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# MS4A1 expression and function in T cells in the colorectal cancer tumor microenvironment

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## Abstract

The majority of human colorectal cancer remains resistant to immune checkpoint inhibitor (ICI) immunotherapy, but the underlying mechanism is incompletely understood. We report here that *MS4A1*, the gene encoding B cell surface marker CD20, is significantly downregulated in human colorectal carcinoma. Furthermore, *MS4A1* expression level in colorectal carcinoma is positively correlated with patient survival. Analysis of scRNA-Seq dataset from public database revealed that MS4A1 is also expressed in subsets of T cells. A $CD8^+CD20^+$ subset of T cells exists in the neighboring non-neoplastic colon but disappears in tumor in human colorectal carcinoma. Furthermore, analysis of a published nivolumab treatment dataset indicated that nivolumab-bound T cells from human patients during anti-PD-1 immunotherapy exhibit significantly higher MS4A1 expression. Our findings indicate that $CD8^+CD20^+$ T subset functions in host cancer immunosurveillance and tumor microenvironment suppresses this T subset through a PD-L1-dependent mechanism.

### Keywords

MS4A1; CD20; T cells; colon carcinoma; PD-L1; Immune suppression; immune checkpoint

## Introduction

Under physiological conditions, immune checkpoint mechanisms prevent the activation of autoreactive T cells via receptor-ligand interactions. One well-characterized immune checkpoint is mediated between programed cell death protein 1 (PD-1) expressed on T cells and programed cell death protein ligand 1 (PD-L1) expressed on the surface of antigen

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presenting cells (APCs) [1, 2]. Under pathological conditions such as cancer, malignant cells can evade immunosurveillance by taking advantage of this mechanism by expressing PD-L1 [3]. The tumor expressed PD-L1 binds to PD-1 on T cells to inhibit activation of tumor infiltrating cytotoxic T lymphocytes (CTLs) in the tumor microenvironment, resulting in impaired host anticancer immune response and consequent tumor immune escape and progression. Based on this mechanism, PD-1 neutralizing monoclonal antibodies (mAb, e.g., pembrolizumab and nivolumab) have been developed for blocking PD-L1/PD-1 interaction to unleash tumor-suppressed T cells to kill tumor cells and approved for human cancer immunotherapy [4, 5]. PD-1 mAb based immune checkpoint inhibitor (ICI) immunotherapy represents a recent breakthrough in human cancer treatment and has achieved durable efficacy in many types of human cancers [6, 7]. However, human colorectal cancer stands out as one of the few human cancers that does not respond to ICI immunotherapy [8, 9]. Non-response to ICI immunotherapy is currently a significant challenge in human colorectal cancer treatment [5, 10, 11]. Currently, only the microsatellite unstable (MSI) subtype of colorectal cancer responds to anti-PD-1 immunotherapy [8, 9]. However, recent study determined that PD-L1 is expressed in human colon carcinoma and $CD8^+$ CTLs are present in both MSI and microsatellite stable (MSS) subtypes of colon carcinoma [12]. This combination of PD-L1 expression and CTL presence in the tumor microenvironment would suggest that CTL functional deficiency in the colorectal tumor microenvironment may underlie human colorectal cancer non-response to ICI immunotherapy.

The *MS4A1* gene encodes for the surface molecule CD20 which is widely considered a B cell lineage marker [13, 14]. CD20 associates with a wide variety of B cell surface molecules. including the B cell receptor, and is thought to function for B cell activation, proliferation, and differentiation [15-17]. Additionally, CD20 dysregulation and a $CD20^{-/-}$ genotype have been reported in patients with common variable immunodeficiency disorder [18, 19]. Furthermore, CD20 expression on tumor infiltrating lymphocytes provided a positive prognostic marker in ovarian cancer, while decreased MS4A1 expression was part of a novel blood-based biomarker panel developed for colorectal detection [20, 21]. We report here the identification of MS4A1 expression and function in T cells. We identified a $CD8^+CD20^+$ subset of CTLs in the colon carcinoma neighboring non-neoplastic colon epithelium and that this CTL subset is suppressed in colon carcinoma. We further determined that CD20 expression level is significantly correlated with colorectal cancer patient survival. Our data indicates that this $CD20^+$ CTL subset plays a key role in host cancer immunosurveillance and colorectal tumor cells may use suppression of $CD20^+$ CTLs as a mechanism to evade CTL-mediated cytotoxicity to advance the disease.

## Methods

### Human colorectal carcinoma specimens.

Human colon carcinoma tissues and matched adjacent non-neoplastic colon and rectal tissues were collected from consented patients at Augusta University Medical Center and provided by the Cooperative Human Tissue Network Southern Division (Duke University Medical Center). Five matched pairs of fixed human colon carcinoma tissues and adjacent non-neoplastic colon tissues from five colon cancer patients were used for

immunohistochemical analysis of CD20 (Table S1). Two pairs of surgically resected fresh colon carcinoma tissues and adjacent non-neoplastic colon tissues from two colon cancer patients were used for analysis of tumor infiltration T cells (Tabel S2). All studies with human specimens were performed according to protocol approved by Augusta University Institutional Review Board.

