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MINI-REVIEW

Novel targeted cancer therapy based on β-hydroxybutyric acid associated energy metabolism regulated by intestinal flora

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ABSTRACT

β-hydroxybutyric acid (β-HBA) is a water soluble small molecule and the main component of ketone body. Upon facing energy shortage, free fatty acids in liver are oxidized and decomposed in mitochondria to produce β-HBA. β-HBA is a carbon source providing energy for extrahepatic tissues such as brain, heart, and skeletal muscles. Intestinal flora is the key component of regulating the host lipid metabolism and other metabolic activities of human body. The imbalance of intestinal flora may lead to the disorders of fatty acid metabolism having impact on cardiovascular, nervous, metabolic systems, etc. This work discusses the potential regulatory mechanism of intestinal flora involved in producing β-HBA through metabolic pathway, molecular mechanism of β-HBA production, physiological effects in animals, and relation between intestinal flora and fatty acid metabolism. These outcomes can provide reference for further work on β-HBA production in treating diseases, especially for cancer treatment in terms of the energy metabolism.

Keywords: β-hydroxybutyric Acid; Intestinal Flora; Fatty Acid Metabolism; Histone Deacetylase Inhibitors; G Protein-coupled Receptor 109A

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1. Introduction

β-hydroxybutyric acid (β-HBA) is an endogenous product of fat metabolism. Its uptake and utilization are active in human heart, kidney, muscle tissue, brain neurons and nerve keratinocytes[1,2]. It reaches tissues and organs and performs functions by crossing the blood-brain barrier and capillary walls. In hypoglycemia, free fatty acids in liver are oxidized and decomposed to produce β-HBA in mitochondria. β-HBA is a carbon source and supplies energy for transport to extrahepatic tissues such as brain, heart, and skeletal muscle[3].

Intestinal flora as the "second genome" of human is crucial to human health and is involved in regulating the metabolic processes of host including energy homeostasis, glucose and lipid metabolisms[4]. β-HBA reaches intestine through the circulatory system and affects intestinal homeostasis and permeability[2]. It causes harmful substances to escape by the action of intestinal flora. The metabolites of intestinal flora also affect the processing and packaging of fatty acids which in turn affect the oxidation and decomposition of fatty acids in liver and regulate the β-HBA production.

The potential interaction between β-HBA and intestinal flora has been focused in recent years and is important to understand the regulatory mechanism of intestinal flora involved in β-HBA production.

2. Metabolism of β-HBA

Fatty acids in mammals undergo β oxidation in liver mitochondria to form acetyl-CoA (Ac-CoA), and two Ac-CoA condense to produce AcAc-CoA via the thiolytic enzymes. AcAc-CoA condenses with AC-CoA to generate β-hydroxy β-methylglutaryl-CoA (HMG CoA) by HMG CoA synthetase. HMG CoA is cleaved by HMG CoA lyase to form Ac-CoA and acetoacetic acid. The free acetoacetic acid is catalyzed by β-HBA dehydrogenase in mitochondrial matrix and reduced to β-HBA by nicotinamide adenine dinucleotide (NADH)[5].

The liver has strong system of β-HBA synthetase however lacks the enzyme that uses β-HBA. Extrahepatic tissues such as brain, myocardium, and skeletal muscle have abundant β-HBA degenerating enzymes, and generated β-HBA is transported out of mitochondrial membrane and liver cytoplasmic membrane. β-HBA then crosses blood-brain barrier and capillary wall and reaches tissues and organs to perform functions[6].

β-HBA is oxidized to acetoacetic acid by β-HBA dehydrogenase in mitochondria. β-ketoyl-CoA transferase catalyzes acetoacetic acid reaction with succinyl-CoA, an intermediate product of citric acid cycle, to form AcAc-CoA. AcAc-CoA is cleaved by thiolyase to form two molecules of Ac-CoA which enter the citric acid cycle for oxidation[7]. One molecule of acetyl-CoA produces 10 ATPs for providing energy to extrahepatic tissues (**Figure 1**).

