cells under oxygen stress, as hypoxia also tends to raise the MCT1 protein levels [30]. Besides, because cancer cells display high rates of aerobic glycolysis-producing lactate, also transported by MCTs, targeting these proteins has shown promising therapeutic effects for breast, colon cancer [31], and osteosarcoma [30].

We also confirmed the basal mRNA and protein expression of the ketolytic enzymes OXCT1 and ACAT1 in CS cells. However, the absence of BDH1 expression, the enzyme involved in the first step of KBs degradation, was inconsistent with the idea of β-HB as an energy source for SW 1353 proliferation. Thus, we evaluated the expression of the other member of the short-chain dehydrogenases/reductases, the R-β-hydroxybutyrate dehydrogenase type 2 (BDH2), encoded by the gene DHRS6 [32]. BDH2 is the cytosolic
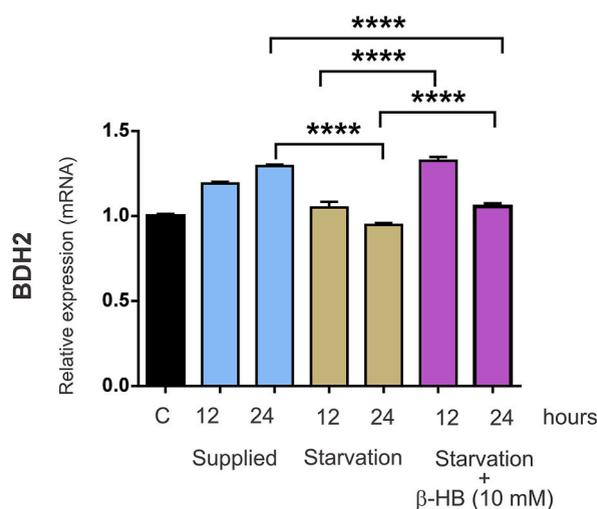
Fig. 4. Effects of β-HB supplementation on expression of BDH2 in SW 1353 cells under starvation. Relative mRNA expression of BDH2 determined by RT-PCR, and normalized to GAPDH. The data are expressed as the mean ± standard deviation, n = 3 independent experiments. Statistical analysis was performed by one-way ANOVA multiple comparison and a post hoc Bonferroni multiple comparison test, ****$p < 0.0001$.
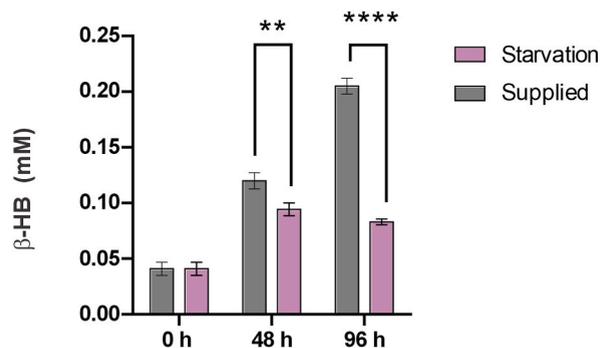
Fig. 5. Differences in quantified levels of ®-HB (mM) in SW 1353 cells under normal and starvation conditions. The data are expressed as the mean ± standard deviation, n = 3 independent experiments. Statistical analysis was performed using one-way ANOVA multiple comparison and a post hoc Bonferroni multiple comparison test. **$P < 0.05$, ****$P < 0.0001$.

counterpart of mitochondrial BDH1. It is responsible for utilizing KBs for energy supply and generating lipid and sterol precursors [33].

