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β-Hydroxy-β-methylbutyrate Suppresses NF-κB Activation and IL-6 Production in TE-1 Cancer Cells

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Abstract. *Background/Aim: Stress reactions, especially those related to surgery, cause poor convalescence of cancer patients. β-Hydroxyβ-methylbutyrate (HMB) is known to regulate excessive inflammation in the body. The objective of this work was to investigate the capacity of HMB to suppress activation of nuclear factor-kappa B (NF-κB) and production of interleukin-6 (IL-6) in a human esophageal squamous cell carcinoma cell line (TE-1). Materials and Methods: Cell proliferation was measured using the water-soluble tetrazolium-1 method, while tumor necrosis factor alpha (TNFα)-induced IL-6 production was measured using an enzyme-linked immunosorbent assay (ELISA) assay. Nuclear translocation of NF-κB was detected by immunofluorescence staining. Results: HMB did not affect cell proliferation. However, HMB suppressed the TNFα-induced increase in IL-6 production in TE-1 cells by inhibiting NF-κB activation. Conclusion: HMB did not influence TE-1 cell proliferation, but inhibited activation of NF-κB and IL-6 production. This result may be useful for improving excessive stress reactions during and after surgery.*

During and after cancer surgery, operative stress can promote tumor metastasis and result in poor prognoses. Notably, stress related to thoraco-laparotomy for esophageal cancer carries an extremely high risk of postoperative complications. Excessive post-surgical stress or postoperative complications, so-called second attacks, cause a cytokine storm that promotes tumor metastasis (surgical oncotaxis). Reported mechanisms underlying surgical oncotaxis include increased adrenal corticoid levels, blood coagulability, immunosuppression, and reactive oxygen species production (1-3).

Usually, increased levels of cytokines, such as interleukin-1 (IL-1) or tumor necrosis factor alpha (TNFα), are observed several hours after surgery, followed by increased IL-6 or IL-8 (4, 5). TNFα, a representative inflammatory cytokine secreted in the tumor microenvironment, elicits secretion of other cytokines by activating nuclear factor-kappa B (NF-κB) signaling (6). NF-κB plays a central role in inflammatory reactions, and is the primary activator of cytokine storms (7, 8). Target genes of NF-κB include adhesion factors, iNOS (inducible nitric oxide synthase), and other factors (9). Thus, NF-κB plays an important role in tumor survival, metastasis, and chemotherapy resistance. NF-κB is essential for promoting inflammation-related cancer. This mechanism can significantly impact chemotherapy and cancer-related surgeries (10). Therefore, NF-κB regulation is extremely important for cancer therapies.

β-hydroxyβ-methylbutyrate (HMB), a metabolite of the branched-chain amino acid leucine that obstructs NF-κB (p65) function, has attracted attention for its ability to control excessive inflammatory reactions. HMB reportedly reduces inflammatory cytokine production, apoptosis, and activity of proteolysis-inducing factor (11). Thus, we hypothesized that HMB regulates activation of the ubiquitin-proteasome pathway to control NF-kB activation and excessive inflammatory reaction in cancer cells.

Materials and Methods

Cell culture. TE-1 cells (RIKEN Bioresource Center Cell Bank, Tsukuba, Japan) were incubated in RPMI-1640 culture medium (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (Nichirei Biosciences, Tokyo, Japan) in a humidified 37˚C incubator with 5% CO_2. TNFα [Cell Signaling Technology (CST), Danvers, MA] and HMB (Alfa Aesar, Lancaster, UK) were dissolved in distilled water to generate stock solutions.

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Cell viability assay. TE-1 cells were seeded in 96-well culture plates (IWAKI, Chiba, Japan) at a density of 10^4 cells/ml (100 μl/well) and incubated overnight. HMB diluted in RPMI-1640 to 0-1 mM was added the following day and cells were cultured for an additional 24 h. Similarly, TNFα diluted to 0-100 ng/ml was added to cells for 24 h. Cell survival was measured using a water-soluble tetrazolium-1 (WST-1) cell proliferation assay kit (Takara, Tokyo, Japan). WST-1 reagent (10 μl) was added to each well for incubation at 37˚C for 4 h. Plates were measured at 450 nm and 690 nm using a microplate reader (Thermo Fisher Scientific, Tokyo, Japan).

