Activation of MAT2A-RIP1 signaling axis reprograms monocytes in gastric cancer

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ABSTRACT

Background The activation of tumor-associated macrophages (TAMs) facilitates the progression of gastric cancer (GC). Cell metabolism reprogramming has been shown to play a vital role in the polarization of TAMs. However, the role of methionine metabolism in function of TAMs remains to be explored.

Methods Monocytes/macrophages were isolated from peripheral blood, tumor tissues or normal tissues from healthy donors or patients with GC. The role of methionine metabolism in the activation of TAMs was evaluated with both in vivo analyses and in vitro experiments. Pharmacological inhibition of the methionine cycle and modulation of key metabolic genes was employed, where molecular and biological analyses were performed.

Results TAMs have increased methionine cycle activity that are mainly attributed to elevated methionine adenosyltransferase II alpha (MAT2A) levels. MAT2A modulates the activation and maintenance of the phenotype of TAMs and mediates the upregulation of RIP1 by increasing the histone H3K4 methylation (H3K4me3) at its promoter regions.

Conclusions Our data cast light on a novel mechanism by which methionine metabolism regulates the anti-inflammatory functions of monocytes in GC. MAT2A might be a potential therapeutic target for cancer cells as well as TAMs in GC.

INTRODUCTION

Gastric cancer (GC) is the fourth most frequent malignancy and the third leading cause of cancer-related mortality around the world. Despite the great advances in GC screening and therapy, the prognosis of patients with GC at advanced stages remains to be dismal. The advent of immunotherapy has greatly changed the landscape of cancer therapy. Cancer immunotherapies, including immune checkpoint inhibitors and adoptive cell transfer, have demonstrated therapeutic benefits in a variety of tumors. However, the proportion of patients who respond to immunotherapy remains modest (about 15%) because of resistance and tumor immune evasion. An ideal immunotherapy for GC would be relieving immunosuppression through remodeling the tumor microenvironment (TME). Thus, further research on the molecular mechanisms underlying tumor immune evasion is required.

Monocytes/macrophages constitute the major component of tumor-infiltrating immune cell population. Monocytes/macrophage are commonly educated by the TME and promote tumor progression via diversified mechanisms, including immune evasion. Previous studies have demonstrated that GC is highly infiltrated with monocytes with activated phenotype (mainly M2 subtype). The functional phenotype of monocytes/macrophage can be influenced by surrounding milieu. However, the underlying molecular mechanisms are largely unclear.

A growing volume of literature have demonstrated the important role of cell metabolism in the activity and function of immune cells, including macrophage, and dendritic cells. Two main subtypes of macrophages have been defined with distinct metabolic profiles. The anti-inflammatory M2 macrophage is characterized by increased oxidative phosphorylation and fatty acid oxidation, while the classical proinflammatory M1 macrophage is characterized by enhanced glycolysis. These metabolic characteristics have been shown to be vital in sustaining the functional phenotypes of macrophages; however, the precise molecular mechanism remains to be explored.

Cell metabolism provides energy and material basis for cellular signaling transduction. Methionine, an essential amino acid, has been shown to play a vital role in the maintenance of stemness of embryonic and pluripotent stem cells as well as carcinogenesis. S-adenosylmethionine (SAM) is generated from methionine by a family of conserved methionine adenosyltransferase (MAT) enzymes. The catalytic subunits of MAT enzymes are...
derived from two genes, the ubiquitously expressed methionine adenosyltransferase II alpha (MAT2A) genes and the liver-specific MAT1A. There has been an established link between methionine adenosyltransferases and the progression of cancer, including GC. However, whether MAT enzymes are also involved in tumor-associated macrophage (TAM) polarization remains unknown. Interestingly, Covarrubias et al performed unbiased metabolic profiling of M2 macrophages and found that methionine metabolism was among the top enriched pathways. This bioinformatics analysis result makes us consider the effects of methionine metabolism on TAM. SAM is a major biological methyl donor, which is required for methylation. Recent studies have shown that epigenetic modifications, including acetylation and methylation modulate the polarization and activation of macrophages. While much attention has been paid to the effect on glycolysis and lipid metabolism in the macrophage, little is known about the role of its methionine metabolism.

In this study, we have found that the upregulated MAT enzyme MAT2A regulates the cellular SAM levels and histone methylation pattern in monocytes/macrophages. MAT2A epigenetically induces RIP1 expression and a protumor phenotype in macrophages.

## MATERIALS AND METHODS

### Patients and clinical samples

The study was censored by the Clinical Research Committee of Yijishan Hospital, Wenzhou People’s Hospital and the Fudan University Shanghai Cancer Center. The GC tissues were confirmed pathologically. A cohort of patients involving 42 paired blood samples, GC tissues and normal tissues were obtained from Yijishan Hospital who underwent surgery in Yijishan Hospital between June 2019 and October 2019 (n=32) and October 3, 2020 and October 17, 2020 (n=4 from Yijishan Hospital, n=6 from Wenzhou People’s Hospital). These samples were used for the purification of peripheral blood-infiltrating and tumor-infiltrating leukocytes. Written consent was obtained from all patients. All of these patients were preoperative chemotherapy or radiotherapy naive. No data that could identify patients were provided. Blood samples were collected from 31 healthy donors who worked at Yijishan Hospital. Heparin tubes (BD Biosciences, San Jose, California, USA) were used for the collection of blood samples, and all blood-related experiments were carried out with fresh cells. The clinicopathological characteristics of the patients with GC in this study are provided in online supplemental table S1.

