

Immune signature as a potential marker for predicting response to immunotherapy in obesity-associated colorectal cancer

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Abstract

Background and Aim: It remains unclear whether immunotherapy, which is not generally considered for microsatellite stable (MSS) colorectal cancer (CRC), can be used to effectively treat select CRC patients. We investigated the feasibility of obesity-associated MSS CRC patients for immunotherapy based on genomic alterations.

Methods: We evaluated differences in genomic alteration types and immune signatures between obese and non-obese patients with MSS CRC. We performed genomic analyses using 434 CRC patients from The Cancer Genome Atlas (TCGA). Patients with MSS CRC were stratified into subgroups based on their BMI and numbers of nonsynonymous single nucleotide variants (nsSNVs) and frameshift insertions and deletions (fs INDELs) using machine learning.

Results: The obese subgroup showed higher incidences of single nucleotide variants (SNV) and insertions and deletions (INDELs) in comparison to healthy weight patients with MSS CRC. The subgroup, who had higher numbers of nsSNVs and fs INDELs, exhibited increased immune signatures, increased number of SNV-derived neoantigens, and had up-regulated two immune checkpoint genes in comparison to healthy weight patients with MSS CRC, reflecting interactions between the cancer genome and immune system.

Conclusions: This study suggests that immunotherapy may be suitable for some obesity-associated CRC patients.

Keywords: Colorectal cancer; Obesity; Immunotherapy; Immune signature

Introduction

Colorectal cancer (CRC) is the third most common cancer in the world and the fourth-leading lethal cancer, with more than 800,000 deaths in 2018.¹⁻³ During the decades, clinicians have witnessed remarkable progress in the treatment of colon cancer, declining mortality from CRC continuously. Effective combinations of chemotherapy regimens⁴⁻⁸ have been yielded higher response rates and prolonged survival.⁹ In recent years, the paradigm shift in cancer treatment has been immunotherapy.^{10, 11} It has shown good efficacy in gastrointestinal-related cancers¹¹ and expected promising efficacies in CRC. Immune checkpoint inhibitors have been approved by the United States Food and Drug Administration (FDA) for high microsatellite instability (MSI-H) and DNA mismatch repair deficiency CRC patients.¹² Recently, multiple studies undergo evaluation immune checkpoint inhibitors in combination with PD-1 for the microsatellite stable (MSS) CRC, speculating combined modalities can provoke an immune response.¹³

The prevalence of obesity will become a health problem.¹⁴⁻¹⁶ Its incidence continues to rise in the young population,¹⁴ along with the increasing incidence of obesity-associated diseases, rendering obesity-associated CRC a critical health problem.¹⁷ Obesity is thought to be a major risk factor for CRC. Interestingly, a recent report has indicated that obesity-associated CRC more commonly involves the MSS type, relative to the genetic types with MSI-H.¹⁷ Immune checkpoint inhibitors (e.g., nivolumab and pembrolizumab) have only been approved by the FDA for MSI-H tumors, which only account for 10–15% of CRC cases.^{18, 19} In contrast, the majority of CRC cases involve the MSS type, which is not expected to respond to immune checkpoint inhibitors. However, approximately 4-10% of patients with MSS tumors and DNA mismatch repair proficiency will experience a clinical benefit from treatment using immunotherapy.^{20, 21} Therefore, it is important to determine whether there are genetic or immune signatures in obesity-associated CRC that may predict response to immunotherapy. This information might help guide the use of immunotherapy for select patients with MSS CRC (>80% of all CRC cases), especially as obesity and obesity-associated CRC become increasingly serious health problems.

In obesity, adipose tissue secretes various growth factors and cytokines associated with mild chronic inflammation.²² In particular, tumor microenvironment resident adipocytes affect immune surveillance and resistance to cancer treatment.

As a result, factors such as energy imbalance, inflammation, and changes in immune surveillance appearing in obesity may induce molecular changes in tumor and may indicate different responses to immunotherapeutic agents.^{23, 24}

Recent studies have reported that immunotherapy could work in approximately 10% of MSS-CRC cases.^{20, 21, 25, 26} Therefore, the identification of suitable target patient subgroups for immunotherapy will provide a breakthrough opportunity for obesity-associated MSS-CRC treatment. In this study, we assessed the genetic mutation profiles of obese MSS-CRC patients. Based on the weight status, we evaluated whether immune signatures could be associated with the types of mutations (i.e., non-synonymous single nucleotide variants [nsSNVs] and frameshift insertions and deletions [fs INDELs]) that might potentially predict their response to immunotherapy.

