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Changes in DNA methylation after 6-week exercise training in colorectal cancer survivors: A preliminary study

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Abstract

Aim: Behavioral interventions such as exercise may induce epigenetic changes. Only few studies investigated the effects of exercise on epigenetic alterations in colorectal cancer survivors. The aim of this study was to explore the changes of genome-wide DNA methylation after 6-week exercise training in colorectal cancer survivors.

Methods: This preliminary study used a subset of data from a randomized controlled trial in 15 colorectal cancer survivors. Participants were randomized either to the 6-week exercise group or control group. The exercise intervention consisted of a weekly, group-based, supervised resistance exercise program and a home-based same resistance exercise plus walking six times per week. Blood samples were collected at baseline and after the intervention and data from eight subjects were analyzed for genome-wide DNA methylation on 865,918 CpG sites.

Results: Compared to the control group, the exercise group shows notable methylation changes in 756 CpG sites (22.7–25.2%). Gene ontology and disease annotation analysis showed that the genes targeting 81 CpG sites in promoter region with significant group-difference were linked in biological process such as immune response and transcription and related to metabolic and immune diseases. Also, hypermethylation on genes related to disease prevention seemed to be inhibited in the exercise group compared to the control group, indicating a likelihood of transcriptional activity of these genes.

Conclusion: We found a preliminary evidence of the positive effects of exercise intervention on epigenetic markers in colorectal cancer survivors. Larger scale randomized controlled trials are warranted to further investigate our findings.

KEYWORDS
cancer survivor, colorectal cancer, DNA methylation, epigenetics, exercise

1 | INTRODUCTION

Abnormal epigenetic modification evokes health problems related to immune diseases, cardiovascular diseases, metabolic disorders, neurological disorders and cancer[1–3]. DNA methylation levels alter a degree of compaction of chromatin and regulate gene expression.[4] In a disease status, abnormal methylation patterns appear and those are related to genomic instability and inhibition of gene expression, which could influence an important biological processes.[2,5] Especially in cancer, aberrant DNA methylations in oncogenes, tumor suppressor

genes and genome-wide regions have been shown to be an indicator for cancer prognosis and a tool for early diagnosis and treatment of cancer.[5–8]

Previous systematic reviews reported that a higher level of physical activity (PA) was associated with reduced risk and mortality in various types of cancers, particularly in breast and colon cancers.[9] Colorectal cancer (CRC) survivors who were engaged in any amount of PA before and after cancer diagnosis had a reduced CRC-specific mortality by 25% compared to sedentary counterparts.[10] Furthermore, exercise can improve metabolism/immune-related biomarkers in CRC survivors.[11] Based on these results from observational and experimental studies, the American Cancer Society (ACS) and American College of Sport Medicine (ACSM) recommend that cancer patients and survivors should be physically active during and following cancer treatment.[12]

In recent years, many studies began to explore mechanisms underlying exercise-induced physiological changes and epigenetic modifications.[13–16] Some observational studies showed a weak correlation between PA and DNA methylation status regardless of the health status of subjects.[17–22] We have conducted a systematic review of interventional studies that examined exercise training-induced changes in DNA methylation and found that both aerobic and resistance training seem to induce a considerable change in genome-wide and gene-specific DNA methylation (not published).[23–39] Although there are a number of studies that investigated the effects of exercise on circulating biomarkers in cancer patients, few studies examined the effects of exercise on DNA methylation in cancer patients. Therefore, the purpose of the current preliminary study was to explore the effects of 6-week structured exercise training on genome-wide DNA methylation in CRC survivors.