### Isolation of monocytes from peripheral blood and tissue samples

Leukocytes were isolated from the peripheral blood using Ficoll density gradient centrifugation assay (AS1114547; Axis-Shield) as previously described. GC tissues or paired normal tissues were cut into small pieces and digested in RPMI 1640 (HyClone, Invitrogen, Camarillo, California, USA) supplemented with 20% fetal bovine serum (FBS, HyClone, Invitrogen, Camarillo, California, USA), 0.05% collagenase IV (Sigma, St. Louis, Missouri, USA) and 0.005% DNase I (Roche Diagnostics, Indianapolis, Indiana, USA) for 1 hour at 37°C. The dispersed monocytes were filtered through anylon mesh of 150 mm pore size and separated by Ficoll centrifugation. The obtained monocytes were washed with phosphate buffered solution (PBS) three times and resuspended in RPMI 1640 supplemented with 10% FBS. CD14+ monocytes and autologous T lymphocytes were isolated using magnetic beads (130-050-201/130-095-130, Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer’s instructions and further verified for CD14 expression using flow cytometry analysis with a purity of >88% CD14+.

### Preparation of methionine starvation medium

Methionine starvation media were generated from RPMI 1640 powder (US Biomedical) lacking methionine as described previously.

### Preparation of tumor culture supernatants (TSNs) from gastric cancer cells and tumor-conditioned monocytes

The origin and characterization (including DNA fingerprinting, cell vitality and mycoplasma detection) of human gastric cell lines (BGC823 and MGC803) used in this study were performed as described previously. The latest cell characterization was performed in September 2019. TSNs were prepared as described previously. 5×10^6 GC cells in 10 ml Dulbecco’s modified Eagle’s medium (DMEM, Gibco BRL, Grand Island, New York, USA), were seeded into 100 mm tissue culture dishes for 24 hours thereafter changing the medium to complete medium supplemented with 10% FBS (HyClone, Invitrogen, Camarillo, CA, USA).

The supernatant was then collected, centrifuged (10,000 g, 15 min) and stored in aliquots at −80°C.

### In vitro culture of monocytes

Purified CD14+ monocytes were cultured in DMEM supplemented with 10% FBS in the presence or absence of 30% TSN. In some cases, purified CD14+ monocytes were treated with medium or TSN in the presence or absence of FIDAS-5 (10 µM, Merck Millipore, USA), RIP1 inhibitor (GSK547, 50 nM, GlaxoSmithKline, Brentford, UK).

In other experiments, monocytes were pretreated with DMSO, 2-aminobicyclo-(2,2,1)-heptane-2-carbo xylic acid (BCH) (5 mM, Sigma, St. Louis, Missouri, USA), Trichostatin A (TSA) (200 ng/mL, Sigma, St. Louis, Missouri, USA), chaetocin (0.5 µM, Sigma, St. Louis, Missouri, USA), OICR-9429 (5 µM, Sigma, St. Louis, Missouri, USA), BIX-01294 (5 µM, Sigma, St. Louis, Missouri, USA), OGL-002 (50 µM, Sigma, St. Louis, Missouri, USA) and DZNep (1 µM, Sigma, St. Louis, Missouri, USA) for 1 hour before their exposure.
to medium or TSN for indicated times. In some cases, monocytes were transfected with desired siRNA before exposure to medium or TSN.

ELISA
The concentration of the cytokines in the culture supernatants of monocytes was determined with the ELISA assay following the manufacturers’ instructions (eBioscience, Santiago, California, USA).

Coculture of monocytes and T cells
The coculture of monocytes and T cells was performed as described previously.33 The CD14⁺ cells purified from peripheral blood of healthy donors were treated with medium or TSN for 24 hours. Then, the CD14⁺ cells were washed with PBS three times and cocultured with autologous T cells at a 1:2 ratio for 24 hours. They were cultured in DMEM supplemented with anti-CD3 (2 µg/mL) and anti-CD28 antibodies (2 µg/mL).

RNA and protein extraction, real-time qPCR analysis, western blot analysis and chromatin immunoprecipitation (ChIP)
RNA and protein extraction, real-time qPCR analysis, western blot and ChIP analysis were performed as described previously.35 Primers used in this study are provided in online supplemental table S3. Information about antibodies is provided in online supplemental table S4.