### Human colorectal cancer genomic database mining.

The Cancer Genome Atlas (TCGA) Colon Adenocarcinoma (COAD) datasets were retrieved from the TCGA database. High through-Seq (HTSeq)-Counts for the genes that encode cell surface receptors and ligands with known expressions in immune cells were downloaded using the UCSC Xena Genomics browser and sorted into normal and tumor sample sets based phenotypic characterization within the database [22]. Null values for HTSeq-Counts were removed from the datasets. Data was then graphed in R.4.0.2 using the ggplot2 package.

### Determination of gene expression and colorectal patient survival.

The gene expression level in tumor tissues and non-neoplastic colon tissues was run using OncoLnc [23]. Kaplan-Meier curves were plotted using TCGA COAD database data. High and low expression cohorts were determined using the median expression value and dividing the upper and lower $50^{th}$ percentiles for both MS4A1 and CD19 expression into two groups. Even when MS4A1 expression level percentiles were adjusted, the MS4A1 low expression cohort exhibited worse overall survival. When the CD19 expression level percentages were adjusted, there remained no statistically significant difference in survival between the CD19 high and low expression cohorts.

### Immunohistochemistry.

Immunohistochemistry was performed essentially as described previously [24]. Human tissue sections were probed with Anti-CD20 antibody (R and D System) overnight at 4°C, followed by incubation with HRP Universal Anti-Mouse/Anti-Rabbit IgG Antibody (Vector Lab, Burlingame, CA) according to the manufacturer's instructions. Patient clinical data is presented in Table S1.

### Analysis of MS4A1 expression profiles in B cells.

B cell specific expression data for MS4A1 was extracted from the GSE118254 genomic dataset within the Gene Expression Omnibus (GEO) genomic data repository [25, 26]. Datasets were screened, using the parameters "B cell", "Homo sapiens", and "expression profiling by high throughput sequencing", for expression data that identified B cell subtypes as a phenotypic characterization. RNAseq data from GEO entry GSE118254 was extracted and analyzed for expression of MS4A1 [27]. This dataset featured B cell expression data from patients with Systemic lupus erythematosus (SLE) and healthy control subjects. The dataset was downloaded and expression data from SLE patients was removed during analysis so that only healthy control samples remained. B cells were sorted into subtypes as previously characterized [27]. B cell subtype *MS4A1* expression was then graphed using R.4.0.2 and the ggplot2 package.

### MS4A1 expression profile analysis in human colorectal tumor-infiltrating immune cells.

Single Cell RNAseq data for MS4A1 was extracted from the GSE146671 genomic dataset within the GEO genomic data repository [25, 26]. scRNAseq data from GEO entry GSE146671 was extracted and analyzed for expression of MS4A1 [28]. The dataset contained scRNAseq data for tissue samples recovered from colorectal cancer patients. Cells were sorted based on cell phenotyping from the original study. Single-cell MS4A1 expression was then analyzed and graphed using R.4.0.2 and the ggplot2 package.

### Analysis of MS4A1 expression profile in T cells.

T cell specific single cell RNAseq expression data for MS4A1 was extracted from the GSE146671 genomic dataset found within the GEO genomic data repository [25, 26]. scRNA seq data from GEO entry GSE146671 was extracted and analyzed for expression of MS4A1 [28]. The dataset contained scRNAseq data for tissue samples recovered from colorectal cancer patients. T cells were sorted based on cell phenotyping from the original study. T cell subtype specific single-cell MS4A1 expression was then analyzed and graphed using R.4.0.2 and the ggplot2 package.

### Flow cytometry analysis of colon- and tumor-infiltrating CTLs.

Surgically dissected colon and rectal carcinoma tissues and the matched adjacent non-neoplastic colon tissues were digested in collagenase solution to make single cells as previously described [29, 30]. The cell mixtures were stained with CD8−, PD-1-, and CD20 (clone 2H7)-specific antibodies (Biolegend, San Diego, CA), and analyzed by flow cytometry. Live cells and $CD8^+$ cells were gated and analyzed for CD20 expressing cells. Patient clinical data is presented in Table S2.

### Analysis of MS4A1 expression in T cells and patient response to nivolumab immunotherapy.

T cell MS4A1 expression change data in response to anti-PD-1 immunotherapy (nivolumab) was extracted from the GSE100860 genomic dataset within the GEO genomic data repository [25, 26]. Datasets were screened, using the parameters “PD-1”, “Homo sapiens”, and “expression profiling by high throughput sequencing”, for T cell expression data in patients treated with anti-PD-1 ICI immunotherapy (mAbs such as pembrolizumab and nivolumab). RNA-Seq data from GEO entry GSE100860 was extracted and analyzed for expression of MS4A1 [31]. Expression data from nivolumab-bound and nivolumab-unbound peripheral blood T cells of patients with non-small cell lung cancer who were treated with nivolumab was analyzed and graphed using R.4.0.2 and the ggplot2 package. Additionally, the difference in MS4A1 expression in nivolumab-unbound and nivolumab-bound cells for each individual patient was analyzed and graphed using R.4.0.2 and the ggplot2 package.