Methods

Gil Medical Center colorectal cancer cohort

We reviewed the data for 1,081 patients with colorectal cancer (CRC) who underwent surgical treatment at the Gachon University Gil Medical Center (GMC), South Korea between November 2011 and March 2016. To ensure a relatively homogenous sample of patients who were initially diagnosed with CRC, we excluded 14 patients with multiple CRCs, 6 patients with recurrent CRC, and 1 patient with HNPCC. Patients who received chemotherapy or radiotherapy were also excluded, as these treatments can alter the characteristics of CRC. Furthermore, we excluded 8 patients with insufficient data to confirm their body mass index (BMI). Therefore, the analyses of microsatellite stable (MSS) status according to BMI evaluated a total of 956 patients with CRC (Table 1). Healthy weight patients with CRC were categorized according to whether they had MSS characteristics (79.1%) or did not have MSS characteristics (20.9%). Obese patients with CRC were also characterized according to whether they had MSS characteristics (84.7%) or did not have MSS characteristics (15.3%) (Figure 1). Obesity was defined as a BMI of $>30 \text{ kg/m}^2$ in the TCGA cohort, although obesity was defined as $>25 \text{ kg/m}^2$ in the GMC cohort based on the World Health Organization's recommendation for the different BMI cut-off point, 25, for the Asian population.^{27, 28}

The Cancer Genome Atlas (TCGA) colorectal cancer (CRC) cohort

Mutational profiles and immune signatures²⁹ were inspected using mutation and expression data of the TCGA from UCSC Xena Functional Genomics Explorer (last accessed on January 28, 2019).³⁰ To identify patient subgroups, we performed t-distributed stochastic neighbor embedding and k-means clustering. Furthermore, expression patterns of immune signatures were assessed in each subgroup.

Data processing

We selected CRC samples from the TCGA dataset with information regarding microsatellite status, height, and weight. The flow chart of our exclusion criteria for the inclusion of TCGA CRC samples is presented in Supplementary Figure S1. The CRC samples were considered eligible if they had a microsatellite stable (MSS) status, and the body mass index (BMI) was calculated as:

$$BMI = \frac{weight \text{ (kg)}}{height \text{ (m)}^2} \quad (1)$$

The MSI statuses for the TCGA CRC samples were obtained from the “TCGA COAD mutation dataset 2015-02-24”, which was downloaded from the University of California, Santa Cruz (UCSC) Cancer Genomics Browser (last accessed on February 21, 2018).³¹ The weight and height for the TCGA CRC samples were obtained from the “phenotype” dataset of the GDC TCGA Colon Cancer sample using UCSC Xena Functional Genomics Explorer (last accessed on January 28, 2019).³⁰ The selected samples were grouped according to their BMI as healthy weight (HW) or obese:

$$Group = \begin{cases} HW, & < 30 \text{ kg/m}^2 \\ Obese, & \geq 30 \text{ kg/m}^2 \end{cases} \quad (2)$$

Mutational analyses (identifying and counting mutational types, single nucleotide variants [SNVs], and insertion and deletion [INDEL]) were identified using the “somatic mutation (SNPs and small INDELs)” dataset, which was based on the “MuTect2 Variant Aggregation and Masking” version of the “GDC TCGA Colon Cancer” dataset from UCSC Xena Functional Genomics Explorer (last accessed on

January 28, 2019).

Expression levels of immune checkpoint genes and gene sets for immune signatures (listed below) were evaluated using the “gene expression RNAseq” dataset, which was based on the “HTSeq – FPKM” version of the “GDC TCGA Colon Cancer” dataset from UCSC Xena Functional Genomics Explorer (last accessed on January 28, 2019). The percentage change (d_{AB}) between the expression levels of two genes (A and B) was calculated as follows:

$$d_{AB} = \frac{Med_A - Med_B}{\left(\frac{Med_A + Med_B}{2}\right)} \times 100 \quad (3)$$

where Med_A and Med_B are the median expression levels of A and B.

We performed t-distributed stochastic neighbor embedding and k-means clustering using a machine learning framework (Scikit-learn, version 0.20.3).³²

Tumor mutation burden (TMB)

We calculated TMB with the sum of somatic, coding and base substitutions, and INDEL mutations per megabase (Mbp).^{33, 34} We set 38 Mb as the estimated exome size.³⁵

Immune signatures

The gene sets for the eight immune signatures were obtained from the report by Rooney et al.²⁹: pDCs (*LILRA4*, *CLEC4C*, *PLD4*, *PHEX*, *IL3RA*, *PTCRA*, *IRF8*, *IRF7*, *GZMB*, and *CXCR3*), co-inhibition APC (*PDCD1LG2*, *CD274*, *C10orf54*, *LGALS9*, and *PVRL3*), type II IFN response (*GPR146*, *SELP*, and *AHR*), cytolytic activity (*GZMA* and *PRF1*), MHC Class I (*HLA-A*, *B2M*, and *TAP1*), CD8+ T cells (*CD8A*), co-inhibition T-cell (*LAG3*, *CTLA4*, *CD274*, *CD160*, *BTLA*, *C10orf54*, *LAIR1*, *HAVCR2*, *CD244*, and *TIGIT*), and co-stimulation APC (*ICOSLG*, *CD70*, *TNFSF14*, *CD40*, *TNFSF9*, *TNFSF4*, *TNFSF15*, *TNFSF18*, *TNFSF8*, *SLAMF1*, and *CD58*).