UPLC-MS analysis
Untargeted liquid chromatograph-mass spectrometer (LC-MS) analysis of polar metabolites was performed by using an ultra performance liquid (UPLC) system (ACQUITY, Waters) interfaced with a mass spectrometer (LTQ-Orbitrap, Thermo Scientific) as described previously.71 Elution conditions for LC/MS analysis are provided in online supplemental table S5. Optimized compound-dependent MS parameters are provided in online supplemental table S6.

Tumor cell apoptosis assay
CD14⁺ cells purified from the peripheral blood were treated with medium (MO) or TSN (TMO) for 24 hours in the presence or absence of MAT2A inhibitor FIDAS-5 (10 µM, Merck Millipore, USA). The monocytes were then washed with cold PBS three times and exposed to autologous CD8⁺ T cells (with a 1:2 ratio) for 24 hours. These cells were collected and cocultured with Carboxyfluorescein diacetate, succinimidyl ester (CSFE)-labeled MGC803 cells (with a 10:1 ratio) for 12 hours. Flow cytometry assay was used to determine the apoptosis of tumor cell.

Quantitative histone methylation proteomics
Quantitative histone methylation proteomics were performed as described previously.36

Plasmid construction and cell transfection
Plasmid construction and cell transfections were performed as described previously.35

Cell viability assay
Cell viability was determined with the Cell Counting Kit 8 (CCK-8, Donjindo).

In vivo experiments
Nude mice (age 4–5 weeks) were purchased from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences, Shanghai, and housed in a pathogen-free facility in the Experimental Animal Centre of Vijnishan Hospital. All animal experiments of laboratory animals were performed by the Guide for the Care and Use published by the US NIH (NIH publication number 85–23, revised 1996). 1×10⁶ MGC803 cells in 0.1 mL PBS were injected subcutaneously into unilateral flank areas of nude mice. 5×10⁶ purified autologous T cells conditioned with monocytes or TSN treated monocytes in the presence or absence of MAT2A inhibitor were subsequently injected into the peritoneum in 0.1 mL PBS every 5 days 1 day after inoculation. Tumor growth was determined every 5 days for 30 days using calipers fitted with a vernier scale. Then, tumor-bearing mice were sacrificed. The tumors formed were surgically removed and weighed. The length (L) and width (W) were measured with calipers, and tumor volumes were calculated using the following formula: \((L^2W)/2\).

Statistical analysis
All statistical analyses were performed using SPSS software (V.17.0, Chicago, Illinois, USA). All data are presented as the mean±SD from three independent repeats. The correlation between MAT2A and RIP1 expression levels was evaluated with Spearman rank correlation test. The differences between two groups were analyzed using the Student’s t test. P values were two-sided, and a value of <0.05 was considered to be statistically significant. Detailed descriptions of Materials and methods can be found in the online supplemental file 2.

RESULTS
Tumor-infiltrating monocytes/macrophages in GC had enhanced level of methionine cycle activity
To explore the role of methionine metabolism in GC, we determined the expression of a panel of genes involved in the methionine metabolism (online supplemental figure S1A) with qRT-PCR in CD14⁺ cells purified from tumor tissues and peripheral blood of 15 patients with GC (online supplemental table S2). We found that the key methionine metabolism-related genes (SHMT2, MAT2A, MTHFR, MTR) were significantly dysregulated in CD14⁺ cells purified from GC tissues compared with that from paired peripheral blood in 15 patients with GC (figure 1A). Among the key methionine metabolism-related genes, MAT2A was consistently upregulated and demonstrated the highest fold-change. A significant correlation between MAT2A expression in CD14⁺ cells purified from GC tissues and from paired peripheral blood was observed (\(r^2=0.592, p<0.001\), online supplemental figure 1B).
Moreover, CD14+ cells isolated from tumor tissues had elevated levels of methionine, Sadenosyl methionine (SAM) and S-adenosyl homocysteine (SAH) (figure 1B), and the ratio of SAM to methionine (the indicator of the capacity for methylation reactions) was increased in CD14+ cells derived from tumor tissues (figure 1B). To further characterize the phenotype of monocytes purified from GC tissues, we used CD86 (M1 marker) and CD163 (M2 marker).10–12 We found that the costimulatory molecule CD86 was almost absent, while strong appearance of CD163 was observed in monocytes isolated from GC tissues, indicating an overwhelming majority of the M2 phenotype. However, the expression of CD163 was significantly downregulated in CD14+ cells purified from peripheral blood of healthy donors (online supplemental figure S1C). To further investigate the role of methionine cycle metabolites in the function of the tumor-infiltrating monocytes/macrophages, we exposed the CD14+ cells purified from GC tissues to transient 48 hours methionine starvation. The methionine starvation for 48 hours reduced methionine cycle activity, as exemplified by a drastic decrease in SAM levels and a slight decrease in SAH levels (figure 1C). Forty-eight hours methionine starvation led to a significant decrease in the expression...
of M2 genes (Mgl2, Ym1 (Chi3I3), Relma (Fizz1), Arg1 (-marginally significant, p=0.045)), while CD36 and FABP4 were not affected or even inducible (figure 1D). It is worth noting that shorter-term starvation (24 hours) was sufficient to induce significant change in the expression of M2 genes (Mgl2, Ym1 (Chi3I3), Relma (Fizz1)) (online supplemental figure S1D), which highlighted the dependence of TAM on methionine. Furthermore, there was no significant difference in cell viability after 48 hours methionine starvation (online supplemental figure S1E).