## Result

### MS4A1 is downregulated in human colorectal carcinoma.

Cell surface receptors and ligands play an essential role in regulation of T cell activation and function in the context of host cancer immunosurveillance. To identify new T cell surface

markers with a function in CTL tumor suppression function, we took a genomic approach and screen the GDC COAD original data in the TCGA database for genes with known expression as cell surface receptor or ligand in immune cells. We analyzed the differential expression of the known immune cell surface markers between colorectal carcinoma and the adjacent non-neoplastic colon tissues. The rationale is that the tumor microenvironment should induce repression of immune cell surface makers that function in immune cell activation and antitumor immune response. One of these identified genes is the B cell marker *MS4A1* that was found to be significantly downregulated in colorectal carcinoma as compared to the adjacent non-neoplastic colon (Fig. 1A). *MS4A1* encodes for the surface molecule CD20, which is thought to play a role in a variety of immune functions [32], so we hypothesized that this loss of MS4A1 expression would correlated with poorer outcomes in colorectal cancer patients. Kaplan Meier survival analysis of data from the COAD database comparing MS4A1 expression level in patients with colorectal cancer revealed that decreased expression of MS4A1 in patient tumor samples is significantly correlated with decreased patient survival (Fig. 1B). Since *MS4A1* is a marker of B cells, we examined the difference in expression levels of CD19, an additional B cell marker, between colorectal carcinoma and adjacent non-neoplastic colon and found CD19 to be significantly downregulated in colorectal carcinoma (Fig. 1C). However, Kaplan Meier survival analysis of data from the COAD database comparing CD19 expression level in patients with colorectal cancer revealed that there was not a statistically significant difference in survival between high and low CD19 expression in patient tumor samples (Fig. 1D).

### MS4A1 expression profiles in human colon carcinoma and non-neoplastic colon.

We next used fixed surgically resected human colon carcinoma and matched adjacent non-neoplastic colon tissues from five colon cancer patients (Table S1) to analyzed the level of CD20, the surface protein that MS4A1 encodes for. Immunohistochemical analysis indicate that $CD20^+$ cells are leukocytes (Fig. 2). $CD20^+$ cells are primarily localized in cells within the tertiary lymphoid structures in four of the five patient colon tissues (Fig. 2). There were dramatically less $CD20^+$ cells in the colon carcinoma tissues and these CD20+ cells are also form tertiary lymphoid structures in the tumor (Fig. 2).

### MS4A1 is highly expressed in B cells in human colorectal cancer patients.

MS4A1 is a B cell marker [33-35]. To gain a general understanding of MS4A1 expression profile under pathological conditions, we sought to determine MS4A1 expression profiles in B cells in healthy donors and colorectal cancer patients. We first searched the GEO genomic data repository for B cell MS4A1 expression data and identified a dataset containing B cell subtype expression data from peripheral blood of healthy donors [27]. As expected, MS4A1 was abundantly expressed on all B cell subtypes, including activated naïve, double negative, resting naïve, switched memory, and transitional 3 B cells (Fig. 3A). Additionally, CD20 is lost when B cells differentiate into antibody-producing plasma cells (Fig 3A).

We then extended our study to colorectal cancer patients and analyzed MS4A1 expression in B cells in human colorectal carcinoma. We identified a scRNA-Seq dataset from tissue samples of colorectal cancer patients [28]. Analysis of MS4A1 expression level at the single B cell level indicates that MS4A1 is indeed abundantly expressed in B cells in human

colorectal carcinoma tissue and circulating peripheral blood (Fig. 3B). We then analyzed the expression of MS4A1 in tumor-infiltrating B cells in the single cell level and observed that MS4A1 is also abundantly expressed in tumor-infiltrating B cells (Fig. 3C). Further analysis of scRNA-Seq data determined that MS4A1 is expressed not only in the tumor-infiltrating B cells but also in B cells of peripheral blood and the adjacent non-neoplastic colon (Fig. 3D). Surprisingly, MS4A1 expression levels in tumor-infiltrating B cells is significantly higher than MS4A1 expression level in B cells in the adjacent non-neoplastic colon (Fig. 3D). These findings indicate that MS4A1 is expressed in B cells, but MS4A1 expression level in B cells is not suppressed in colorectal carcinoma, suggesting that B cell-expressed MS4A1 may not contribute the decreased MS4A1 expression in colorectal carcinoma and $CD20^+$ B cells unlikely plays a role in colorectal cancer patient survival.

### MS4A1 is expressed in T cells in human colorectal carcinoma.

We then analyzed MS4A1 expression in T cells. scRNA-Seq data analysis indicates that MS4A1 is expressed in both $CD4^+$ and $CD8^+$ T cells in the peripheral blood (Fig. 3B) and tumor (Fig. 3C), albeit at a lower level than that in B cells (Fig. 3B & C). To further determine the expression profiles of MS4A1 in T cells, we analyzed scRNAseq data of T cell subsets from the previously mentioned dataset containing single cell data from tissue recovered from colorectal carcinoma patients [28]. We extracted $CD4^+$ and $CD8^+$ T cell subtype specific expression data and analyzed MS4A1 expression. Specific subtypes of T cells exhibited statistically significant differences in MS4A1 expression. In the $CD4^+$ T cell group, CD4-GZMK T cells exhibited statistically significantly higher MS4A1 expression levels while CD4-CXCR5, CD4-IL23R, CD4-CXCL13, and CD4-CTLA4 T cells exhibited statistically significantly lower MS4A1 expression levels (Fig. 4A). In the $CD8^+$ T cell group, CD8-GZMK T cells exhibited statistically significantly higher MS4A1 expression levels while CD8-LEF1, CD8-CD6, CD8-CD160, and CD8-LAYN T cells exhibited statistically significantly lower MS4A1 expression levels (Fig. 4B). Of note, the $CD20^+$ T cell subset comprises a small population of cells explaining the diminished sensitivity of scRNA-Seq analysis for low expression genes.