2 | METHODS

2.1 | Study design and participants

This study was an exploratory analysis from a randomized controlled trial in CRC patients and data from 15 participants were included in this study. Eligible participants were: (1) aged 19 and over, (2) pathologically diagnosed with stage I–IV colon or rectal cancer, (3) those who underwent surgery for the primary treatment, (4) those who completed adjuvant chemotherapy or radiotherapy at least five years prior to study participation, (5) physically able to participate an exercise program defined as 0–2 level of Eastern Cooperative Oncology Group (ECOG) performance status and (6) willing to participate in the present study by voluntarily signing the informed consent. Participants were randomly assigned to the exercise group or the control group with 1:1 ratio. The study was conducted at Yonsei University and the Severance Hospital, Seoul, South Korea. Ethics approval was obtained from the Severance Ethics Committee prior to the study initiation and the study was registered in the Clinical Research Information Service (KCT0004796; cris.nih.go.kr). Written informed consent was obtained from all study participants. All methods in the study were performed in accordance with CONSORT 2020 guidelines.

2.2 | Exercise intervention

The exercise group was given a 6-week supervised plus home-based exercise program (Table S1). In brief, participants in the exercise group were provided with supervised exercise sessions once a week and asked to conduct home-based exercise six times per week for the rest of the week. The supervised exercise program consisted of resistance exercises including push-up, squat, shoulder press, sit-ups, pelvic tilt, shoulder bridge and bird-dog)for 20 min and additional individualized exercises based on their symptoms including pelvic abduction, squeezing ball with knees, calf raise and superman for 10 min, followed by waist and shoulder stretching. The number of repetitions was 10 times for each exercise and sets were progressed from 1 to 2 sets for Week 1 to 2, 2 to 3 sets for Week 3 to 4 and 3 sets for Week 5 to 6. Intensity was modified to enable participants to complete each given reps and sets. For instance, the intensity of push-up was modified by changing the incline such as wall or chair height for those who were not able to do it on the flat level. Participants in the exercise group were also given workout logs and daily homework to conduct same resistance exercise achieve their daily step goal and the exercise specialists checked their records once a week. Exercise sessions were conducted in the Sport Science Complex Fitness Center at Yonsei University or in the exercise facility at Yonsei Cancer Center under the guidance of qualified exercise specialists. Participants in the control group were managed under the standard of care and not given any type of exercise nor materials.

2.3 | Physical fitness and body composition measurements

This study analyzed the secondary outcomes of epigenetic markers from the clinical trial (KCT0004796). All outcomes were measured at baseline and 6-week postintervention for both the exercise and control groups. Physical fitness measurements included cardiopulmonary fitness, and muscle endurance. Muscle endurance consisted of core stability, upper body flexor endurance, lower extremity muscle endurance and grip strength. Cardiopulmonary fitness was assessed by the Tecumseh Step Test.[40] The subject wore a heart rate (HR) monitor (Polar FT1; Polar USA, Port Washington, NY, USA) on the chest and performed 24 steps/min using a 20.3 cm-high step for 3 min at 96 beats/min. The assessor recorded the HRs at the end of test and at 1 min after the test. The cardiovascular fitness was then predicted using the HR recovery. For core stability, the subject lied on the floor, bent the knee 90 degrees, and attached the soles of the feet to the floor, and an assessor placed a pressure biofeedback unit (PBU) on the arch under the waist. The subject then pushed the PBU with the lower back while lying down and repeated three times. The initial pressure

was set 40 mmHg and the average value of the three trials was used. For upper body flexor endurance measure, the subject sat on the floor while the knees bent 90° and both hands on the shoulders and then kept the upper body at 45° to the floor.[41,42] The subject had one trial and the duration was recorded in seconds. Lower extremity muscle endurance was assessed during the Chair Stand Test.[43] The subject performed sit-to-stand as many time as possible for 30 seconds. Grip Strength was measured using a hand dynamometer (T.K.K.5401; Takei Scientific Instruments Co., Ltd., Niigata, Japan). The measurement was performed twice for each hand and values were recorded in 0.1 kg units. Blood pressure and pulse were measured at rest using an automatic blood pressure monitor (MD-2070; MEDITEC, Seongnam, South Korea). Height was measured using DS-103 (Dongshan JENIX Co., Ltd., Seoul, South Korea) and body composition using the bioelectrical impedance analysis (BIA) method (InBody Co., Ltd., Seoul, South Korea). Waist circumference was measured at the umbilicus using tapeline.[44]

