Review

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Role of Branched-chain Amino Acid Metabolism in Tumor Development and Progression

Min Kyu Jung[1,*], Akinkunmi Paul Okekunle[2,3,*], Jung Eun Lee[2,3], Mi Kyung Sung[4], Yun Jeong Lim[5]

[1]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kyungpook National University Hospital, Daegu, [2]Department of Food and Nutrition, College of Human Ecology, Seoul National University, [3]Research Institute of Human Ecology, Seoul National University, [4]Department of Food and Nutrition, Sookmyung Women's University, Seoul, [5]Department of Internal Medicine, Dongguk University Ilsan Hospital, Dongguk University College of Medicine, Goyang, Korea

Branched-chain amino acids (BCAAs), isoleucine, leucine and valine, are essential amino acids with vital roles in protein synthesis and energy production. We reviewed the fundamentals of BCAA metabolism in advanced cancer patients. BCAAs and various catabolic products act as signalling molecules, which activate mechanisms ranging from protein synthesis to insulin secretion. Recently, BCAA metabolism has been suggested to contribute to cancer progression. Of particular interest is the modulation of the mTOR activity by BCAAs. There are likely multiple pathways involved in BCAA metabolism implicated in carcinogenesis. Understanding the mechanism(s) underlying altered BCAAs metabolism will significantly advance the current understanding of nutrient involvement in carcinogenesis and direct future studies to unravel the significance of BCCA metabolites in tumor development and progression.

Key Words Amino acids, branched-chain, Isoleucine, Leucine, Valine, Neoplasm

INTRODUCTION

Tumor cells often potentially undergo uncontrolled and unlimited evolution which is dependent on the availability of some metabolites in the context of tumor microcosm for survival [1,2]. Several nutrients [3-5] have been documented to promote tumor growth through complex metabolic pathways that are modulated by environmental factors, lifestyle changes, genetic predispositions, cellular interactions, and metabolic manipulations [6-8].

The significance of branched-chain amino acids (BCAAs) has been documented in multiple chronic diseases [9-13]. Whether the pathophysiology of BCAA metabolism in chronic diseases is potentially associated with cancer biology is yet to be clearly understood. The vitality of such association cannot be underestimated in proposing novel strategies for tumor management and treatment to improve the quality of life and wellbeing of populations. This short review summarizes role for BCAA metabolism in tumor development and progression.

THE BIOCHEMISTRY OF BCAAS

BCAAs including isoleucine, leucine and valine (Fig. 1) are essential amino acids synthesized in low quantities, and they constitute approximately 35% of all essential amino acids [14]. BCAAs regulate protein metabolism via multiple pathways, primarily those involving the mTOR [15]. They are the mostly hydrophobic amino acids with a vital role in proteins' structural and membrane integrity [16]. BCAAs have unique similarities that are most often studied together (dietary consumption, combustion, and metabolism). However, they differ significantly in terms of morphology, hydrophobicity and biological effects [17].

BCAAs are abundantly synthesized in bacteria, plants, and fungi but not in animals [18]. Similar enzymes synthesize valine and isoleucine, but leucine is usually produced from α-ketoisovaleric acid, a transamination precursor of valine [19]. Also, carbon elements in valine and leucine are derived from pyruvate, but isoleucine carbons are derived

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Correspondence to Yun Jeong Lim, E-mail: drlimyj@gmail.com, https://orcid.org/0000-0002-3279-332X
*These authors contributed equally and are joint first authors for this work.

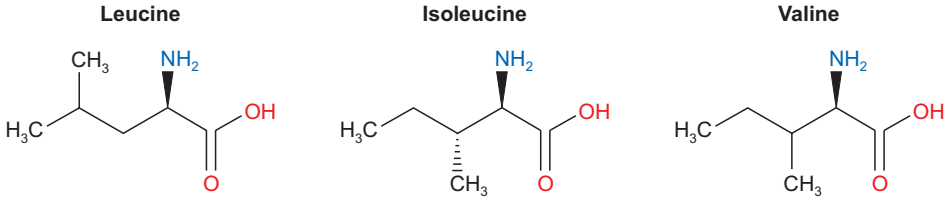

Figure 1. Chemical structures of branched-chain amino acids.

from the relatively rare threonine, reflecting the conserved protein abundance ratio [18]. Similarly, valine and isoleucine are more rampant in β-sheets, but leucine features more in the α-helices [20]. In mammals, BCAAs are primarily catabolized in the hepatic tissues or skeletal muscles [21-23] by branched-chain aminotransferases (BCATs) to branched-chain α-keto-acids (BCKAs) [24].