### Tumor-infiltrating CTLs lose MS4A1 in human colorectal carcinoma.

Cytotoxic T lymphocytes are the primary immune cells that function in host cancer immune surveillance to suppress tumor development [36, 37]. Our data indicate that MS4A1 expression level is down-regulated in human colorectal carcinoma as compared to the adjacent non-neoplastic colon and that MS4A1 expression level in colorectal carcinoma is positively correlated with patient survival (Fig. 1). We therefore hypothesized that MS4A1 expression is down-regulated in CTLs in the tumor microenvironment. To test this hypothesis, we performed flow cytometry analysis of human colon carcinoma and adjacent non-neoplastic colon. The rationale is that if $CD20^+$ CTLs function to suppress tumor growth in the colon microenvironment, then CD20 should be expressed on CTLs in the neighboring normal colon epithelium but repressed in the tumor-infiltrating CTLs. Flow cytometry analysis of the adjacent normal colon and colon tumor revealed that indeed there exists a $CD8^+CD20^+$ CTL subpopulation in the adjacent non-neoplastic colon, but this subpopulation disappears in the colon carcinoma (Fig. 5A & B).

### Anti-PD-1 immunotherapy increases MS4A1 expression in T cells in human cancer patients

Our above findings suggest that a $CD8^+CD20^+$ subset of CTLs may function as a tumor-suppressive CTLs and colorectal carcinoma may use suppressive mechanisms such as PD-L1 to suppress this CTL subset as a mechanism to escape from host cancer immunosurveillance and to confer resistant to ICI immunotherapy. To test this hypothesis, we sought to determine the effect that anti-PD-1 immunotherapy on MS4A1 expression in T cells. The rationale is that if MS4A1 expression in T cells is repressed by tumors, then T cells from patients treated with anti-PD-1 mAb should have higher MS4A1 expression level if it is bound by the antibody. We identified a original dataset in GEO database from a study on the expression changes with nivolumab binding in T cell of non-small cell lung cancer patients [31]. We analyzed MS4A1 expression in patient peripheral blood T cells that were unbound and bound by nivolumab. T cells that were nivolumab-bound had significantly higher expression levels of MS4A1 as compared to unbound T cells (Fig. 6A). Additionally, when dividing the data based on samples recovered from each individual patient, nivolumab-bound T cells exhibited higher expression levels of MS4A1 as compared to nivolumab-unbound T cells (Fig. 6B). We therefore conclude that tumor cell-expressed PD-L1 engaged CTL-expressed PD-1 to repress MS4A1 expression to suppress the $CD8^+CD20^+$ CTL subset in the tumor microenvironment in human cancer patients.

## Discussion

$CD8^+$ CTLs provide essential antitumor functionality [37]. Due to this function, CTL infiltration into the tumor of colorectal tumors has been evaluated as a prognostic indicator for patient survival and tumor progression [38, 39]. Comparison of metastatic and non-metastatic colorectal cancer found that immune cell infiltrates, T cell marker mRNA, and $CD8^+$ CTLs were increased in non-metastatic tumors indicating that CTL infiltration into the colorectal tumor microenvironment prevents tumor progression and metastasis [39]. Additional studies found that CTL infiltration into human colorectal tumors was directly correlated with more favorable survival, highlighting the essential function CTLs provide to suppress tumor development [38, 40]. Human colorectal cancer is therefore a type of highly immunogenic cancer [10].

However, colon tumorigenesis is not naturally suppressed in humans and human colorectal cancer does not respond to anti-PD-1 immunotherapy[9, 10]. One notion underlying human colorectal carcinoma immune evasion is that tumor-reactive CTLs are immunologically suppressed in the tumor microenvironment [11, 41]. Myeloid cell such as myeloid-derived suppressor cells (MDSCs) are abundant in colon carcinoma [11, 30, 41]. In addition to their potent immune suppressive activity [42], emerging experimental data indicates that myeloid cells use ROS to induce colon epithelial cell mutagenesis[43]. These somatic mutations in colon epithelial cells may drive colon tumorigenesis in the absence of a carcinogen challenge in a paracrine manner [43]. At the same time, these somatic mutations may also serve as neoantigens to activate tumor-reactive CTLs [44-47]. Consistent with this phenomenon, it was recently reported that colorectal tumor adjacent non-dysplastic mucosa has considerable mutation burden, including mutations shared with the neighboring

colorectal carcinoma, indicating a precancer mutational field in both the colorectal carcinoma and the adjacent “normal” epithelial cells [44]. Therefore, it is reasonable to assume that tumor-reactive CTLs are present in the neighboring non-neoplastic colon epithelium, but these CTLs are suppressed in the tumor microenvironment. In this study, we identified MS4A1/CD20 as a gene whose expression is significantly down-regulated in human colorectal carcinoma as compared to the adjacent non-neoplastic colon. We further determined that MS4A1 expression level in significantly correlated with increased colorectal cancer patient survival. We further extended MS4A1 expression to T cells and identified a $CD8^+CD20^+$ CTL subset that is present in the neighboring non-neoplastic colon, but not in the human colon carcinoma.