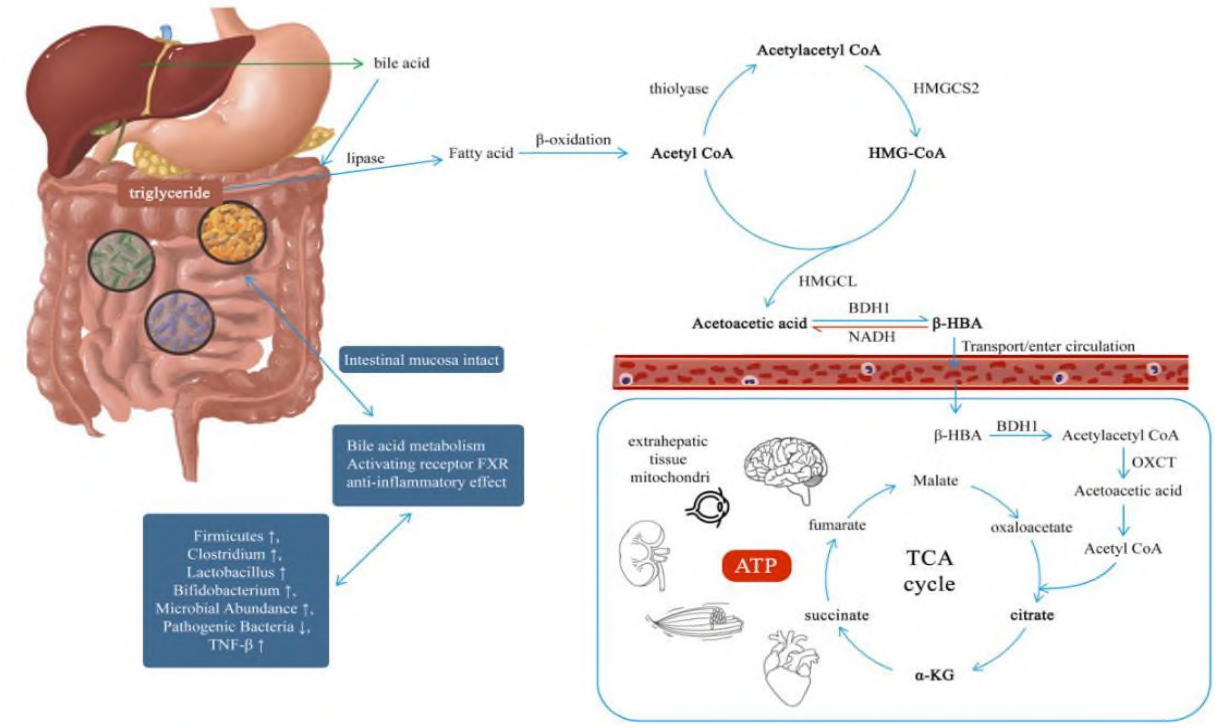

Figure 1. Regulate and control mechanism of intestinal microbiota—fatty acids—β-HBA.

3. Molecular mechanism of β-HBA action

3.1 Histone deacetylase inhibitors (HDACi)

Histone deacetylases (HDACs) regulate the chromatin remodeling and maintain the dynamic acetylation balance of DNA damage-associated proteins[8]. β-HBA is an endogenous HDACi which affects the transcriptional levels of genome by regulating the histone acetylation modification and is involved in the occurrence and development of tumors.

β-HBA provides neuroprotection by inhibiting HDAC1 and reduces oxidative stress during spinal cord injury in rats[9]. β-HBA inhibits HDACs activity of nasopharyngeal carcinoma cells, promotes acetylation of histone in CDH1 promoter region of cells, up-regulates transcription and expression of CDH1, and inhibits the migration and invasion of tumor cells[10].

β-HBA plays role as HDACi in muscle stem cells under starvation which leads to acetylation and activation of HDAC1 target protein p53 and promotes proliferation of muscle stem cells and reverses the muscle atrophy[11]. β-HBA inhibits HDAC by activating FoxO3a/MT2 antioxidant pathway, relieves myocardial oxidative stress and enhances the antioxidant capacity of cells[12].

3.2 Membrane receptor-mediated effects

GPR109A is also known as hydroxy-carboxylic acid receptor 2 (HCAR2) which is G-protein coupled receptor and β-HBA specific receptor distributed in central and peripheral tissues for sensing endogenous metabolic intermediates. It reduces metabolic rate by reducing lipolysis and sympathetic tension[13]. β-HBA binds with adipocyte GPR109A for regulating the excitability of cell membrane and exerting a feedback regulatory mechanism.

β-HBA regulates the hypothalamic secretion function by binding to GPR109A receptor as signaling molecule and activates its downstream signaling pathway[14]. In the mouse models of Alzheimer's disease, β-HBA reduces amyloid beta (Aβ) peptide accumulation and microglial overactivation through GPR109A/NF-κB signaling pathway, while enhancing the mitochondrial respiratory function of hippocampal neurons and protecting them from Aβ toxicity[15].