To further clarify the role of methionine cycle activity in the maintenance of the phenotype of tumor-infiltrating macrophages, we attempted to rescue the methionine-starved cells with homocysteine (Hcy, 250 µM), SAM (500 µM) or non-starvation reversal. As would have been anticipated, homocysteine supplementation (250 µM) was unable to attenuate the effects of methionine starvation, which suggested that tumor-infiltrating monocytes/macrophages required exogenous methionine for phenotype maintenance (figure 1E). SAM supplementation strategy was used to bypass the requirement of methionine for tumor-infiltrating macrophages. The downregulation of M2 genes induced by methionine starvation was greatly ameliorated with SAM supplementation (500 µM) (figure 1E). However, the attenuation level of M2 gene expression when macrophages were recovered in complete medium was not as dramatic as that with SAM supplementation (figure 1E). Surprisingly, we found that while methionine starvation markedly decreased the production of IL-10 and TGF-β, SAM supplementation significantly rescued the production of IL-10 and TGF-β in tumor-infiltrating monocytes/macrophages (figure 1F).

**MAT2A modulates methionine metabolism-induced RIP1 expression on monocytes**

To further confirm the relationship between increased methionine cycle activity and cancer microenvironment, we treated with monocytes purified from healthy peripheral blood with GC cell line MGC803 derived tumor cell supernatants (TSN). TSN treatment resulted in significantly enhanced levels of SAM, SAH and SAM:methionine ratio compared with those in the control medium-treated monocytes (figure 2A). TSN-exposed monocytes also demonstrated higher transcripts levels of key methionine metabolism-related genes (figure 2B). As anticipated, TSNs derived from BGC823 cells markedly increased the intracellular levels of SAM, SAH and SAM:methionine ratio (online supplemental figure 2A).

As TAMs have been reported to promote immune escape within the cancer microenvironment via diverse mechanisms, we would like to explore the role of methionine metabolism in TAM-associated antitumor immunity. TSN induced marked upregulation of Mgl2, Ym1 (Chi3I3) and Relma (Fizz1) (figure 2C) as well as IL-10 and TGF-β production (figure 2D), while transient methionine 24 hours starvation before TSN treatment significantly blunted the effects of TSN (figure 2C,D).

Expected, SAM supplementation attenuated the suppressive effect of methionine starvation (figure 2C,D). Furthermore, the expression of amino acid transporter SLC7A5 was increased on TSN treatment (figure 2E). The competitive blocker for system L transporters (BCH, 5 mM) markedly blunted the promoting effects of TSN on Mgl2, Ym1 (Chi3I3) and Relma (Fizz1) (figure 2F).

Among the key methionine metabolism-related genes dysregulated in tumor-infiltrating or TSN-exposed monocytes, MAT2A demonstrated the highest fold-change (figure 2B). TSN increased the expression of MAT2A in a time-dependent manner, which peaked at 18 hours (figure 3A). Furthermore, TSN also induced a significant increase in the protein level of MAT2A (figure 3B). To investigate whether MAT2A played a role in the establishment of the phenotype of TAM, we treated CD14+ cells derived from normal blood with TSN in the presence or absence of MAT2A inhibitor FIDAS-5. FIDAS-5 treatment (10 µM) significantly attenuated the increase in the MAT2A protein level in TSN-exposed monocytes (figure 3C). Furthermore, we observed that the MAT2A inhibitor FIDAS-5 (10 µM) obviously downregulated the intracellular levels of SAM and SAH (figure 3D). Transient exposure to FIDAS-5 did not result in significant differences in cell viability in TSN-treated CD14+ cells (online supplemental figure 2B). As anticipated, FIDAS-5 inhibitor significantly attenuated the IL-10, TGF-β, Mgl2 and Relma (Fizz1) production/expression in/on TSN-exposed monocytes (figure 3E), while supplementation with SAM (500 µM) in the context of FIDAS-5 treatment markedly rescued the IL-10, TGF-β, Mgl2 and Relma (Fizz1) production/expression (figure 3E). To further confirm the role of MAT2A in the TAM phenotype, we employed the RNA interference strategy to modulate the expression level of MAT2A. As illustrated in figure 3F, si-MAT2A obviously abrogated the upregulation of MAT2A in TSN-exposed monocytes. MAT2A silencing led to suppression on M2 markers, which could be phenocopied by SAM supplementation (online supplemental figure 3).