Measurement of IL-6 production. TE-1 cells (10^5 cells/ml) were seeded in 24-well culture plates (IWAKI) and incubated for 24 h. After incubation, supernatants were removed and new media were added; this point was set as 0 h. After 1, 3, or 6 h of culture with TNFα (50 ng/ml) and HMB (0.03, 0.3, 0.5, or 1 mM), supernatants were collected and measured at 450 nm and 570 nm using a Human IL-6 ELISA Kit (Thermo Fisher) and microplate reader.

Western blot analysis. TE-1 cells isolated from each condition were washed in phosphate-buffered saline (PBS) before nuclear and cytoplasmic extraction was performed using the NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher). Protein quantification of each sample was performed using a Pierce® BCA Protein Assay Kit (Thermo Fisher). After adding a 4× volume of protein sample buffer and heating samples at 95˚C for five min, samples were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Immun-Blot® PVDF membranes (BioRad, Hercules, CA, USA). After blocking in 1×Tris-buffered saline with 0.1% Tween-20 (TBS-T) containing 5% skim milk for 1 h at room temperature, gels were washed three times for five min each. Rabbit anti-NF-kB-p65 (D14E12, CST), mouse anti-IκB (L35A5, CST), and rabbit anti-β-actin (13E5, CST), were diluted to 1:1,000 in TBS-T containing 5% bovine serum albumin in or 5% non-fat dry milk, and incubated at 4˚C overnight. Membranes were washed three times in TBS-T and then incubated with 1:2,000-3,000 solution of horseradish peroxidase anti-rabbit IgG (CST) in TBS-T with 5% skim milk for 1 h at room temperature. After incubation, gels were washed with TBS-T and prepared for chemiluminescence with ECL western blotting detection reagents (GE Healthcare, Tokyo, Japan). Membranes were visualized with an ImageQuant LAS 4000mini and analyzed with ImageQuant TL software (GE Healthcare).

Immunofluorescence staining of NFκ-B in TE-1 cells. TE-1 cells (2×10^4 cells/ml) were seeded onto 13-mm cover glasses (Matsunami Glass, Osaka, Japan) in 24-well culture plates. After incubation for 24 h, culture medium was changed to control medium or medium including TNFα (50 ng/ml) or HMB (0.5 mM) for 6 h. After fixing in 4% formaldehyde for 15 min, samples were blocked in PBS containing 5% normal goat serum (Wako, Osaka, Japan) and 0.2% Triton®X-100 (Wako). After removing the blocking solution, cells were incubated with NF-κB-p65 primary antibody at 4˚C overnight. Subsequently, an Alexa Fluor®488-conjugated secondary antibody (CST) was added and incubated for 1 h, and nuclei were counterstained with ProLong®Gold Antifade Reagent with DAPI (CST). Samples were observed by confocal laser-scanning microscopy (CLSM 700; Carl Zeiss Microscopy, Tokyo, Japan) with an oil-immersion objective.

Statistical analysis. Each experiment was repeated three times. All numerical values represent mean±standard error of the mean (SEM). Statistical analysis of group differences was performed using one-way analysis of variance (ANOVA) and multiple comparison (Tukey-Kramer) tests. *p*-Values were two-sided and $p<0.05$ was considered significant.