THE PHYSIOLOGY OF BCAAS

Under normal conditions, there is a precise balance between the intake and expenditure of BCAAs [22]. Diet is likely the only significant source of BCAAs, even though gut microbiota could synthesize BCAAs in relatively minimal and insignificant amounts [25]. Most BCAAs are lost through oxidative catabolism in the body, but the amount of BCAAs in urine is usually negligible to juxtapose the loss. Average circulating levels of BCAAs in the fasting state, i.e. approximately 200 μM of valine, 100 μM of leucine, and 60 μM of isoleucine [23], are usually well maintained within hours after feeding [26-28]. BCAAs (derived from the diet or released from protein breakdown that appears in the circulation) are released into tissues where they can be oxidized or incorporated into newly synthesized proteins [23,29,30].

BCAA intake and utilization are likely to vary depending on age and sex differences of individuals as observed in protein intake. The usual daily minimum requirement of protein intake to maintain muscle mass is 0.8 g/kg/day, though recent recommendations for a healthy diet are higher [31]. The average protein intake among males is 1.7 g/kg/day, which is equivalent to 88, 145, and 66 mg/kg/day intakes of valine, leucine, and isoleucine, respectively. However, protein intake is lower among females and declines with age [32].

Most of the BCAAs (Fig. 2) in the circulatory pool are reincorporated into newly synthesized proteins, typically accounting for 7,090% of disposal in the fasting state [33-35]. Protein synthesis requires an anabolic signal for amino acid assembly into new proteins [23]. Importantly, leucine plays the role in an anabolic signal transduction [36]. In skeletal muscles, oral administration of leucine (but not isoleucine or valine) stimulates protein synthesis [37,38]. Many of these effects are likely to be initiated by hormonal (e.g., insulin or insulin-like growth factor [IGF]-I) and amino acid signals primarily mediated by mTOR [39]. Hence, whole-body BCAA metabolism reflects a balance among the complex network of protein intake, protein synthesis cycle, breakdown and oxidation of BCAAs, etc.

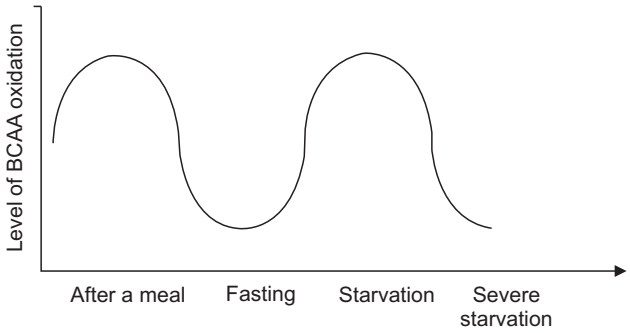

Figure 2. Relationship between BCAAs oxidation and diet. Intracellular BCAA oxidation is dependent on a constellation process manipulated by several states of dietary availability; after a meal, fasting, starvation and severe starvation. BCAAs oxidation increases after a meal but falls in a fasting state. BCAA oxidation increases in the liver during starvation and is primarily driven by gluconeogenic precursors in the TCA cycle. In severe starvation, BCAAs oxidation falls, likely to preserve essential amino acids for other metabolic functions critical for functional survival in the cytosol. BCAA, branched-chain amino acid; TCA, tricarboxylic acid.

THE PATHOPHYSIOLOGY OF BCAA METABOLISM IN MULTIPLE CANCERS

Since BCAAs are essential amino acids, they are also utilized by cancer cells: derived either from circulation or surrounding tissues. Alterations in circulating BCAAs levels in patients diagnosed with cancer have long been noted in observational studies [40-43]. A retrospective metabolomic study demonstrated that elevated plasma BCAA levels are associated with more than a two-fold increase in the risk of pancreatic cancer independent of the intermediate development of diabetes [40]. Similarly, mice bearing *Kras*-driven pancreatic tumors displayed higher plasma levels of BCAAs before the manifestation of subclinical cancers [40]. Interestingly, the same appears not true for other tumors, even when driven by the same mutations in *Kras* [44]. It remains unclear whether these alterations in systemic BCAAs metabolism contribute to tumor growth or metastasis.

Leucine is a well-described mTOR agonist [45]. In addition, Sestrin2 was identified as a direct intracellular leucine sensor and an mTOR complex 1 (mTORC1) regulator [46,47]. Many studies on BCAA metabolism in tumor manifestations have focused on BCAT1. The expression of BCAT1 is altered in

numerous cancers (Fig. 3) and correlates with poor tumor outcomes [48-51]. BCAT1 expression in glioblastoma, an aggressive type of cancer that can occur in the brain or spinal cord, is specific to those carrying wild-type isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) [52]. Mutations in either IDH1 or IDH2 can suppress BCAT1 through DNA methylation and epigenetic silencing [52]. By means of depleting α-ketoglutarate, the mutation in IDH generates the oncometabolite, 2-hydroxyglutarate that potently inhibits α-ketoglutarate-dependent dioxygenases, including histone demethylases [53] and ultimately promotes growth of cancer (stem) cells via hypoxia-inducible factor-1α stabilization and by altering the epigenetic mechanism [54]. Conversely, 2-hydroxyglutarate can limit the supply of glutamate by inhibiting 2-oxoglutarate-dependent transaminases such as BCAT1 and BCAT2, thereby promoting dependence on glutaminase for glutamate biosynthesis [55] and conferring vulnerability to repression of cancer cell proliferation by glutaminase inhibitors [56]. Also, BCAT1 overexpression can promote mTORC1 activity in breast cancer through complex mechanisms [57].