As TSN treatment induced the antitumor immunity in monocytes, we then screened the reported the regulators of the immunosuppressive effects of TAN (online supplemental table S7). Among the dysregulated regulators of the immunosuppressive effects of TAM, RIP1 exhibited the most significant fold-change in TSN-exposed monocytes pretreated with MAT2A inhibitor (figure 4A). The increase in the mRNA level of RIP1 in response to TSN could be significantly attenuated by MAT2A inhibitor (FIDAS-5, 10 µM), siRNA mediated-MAT2A silencing, the competitive blocker for system L transporters (BCH, 5 mM) and transient methionine starvation. However, SAM supplementation could rescue the decrease induced by MAT2A inhibition (figure 4B,C). MAT2A suppression also abrogated the RIP1 expression on monocytes exposed to TSN from BGC823 cells (online supplemental figure 4A). Furthermore, RIP1 inhibitor (GSK547, 50 nM) obviously suppressed the upregulation/production...
of M2 gene in response to TSN (online supplemental figure 4B,C). Functionally, MAT2A inhibitor antagonized the suppressive effect on the antitumor activity of autologous CD8+ T lymphocytes exerted by TSN (figure 4D,E).

Higher expression levels of MAT2A/RIP1 are correlated with a significantly poorer overall survival and progression-free survival in GC according to data from the KMPlot database (online supplemental figure 5A,B). According to data from TIMER (http://cistrome.org/TIMER/), a significant correlation between MAT2A and RIP1 expression was observed in GC (online supplemental figure 5C). Correlation results between MAT2A and RIP1 in GEPIA (http://gepia.cancer-pku.cn/) were similar to that in TIMER (online supplemental figure 5D). Such a correlation could also be found in other cancers (online supplemental figure 6A,B). A positive correlation between the transcript levels of MAT2A and RIP1 was observed in monocytes purified from GC tissues (n=32, R^2=0.501, p<0.001, figure 4F).

**MAT2A leads to epigenetic activation of RIP1 expression on monocytes**

It was well known that cellular SAM levels could affect the histone methylation. Thus, we hypothesized that induction of MAT2A drives gene expression changes and acquisition of M2 phenotype through epigenetic activation/repression of vital regulators. To further clarify the contribution of epigenetic mechanisms, we transfected CD14+ monocytes purified from healthy peripheral blood with siRNA-MAT2A or siRNA-NC, then exposed to TSN.
for 14 hours, a time course for RNA stability was started after treatment with actinomycin D (0.5 mg/mL). Our data showed that the degradation rate of RIP1 appeared to be similar to MAT2A silencing (figure 5A). The CD14+ monocytes purified from healthy peripheral blood were pretreated with histone methyltransferase (HMT) inhibitor (chaetocin, 0.5 µM) or histone deacetylases inhibitor (TSA, 200 ng/mL) for 1 hour before exposure to TSN. We found that HMT inhibitor significantly abolished the induction of RIP1 by TSN, while TSA had no such effect (figure 5B). Furthermore, chaetocin treatment greatly suppressed the induction of M2 genes induced by TSN, which directly supported the role of histone methylation in the regulation of TAM (online supplemental figure 7). Altogether, these data suggested that histone methylation might play a role in the modulation of RIP1, which was consistent with the previous studies that methionine metabolism could influence histone methylation of the epigenome.40
To explore how methionine metabolism affected histone methylation in TAM, we used targeted histone proteomic analyses using a multireaction monitoring approach to quantify relative levels of histone lysine and arginine methylation. MAT2A inhibitor decreased the histone methylation at residues associated with

Figure 4  MAT2A modulates the RIP1 expression on monocytes. (A–E) CD14+ cells were isolated from peripheral blood of healthy donors. (A) CD14+ cells were treated with MGC803 TSN for 20 hours in the presence or absence of FIDAS-5 treatment (10 µM). qRT-PCR analysis of RIP1. (B,C) CD14+ cells transfected with siRNA-NC or siRNA-MAT2A were left untreated or treated with MGC803 TSN for 20 hours in the presence or absence of FIDAS-5 treatment (10 µM), system L transporters (BCH, 5 mM), starved for 48 hours for methionine or SAM supplementation (500 µM). qRT-PCR analysis (B) and western blotting of RIP1 (C). (D,E) CD14+ cells were isolated from peripheral blood of healthy donors and left untreated (MO) or treated with MGC803 TSN (TMO) for 20 hours in the presence or absence of FIDAS-5 treatment (10 µM). These cells were then washed with PBS and exposed to autologous CD8+ T cells for 20 hours (with a 1:2 ratio), (D) before the latter were harvested and cocultured with CSFE-labeled MGC803 cells for another 12 hours (with a 10:1 ratio). The death of tumor cells was analyzed through flow cytometry. T+MO: Tumor cells treated with MO-primed T cells; T+different TMO: tumor cells treated with different TMO-primed T cells. (E) These cells (treated as described in D) were then washed and injected into the established NOD/SCID mice bearing MGC803-derived gastric cancer with autologous CD8+ T cells. Top left, representative images of tumors formed (n=6 per group). Top middle, tumor growth curves. Top right, tumor weights. (F) The correlation between MAT2A and RIP1 mRNA levels was measured in CD14+ cells isolated from tumor tissues of 32 patients with GC. The ΔCt values (normalized to β-actin) were subjected to Spearman rank-correlation analysis. Data are presented as the mean±SD; *p<0.05. N=3 biologically independent experiments. GC, gastric cancer; MAT2A, methionine adenosyltransferase II alpha; NOD/SCID, non-obese diabetic/severe combined immunodeficiency; SAM, S-adenosylmethionine; TSN, tumor culture supernatant.
methionine metabolism, especially the concentration of SAM, regulated histone methylation. It mainly had effect on trimethylation at lysine-4 on histone H3 (H3K4me3) and at lysine-27 on histone H3 (H3K27me3). Immuno-blot analysis confirmed that H3K4me3 and H3K27me3 were decreased with MAT2A suppression (figure 5D). Moreover, we found high enrichment of H3K4me3 at the promoter region of RIP1 according to University of California (UCSC, Santa Cruz) Genome Bioinformatics Site (http://genome.ucsc.edu/) (figure 5E). It prompted us to determine whether it was H3K4me3 that was responsible for the transactivation of RIP1. As predicted, TSN exposure greatly increased global H3K4me3 levels in CD14+ monocytes derived from healthy peripheral blood, while MAT2A suppression, methionine starvation and system L transporters markedly decreased the H3K4me3 levels (figure 5F). SAM supplementation rescued the effects of MAT2A suppression on histone methylation (figure 5F).