β-HBA has anti-inflammatory role in diabetic retinal pigment epithelial cells via GPR109A/NF-κB signaling pathway[16]. β-HBA alleviates mouse atherosclerosis by acting on macrophages through GPR109A receptors for promoting cholesterol effluence[17]. The expression and activation of β-HBA receptor HCAR2/GPR109A promote maintenance of retinal endothelial cell barrier function, thereby preventing and treating retinal diseases[18].

PPARα is the ligand activated receptor in proliferator receptor family that control the intracellular metabolic processes and are expressed in liver, skeletal muscle, kidney, heart, and vascular walls[19]. β-HBA shows antiepileptic effect by promoting AMPK signaling pathway and the expression of PPARα protein and by inhibiting the neuronal oxidative stress damage mediated by apoptosis[20]. By adding β-HBA to bovine endometrial cell culture medium, the protein expressions of PPARα, PPARβ and PPARγ are elevated with the increase of β-HBA concentration[21]. β-HBA reduces gene and protein expressions of FoxO1, inhibits its nuclear translocation, promotes expression of PPARα protein, and promotes liver glycogen storage in diabetic rats[22].

NLRP3 inflammasome is a complex composed of multiple proteins in the solute of cells. It is produced by endogenous inflammatory immune response of cells and expressed in macrophages. The formation of NLRP3 inflammasome activates inflammatory protease Caspase-1, promotes maturation, releases inflammatory cytokines interleukin-1β (IL-1β) and IL-18, and triggers the death of inflammatory cells[23]. β-HBA inhibits the activation of NLRP3 inflammatorome and controls inflammatory diseases. The inhibitory effect of β-HBA on NLRP3 inflammasome is independent of GPR109A and HDAC inhibition.

β-HBA inhibits the activation of NLRP3 inflammatories and down-regulates the placental absorption and IL-1β production in pregnant mice induced by lipopolysaccharide (LPS), thereby reducing placental inflammation and pregnancy complications[24]. The inflammasome complex (caspase-1, ASC and NLRP3) and inflammation-related proteins (TNF-α and NF-κB) levels are decreased which result in the decrease of amylase, lipase, IL-18 and IL-1β in patients with acute pancreatitis after exogenous supplementation of β-HBA. β-HBA improves acute pancreatitis in rats by inhibiting NLRP3 inflammasome pathway[25]. β-HBA also inhibits osteoclast differentiation and alleviates osteolysis by regulating NLRP3 inflammatories[26].

β-HBA lessens retinal damage in diabetes by inhibiting NLRP3 inflammatorome and reducing the levels of IL-1β and IL-18 related pro-inflammatory cytokines[27].

3.3 Relieving endoplasmic reticulum stress

When cells are externally stimulated, the intracellular environment is imbalanced and calcium metabolism is disordered which cause the accumulation of unfolded or misfolded proteins in the cells and leads to endoplasmic reticulum stress and induces cell apoptosis. β-HBA has the protective role in early subarachnoid hemorrhage via inhibiting the endoplasmic reticulum stress caused in brain injury. It reduces apoptosis rate of nerve cells by decreasing protein expressions of GRP78 and CHOP, and down-regulating the protein expressions of p-PERK, p-eIF2 and ATF4[28].

β-HBA reduces the expression levels of endoplasmic reticulum stress and provides neuroprotection to PD model rats via decreasing the quantity of GRP78 and CHOP, and p-IRE1 and XBP1 proteins in striatum inhibit IRE1-XBP1 signaling pathway[29]. In myocardial infarction patients, exogenous supplementation of β-HBA improves acute myocardial infarction in rats by activating Notch1/Hes1 pathway and inhibiting the endoplasmic reticulum stress[30].

4. Physiological effects of β-HBA

β-HBA is about 0.1 mmol/L in the body under normal conditions. The physiological doses of β-HBA have role in the energy metabolism of animals. β-HBA as signaling molecule is related to the prevention and treatment of diseases. β-HBA is anti-inflammatory, antioxidant, and anti-aging with some other protective effects. It improves nervous system, cardiovascular system, diabetes, obesity, and enteritis by activating signaling pathways and regulating expressions of related genes.