2.4 | Epigenetic analysis

2.4.1 | Genomic DNA extraction and bisulfite conversion

Circulating blood was drawn from the antecubital vein into a two SST tubes (5 mL) and a one EDTA tube (3 mL) with seated position. All resting blood samples were collected from participants in the morning following an overnight fast. Blood samples were preserved at −70°C before further processing. In order to identify the changes in genome-wide DNA methylation following 6-week exercise intervention, a genomic DNAs (gDNAs) were extracted from blood samples using DNeasy 96 Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's guidelines. After obtaining the gDNAs, a step of bisulfite conversion was required to distinguish between unmethylated cytosine and methylated cytosine through sodium bisulfite treatment using EZ DNA Methylation Gold Kit (Zymo Research, Inc., Irvine, CA, USA). The principle of bisulfite conversion is that unmethylated cytosine is deaminated and converted to uracil, while methylated cytosine remains as cytosine when being treated with sodium bisulfite.

2.4.2 | Genome-wide DNA methylation analysis

Genome-wide DNA methylation was quantified using an Infinium MethylationEPIC BeadChip Kit (Illumina, Inc., San Diego, CA, USA) according to the manufacturer's guidelines. This provided coverage throughout gene regions [45]. Scan protocol was followed standard Illumina procedures using Illumina iScan scanner. Array data export processing was performed using Illumina GenomeStudio v2011.1 (Methylation Module v1.9.0). Each methylation data point is represented by fluorescent signals from the M (methylated) and U (unmethylated) alleles. Background intensity computed from a set of negative controls was subtracted from each analytical data point. The ratio of fluorescent signals was then computed from the two alleles $\beta = \frac{\text{Max(M,0)}}{\text{Max(M,0)+Max(U,0)+100}}$. A β-value of 0–1 reflects the methylation level of each CpG site and it was reported signifying percent methylation from 0% to 100%, respectively, for each CpG site.

2.5 | Statistical analysis

Subject characteristics were assessed using descriptive statistics. Continuous variables were expressed as mean ± SD. Independent *t*-test between two groups and paired *t*-test within each group were used to identify significant DNA methylation changes in each CpG site. Statistical significance in methylation changes was set at the absolute value of mean $\Delta\beta$ ($= \beta_{\text{after}} - \beta_{\text{before}}$) ≥0.2 and $P < 0.5$. Descriptive statistical analysis was conducted using SPSS 23.0, and array data were analyzed using the statistical software R 3.0.2. Functional annotation analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 and statistical significance was set at $P < 0.05$.

3 | RESULTS

Of 15 participants (exercise group [$n = 9$] and control group [$n = 6$]), five from the exercise group and three from the control group who were considered to adhere to the intervention as per protocol were included for exploratory epigenetic analyses. The criteria for adherence to the exercise intervention were an improvement in high-density lipoprotein cholesterol (HDL-C) and/or triglyceride Therefore, four participants in the exercise group who did not meet the criteria were excluded. Also, three participants in the control group who had an unplanned intake of anti-virus medication for herpes zoster, an excessive increase in high-sensitivity C-reactive protein, or any incomplete postintervention measurements were excluded. The demographic and clinical characteristics of subjects are shown in Table 1. No imbalances in baseline characteristics between groups were observed. The changes on PA, body composition, physical fitness and blood-related variables at postintervention are presented in Table 2. The participants in the exercise group significantly increased the amount of leisure-time PA and resistance exercise participation. Furthermore, a significant reduction in triglyceride and increase in HDL-C were observed in the exercise group (*P* values not presented).

3.1 | Exercise-induced DNA methylation changes

The methylation profiles of 865,918 CpGs were examined at baseline and postintervention. A significant change in methylation was defined as ≥20% changes of the CpGs methylation for 6 weeks ($\Delta\beta = \beta_{\text{postintervention}} - \beta_{\text{baseline}} \geq 0.2$ or ≤ -0.2; $P < 0.05$). A total of 756 CpGs identified within 524 genes showed a significant between-group difference of $\Delta\beta$ ($\Delta\beta_{\text{exercise group}} - \Delta\beta_{\text{control group}} \geq 0.2$ or ≤ -0.2).