Results

Increasing obesity-associated CRC is positively associated with the proportion of the MSS type in CRC

Epidemiological analysis of the Gachon University Gil Medical Center (GMC) cohort showed that the MSS type was more common among obese patients than among healthy weight patients (HW; GMC: 84.7% vs. 79.1%, p -value < 0.05, Figure 2A), and The Cancer Genome Atlas (TCGA) cohort also showed similar trend (TCGA: 73.6% vs. 61.7%, p -value < 0.1, Figure 2A). Furthermore, MSS-CRC patients tended to have a greater increase in BMI (Figure 2B). Moreover, between 2012 and 2016, MSS-CRC patients had an increasing average BMI value in the GMC cohort (Figure 2C; detailed inclusion criteria for study participants at the GMC are presented in the Materials and Methods section). These results suggested that the increasing proportion of obesity-associated CRC was positively associated with the proportion of the MSS type among all CRC cases.

Obese MSS-CRC patients show different mutation type

The number of mutations per mega-base pair is recognized as a biomarker for predicting the prognosis of immunotherapy.³⁶ In this study, we inspected mutational profiles and immune signatures to explore the immunogenic difference between obese and HW patients with MSS-CRC. In the TCGA cohort, obese CRC patients displayed different types of mutations than HW patients, more specifically in the number of nsSNVs and fs INDELs. The overall numbers of mutated genes were not significantly different between obese and HW patients with MSS-CRC (Figure 3A). However, insertions (INS) and deletions (DELs) were more common among obese patients than among HW patients (INS: 25.6% vs. 2.3%, DEL: 4.1% vs. 2.8%) (Figure 3B). Obesity is reportedly associated with deficiencies in DNA damage and mismatch repair mechanisms;³⁷ however, we did not observe significant differences in mutations involving the *BRAF* and DNA mismatch repair-related genes when comparing obese and HW MSS-CRC patients (Supplementary Table S1).

Recently, immune response gene signatures²⁹ were used to measure immune activation, which was classically associated with single nucleotide variants (SNVs) and insertion and deletion (INDEL)-derived immunogenic neoantigens.³⁸ Considering the proportional differences of SNVs and INDELs between obese and patients with HW MSS-CRC, we speculated that these differences may be related to different

immune activations. However, immune activation was not observed in obese patients (Figure 3C) in the immune response gene signatures²⁹ of obese versus HW patients.

Obese MSS-CRC patients having a large number of nsSNV and fs INDELs indicate enriched immune signatures

We suspected that different occurrences of SNVs and INDELs among CRC patients may reveal different immune activations. Therefore, to identify patient subgroups, we applied machine learning based on the types of mutations that occurred (e.g., nsSNVs and fs INDELs), as shown in Figure 4A. The results revealed that MSS-CRC patients were categorized into two groups (G1 and G2) based on t-distributed stochastic neighbor embedding and k-means clustering. Patients in G1 had a higher number of nsSNVs than other patients in G2. G1 had a significantly higher number of SNV-derived neoantigens (Figure 4A). However, only the obese patients in G1 (henceforth, obese (G1)) showed a significantly higher number of fs INDELs than the healthy weight patients in G1 (henceforth, H.W. (G1)), the obese patients in G2 (henceforth, obese (G2)), and the healthy weight patients in G2 (henceforth, H.W. (G2)) (Figure 4A). We also inspected TMB among these subgroups, and patients in the G1 had a higher TMB than other patients in the G2.

The obese (G1) had an upregulated immune signature that involved cytolytic activity, CD8+ T-cells, plasmacytoid dendritic cells, co-inhibition of T-cells, and co-stimulation of antigen-presenting cells compared with H.W. (G1), obese (G2), and H.W. (G2) (Figure 4B). We calculated the Pearson's correlation coefficients (PCCs) of the numbers of nsSNVs and fs INDELs with immune signatures (Supplementary Figure S2). Obese (G1) exhibited higher PCCs between the numbers of nsSNVs and fs INDELs with immune signature scores, especially cytolytic activity and CD8+ T cells compared to the other subgroups.