4.1 Protective effect of β-HBA on nervous system

β-HBA is used as energy material for brain when glucose function of brain tissue is impaired after energy deficit or traumatic brain injury. This adjusts the balance of microenvironment and reduces metabolic disorder of nerve cells to protect the brain nerve[31]. β-HBA has neuroprotective role in neurodegenerative diseases by negatively regulating STAT3/NLRP3/GSDMD signaling pathway which inhibits microglia apoptosis and downregulates IL-1β and IL-18 in patients[32]. β-HBA assists nutritional treatment of chronic demyelinating diseases by activating microglia production and upregulating the brain-derived neurotrophic factors in corpus callosum and hippocampus to prevent myelin loss[33]. β-HBA reduces heat stress-induced neuroinflammation by inhibiting TLR4/p38MAPK and NF-κB pathways in hippocampus[34].

Exogenous β-HBA reduces apoptotic process and increases levels of Bcl-2 by decreasing Bax and calpsin Ⅰ in spinal cord of mice with autoimmune encephalomyelitis[35]. β-HBA alleviates oxidative stress, inhibits mitochondrial apoptosis, and lessens ischemic stroke by activating Erk/CREB/eNOS pathway[36]. After treatment with β-HBA, there is an increase in integrated neurons in hippocampus of epileptic mice, and decrease in apoptotic cells and GSH-Px activities. This inhibits the epileptic-mediated oxidative stress and inflammatory damage in hippocampus and plays protective role[37].

4.2 Protective effects of β-HBA on cardiovascular system

β-HBA penetrates and diffuses into peripheral tissues for improving protein metabolism. Increasing β-HBA metabolism to supplement energy in the occurrence and development of heart disease has important role in heart failure, atrial fibrillation, and myocardial damage. β-HBA metabolism reduces oxidative stress response of cardiomyocytes, blocks overgeneration and accumulation of ROS, inhibits lipid peroxidation, increases antioxidant capacity of proteins, and thus improves mitochondrial function and promotes the efficiency of ATP synthesis[38].

β-HBA provides energy to the heart. It improves endothelial function, reduces inflammation and oxidative stress, improves mitochondrial function, and delays cardiac remodeling. β-HBA inhibits histone deacetylase activity and alleviates oxidative stress in vascular endothelial cell mitochondria

by activating antioxidant pathways[39]. β-HBA is upregulated to compensate for the insufficient energy supply to heart in early stages of heart failure[40]. Intravenous administration of β-HBA during reperfusion in mice with heart failure preserves systolic function as β-HBA reduces infarct size by increasing autophagy flow and maintaining the mitochondrial homeostasis[41]. Impaired autophagy flow in diabetic cardiomyopathy leads to mitochondrial dysfunction and lipid accumulation. β-HBA improves myocardial cell damage with high glucose and reduces oxidative stress and apoptosis of myocardial cells by promoting autophagy flow[42]. Trx1 is an antioxidant that protects heart during the development of diabetic cardiomyopathy. β-HBA enhances antioxidant defense of cardiomyocytes by inhibiting HDAC1 and increasing acetylation and stabilizing Trx1[43].

4.3 Regulation of β-HBA on metabolic diseases

Hyperketonemia inhibits innate immune function of neutrophils in dairy cows. β-HBA increases the expression levels of IL-1β, IL-6, TNF-α and NF-κBp65 mRNA, and NF-κBp65 protein and inhibits the activation of NF-κB signaling pathway in dairy neutrophils induced by LPS. It has a certain anti-inflammatory function[44]. β-HBA inhibits renal tubule reabsorption via AKT/DAB2 megaloblastic glycoprotein signaling pathway[45] and alleviates diabetic neuropathy by restoring aquaporin-4 polarity in spinal lymphatic system[46]. β-HBA contributes to lymphangiogenesis and facilitates the release of excess lymph fluid to alleviate lymphedema[47]. Exogenous supplementation of β-HBA lowers blood glucose by lowering L-alanine in gluconeogenic substrates[48].

4.4 Regulatory effect of β-HBA on tumors

β-HBA as signaling molecule has role in treating neurological, cardiovascular, and metabolic diseases. It has therapeutic effects on cancers through metabolic changes and apoptosis of cancer cells[49]. β-HBA inhibits proliferation of renal clear cell carcinoma cells, induces cell apoptosis, and reduces expressions of glycolytic enzyme and c-myc[50]. β-HBA inhibits the proliferation of diffuse intra-pontine glioma cells and effects through the combination of radiotherapy and β-HBA[51]. β-HBA promotes cells proliferation by stimulating the DNA synthesis. It also has potential of cancer treatment by inhibiting the proliferation of melanoma, colon, prostate, breast, cervical, and liver cancers[49,52–54].