TABLE 1 Demographic and clinical characteristics of subjects included for analyses

	Total ($n = 8$)	Exercise ($n = 5$)	Control ($n = 3$)
Age (year)	57.1±11.3	54.2±10.3	62.0±13.1
Male (*n*)	3	2	1
Site of cancer (*n*)			
Colon	5	3	2
Rectal	3	2	1
Stage of cancer (*n*)			
Stage	2	1	1
Stage	5	4	1
Stage	1	0	1
Duration after diagnosis (year)	4.7±2.1	3.8±2.4	6.3±1.5
Comorbidities (*n*)			
Hypertension	1	1	0
Diabetes	1	0	1
Other	3	2	1

Of the changes in 756 CpGs, 377 were in gene body, 208 in intergenic regions, 81 in the promoter region (TSS1500 and TSS200), 66 in 5′UTR, 21 in 3′UTR and 3 CpGs in exon (first exon and ExonBnd; Figure 1).

3.2 | Functional annotation analysis

Since the methylation changes in promoter region affects to gene expression directly, the functional annotation analysis of 74 genes targeted by 81 CpGs belonging to the promoter region was performed using DAVID v6.8.

3.2.1 | Gene ontology annotation

To examine biological impact of the methylation changes of CpGs in promoter following exercise training, gene ontology annotation analysis was performed. Most common genes that showed altered methylation were the genes related to innate immune response, transcription from RNA polymerase II promoter, inflammatory response and neurological process (Figure 2).

3.2.2 | Disease annotation

Disease annotation analysis showed that genes with altered methylation were mostly related to metabolic diseases and immune diseases. We presented the top 10 CpG sites showing the largest $\Delta\beta$ difference between groups (Table 3) and their correspondent genes were identified to involve in several immune and metabolic processes. In brief, STK38L (cg05171937; −25.2%) which acts as a pro-apoptotic kinase promotes apoptosis, LTBP1 (cg11918450; −24.7%) which involve in regulation of TGF-β1 activation plays an important role in controlling the immune system and cellular protein metabolic process, FOXD3 (cg14668802; −24.7%) which is a transcriptional regulator acts as a tumor suppressor, CHI3L1 (cg19081101; −22.7%) plays a role in inflammatory response and inflammatory cell apoptosis and ARG1 (cg01699630, cg06975018; −23%) plays a role in arginine metabolism which is a critical regulator of innate and adaptive immune responses. Furthermore, UBE3C (cg04185197; −23.5%) degrades target substrates and FGF23 (cg03581638; −20.5%; data not shown) involves in metabolism of phosphate and calcium.

4 | DISCUSSION

This study was conducted to explore the effects of 6-week combined exercise training on genome-wide DNA methylation in CRC survivors. As a result of analyzing methylation changes in 865,918 CpGs, a noticeable difference between exercise and control groups in gene methylation were identified in 756 CpGs. Among them, 81 CpGs were located in promoter region and used for functional annotation analysis. These 81 CpGs in promotor region were responsible for 74 genes and these genes were related to immune response, transcription, inflammatory response, neutrophil chemotaxis, synaptic vesicle clustering, neuronal signal transduction and antigen receptor-mediated signaling pathway. Furthermore, CpG sites showing remarkable $\Delta\beta$ differences between groups before and after the exercise intervention were largely related to metabolic diseases and immune diseases. Interestingly, a hypermethylation in genes associated with diseases seemed to be inhibited in the exercise group compared to the control group, indicating that these genes may have been transcriptionally active.