Obese MSS-CRC patients having a large number of nsSNV and fs INDELs show enriched CTLA4 and HAVCR2

The upregulation of CD8+ T-cells and cytolytic activity in the obese (G1) implies that this subgroup may respond to immunotherapy. High cytolytic activity is reportedly associated with an increased expression of immune checkpoint genes, which triggers a response to immune checkpoint blockade.³⁹ Interestingly, this study revealed that the obese (G1) had significantly higher expressions of immune

checkpoint genes (Figure 5 and Supplementary Figure S3). For cytotoxic T-lymphocyte associated protein 4 (*CTLA4*), The obese (G1) showed significantly higher expression levels than in adjacent normal tissues obtained from CRC patients, H.W. (G1) and H.W. (G2). For hepatitis A virus cellular receptor 2 (*HAVCR2*; also known as T-cell immunoglobulin and mucin domain 3 [*TIM-3*]), The obese (G1) showed significantly higher expression levels than the H.W. (G1). But the obese (G1) has not observed significantly different expression levels in programmed cell death 1 (*PDCD1*; PD-1 encoding gene) and *CD274* (PD-L1 encoding gene) in comparison to adjacent normal tissues obtained from CRC patients, obese (G2), H.W. (G1) and H.W. (G2) (Figure 5 and Supplementary Figure S3).

We also inspected the expression levels of ligands of the *CTLA4*. *CD80* and *CD86* are natural ligands for CTLA-4 and found on antigen-presenting cells such as dendritic cells, monocytes and activated B cells.⁴⁰ The lack of *CD80* expression have been suggested to evade immune surveillance and lead for failure of immune recognition.⁴¹ *CD80* can costimulate the CD8 T cells to produce IL-2, proliferate, and acquire cytolytic activity.⁴² The function of *CD86* is yet to elucidate. *CD80* has a higher affinities and avidities for CTLA-4 than *CD86*.⁴³ However, *CD86* was suggested that is more important in T cell activation than *CD80*.⁴⁴ Like expression patterns of *CTLA4* between subgroups, *CD80* was increased in the obese (G1) than other subgroups. *CD86* also showed increased expression levels in the obese (G1) than other subgroups like *CD80*, however, could not pass the statistical test (Supplementary Figure S4).

Discussion

In this study, we presented that patients with a large number of nsSNVs and fsINDELs show enriched immune signatures among obese MSS-CRC patients. Moreover, this subgroup shows enriched immune checkpoint genes compared to other MSS-CRC patients, especially higher *CTLA4* than that of (adjacent) normal control. From these observations, this subgroup could be considered as a new immunotherapy target.

Although heavily mutated (i.e. MSI-H) patients with CRC are considered to have a great benefit in immunotherapy in comparison to patients with MSS CRC having an overall low number of mutations. We observed the upregulation of *CTLA4* in the patients with MSS CRC of the obese (G1). Elevated messenger RNA levels of

CTLA4 and *HAVCR2* have been observed in exhausted T cells of CRC tissues, especially in advanced stages.⁴⁵ *CTLA4* and *HAVCR2* are participants of signaling pathways linked to suppressed antigen-presenting cells, T effector cells, and T regulatory cells.^{46, 47} FDA has approved anti-PD1 antibodies and the anti-CTLA4 antibody only for high levels of microsatellite instability or mismatch-repair-deficient CRC patients.¹² However, a recent clinical trial⁴⁸ suggested that combining anti-CTLA4 and other agents has a beneficial effect for MSS-CRC patients to overcome resistance to immunotherapy. Blockade of *HAVCR2* also showed improving anti-tumor immunity in preclinical stages,⁴⁹⁻⁵¹ and several clinical trials have been initiated for solid tumors (NCT02817633, NCT03489343, NCT03311412, NCT03744468, NCT03652077, and NCT03446040). We also observed the upregulation of CD8+ T-cells and cytolytic activity in the obese (G1) suggesting the presence of an anti-tumor mechanism that could provide the benefit of the immunotherapy and a clue to developing effective immunotherapy through different immune checkpoint pathways other than PD-1 and/or PD-L1, including *CTLA4* and *HAVCR2*.

Obesity and CRC constitute a significant global burden and lack effective therapies. Immunotherapy has not been considered for MSS cases, the most common CRC cases. However, our study suggests the possibility of selecting target patients, among obesity-associated CRC patients, suitable for immunotherapy. Multiple clinical trials undergo evaluation of immune checkpoint inhibitors in MSS-CRC patients, however, existing studies have not investigated the presence of clinical and/or molecular features that may vary immune signatures and immune checkpoint genes among MSS-CRC patients. This study suggested the possibility that obesity and the numbers of nsSNVs and fs INDELs in MSS-CRC patients could serve as criteria for finding subgroups with different activation of immune signatures for immunotherapy.

In this study, the 20 obese patients with MSS CRC in TCGA CRC cohort were assigned G1 (higher numbers of nsSNVs and fs INDELs than G2). And these 20 patients in the obese (G1) out of 133 patients with MSS CRC showed higher activity scores of immune signatures, especially, cytolytic activity and CD8+ T cells (Figure 4B). Thus, we assumed about 15% (20/133) of patients with MSS CRC could be possible target candidates of immunotherapy.