WDR5, a vital component of SET/MLL (SET-domain/mixed-lineage leukemia) histone-methyltransferase complexes, played a key role in H3K4me3 and subsequent transactivation of target genes.32 We constructed the luciferase reporter vector containing the 5’ flanking DNA fragment (~3 kbp) of RIP1 promoter region. We found that TSN treatment enhanced the luciferase activity of pGL3 plasmid of RIP1 induced by TSN exposure in monocytes (figure 6A). To determine the specific type of HMT that took part in the transcriptional regulation of RIP1, we pretreated monocyte cells with three pharmacological HMT/demethylases inhibitors: OICR-9429 (an inhibitor of WDR5, 5 µM), BIX-01294 (an inhibitor of G9a HMT, 5 µM), DZNep (an inhibitor of EZH2, 1 µM) and OG-L002 (an inhibitor of LSD1, 50 µM). We observed that OICR-9429 treatment ameliorated the elevated luciferase activity of pGL3 plasmid of RIP1 induced by TSN exposure in monocytes (figure 6A). It indicated that WDR5 might take part in the regulation of RIP1. Furthermore, none of the TSN exposure, methionine starvation, MAT2A suppression had any significant effect on the protein level of WDR5 (figure 6B). We found that WDR5 silencing (figure 6C) abolished the effect of SAM supplementation on and RIP1 levels (figure 6D). The chromatin IP (ChIP) assay illustrated that obvious enrichment of H3K4me3 was found at the promoter region of RIP1 (figure 6E). TSN treatment greatly increased the H3K4me3 levels at the promoter region, while MAT2A suppression abolished these effects (figure 6E). SAM attenuated the effects of MAT2A suppression; however, such effects disappeared with WDR5 silencing (figure 6E). These data suggest that MAT2A leads to epigenetic activation of RIP1 expression on monocytes.

**DISCUSSION**

The polarization of monocytes/macrophages requires distinct transcriptional profiles, in which epigenetic regulation plays an important role. It is well known that cell
metabolism is vital for the polarization of macrophages. Metabolic reprogramming may support macrophage polarization by increasing uptake of certain nutrients (glutamine, glucose and lipid) and reprogramming of cellular biosynthetic and biogenetic pathways.20 21 In this study, we established a link between methionine metabolism and epigenetic reprogramming to support M2 polarization. We found that depletion of exogeneous methionine or inhibition of methionine adenosyltransferase enzyme (MAT2A) impaired the polarization of macrophages. Methylation patterns demonstrated great dependence on SAM content, with genes involved in M2 polarization showing decreased levels of H3K4me3 on the promoter region on SAM starvation. The effect of methionine metabolism on immune surveillance was confirmed by both in vitro and ex vivo findings.

There has been an established link between methionine adenosyltransferases and the progression of cancer, including GC.26 27 However, the role of methionine metabolism in TAM remains unknown. Yu et al43 reported that SAM generation maintains a high SAM:methionine ratio to support histone H3K36me3 for macrophage IL-1β production. The discrepancy between our study and the study by Yu et al43 may be due to differences in stimulation conditions (ie, lipopolysaccharide (LPS) by Yu et al) for limited SAM availability. It suggests that the impact of SAM bioavailability on methylation pattern depends both on stimulation conditions and Km of HMTs.