In our study, we found that exercise seemed to affect methylation changes in CpG sites linked to metabolism and innate immunity. It is well known that exercise is associated with metabolic diseases, especially excessive fat-related metabolic diseases. Exercise may have favorable effects on lipogenesis, fat distribution and nutrient metabolism such as free fatty acids.[46] Also, previous studies reported that regular exercise clearly improves risk factors for metabolic syndrome.[47,48] The possible explanations which support these results are improvement in obesity and insulin resistance induced by exercise.[49–51] In addition, previous studies have reported that exercise improve various immune function such as natural killer cell cytotoxic activity,[52] monocyte function, proportion of circulating granulocytes[53] and macrophage phagocytic activity.[54] It is clear that these factors are related to many diseases, especially cancer.[55] Moreover, exercise regulates the secretion of various cytokines and myokines which are mediators of immune responses and inflammation. Exercise increases the amount of anti-inflammatory cytokines, which serve to reduce inflammation and promote healing, such as Interleukin 6 (IL-6), Interleukin 1 receptor antagonist (IL-1ra), Interleukin 10 (IL-10) and Transforming growth factor beta 1 (TGF-α1). While, exercise inhibits the production of pro-inflammatory cytokines, which act to

TABLE 2 Changes on physical activity, body composition, physical fitness and blood-related variables after 6 weeks

	Exercise ($n = 5$)		Control ($n = 3$)	
	Baseline	6-Week	Baseline	6-Week
Physical activity (PA)				
Vigorous LTPA (min/week)	0	84.0 ± 156.5	0	0
Moderate LTPA (min/week)	192.0 ± 147.9	414.0 ± 249.6	289.2 ± 114.1	110.0 ± 121.2
Transport PA (min/week)	198.0 ± 135.4	195.0 ± 75.3	180.0 ± 158.7	233.3 ± 208.2
Resistance exercise (day/week)	0.8 ± 1.3	6.0 ± 1.7	1.0 ± 1.7	0.7 ± 1.2
Body composition				
Weight (kg)	61.9 ± 7.9	62.0 ± 7.3	55.9 ± 5.4	55.0 ± 6.5
Body mass index (kg/m^2)	22.5 ± 2.6	22.5 ± 2.6	21.7 ± 3.1	21.5 ± 3.6
Waist circumference (cm)	77.6 ± 6.3	79.8 ± 5.5	79.0 ± 3.6	79.0 ± 6.0
Skeletal muscle mass (kg)	24.8 ± 4.4	25.2 ± 4.0	22.0 ± 1.8	21.4 ± 1.5
Body fat percentage (%)	27.1 ± 7.0	26.1 ± 6.9	26.5 ± 10.0	27.0 ± 10.4
Physical fitness				
HR at rest before 3 min step test	65.6 ± 4.8	68.0 ± 5.7	61.3 ± 4.2	62.0 ± 8.9
HR at 1 min after 3 min step test	79.2 ± 13.4	81.6 ± 9.9	76.3 ± 8.7	79.3 ± 11.4
Chair stand test, times/30 s	26.6 ± 4.7	26.4 ± 1.9	20.7 ± 0.6	26.7 ± 5.5
Core muscle strength (mmHg)	92.8 ± 12.7	103.3 ± 11.7	73.6 ± 16.4	83.3 ± 5.2
Core muscle endurance (s)	63.4 ± 29.0	76.6 ± 25.4	41.0 ± 30.8	29.3 ± 6.0
Handgrip strength (kg)	31.6 ± 7.4	33.3 ± 7.4	25.9 ± 4.1	27.2 ± 5.8
Blood-related variables				
Systolic blood pressure (mmHg)	115 ± 13.2	114.8 ± 14.0	126.3 ± 9.2	131.0 ± 11.5
Diastolic blood pressure (mmHg)	73.4 ± 13.2	72.4 ± 14.4	83.0 ± 10.1	85.3 ± 11.9
Resting heart rate	67.0 ± 6.7	67.4 ± 6.4	61.3 ± 5.1	67.3 ± 9.6
Glucose (mg/dL)	78.2 ± 4.3	87.2 ± 7.4	85.0 ± 21.0	83.3 ± 9.0
Insulin (mg/dL)	4.8 ± 0.9	5.3 ± 2.1	3.6 ± 1.0	5.2 ± 4.0
Total cholesterol (mg/dL)	169 ± 20.3	192.2 ± 39.0	175.3 ± 25.1	193.7 ± 31.3
Triglyceride (mg/dL)	116.2 ± 77.5	86.4 ± 43.5	79.3 ± 53.8	94.3 ± 47.4
HDL-cholesterol (mg/dL)	54.2 ± 18.0	66.8 ± 19.6	67.8 ± 32.2	72.5 ± 24.4
hs-CRP (mg/dL)	1.1 ± 1.6	0.4 ± 0.3	0.6 ± 0.6	0.5 ± 0.2