Currently, immune checkpoint, especially, the expression level of PD-L1 and MSI-H are the only approved biomarkers for immune therapy. However, in some

cancers (late staged melanoma, non-small cell lung cancer, and MSI-H CRC), the outcomes of immune checkpoint inhibition have been observed positive correlation with the numbers of mutations and neoantigens.^{21, 52, 53} Body status, mutation, and response of checkpoint inhibitor data are not available yet for MSS CRC. Therefore, we could not inspect an association among body status, burdens of nsSNVs and fs INDELs, and the response of checkpoint inhibition. However, in the study of CTLA-4 inhibition in metastatic melanoma, burdens of nsSNVs and fs INDELs showed statistical significant association with the response of CTLA-4 inhibition.⁵⁴ And in our study, we observed the upregulations of CD8⁺ T cell and cytolytic activity signatures in the obese (G1) that presents higher burdens of nsSNVs and fs INDELs (Figure 4B). And these signatures were more closely correlated with the numbers of nsSNVs and fs INDELs in obese (G1) than other subgroups (Supplementary Figures S2). In addition, the expression levels of *CTLA4* and the ligands of CTLA4 (*CD80* and *CD86*) were also upregulated in obese (G1) in comparison with the other subgroups (Supplementary Figure S4). These pieces of indirect evidences suggest that the obese patients with MSS CRC having higher burdens of nsSNVs and fs INDELs (i.e., obese (G1)) could be candidate for immunotherapy.

There are limits to this study. Le and colleagues reported that MSS-CRC did not seem to benefit from anti-PD1 antibodies.²¹ Although multiple studies are ongoing for evaluating immune checkpoint inhibitors in combination with various regimens in MSS-CRC, however, there have been unsuccessful results for drawing out the immunogenic response in trials.^{55, 56} However, we showed that there is a certain subgroup showing enriched signatures in obese MSS-CRC patients and they have enriched immune checkpoint genes. Also, MSI-H CRC is more prevalent on the right-side location, and there are different molecular mechanisms between the left- and right-sided CRC patients.⁵⁷ Also, patients with right-sided CRC showed significant weight loss compared to left-sided CRC patients.⁵⁸ Therefore, it is critical to consider the weight (i.e., BMI), along with the number of mutations, and the tumor location, when searching for the subgroup capable of immunotherapy. However, in Supplementary Table S2, total numbers of left- and right-sided CRC patients were 9 (4.09%) and 10 (4.55%), respectively, in the TCGA COAD dataset in this study. Due to fewer left- and right-sided CRC patients and, subsequently, lack of statistical power, it was not feasible to adjust the tumor site for subgrouping of TCGA MSS CRC by using nsSNVs and fs INDELs. In this study, we showed the correlations

between immune signature scores, BMI status, and numbers of nsSNVs and fs INDELs (Supplementary Figure S2). However, further study is needed to elucidate the direct link of obesity-related MSS-CRC and immune signature. Further investigation should explore the applicability of the obesity differing immune signatures indicating promising outcomes for the use of immunotherapy in MSS-CRC. If further studies will confirm, immunotherapy may be beneficial for select obesity-associated CRC patients.

In conclusion, further studies evaluating immune checkpoint markers for obesity-associated CRC, as well as the potential use of immunotherapy for select patients with these markers, are urgently needed.

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Authors' contributions

SN and JHK designed and coordinated the study; SP, JA, SYH, SN, and JHK acquired, analyzed, and/or interpreted of data; SP and JHK wrote the manuscript; SP performed statistical analysis; SN and JHK provided administrative, technical, or material support; SN and JHK supervised this study.

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Table 1. Baseline demographic and clinical characteristics of patients

Characteristics	GMC colorectal cancer cohort
Total participants	956
Age (yr)	64.3±11.9
Male/Female	565 (59.1%)/391 (40.9%)
Laboratory findings	
Hemoglobin (g/dL)	12.5±2.4
WBC (/mm ³)	7,8367.2±3,3336.4
CEA (ng/ml)	108.1±366.9
BMI (kg/m ²)	23.1±3.4
Tumor size (mm)	46.1±23.3
Tumor location	
Cecum	34 (3.6%)
Ascending colon	129 (13.5%)
Hepatic flexure	42 (4.4%)
Transverse colon	47 (4.9%)
Sigmoid flexure	6 (0.6)
Descending colon	30 (3.1%)
Sigmoid-descending colon	15 (1.6%)
Sigmoid colon	260 (27.2%)
Recto-sigmoid colon	195 (20.4%)
Rectum	198 (20.7%)
Histology	
WD	78 (8.2%)
MD	815 (85.3%)
PD	48 (5.0%)
MC	10 (1.0%)
SRC	5 (0.5%)
Molecular features	
MSS	773 (80.9%)
MSI-L	63 (6.6%)
MSI-H	65 (6.8%)
NA	55 (5.8%)
TNM stage	
Stage 0	16 (1.7%)
Stage 1	209 (21.9%)
Stage 2	292 (30.5%)
Stage 3	337 (25.3%)
Stage 4	102 (10.7%)