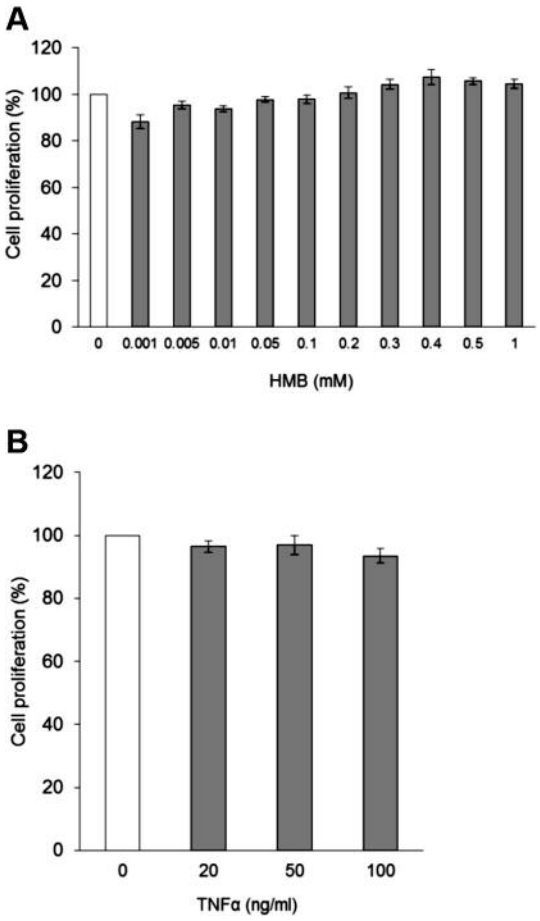

Figure 1. *TE-1 cell proliferation. (A) Cell proliferation after treatment with the indicated concentrations of β-hydroxy-β-methylbutyrate (HMB) for 24 h. The white column shows the control. (B) Cell proliferation after treatment with the indicated concentrations of tumor necrosis factor-α (TNFα) for 24 h. Results are presented as the mean±SEM of three experiments.*

Results

Cell viability. The capacity of HMB to suppress cell proliferation of TE-1 cells was investigated. Inhibition rates were calculated based on 0 μM HMB. HMB did not suppress cell proliferation (Figure 1A). Next, the effect of TNFα on cell proliferation was investigated. TNFα did not influence the proliferation of TE-1 cells after a 24-h incubation (Figure 1B). Based on these results, 50 ng/ml TNFα was used to stimulate TE-1 cells.

HMB suppressed TNFα-induced IL-6 production. We next determined if HMB was able to suppress TNFα-induced

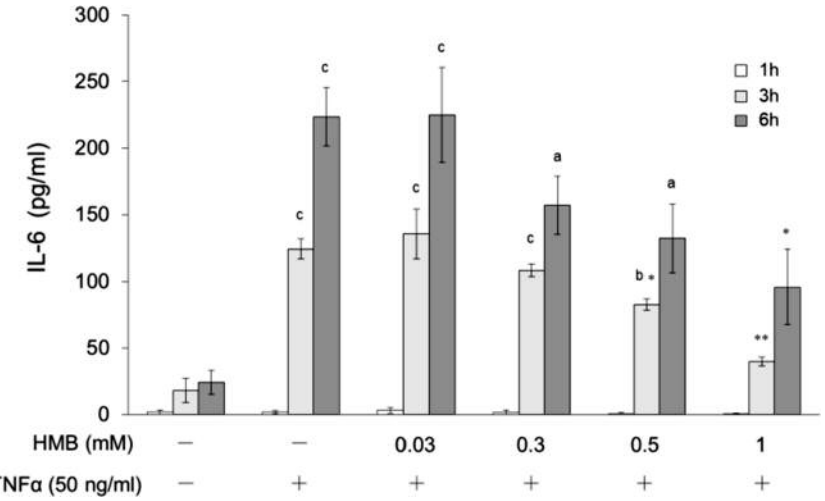

Figure 2. *The effects of β-hydroxy-β-methylbutyrate (HMB) and tumor necrosis factor-α (TNFα) on interleukin-6 (IL-6) production in TE-1 cells. HMB and TNFα were added to TE-1 cells at the same time and incubated for 1, 3, or 6 h. Results are presented as the mean±SEM of three experiments. Differences from the control (HMB (–), TNFα (–)) are represented by different letters: a, p<0.05; b, p<0.01; c, p<0.001. Differences from HMB (–), TNFα 50 ng/ml (+) are represented by asterisks: *p<0.05; **p<0.001. Statistical differences were assessed using ANOVA and Tukey post hoc tests.*

IL-6 production in TE-1 cells (Figure 2). IL-6 production was conspicuously increased by TNFα (50 ng/ml) (p<0.001). Low concentration HMB (0.03 mM) induced a similar level of IL-6 production as TNFα alone. However, 0.3 mM and 0.5 mM HMB suppressed IL-6 production in a dose-dependent manner, while 1 mM HMB significantly decreased IL-6 production (3 h: p<0.001, 6 h: p<0.05) (Figure 2).