Abbreviations: HDL, high-density lipoprotein; HR, heart rate; hs-CRP, high-sensitivity C-reactive protein.; LTPA, leisure time physical activity.

make disease worse, such as Tumor necrosis factor alpha (TNF-α).[56–60] These changes are also related to protecting against TNF-α-induced insulin resistance.[56]

Metabolism and immune system are closely related to human diseases,[61] especially cancer. Non-insulin-dependent diabetes mellitus is associated with an elevated acute-phase immune response, particularly in those with features of metabolic syndrome.[62] Also, insulin resistance induced by abnormal lipid and glucose metabolism is a cause of Nonalcoholic Fatty Liver Disease.[63] In addition, previous meta-analysis study reported that metabolic syndrome is associated with increased risk of common cancers,[64] and other study reported that colon cancer patients with diabetes had significantly worse disease-free survival compared with patients without diabetes.[65] Furthermore, immune cells are a crucial regulators of cancer development and play a paradoxical role in carcinogenesis.[66]

Therefore, exercise may be a good treatment of numerous diseases through maintaining normal metabolic processes and improving immune response. A previous comprehensive review reported statistically significant improvements in a number of cancer-related immune system components as a result of exercise in cancer survivor.[55] Also, exercise in cancer survivors may reduce the risk of cancer recurrence,[67] and improve circulating insulin level, TNF-α,[11] physical function and body composition.[68] Even though the results of this study could not present a direct interaction with well-known metabolic and immune related factors (i.e., insulin, adiponectin and TNF-α, etc.), these could support the results of previous studies and make it possible to infer an underlying mechanisms of exercise-induced positive influences with observed methylation changes in genes which involve numerous metabolic processes and immune responses.