Continuous variables are expressed as mean with standard deviation (SD), whereas categorical variables are shown as absolute numbers and percentage

BMI: Body mass index; CEA, Carcino-embryonic antigen; MC, Mucinous carcinoma; MD, Moderately differentiated carcinoma; MSI-H, Microsatellite instability-high; MSI-L, Microsatellite instability-low; MSS, Microsatellite stable; NA, Not applicable; PD, Poorly differentiated carcinoma; SRC, signet ring cell carcinoma; TNM stage: Tumor,

Node, Metastasis staging system; WBC: White blood cell; WD, well differentiated carcinoma

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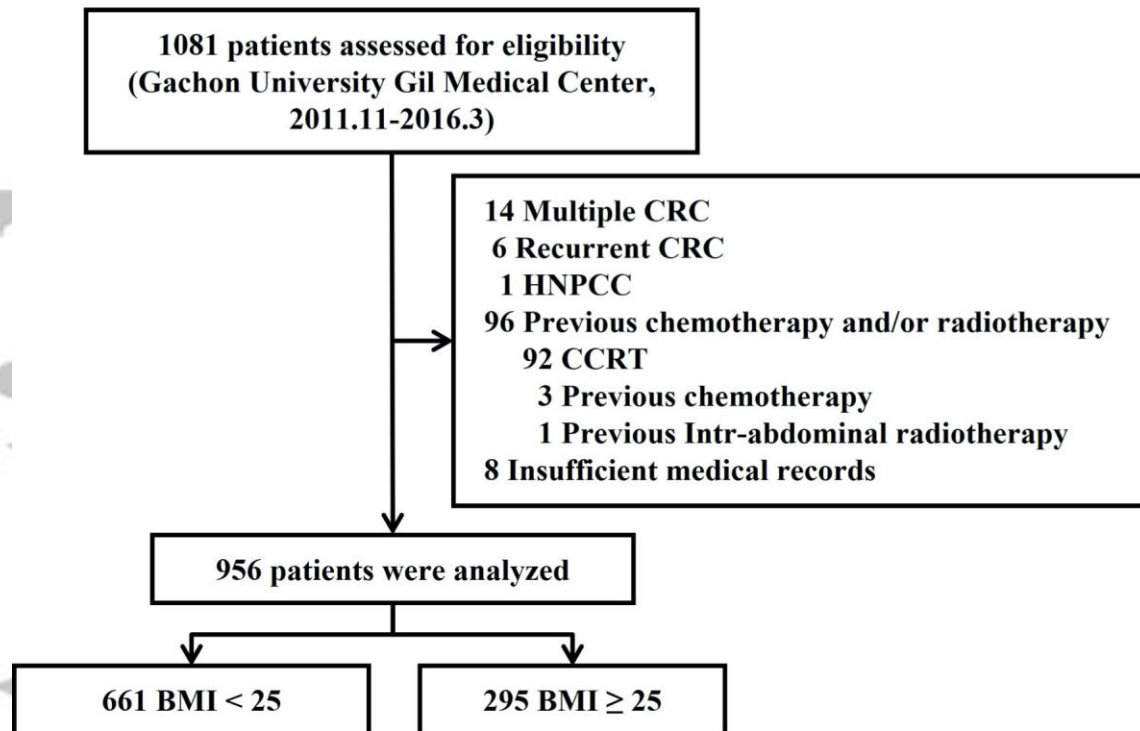


Figure 1. Inclusion criteria for study subjects. BMI; body mass index, CRC; colorectal cancer, HNPCC; Hereditary nonpolyposis colorectal cancer.