HMB suppressed TNFα-induced NF-κB activity. Nuclear translocation of NF-κB was quantified by western blot analysis. HMB (0.5 mM) suppressed NF-κB activation (p<0.001, Figure 3A and B), as changes in p65 and IκB protein expression were detected. Indeed, TNFα stimulation induced the expression of IκBα, which was suppressed by 0.5 mM HMB (p<0.01, Figure 3C and D).

Immunofluorescence staining of NF-κB activity. To confirm NF-κB activation, nuclear translocation of the NF-κB p65 subunit was observed using confocal laser-scanning microscopy. Upon addition of 50 ng/ml TNFα, the green fluorescence signal of p65 overlapped with the nuclear blue fluorescence signal (Figure 4A), confirming the translocation of p65 protein into cell nuclei in response to TNFα stimulation. Combining 0.5 mM HMB with TNFα reduced nuclear translocation of NF-κB. Furthermore, the density of nuclear NF-κBp65 was 7.3%±1.1% in the control, but increased to 79.8%±7.3% with TNFα stimulation, and decreased to 11.3%±1.2% with combined administration of TNFα and 0.5 mM HMB (Figure 4B). Thus, HMB significantly suppressed NF-κB activation induced by TNFα stimulation (p<0.001).

Discussion

The nutritional state of cancer patients has a serious influence on prognosis and quality of life (QOL). With cancer progression, many patients experience loss of appetite and weight, thus falling into an undernourished state. Excessive surgical stress and post-operative complications can cause a cytokine storm, which promotes malnourishment and tumor metastasis, resulting in a poor prognosis for cancer patients (2, 4). In particular, thoraco-laparotomy for esophageal cancer causes a high amount of stress on patients, which frequently causes post-operative complications (so-called second attack) and harmful cytokine storms. This phenomenon leads to a poor prognosis of esophageal cancer patients (1, 3). In addition, chemotherapy and radiotherapy activate NF-κB signaling, which may contribute to therapeutic resistance (12, 13).

Constant NF-κB activation has been reported in many human tumors, whereby it participates in various processes including inflammation, immunity, cell cycle, apoptosis, and metastasis (14-16). TNFα, a representative inflammatory cytokine secreted in cancer microenvironments, as well as activation of NF-κB, are thought to cause secretion of other cytokines. As the persistence of hypercytokinemia is strongly related to an outbreak of infectious complications after surgery