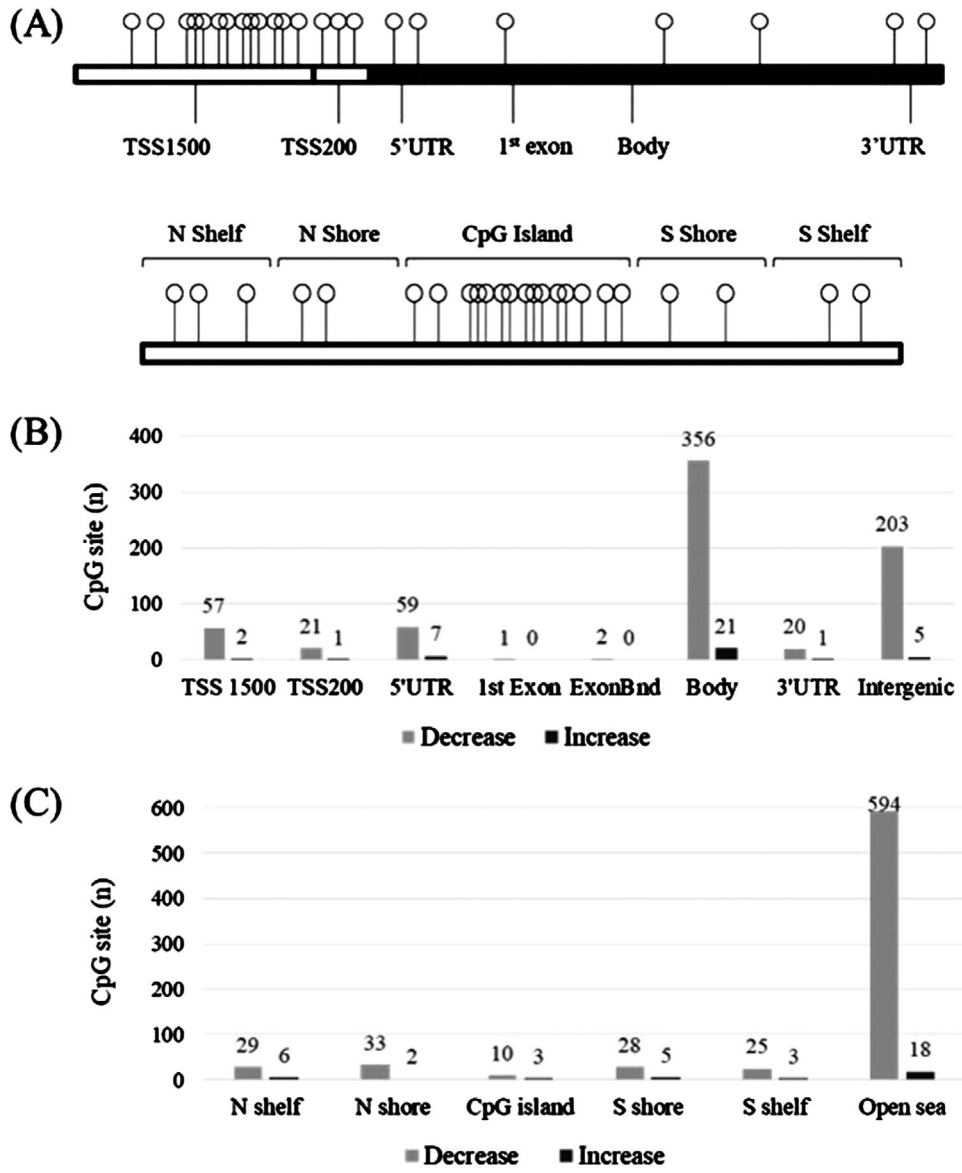

FIGURE 1 Distribution of CpG sites in relation to gene and CpG islands. (A) Gene region and CpG island region (2012, Illumina, Inc., https://cancergenome.nih.gov/abouttcga/aboutdata/platformdesign/illuminamethylation450). Methylation changes at CpG sites in relation to the (B) nearest gene and (C) CpG islands

The findings of our study are in line with previous evidence showing that alterations in DNA methylation may be one of the underlying mechanisms which could explain the favorable health adaptations following exercise in cancer survivors. According to previous studies, both aerobic and resistance training had a marked influence on the methylation patterns. Until now, many studies had focused on skeletal muscle and blood sample, but DNA methylation patterns have tissue-specific characteristics, therefore future studies should be conducted with more diverse samples such as adipose tissue and germ cell. Moreover, it is necessary to conduct RCT study for proving that exercise-induced methylation changes do not occur by simple time-related effect and to study how exercise manipulates DNA methylation in cancer population.

This study has several limitations. First, our study provides exploratory findings with a small sample size and the results are difficult to generalize. Second, DNA methylation is mostly tissue-specific, and a caution is needed to interpret the results. For example, our findings of DNA methylation using circulating blood may not fully reflect the effects of exercise training. Thirdly, RNA expression and gene-specific DNA methylation analyses were not conducted, which might have been helpful to interpret our findings. Finally, this study did not control the diet which might have had a great effect on epigenetic alteration. Even with the aforementioned limitations, this study will provide preliminary evidence regarding the underlying mechanisms of the effects of exercise on the human body, especially according to metabolism and immune response.