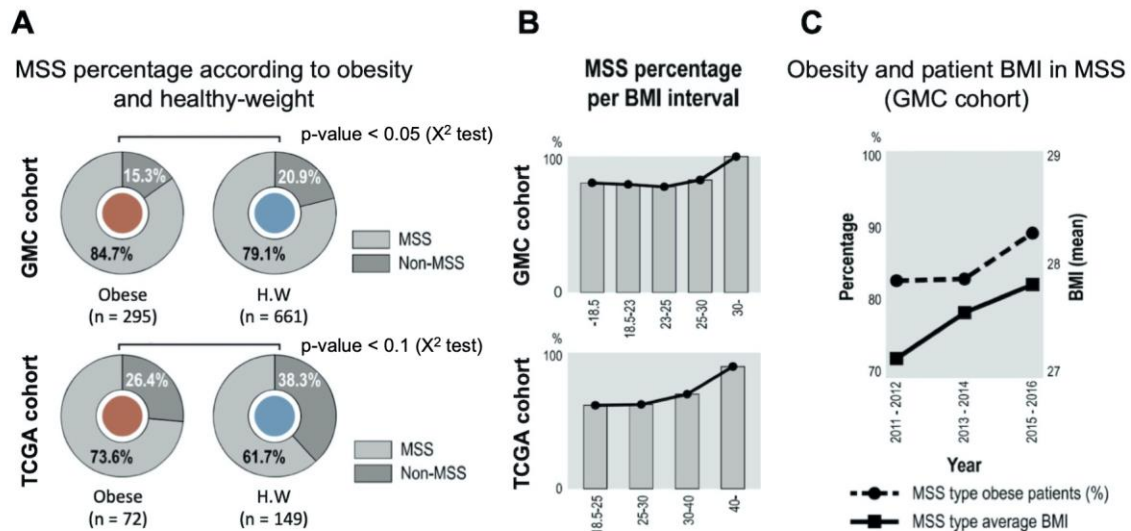


Figure 2. Increasing cases of microsatellite stable (MSS)-colorectal cancer (CRC) patients. The two cohorts (TCGA and our GMC cohorts) revealed that obesity was increasing among patients with colorectal cancer (CRC). A: Our GMC and TCGA cohorts show the MSS type is more common among obese CRC patients. B: MSS-CRC patients have tended to show a greater increase in BMI. C: In our GMC cohort, MSS-CRC patients have shown an increasing average BMI.

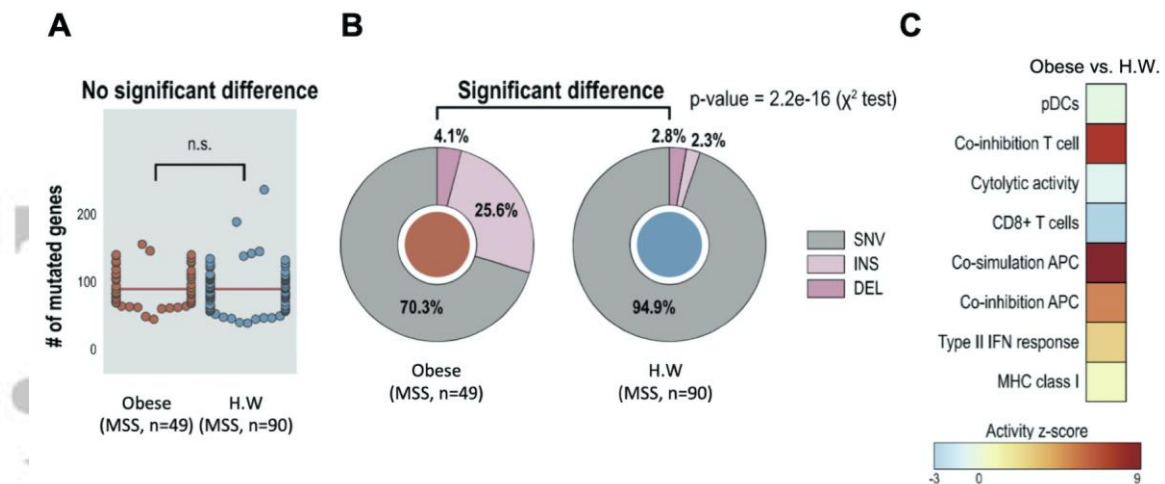


Figure 3. MSS-CRC patients showed different mutation types according to body weight status. A: No significant difference was found in the numbers of mutations between obese and healthy weight (H.W.) MSS-CRC patients. B: However, significant differences were observed in the mutation types. In The Cancer Genome Atlas (TCGA) cohort, insertions (INS) and deletions (DEL) were more common in obese patients than in HW patients. C: Cytolytic activity and CD8+ T cells signatures were suppressed obese group versus H.W. group.