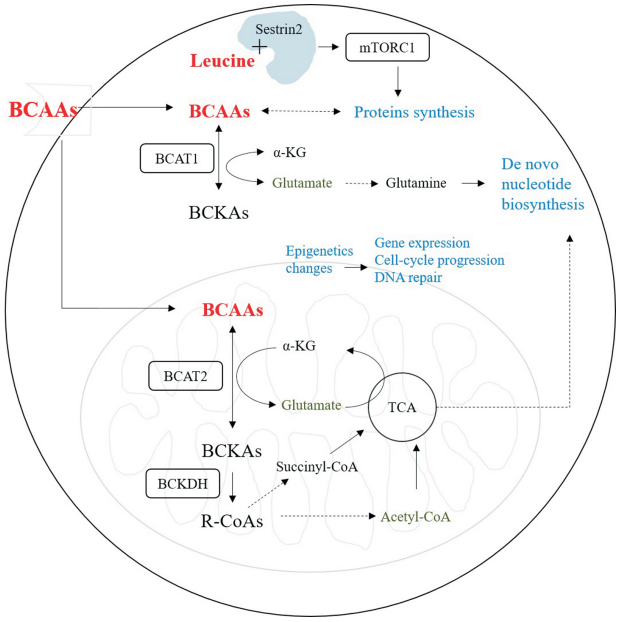

Figure 3. A model of BCAA metabolism in tumor manifestation. Cancer cells are likely to obtain BCAAs from the tumor microenvironment or protein degradation as essential amino acids. BCAAs play distinct roles in cancer cells. Thus, BCAAs can activate the mTORC1 signalling, which stimulates protein translation, growth, and survival. They also serve as building blocks in protein synthesis and can be metabolized into BCKAs in the cytosol by BCAT1 and/or mitochondria by BCAT2, a process involving the conversion of α-KG to glutamate. BCAAs are also used as indirect nitrogen sources for nucleotide and non-essential amino acid biosynthesis via the glutamate-glutamine axis, and further catabolized to yield acetyl-CoA and succinyl-CoA that feed into the TCA cycle, thereby contributing to energy production. The acetyl-CoA levels have an impact on the epigenetic changes of cells. It can influence diverse cellular processes, such as gene expression, cell-cycle progression and DNA repair. In some cancers such as chronic myeloid leukemia, BCAT1 is thought to convert BCKAs back to BCAAs. mTORC1, mTOR complex 1; BCAAs, branched-chain amino acids; BCAT, branched-chain aminotransferase; α-KG, α-ketoglutarate; BCKAs, branched-chain α-keto acids; TCA, tricarboxylic acid; BCKDH, branched-chain α-keto acid dehydrogenase; R-CoAs, branched-chain acyl-CoAs.

Mammalian lethal giant larvae homolog 2 (LLGL2) is a scaffolding protein that plays a role in regulating the mobilization of apical-basal polarity in epithelial cells and solute carrier family 7 member 5 (SLC7A5) [58]. LLGL2 has been implicated in leucine uptake and proliferation of estrogen-sensitive breast cancer cells under excessive leucine stress [59]. However, data from epidemiologic studies alluding to the significance of dietary and plasma BCAAs in breast cancer manifestation are divergent [43,60].

Compared to healthy controls, a negative differential fold difference of plasma valine and leucine has been reported among colorectal cancer (CRC) patients in China [61]. Similarly, plasma leucine and valine concentrations were inversely related to odds of CRC among Japanese adults, particularly males [42]. In another study among CRC patients, BCAAs were unrelated to CRC-specific survival, but 2-ethylhydracrylic acid, a BCAA metabolite, was significantly associated with reduced CRC-related mortality [62]. Likewise, another BCAA metabolite, leucyl-leucine was inversely associated with CRC occurrence in a study on metabolomic profiling in the United States [63]. The mechanisms responsible for this association are yet to be clearly understood, but manipulating the IGF-I expression might be a plausible route. First, IGF-I can promote colon carcinogenesis by facilitating the formation of premalignant lesions such as β-catenin accumulated crypt and aberrant crypt foci [64-66]. However, lower expression of IGF-I has been reported in BCAA-supplemented C57BL/KsJ-*DB/db* mice treated with azoxymethane [67]. Second, BCAA turnover was higher in primary colon carcinoma tissues than normal colon mucosa [68]. Third, an ex vivo/in vitro report [69] demonstrated that isoleucine, in a mouse liver metastatic colon cancer model, partly exerted antiangiogenic effects via the mTOR pathway by inhibiting (VEGF) synthesis. In line with these observations, two recent epidemiological reports from Italy [70] and the United States [71] found a modest inverse relationship between dietary BCAA intake and CRC. In another study, branched-chain α-keto acid dehydrogenase (BCKDH) expression has been linked to CRC tumorigenesis via the mitogen-activated protein kinase (MAPK) pathway ex vivo [72].