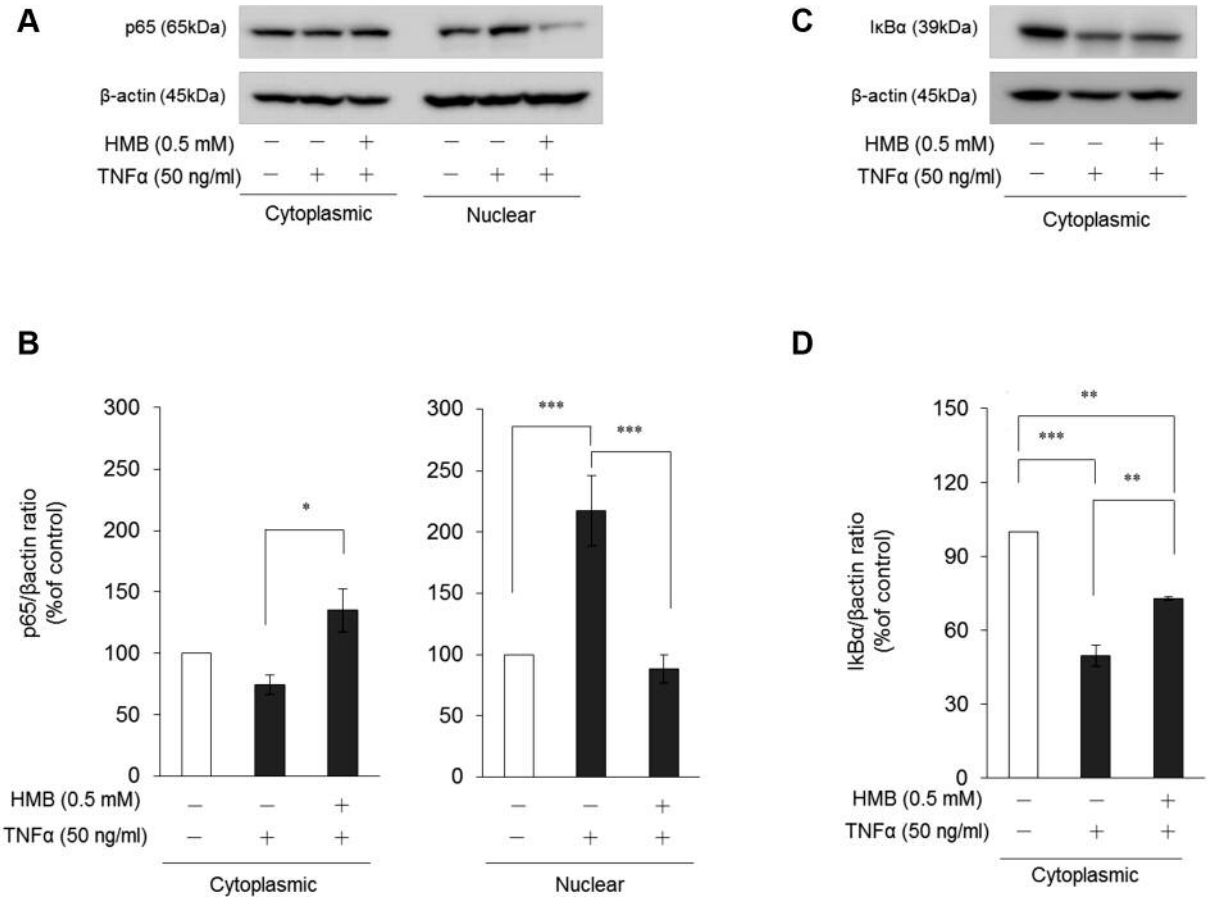

Figure 3. *β-hydroxy-β-methylbutyrate (HMB)-mediated inhibition of nuclear factor-kappa B (NF-κB) activation observed by western blotting. The protein levels of p65 (A) and NF-κB inhibitor α (IκBα) (C) in nuclear and cytoplasmic fractions were determined by western blotting. The ratio of p65 (B) and IκBα (D) to β-actin levels are shown normalized to that of the control using combined quantitative data. TE-1 cells were incubated in the presence of 0.5 mM HMB or 50 ng/ml TNFα alone or in combination. Samples were collected for western blot analysis 24 h after the addition of HMB. Results are presented as the mean±SEM of three experiments: *p<0.05; **p<0.01; ***p<0.001 as determined by ANOVA and Tukey post hoc tests.*

and known to increase side effects associated with cancer chemotherapy, control of cytokine signaling might contribute to the improvement of cancer therapy by various mechanisms.

HMB, a metabolite of the essential amino acid leucine, is produced in liver and muscle. In addition, HMB is used as a nutritional supplement to promote muscle strength and lean body muscle (17). Physiological effects of HMB include alterations of protein synthesis, suppression of proteolysis, and anti-inflammatory effects, which maintain muscle in both young and elderly individuals (18-21). Indeed, the effects of HMB on muscle training, muscular hypertrophy, recovery from muscle damage, prevention of age-induced muscle atrophy, and decrease fat content are known (22). In a clinical study, the anti-inflammatory action of HMB improved breathing function in dangerously ill chronic obstructive pulmonary disease patients (23). Furthermore, maintenance or increased lean body mass was observed in debilitated patients with acquired immunodeficiency syndrome (24). In cancer cachexia, a mixture including HMB, arginine, and glutamine showed dose-dependent maintenance or increased lean body mass (25). Thus, HMB can bring about various beneficial effects without harming healthy or ill individuals (26). However, not all clinical studies showed a desirable outcome and the precise molecular mechanism of HMB function remains unclear (27, 28). One mechanism of HMB function is inhibition of the ubiquitin-proteasome pathway, which elicits a protective effect against cancer-associated proteolysis (11).