β-HBA reduces uric acid in wasted muscle to treat muscle atrophy, promotes protein and nucleotide balance, and enhances glutamic acid accumulation by inhibiting the decrease in muscle weight, muscle fiber size, and muscle fiber diameter[55]. β-HBA regulates inflammation in chronic diseases by influencing the chromatin structure and promoting the transcription of multiple genes[56]. Recent studies have found that exogenous supplementation of β-HBA in severe COVID-19 patients increases cytokine expression and secretion, enhances cell lysis, and up-regulates mitochondrial respiratory chain activity for better energy supply[57]. The following table shows the exploration of β-HBA in different diseases in recent years (**Table 1**).

5. Relationship between intestinal flora and fatty acid metabolism

Gut and liver are the two main organs involved in lipid metabolism. The gut not only carries out lipid digestion and absorption but also inhabits trillions of microorganisms[58]. The number of human intestinal microbes is 1,013–1,014 of more than 1,000 species and number of coding genes exceeds 150 times that of human genes. They are involved in physiological and pathological processes of host which include food digestion and absorption, nutrient metabolism, host immunity development, and intestinal inflammation. They have vital role in human metabolism and health[59]. The 98% of microorganisms colonizing in gastrointestinal tract are Bacteroidetes, Verrucomicrobia, Phylum Firmicutes, Proteobacteria and Actinobacteria. Phylum Firmicutes, Bacteroidetes and Proteobacteria greatly influence the fatty acid metabolism[60]. Studies have found that the diversity of intestinal flora in AD

Table 1. Protective effects and molecular mechanisms of β-HBA in disease

No.	Medical field	Functional effect	Molecular mechanism	References
1.	Central nervous system	Neuroprotective effect	HDACi PERK-eIF2α-ATF4 IRE1-XBP1 p38-MAPK	[9] [28] [29] [33,34]
		Anti-epileptic	PPARα	[20,37]
		Reduce cell apoptosis	STAT3/NLRP3 BCL-2/BAX/CASPASE-3	[32] [35]
		Relieve stroke	ERK/CREB/ENOS	[36]
2.	Cardiovascular circulation	Reduce oxidative stress	HDACi	[12,39,41,43]
		Alleviate atherosclerosis	GPR109A	[17,40]
		Improve myocardial infarct	Notch1/Hes1	[30]
3.	Metabolic diseases	Anti-inflammatory	GPR109A/NF-κB	[16,44]
		Relieve retinal damage	HCAR2/GPR109A NLRP3	[18] [27]
		Inhibition of renal reabsorption	AKT/DAB2	[45]
		Relief of lymphedema	HDACi	[47]
4.	Tumor disease	Inhibiting cell proliferation	HDACi Hcar2-Hopx	[10,49,51]
		Inducing cell apoptosis	ACAT1and BDH2	[50]
5.	Skeletal system	Reversing muscle atrophy	HDACi	[11,55]
		Reduce osteolysis	NLRP3	[26]
6.	Others	Reduce placental inflammation	NLRP3	[24]
		Improve pancreatitis	NLRP3	[25]

patients decreases where abundance of phylum firmicutes decreases, while those of Bacteroides and Helicobacter pylori increases[61].

Proteobacteria are more abundant in the intestinal flora of cancer cachexia patients[62]. There is decrease in abundance and diversity of intestinal flora in cardiovascular and metabolic diseases. Beneficial bacteria such as butyrogenic are decreased and pathogenic are increased[63]. The decreased intestinal flora such as Coprofecalis prevotelli and some butyrogenic bacteria are anti-inflammatory and alleviate chronic kidney disease[64]. In type 2 diabetes, the ratio of Bacteroidetes to Escherichia coli increases with decrease of glucose tolerance, and ratio of Firmicutes to Bacteroidetes in intestinal flora of insulin resistant patients increases[65]. The changes in intestinal flora species are associated with the development of diseases linked to lipid metabolism disorders such as fatty liver, obesity, cardiovascular disease, and diabetes.