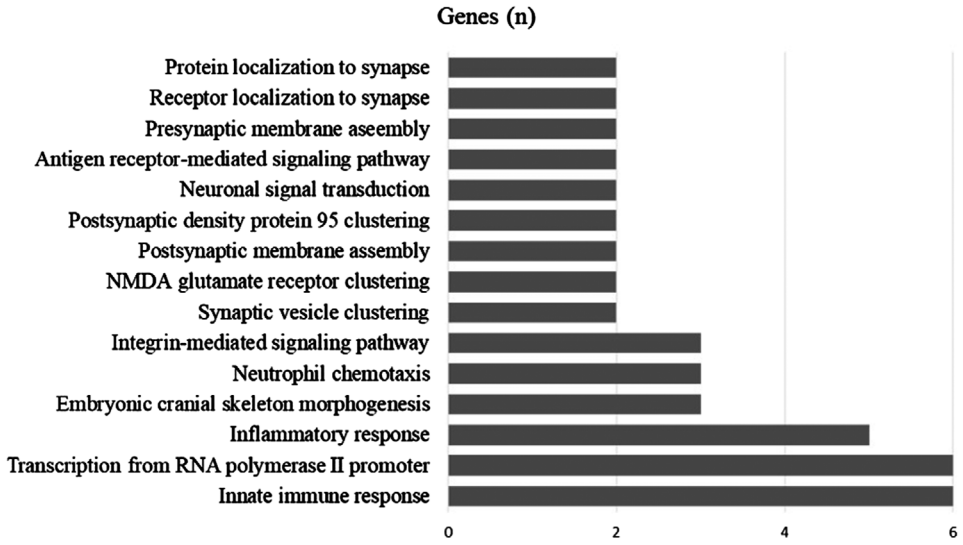

FIGURE 2 Gene ontology annotation analysis for genes targeted by CpG sites in promoter region

TABLE 3 DNA methylation changes after 6-week exercise training (Top 10)

CpG site	Nearest gene	Control group Δ percentage (%)	Exercise group Δ percentage (%)	Difference between groups (%)	Disease class	Gene function
cg05171937	STK38L	22.6	−2.5	−25.2	IM	Promotion of apoptosis
cg13594075	NRXN1	24.7	−0.4	−25.1	MET, IM	Involved in formation or maintenance of synaptic junctions
cg14668802	FOXD3	26.0	1.3	−24.7	IM	Involved in autoimmune disease/tumor suppressive activity
cg11918450	LTBP1	19.3	−5.4	−24.7	MET	Involved in controlling and directing the activity of TGF-β1
cg01586609	HTR3A	19.7	−4.2	−23.9	MET	Biogenic hormone that functions as a neurotransmitter, a hormone, and a mitogen
cg04185197	UBE3C	22.2	−1.3	−23.5	IM	Target substrate degradation
cg01699630	ARG1	20.0	−3.1	−23.0	IM	Catalytic activity for arginine hydrolysis
cg06975018	ARG1	21.3	−1.7	−23.0	IM	
cg06926818	CHN2	20.0	−3.3	−22.8	MET, IM	Regulation of vascular smooth muscle proliferation and migration
cg19081101	CHI3L1	22.3	−0.4	−22.7	MET, IM	Involved in defense against pathogens/tissue remodeling

Abbreviations: IM, immune disease; MET, metabolic disease.

In conclusion, this is the first interventional study which explore the epigenetic changes following exercise training in CRC survivor. Exercise training was associated with inhibited global hypermethylation. The results of this study showed inhibited hypermethylation in promoter region of several genes involved in prevention of human disease such as immune response, indicating a potential transcriptional activities of such genes from exercise. We believe that our findings provide a preliminary evidence of the positive effects of exercise in CRC survivors through epigenetic mechanisms. Larger scale randomized controlled trials are warranted to further investigate our findings.

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DATA AVAILABILITY STATEMENT

Research data are not shared.

FUNDING STATEMENT

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CONFLICTS OF INTEREST DISCLOSURE

We have no conflicts of interest to declare.

ETHICS APPROVAL STATEMENT

Ethics approval was obtained from the Severance Ethics Committee prior to the study initiation and the study was registered in the Clinical Research Information Service (KCT0004796; cris.nih.go.kr).

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all study participants.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

No other resources were reproduced.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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