We investigated whether HMB could suppress upregulated IL-6 production in response to TNFα stimulation in the human esophageal cancer cell line TE-1 through control of the ubiquitin-proteasome pathway. Our data showed an obvious suppression of NF-κB nuclear translocation with concentrations of HMB 0.5 mM or greater. We speculate that HMB may

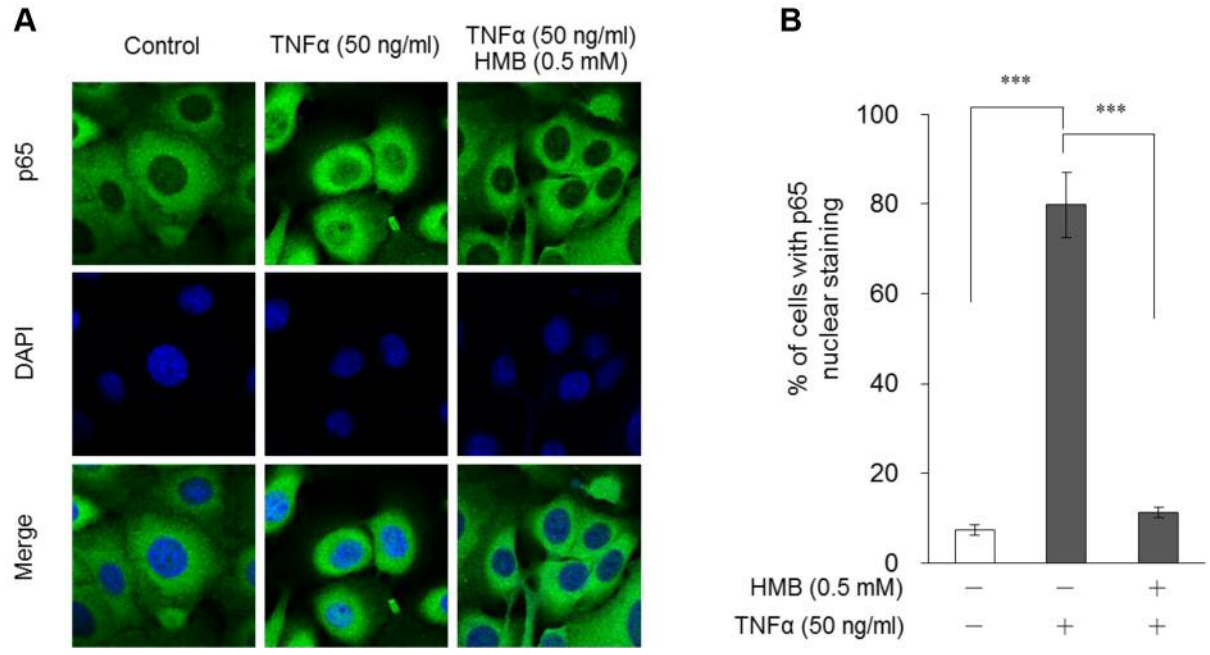

Figure 4. *β-hydroxy-β-methylbutyrate (HMB)-mediated inhibition of nuclear factor-kappa B (NF-κB) activation observed by immunofluorescence. (A) Immunofluorescence staining of nuclear factor-kappa B (NF-κB) in TE-1 cells. (B) The proportion of cells with nuclear p65 staining. Results are presented as the mean±SEM of three experiments: ***p<0.001 as determined by ANOVA and Tukey post hoc tests.*

control IκBα phosphorylation to suppress nuclear translocation of NF-κB/Rela, thereby reducing IL-6 production. Notably, the primary mechanism by which IL-6 production is suppressed is unrelated to cell proliferation or death. Regardless, HMB elicits an anti-inflammatory effect in TE-1 cells.

A compounding agent containing HMB, glutamine, and arginine effectively protected against chemoradiotherapy-induced dermatitis in head and neck cancer patients (29). This effect may be related to the anti-inflammatory activity of HMB. In cancer patients, hyperactive cytokine signaling brings about appetite loss, increased energy consumption, and metabolic disorders. Furthermore, it causes tissue injury, mucosal breaks and, ultimately, cachexia. Therefore, the anti-inflammatory effect of HMB is expected to be beneficial for various cancer therapies.

Anti-cancer agents induce apoptosis of cancer cells, but also activate NF-κB, which elicits an anti-cancer effect (30). NF-κB induces anti-apoptotic activity in response to 5-fluorouracil or cisplatin, two standard chemotherapy agents for esophageal cancer patients. One mechanism by which this occurs is induction of Bcl-2 family members (31). TNFα-induced activation of NF-κB and the mechanistic target of rapamycin (mTOR) is another reported mechanism of anti-cancer resistance (32). Our unpublished flow cytometry data indicated that HMB did not induce apoptosis. From these results, HMB elicits an anti-inflammatory effect by reducing NF-κB activation without inducing apoptosis in cancer cells. This result reveals the usefulness of HMB for various cancer therapies including surgery, chemotherapy, and QOL improvement. However, the precise mechanism by which HMB exerts its effects, for example, participation in mTOR signaling, remains unknown and, thus, requires further investigation.