While CD20 is primarily considered a B cell marker, there is growing evidence that MS4A1/CD20 is also expressed in T cells [48, 49]. $CD20^+$ T cells are found in higher levels in the peripheral blood of patients with rheumatoid arthritis and are linked to rheumatoid arthritis pathogenesis [50, 51]. $CD3^+CD20^{dim}$ T cells are abundant in human patients with multiple sclerosis, and the efficacy of Rituximab and anti-CD20 mAb, in patients is linked to depletion of this $CD3^+CD20^{dim}$ T cell subset [52]. The pathological relevance of this $CD3^+CD20^{dim}$ T cell subset in multiple sclerosis remains to be determined. However, given their potential proinflammatory functionality, depletion of CD20-expressing T cells may also contribute to the therapeutic effect of Rituximab and CD20-neutralizing mAb. These observations revealed a function of $CD20^+$ T cells in proinflammatory response. Anti-PD-1 immunotherapy acts through blocking PD-L1 and PD-1 interactions to activate tumor-reactive CTLs in the tumor microenvironment. We observed in this study that anti-PD-1 antibody-bound T cells express higher level of CD20 as compared to unbound T cells, suggesting that $CD8^+CD20^+$ CTL subset is a target of PD-L1-dependent immune suppression in the human colorectal tumor microenvironment. Based on these findings, we propose that $CD8^+CD20^+$ CTL subset play an important role in cancer immunosurveillance in the colon epithelium to prevent colon tumorigenesis and progression. Human colorectal tumor cells use PD-L1-dependent mechanism to directly suppress this CTL subset. Absence of this $CD8^+CD20^+$ CTL subset in the tumor microenvironment enables colorectal tumor immune evasion and confers colorectal cancer non-response to anti-PD-1 immunotherapy. Reactivating this $CD8^+CD20^+$ CTL subset in the colorectal tumor microenvironment therefore is potentially an effective approach to suppress human colorectal cancer immune escape and to overcome human colorectal cancer non-response to ICI immunotherapy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Highlights

- MS4A1 expression is positively correlated with colorectal cancer patient survival.
- Subsets of T cells express MS4A1 in humans.
- $CD8^+CD20^+$ T cell level is diminished in human colon carcinoma.
- PD-L1 suppresses MS4A1 expression in T cells in human cancer patients.

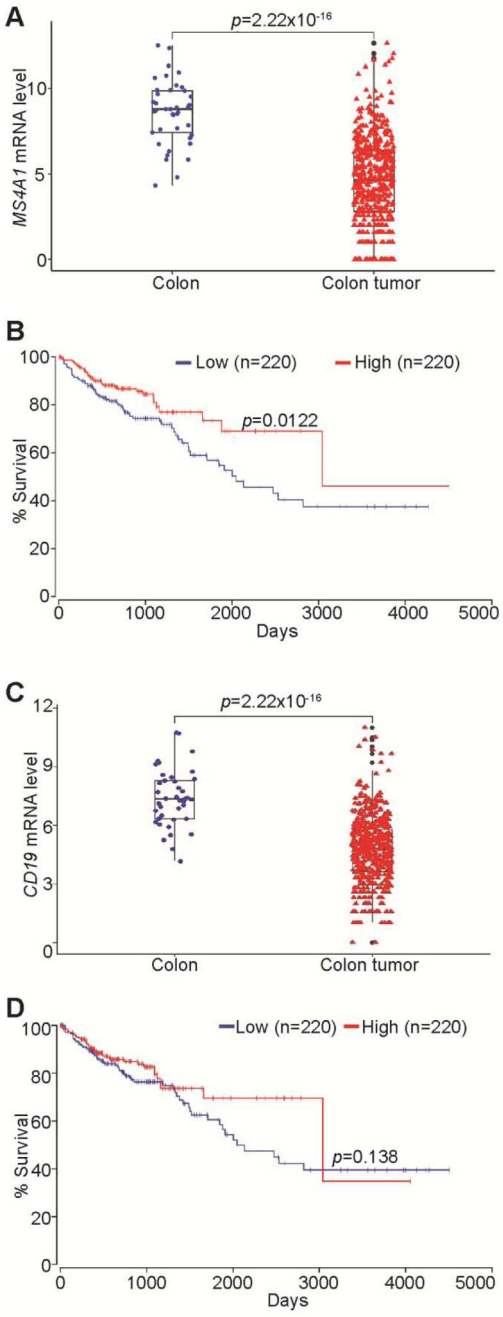


**Figure 1: MS4A1 expression level in colorectal carcinoma is correlated with patient survival.**

**A**. MS4A1 RNA-seq data was extracted from the GDC COAD dataset in TCGA database and analyzed for MS4A1 mRNA level in human colorectal carcinoma (n=384) and the adjacent non-neoplastic colon (n=51). Samples with null values for MS4A1 expression were removed. Each dot represents RNA-seq data from an individual patient. **B.** MS4A1 mRNA levels from colon adenocarcinoma were extracted from the COAD dataset in the TCGA database and analyzed using Kaplan-Meier survival analysis. The groups were divided based on a 50$^{th}$ percentile of high and low expression of MS4A1. **C**. CD19 RNA-seq data was extracted from the GDC COAD dataset in TCGA database and analyzed for CD19 mRNA