Some reports have itemized the role of BCAA in pancreatic cancer. For example, elevated BCAA uptake was related to progression of pancreatic ductal adenocarcinoma (PDAC) [73]. Also, BCKDH knockdown inhibited lipogenesis and reduced the proliferation of PDAC cells. Similarly, the elevated levels of circulating BCAAs are considered an early event in human pancreatic adenocarcinoma development [40]. Furthermore, evidence for the autochthonous (and not *Kras*-driv-

en) tumor manifestation localized to the pancreas associated with elevated BCAA has been reported [40]. These results consistently suggest that BCAAs are associated with PDAC.

In chronic myelogenous leukemia (CML) blast crisis, BCAT1 overexpression resulted in increased intracellular concentrations of BCAAs by aminating branched-chain α-keto acids (BCKAs) to BCAAs [74]. In this study, inhibition of BCAT1 expression reduced mTORC1 activity, presumably by reducing intracellular BCAA concentrations. Importantly, from a therapeutic standpoint, BCAT1 knockdown in a CML mouse model improved survival, while the use of the BCAT1 inhibitor gabapentin suppressed colony formation of CML in human patients [74]. The most well known epigenetic mechanism of BCAT1 expression is the mutation of IDH. Another epigenetic mechanism involving the disruptor of telomeric silencing 1-like (DOT1L) histone methyltransferase was proposed by Oktyabri and colleagues [75]. DOT1L activates BCAT1 gene expression through histone H3 lysine79 methylation of the coding region [75]. In leukemias driven by genetic mutation of the mixed-lineage leukemia 1 (MLL1) gene, DOT1L maintains an open chromatin state and gene transcription [75].

The cancer-specific expression of BCAT1 is likely to be a promising target for therapeutic interventions in cancer treatment. However, the biological functions of BCAT1 in cancer are not well understood, but may be dependent on the cancer tissue of origin [44,76]. Accumulating evidence supports the vital and multifaceted role of BCAT1 in the development and progression of multiple types of cancer, likely via various multiple mechanisms unique to each cancer type.

BCAA oxidation increases after feeding (Fig. 2). Conversely, briefly restricting food reduces oxidation of BCAAs, causing the elevation of their plasma levels. If fasting continues into starvation, BCAAs oxidation increases, providing gluconeogenic precursors to the liver. BCAAs oxidation rates fall again in severe starvation, presumably to conserve essential amino acids [77-79].

CONCLUSION

BCAAs are essential amino acids, and they are likely to be supplied to cancer cells from the tumor microenvironment or through protein degradation. They play prominent roles in tumor development and progression. The most well-defined mechanism of the BCAA and cancer association is the activation of the mTORC1 signalling. Also, BCAAs serve as building blocks in protein synthesis and are indirect sources of nitrogen supply for nucleotide (and non-essential amino acid) biosynthesis via the glutamate-glutamine axis. Aside from the mTOR pathway, other potential pathways within the framework of glucose and lipid metabolism might be a promising route in understanding the significance of BCAAs in cancer.

FUTURE DIRECTIONS

First, further epidemiological studies on the significance of dietary BCAAs in tumor development and progression in populations are needed to significantly improve our understanding of the role of dietary factors in cancer. Second, molecular studies exploring the profiles of interaction between dietary and intracellular plasma BCAAs in the context of tumor manifestation would be worthwhile. Third, whether BCAAs are potential epigenetic markers that can manipulate genetic predisposition to tumorigenesis is yet to be understood. Also, the differential roles of cytosolic BCAT1 and mitochondrial BCAT2 by cancer types cannot be underestimated in expanding our understanding of this topic.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ORCID

Min Kyu Jung, https://orcid.org/0000-0001-8749-408X
Akinkunmi Paul Okekunle, https://orcid.org/0000-0003-4825-4934
Jung Eun Lee, https://orcid.org/0000-0003-1141-878X
Mi Kyung Sung, https://orcid.org/0000-0002-3575-5628
Yun Jeong Lim, https://orcid.org/0000-0002-3279-332X

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