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References

1 Hirai T, Yamashita Y, Mukaida H, Kuwahara M, Inoue H and Toge T: Poor prognosis in esophageal cancer patients with postoperative complications. Surg Today *28*: 576-579, 1998.

2 Hirai T, Matsumoto H, Yamashita Y, Urakami A, Iki K, Yamamura M and Tsunoda T: Surgical oncotaxis-excessive surgical stress and postoperative complications contribute to enhancing tumor metastasis, resulting in a poor prognosis for cancer patients. Ann Thorac Cardiovasc Surg *11*: 4-6, 2005.

3 Mikami Y: NF-κB status under operative stress in rat. Kawasaki Med J *35*: 147-157, 2009.

4 Akira S, Taga T and Kishimoto T: Interleukin-6 in biology and medicine. Adv Immunol *54*: 1-78, 1993.

5 Kishimoto T: Interleukin-6: From basic science to medicine-40 years in immunology. Annual Rev Immunol *23*: 1-21, 2005.
6 Logan TF, Gooding WE, Whiteside TL, Ernstoff MS, Kaplan SS, Miketic L, Vlock DR, Tompkins C, Wood DL, Nadler PI and Kirkwood JM: Biologic response modulation by tumor necrosis factor alpha (TNF alpha) in a phase Ib trial in cancer patients. J Immunother *20*: 387-397, 1997.
7 Karin M: Nuclear factor-kappa B in cancer development and progression. Nature *25*: 431-436, 2006.
8 Zubair A and Frieri M: Role of nuclear factor-κB in breast and colorectal cancer. Curr Allergy Asthma Rep 13: 44-49, 2013.
9 Panwalkar A, Verstovsek S and Giles F: Nuclear factor-kappa B modulation as a therapeutic approach in hematologic malignancies. Cancer *15*: 1578-1589, 2004.
10 Kubota H, Matsumoto H, Higashida M, Murakami H, Nakashima H, Oka Y, Okumura H, Yamamura M, Nakamura M and Hirai T: Eicosapentaenoic acid modifies cytokine activity and inhibits cell proliferation in an oesophageal cancer cell line. Anticancer Res *33*: 4319-4324, 2013.
11 Smith HJ, Wyke SM and Tisdale MJ: Mechanism of the attenuation of proteolysis-inducing factor stimulated protein degradation in muscle by β-hydroxy-β-methylbutyrate. Cancer Res *64*: 8731-8735, 2004.
12 Karin M, Cao Y, Greten FR and Li ZW: NF-kappa B in cancer: from innocent bystander to major culprit. Nat Rev Cancer *2*: 301-310, 2002.
13 Baldwin AS: Control of oncogenesis and cancer therapy resistance by the transcription factor NF-κB. J Clin Invest *107*: 241-246, 2001.
14 Kunnumakkara AB, Diagaradjane P, Anand P, Harikumar KB, Deorukhkar A, Gelovani J, Guha S, Krishnan S and Aggarwal BB: Curcumin sensitizes human colorectal cancer to capecitabine by modulation of cyclin D1, COX-2, MMP-9, VEGF and CXCR4 expression in an orthotopic mouse model. Int J Cancer *125*: 2187-2197, 2009.
15 Karin M and Greten FR: NF-κB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol *5*: 749-759, 2005.
16 Aggarwal BB and Sung B: NF-κB in cancer: A matter of life and death. Cancer Discov *1*: 469-471, 2011.
17 Nissen S, Sharp R, Ray M, Rathmacher JA, Rice D, Fuller JC Jr, Connelly AS and Abumrad N: Effect of leucine metabolite beta-hydroxy-beta-methylbutyrate on muscle metabolism during resistance-exercise training. J Appl Physiol *81*: 2095-2104, 1996.
18 Llakoll P, Sharp R, Baier S, Levenhagen D, Carr C and Nissen S: Effect of beta-hydroxy-beta-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. Nutrition *20*: 445-451, 2004.