level in human colorectal carcinoma (n=471) and the adjacent non-neoplastic colon (n=41). Samples with null values for CD19 expression were removed. Each dot represents RNA-seq data from an individual patient. **D.** CD19 mRNA levels from colon adenocarcinoma were extracted from the COAD dataset in the TCGA database and analyzed using Kaplan-Meier survival analysis. The groups were divided based on a $50^{th}$ percentile of high and low expression of CD19.

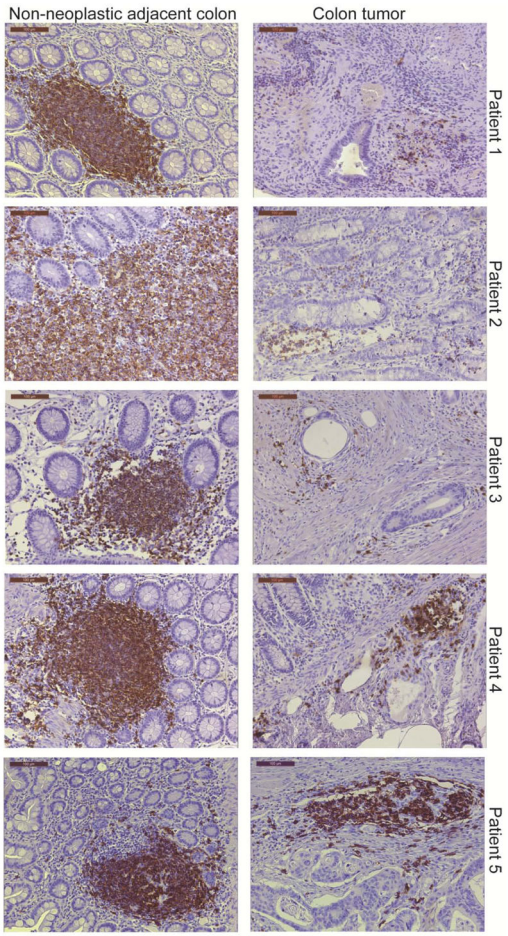


**Figure 2. CD20 expression in human colon cancer.**

Matched pairs of colon carcinoma tissues and adjacent non-neoplastic colon were stained with CD20-specific antibody. Shown are representative images of tissue and tumor-infiltrating $CD20^+$ cells. Scale bar = 100 μm.

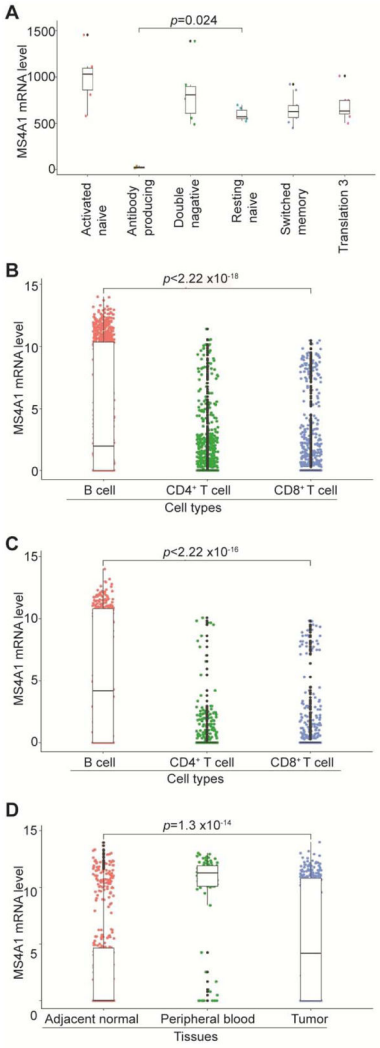


**Figure 3: MS4A1 is expressed in B-cells and T-cell subtypes.**

**A.** A dataset from RNA-Seq data of B cell subsets isolated from healthy human donors as indicated was extracted from GEO database and analyzed for MS4A1 expression level. Each dot represents an individual patient sample. MS4A1 is expressed across B cell types, and MS4A1 expression is lost upon differentiation of B cells into antibody producing plasma cells. **B-D.** A dataset from sc-RNA-Seq data of T and B cells isolated from colorectal cancer patient tissue samples was extracted from GEO database and analyzed for MS4A1 expression level. Each dot represents a single cell. **B.** sc-RNA-Seq analysis of MS4A1 expression in CRC patient immune cells. Cell samples were recovered from tumor, adjacent normal, and peripheral blood tissue sites and cells from all three sites were grouped for analysis. **C.** MS4A1 expression level in CRC patient immune cells recovered from the tumor site. Immune cells from adjacent normal and peripheral blood tissue sites in panel B were removed for analysis of MS4A1 expression in TILs. **D.** MS4A1 expression level in different B cell populations in CRC patients is shown.