The anti-atherosclerotic effects of Lactobacillus rhamnosus probiotics are related to the biosynthetic pathway of β-HBA. Intestinal flora regulates the host brain function through gut-brain axis and affects patients' cognition[66]. By regulating the ratio of Firmicutes and Bacteroidetes in intestine, the proliferation of Lactobacillus, Clostridium and other bacteria is promoted, while proliferation of Enterobacteriaceae is inhibited, which improve the antioxidant capacity of body and maintain intestinal health[67]. Enterococcus faecalis and its metabolite, myristic acid (MA, an unsaturated long-chain fatty acid) reduce obesity and fatty liver in mice by increasing the energy metabolism and activating the brown adipose tissue[68]. Lactobacillus PS128 contributes to the prevention and adjuvant therapy of Parkinson's disease by promoting intestinal motility, mucin production and serotonin signaling[69]. Intestinal flora enhances the expression of anti-inflammatory cytokine TGF-β and inhibits the expression level of IL-17 by altering IL-17 signaling pathway,

thus promoting the recovery of neural function after cerebral ischemia in rats[70]. The increase of Akermannia mucophil improves symptoms of patients with metabolic syndrome, sends signals to the host through GPCRs, triggers cascade of host expression mechanisms, and intervenes in intestinal flora disorders[71]. β-HBA production is mainly from the catabolism of fatty acids. This suggests that gut flora has role in the immunity, possibly by signaling to the host via GPCRs to regulate fatty acid metabolism and β-HBA production.

Bile acid is an amphiphilic metabolite produced by liver cells and secreted into intestine by bile duct. It has role in the emulsification of fats consumed by human body during digestion. Bile acid decomposes triglycerides into fatty acids by lipase[72]. Functional metagenomics has uncovered the microflora gene profile of intestinal microbial metabolites synthesis and signal transduction. The changes in intestinal flora affect the composition of bile acids and activate bile acid receptor signaling pathway, thus regulating the fatty acid metabolism[73]. The metabolites of intestinal flora participate in the systemic inflammation and metabolic diseases by inhibiting histone deacetylase and interacting with GPCRs.

Finally, cancer cells utilize more energy than normal cells due to their adaptive ability to change nutrient demands and have a diversity of energy production pathways. For developing efficient cancer therapy, it is necessary to understand the underlying mechanisms of energy usage which is surprising yet largely unknown. Both cancer and surrounding noncancerous cells, located in the same region, compete to fulfill their energy requirements through various routes. The most efficient cells in energy utilization can develop preferentially. Cancer cells are in a relatively hypoxic environment with decreased energy supply, indicating that their robust proliferation requires a supernatural capacity in capturing energy[74]. β-HBA will possibly facilitate this novel cancer therapy strategy beyond the classical theory of energy metabolism involved in cancer cell proliferation and metastasis.

6. Conclusion

Lipid mobilization is enhanced when source of sugar or oxidative energy supply becomes limited such as in the case of starvation, fasting or diabetic pathology. β-HBA is the body's energy source produced by oxidative decomposition of fatty acids. β-HBA is also used as an endogenous small molecule bioactive substance having protective role in diseases of the nervous system, cardiovascular, metabolic, tumors, and other.

The molecular mechanism, cell levels and tissue levels of β-HBA have been studied, and its effects in cancer prevention and treatment are recognized. The supplements of exogenous β-HBA consist of keto salts, keto esters, and keto acids which are rarely supplied in the market, most of which are chemically synthesized. The only pure β-HBA ketoacid is sold as standard sample that is expensive.

As the second human genome, experimental and clinical data have confirmed that intestinal flora plays role in the interaction between host gut and multiple systems. Microbiota, fatty acid metabolism, and β-HBA are an intercommunicating system. Theoretical studies suggest that fatty acid metabolism is regulated by adjusting the number and type of intestinal flora. Regulating β-HBA production by intestinal flora improves the occurrence and development of disease. More work on molecular mechanism of intestinal flora for regulating the β-HBA production may provide direction for treating nervous system, cardiovascular, and metabolic diseases.

Author contributions

Conceptualization, ZF and YJ; methodology, ZF, ZX and HY; software, LY; validation, ZF, ZX and YJ; formal analysis, ZF, ZX and HY; investigation, ZF and YJ; resources, ZF; data curation, ZF, ZX and YJ; writing—original draft preparation, ZF, ZX, HY and YJ; writing—review and editing, YJ; visualization, LY; supervision, YJ; project administration, ZF, ZX and YJ; funding acquisition, ZF and YJ. All authors have read and agreed to the published version of the manuscript.

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Ethics approval

Not applicable.

Conflict of interest

The authors declare no competing interests.

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