Although this study concentrates on the effect of methionine metabolism on histone methylation pattern, it is worth noting that SAM availability affects a plethora of cellular processes, including DNA/RNA methylation, which may contribute to the M2 polarization induced by MAT2A inhibition. It is reported that SAM depletion reduced the N6-methyladenosine (m6A) in the 3' Untranslated Regions (UTR) of MAT2A, leading to mRNA stabilization.45 46 To exclude the possibility that m6A might be involved in the regulation of RIP1, we tested the effect of MAT2A silencing on the stability of RIP1 mRNA. We found that MAT2A silencing had no effect on the stability of RIP1 mRNA, thus excluding the possibility that m6A is involved in the regulation of RIP1, trancript. Roy et al47 found that acute methionine starvation (6 hours) had limited impact on the level of lineage-specific transcription factors (eg, RORγt expression by Th17 cells). However, we found that methionine starvation (48 hours) suppressed the expression of M2 markers. It still requires investigation whether extended exposure (>6 hours) to methionine starvation of established Th17 cells would result in changes in methylation markers (ie, H3K4me3) that affect Th cell lineage stability.

Previous studies demonstrate that TAMs within the TME (5%–10% of the tumor stromal mass) prominently exhibit an M2-like phenotype and promote tumor growth and metastasis via different mechanisms, including tumor immune evasion.7–9 Thus, modulating macrophage

Figure 6  MAT2A-mediated H3K4me3 modification regulates RIP1 expression. (A–E) CD14+ cells were isolated from peripheral blood of healthy donors. (A) Luciferase reporter vector was constructed by inserting the promoter region (∼2500 to 500 bp) of the RIP1 gene into pGL3 enhancer plasmid. The reporter vectors were then cotransfected into CD14+ cells. Cells were pretreated with DMSO or OICR-9429 (5 µM) or BIX-01294 (5 µM) or DZNep (1 µM) or OG-L002 (50 µM) for 1 hour before exposure to TSN for 20 hours. Cells were harvested and subjected to luciferase activity assay. (B) Western blotting analysis of WDR5 in CD14+ cells after TSN exposure, methionine starvation, MAT2A suppression. (C) Western blot was performed to detect WDR5 expression after transfection of WDR5 siRNA in CD14+ cells. (D) Western blotting analysis of RIP1 in CD14+ cells treated with TSN in presence or absence of MAT2A inhibitor or SAM supplementation or WDR5 silencing. (E) (Left panel) The RIP1 DNA was detected in the chromatin sample immunoprecipitated from CD14+ cells using an antibody against H3K4me3. (Right panel) Real-time PCR of the ChIP samples shows the binding efficacy of H3K4me3 to the RIP1 gene promoter treated with TSN in presence or absence of MAT2A inhibitor or SAM supplementation or WDR5 silencing. Data are presented as the mean±SD; *p<0.05, N=3 biologically independent experiments. ChIP, chromatin immunoprecipitation; GC, gastric cancer; MAT2A, methionine adenosyltransferase II alpha; SAM, S-adenosylmethionine; TSN, tumor culture supernatant.
function represents an attractive approach. Inhibitors targeting MAT2A would modulate the TAM and cancer cells with high methionine metabolism rate, which would provide more ideal TMEs for chemotherapies or immune-therapies.

In conclusion, our study showed the detailed mechanistic insight of MAT2A-WDR5-RP1 axis in TAM. This study suggests a novel cancer immune modulatory strategy.

**REFERENCES**


Supplemental Figure S1. (A) Methionine is an essential sulfur-containing amino acid that is catabolized and recycled in a series of metabolic reactions termed the methionine cycle. (B) The correlation between MAT2A mRNA levels was measured in CD14+ cells isolated from tumor tissues and from paired peripheral blood of 15 GC patients. The ΔCt values (normalized to β-actin) were subjected to Spearman rank-correlation analysis. (C) FACS dot plots depicting CD80 and CD163 expression.
in CD14+ cells purified from peripheral blood of healthy donors or gastric cancer tissues of GC patients.

(D) qPCR analysis of the M2-associated genes Relma, Mgl2 and Ym1 after 24 h methionine starvation. (E) Cell viability determined with CCK8 assay in the presence or absence of 48-h methionine starvation. Values normalized to that of control.
Supplemental Figure S2. CD14^+ cells were isolated from peripheral blood of healthy donors. CD14^+ Cells were left untreated or treated with supernatant (TSN) from BGC823 cells for 20 h. (A) (Left) LC–MS was used to determine the abundance of methionine cycle metabolites in CD14^+ cells purified from tumor tissues 48 h after
methionine starvation. Values were normalized to that in the absence of TSN. (Right)