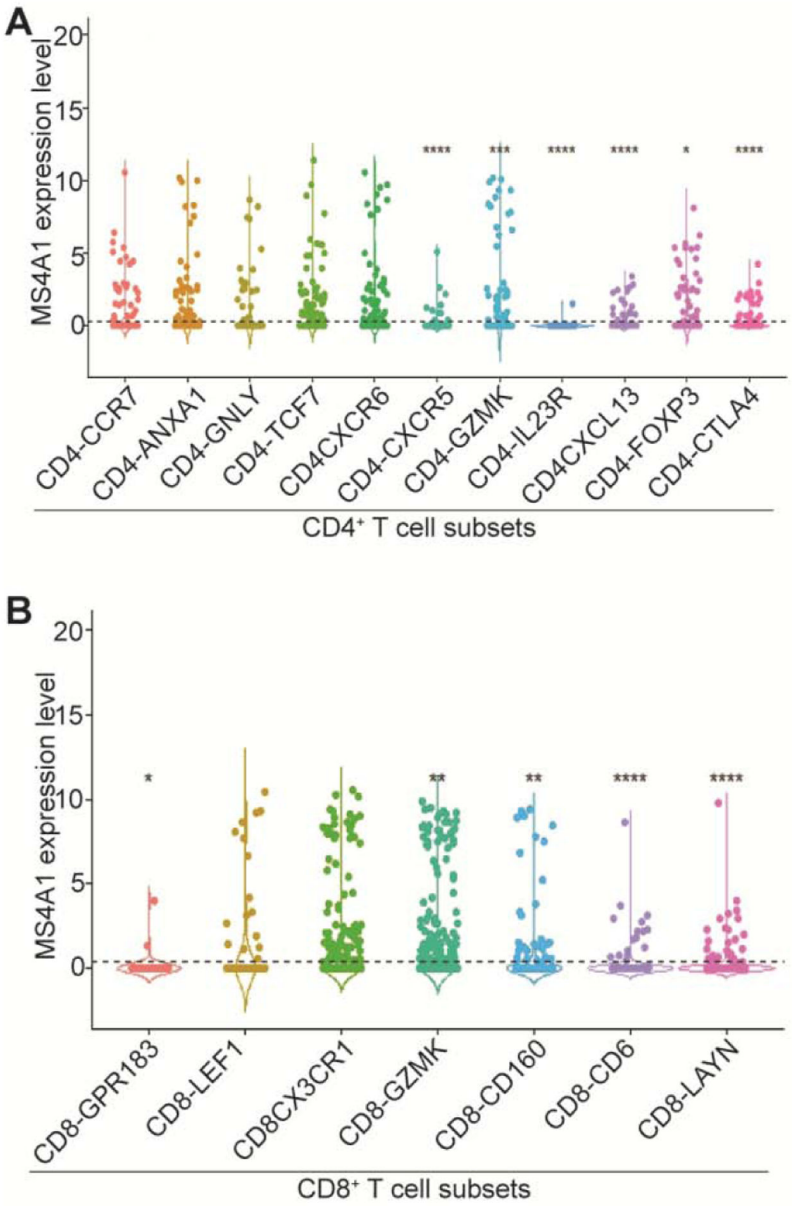


**Figure 4. RNA-Seq analysis of MS4A1 expression levels in human T cell subsets.**

**A**. A dataset from scRNAseq data of T cell subsets isolated from colorectal cancer patients was extracted from GEO database and analyzed for MS4A1 expression. Each dot represents a single cell. **A**. MS4A1 expression in $CD4^+$ T cell subsets. **B.** MS4A1 expression in $CD8^+$ T cell subsets.