19 Deutz NEP, Pereira SL, Hays NP, Oliver JS, Edens NK, Evans CM and Wolfe RR: Effect of β-hydroxy-β-methylbutyrate (HMB) on lean body mass during 10 days of bed rest in older adults. Clinical Nutrition *32*: 704-712, 2013.
20 Wu H, Xia Y, Jiang J, Du H, Guo X, Liu X, Li X, Huang G and Niu K: Effect of beta-hydroxy-beta-methylbutyrate supplementation on muscle loss in older adults: A systematic review and meta-analysis. Arch Gerontol Geriatr *61*: 168-175, 2015.
21 Stout JR, Smith-Ryan AE, Fukuda DH, Kendall KL, Moon JR, Hoffman JR, Wilson JM, Oliver JS and Mustad VA: Effect of calcium β-hydroxy-β-methylbutyrate (CaHMB) with and without resistance training in men and women 65+yrs: A randomized, double-blind pilot trial. Exp Gerontol *48*: 1303-1310, 2013.
22 Wilson JM, Fitschen PJ, Campbell B, Wilson GJ, Zanchi N, Taylor L, Wilborn C, Kalman DS, Stout JR, Hoffman JR, Ziegenfuss TN, Lopez HL, Kreider RB, Smith-Ryan AE and Antonio J: International society of sports nutrition position stand: beta-hydroxy-beta-methylbutyrate (HMB). J Int Soc Sports Nutr *10*: 6, 2013.
23 Hsieh LC, Chien SL, Huang MS, Tseng HF and Chang CK: Anti-inflammatory and anticatabolic effects of short-term β-hydroxy-β-methylbutyrate supplementation on chronic obstructive pulmonary disease patients in intensive care unit. Asia Pac J Clin Nutr *15*: 544-550, 2006.
24 Clark RH, Feleke G, Din M, Yasmin T, Singh G, Khan FA and Rathmacher JA: Nutritional treatment for acquired immunodeficiency virus-associated wasting using β-hydroxy-β-methylbutyrate, glutamine and arginine: a randomized, double-blind, placebo-controlled study. JPEN J Parenter Enteral Nutr *24*: 133-139, 2000.
25 May PE, Barber A, D'Olimpio JT, Hourihane A and Abumrad NN: Reversal of cancer-related wasting using oral supplementation with a combination of β-hydroxy-β-methylbutyrate (HMB), arginine, and glutamine. Am J Surg *183*: 471-479, 2002.
26 Rathmacher JA, Nissen S, Panton L, Clark RH, Eubanks-May P, Barber AE, D'Olimpio J and Abumrad NN: Supplementation with a combination of beta-hydroxy-beta-methylbutyrate (HMB), arginine and glutamine is safe and could improve hematological parameters. JPEN J Parenter Enteral Nutr *28*: 66-75, 2004.
27 Berk L, James J, Scwartz A, Hug E, Mahadevan A, Samuels M and Kachnic L: A randomized, double-blind, placebo-controlled trial of a β-hydroxyl-β-methyl butyrate, glutamine, and arginine mixture for the treatment of cancer cachexia (RTOG 0122). Support Care Cancer *16*: 1179-1188, 2008.
28 Wilson GJ, Wilson JM and Manninem AH: Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex and training experience: a review. Nutr Metab (Lond) *5*: 1-17, 2008.
29 Imai T, Matsuura K, Asada Y, Sagai S, Katagiri K, Ishida E, Saito D, Sadayasu R, Wada H and Saijo S: Effect of HMB/Arg/Gln on the prevention of radiation dermatitis in head and neck cancer patients treated with concurrent chemoradiotherapy. Jpn J Clin Oncol *44*: 422-427, 2014.
30 Nakanishi C and Toi M: Nuclear factor-κB inhibitors as sensitizers to anticancer drugs. Nat Rev Cancer *5*: 297-309, 2005.
31 Reed JC: Dysregulation of apoptosis in cancer. J Clin Oncol *17*: 2941-2953, 1999.
32 Nishida S and Tsubaki M: Exploration of molecular targets in the development of new therapeutics aimed at overcoming multidrug resistance. Yakugaku Zasshi *137*: 145-149, 2017.

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