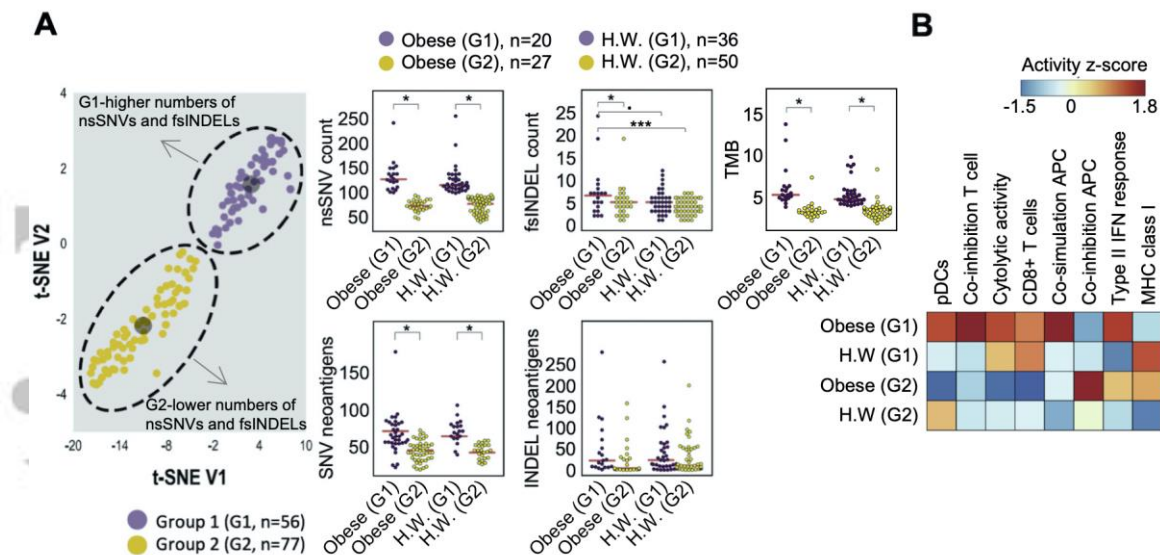


Figure 4. Obese MSS-CRC patients having large numbers of nsSNV and fs INDELs showed different immune signatures A: Two groups (G1 and G2) were identified based on the numbers of nonsynonymous single nucleotide variants (nsSNVs) and frameshift insertions and deletions (fs INDELs) in MSS-CRC patients. Obese patients with MSS-CRC in G1 (i.e. obese [G1]) showed having higher numbers of nsSNVs, fs INDELs, tumor mutational burden (TMB), and SNV neoantigens. B: Obese (G1) exhibited upregulated immune signatures, especially cytolytic activity and CD8+ T cells. Obese (G1) [obese patients in G1]; Obese (G2) [obese patients in G2]; H.W. (G1) [healthy weight patients in G1]; and H.W. (G2) [healthy weight patients in G2]. ∴ p-value < 0.1, *: p-value < 0.05.

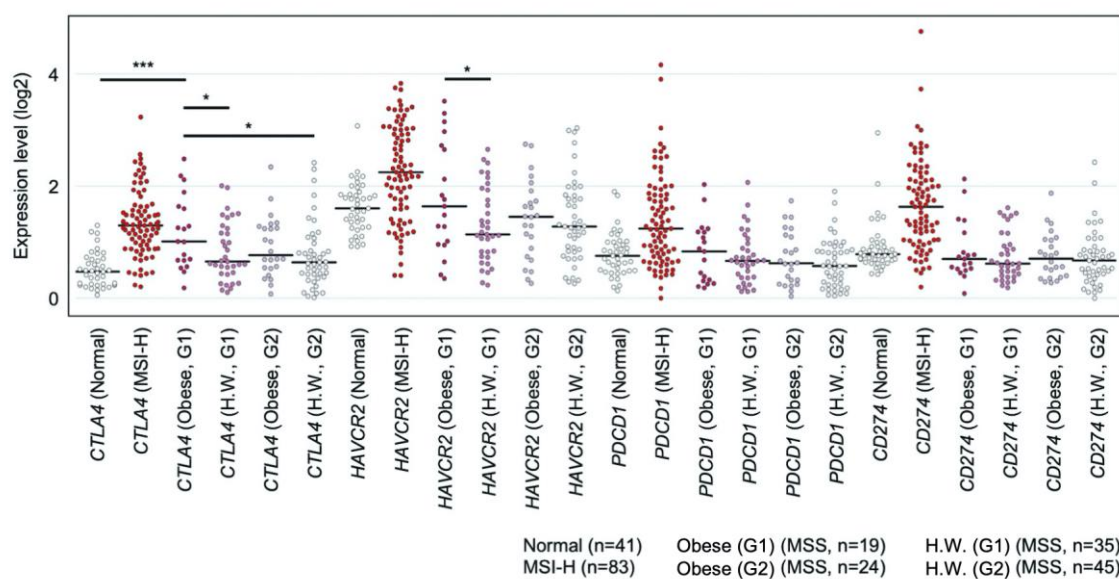


Figure 5. Obese MSS-CRC patients having large numbers of nsSNVs and fs INDELs exhibited enriched immune checkpoint expression patterns. Obese (G1) exhibited upregulated immune checkpoint genes (*CTLA4* and *HAVCR2*). *: p-value < 0.05, **: p-value < 0.01, and ***: p-value < 0.001.