In cancer, the roles of BDH2 differ in several human malignancies. BDH2 mRNA and protein downregulated expression has been demonstrated in lung adenocarcinoma, nasopharyngeal carcinoma, and gastric cancer; in all cases, the increase of BDH2 expression reduces cell proliferation [34–36]. In contrast, a higher expression of BDH2 mRNA has been related to antiapoptotic effects in esophageal squamous cell carcinoma cells and bone marrow from patients with acute myeloid leukemia [37,38]. Thus, the presence of the BDH2 in CS SW 1353 is an exciting finding since it has not been explored its potential role in bone tumors. In contrast, the expression of other ketogenic genes such as HMGCL, HMGCS1, HMGCS2, and OXCT1 has been reported in osteosarcoma biopsies, and U2OS OS cells by in situ hybridization [39,40]. The cytosolic BDH2 in CS might convert high levels of circulating β-HB into acetoacetate in starved cells and further ketolysis by OXCT1 and ACAT1 enzymes. Additionally, the recovery of low mRNA levels of enzymes BDH2, OXT1, and ACAT1 by β-HB supplementation under nutritional stress confirmed CS cells metabolic plasticity, probably activating other metabolic pathways for energy production and cancer progression. The substantial increase of the ACAT1 expression in starvation cells as opposed to the enzyme OXCT1 is a striking fact, and this may be related to ACAT1-dependant cholesterol esterification [41]. Accumulating evidence indicates that intracellular cholesterol synthesis is upregulated in enchondroma-like lesions and CS [7], while it is known that in several cancers, PI3K/Akt pathway activates this synthesis by Sterol regulatory element-binding proteins (SREBPs) [42]. PI3K/Akt route is critical for apoptosis prevention in nutrition-stressed CS cells [43]; therefore, under these circumstances, ACAT1 overexpression could be related to the synthesis of esterified cholesterol in addition to KBs oxidation for the subsistence of CS cells.

Notably, we found discrepancies in the mRNA and protein levels of MCT1, OXCT2, and ACAT1. The general practice of examining mRNA and protein levels is a crucial step in understanding how gene expression influences cell phenotype. However, recent insights have revealed a limited correlation between mRNA and protein levels in eukaryotic systems. It has been suggested that post-transcriptional regulation plays a more significant role in determining protein levels than changes in mRNA levels. Various transcriptional and post-transcriptional regulatory mechanisms have been identified, particularly concerning the gene expression of ketolytic enzymes like MCT1 [44,45]; [29]. MCT1 gene expression is subject to regulation by microRNAs (miRNAs) such as miR-29a, miR-29b, miR-124, and miR-495, which target the 3′ untranslated regions of MCT1 mRNA [29]. Differences observed in our study in MCT1 mRNA and protein levels could be attributed to miRNA-mediated regulation. However, the specific mechanisms through which miRNAs regulate MCT1 expression remain to be fully elucidated [29,46]. Therefore, while mRNA and protein levels provide valuable insights into gene expression dynamics, it is essential to consider the influence of post-transcriptional regulatory mechanisms, such as miRNA-mediated regulation, in interpreting these results comprehensively. Further research is warranted to unravel the intricate regulatory networks governing the expression of ketolytic enzymes in CS cells.

Overall, the results of this work suggest CS cells can activate KBs metabolism and that β-HB is an alternative cell intrinsic or systemic fuel that can promote CS growth. We demonstrated that CS cells can synthesize β-HB at basal levels under both starvation and supplemented conditions. This suggests that β-HB may be used not only as an energy source but also as a signaling metabolite. β-HB induces histone lysine β-hydroxybutyrylation (Kbhb), serving as an epigenetic regulatory mark that links metabolism to gene expression. Recent discoveries have shown that Kbhb inhibits the activity of S-adenosyl-L-homocysteine hydrolase, a rate-limiting enzyme in the methionine cycle. Additionally, Kbhb inhibits the acetylation of p53, reducing its transcriptional activity on target genes such as p21 and PUMA. This reduction diminishes p53's effect on apoptosis and cell cancer growth [47–49]. At the same time, the exogenous KBs can be taken up from the extracellular space and used as a fuel source or signalling molecule for cancer development [50]. Considering these results, it appears essential to further explore the molecular mechanisms regulated by ketolysis in CS that led to CS proliferation. Further investigation into the molecular mechanisms regulated by ketolysis in CS is warranted, as targeting KB metabolism may represent a novel therapeutic strategy for CS treatment.

CRediT authorship contribution statement

Misael Vargas-López: Methodology, Data curation. **Carlos A. Quiroz-Vicente:** Methodology, Data curation. **Nury Pérez-**

Hernández: Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Fernando Gómez-Chávez:** Writing – review & editing, Supervision, Formal analysis, Data curation. **Angel E. Bañuelos-Hernández:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation. **Elizabeth Pérez-Hernández:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30212.

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