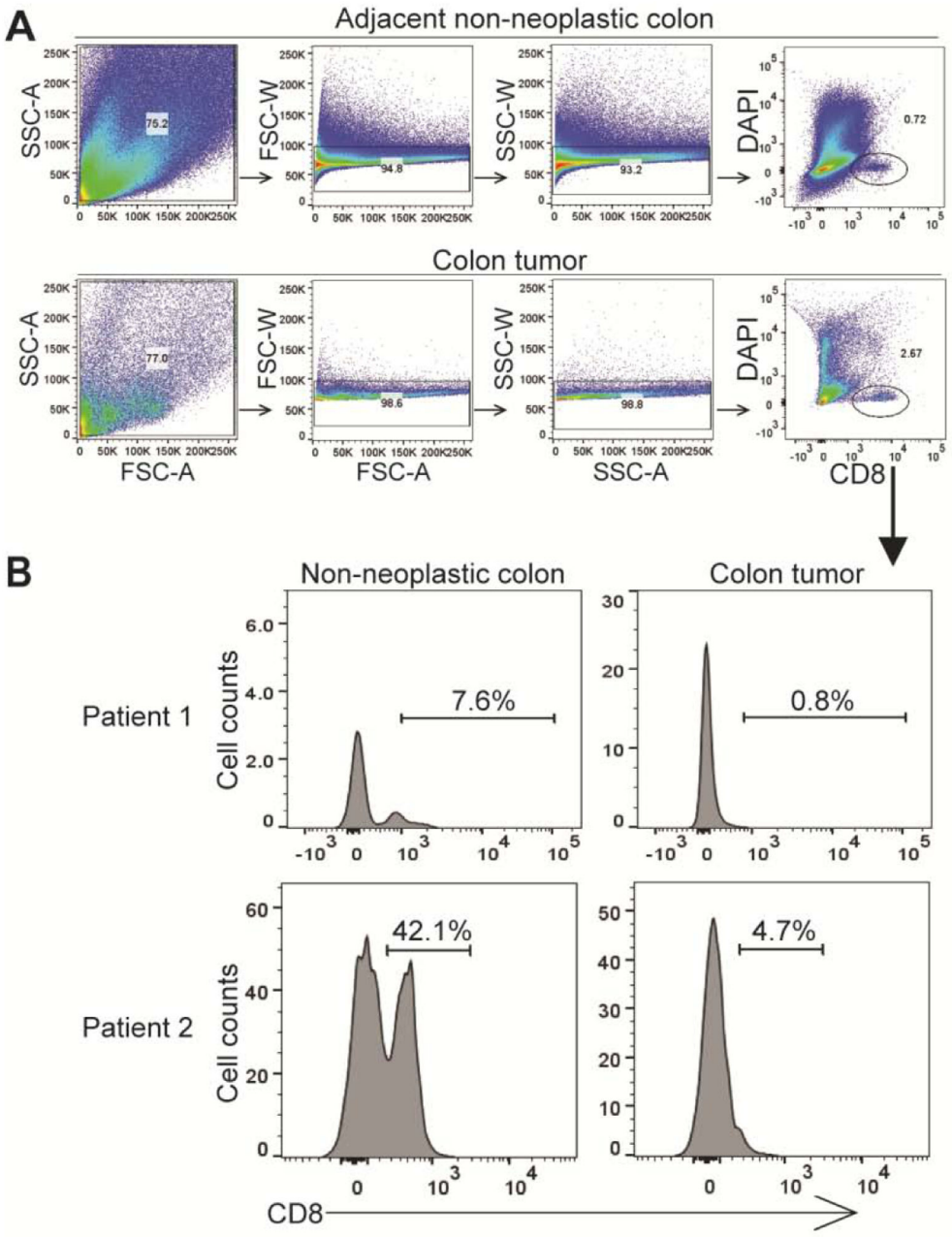


**Figure 5. CD8$^+$CD20$^+$ CTL subset profiles in human colon cancer patients.**

**A**. Surgically dissected colon tumor tissues and matched adjacent non-neoplastic colon tissues were digested with collagenase. The tissue digests were stained with CD8−, and CD20-specific antibodies. The live CD8+ cells were gated and analyzed for CD20 protein level. Shown is the gating strategy of one pair of normal and tumor tissues. **B**. CD8$^+$CD20$^+$ CTL subset in matched pairs of human colon tumor and adjacent non-neoplastic colon from two colon cancer patients.

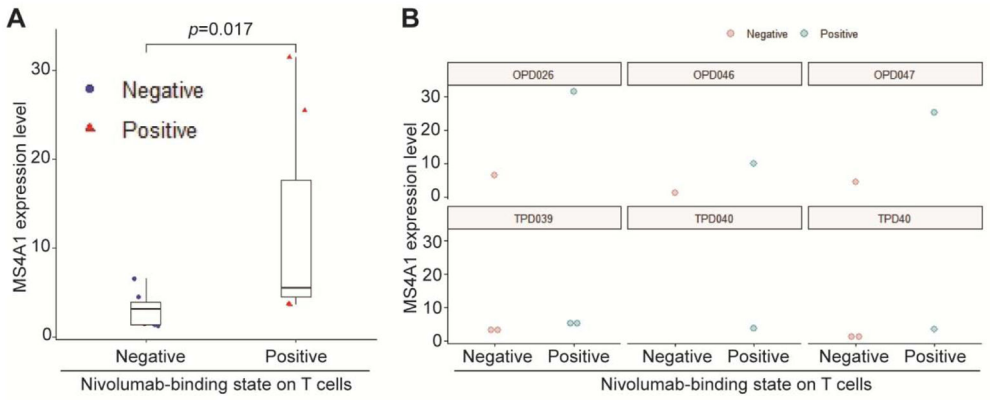


**Figure 6. Blocking PD-1 on T cells increases MS4A1 expression in T cells from human cancer patients.**

MS4A1 expression data was extracted from a dataset from the GEO database of T cells treated with Nivolumab (anti-PD-1 mAb). Each dot represents an individual patient sample. Cells were classified into negative and positive groups based on the presence of Nivolumab binding. **A.** T cells bound by nivolumab had significantly increased expression of MS4A1. **B.** In T cell samples recovered from each individual patient, cells bound by nivolumab exhibited elevated MS4A1 expression.