Ratio of SAM to methionine levels in CD14$^+$ cells. (B) Cell viability determined with CCK8 assay in the presence or absence of FIDAS-5 treatment. Values normalized to that of control.
Supplemental Figure S3. CD14⁺ cells transfected with siRNA-NC or siRNA-MAT2A were left untreated or treated with MGC803 TSN for 28 hours in the presence or absence of SAM supplementation (500 μM). qRT-PCR analysis of the M2-associated genes Mgl2 and Relma (Fizz1). ELISA analysis of IL-10 and TGF-β production.
Supplemental Figure S4. (A) CD14+ cells were left untreated or treated with BGC823 TSN for 20 hours in the presence or absence of FIDAS-5 treatment (10 μM) or SAM supplementation (500 μM). qRT-PCR analysis of RIP1. (B,C) CD14+ Cells were left untreated or treated with supernatant (TSN) from MGC803 cells for 20 h in the presence or absence of RIP1 inhibitor (GSK547, 50 nM). (B) qRT-PCR analysis
of the M2-associated genes Mgl2 and Relma (Fizz1). (C) ELISA analysis of IL-10 and TGF-β production.
Supplemental Figure S5. (A) A higher expression level of MAT2A is correlated with a significantly poorer OS ($p<0.05$) and PFS ($p<0.05$) in GC according to data from the KMPlot database. (B) A higher expression level of RIP1 is correlated with a significantly poorer OS ($p<0.05$) and PFS ($p<0.05$) according to data from the KMPlot database. The HRs and p values were calculated with log-rank tests. (C) Scatterplots of correlations between MAT2A expression and RIP1 expression adjusted by purity according to TIMER (http://cistrome.org/TIMER/) in STAD. (D) Correlation results between MAT2A and RIP1 according to GEPIA (http://gepia.cancer-pku.cn/) in STAD.
Supplemental Figure S6. (A) Scatterplots of correlations between MAT2A expression and RIP1 expression adjusted by purity according to TIMER.
(http://cistrome.org/TIMER/) in BLCA (bladder urothelial carcinoma), BRCA (breast invasive carcinoma), COAD (colon adenocarcinoma), ESCA (esophageal carcinoma), HNSC (head and neck cancer), KIRC (kidney renal clear cell carcinoma), LIHC (liver hepatocellular carcinoma), LUAD (lung adenocarcinoma), OV (ovarian cystadenocarcinoma), PAAD (pancreatic adenocarcinoma). (B) Correlation results between MAT2A and RIP1 according to GEPIA (http://gepia.cancer-pku.cn/) in BLCA, BRCA, COAD, ESCA, HNSC, KIRC, LIHC, LUAD, OV, PAAD.
Supplemental Figure S7. CD14+ Cells were pretreated with histone
methyltransferase inhibitor (chaetocin, 0.5 μM) for 1h before left untreated or treated with supernatant (TSN) from MGC803 cells for 20 h. qRT-PCR analysis of the M2-associated genes Mgl2 and Relma (Fizz1).
Supplemental Table S1. Clinicopathological characteristics of 32 GC patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>32</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21 (65.63%)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (34.37%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38 to 69, mean 57.32</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>1.32 to 14, mean 3.46</td>
</tr>
<tr>
<td>Lymph node metastasis (N stage)</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>9 (28.13%)</td>
</tr>
<tr>
<td>N1</td>
<td>6 (18.75%)</td>
</tr>
<tr>
<td>N2</td>
<td>10 (32.15%)</td>
</tr>
<tr>
<td>N3</td>
<td>7 (21.87%)</td>
</tr>
<tr>
<td>Depth of invasion (T stage)</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>6 (18.75%)</td>
</tr>
<tr>
<td>T2</td>
<td>7 (21.88%)</td>
</tr>
<tr>
<td>T3</td>
<td>13 (40.63%)</td>
</tr>
<tr>
<td>T4</td>
<td>6 (18.75%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>well and morderately</td>
<td>15 (46.88%)</td>
</tr>
<tr>
<td>Poorly and others</td>
<td>17 (53.12%)</td>
</tr>
<tr>
<td>Perineural Invasion</td>
<td></td>
</tr>
</tbody>
</table>
Negative 20 (62.50%)
Positive 12 (37.50%)

Lymphovascular invasion
Negative 24 (75.00%)
Positive 8 (25.00%)

Gross type
EGC 3 (9.38%)
Borrmann type I 2 (6.25%)
Borrmann type II 6 (18.75%)
Borrmann type III 15 (46.87%)
Borrmann type IV 6 (18.75%)


Supplemental Table S2. Clinical characteristics of 15 GC patients.

<table>
<thead>
<tr>
<th>Code</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>TNM stage</th>
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<td>3</td>
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<td>47</td>
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</tr>
<tr>
<td>12</td>
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<td>IIIC</td>
</tr>
<tr>
<td>36</td>
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<td>67</td>
<td>IIIB</td>
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<td>37</td>
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<td>39</td>
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<td>55</td>
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<td></td>
<td>Gender</td>
<td>Age</td>
<td>Stage</td>
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<tr>
<td>-----</td>
<td>--------</td>
<td>-----</td>
<td>-------</td>
</tr>
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<td>71</td>
<td>IIB</td>
</tr>
<tr>
<td>41</td>
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<td>66</td>
<td>IIIC</td>
</tr>
<tr>
<td>42</td>
<td>female</td>
<td>73</td>
<td>IIB</td>
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### Supplemental Table S3. Primer sequence used in this study.

<table>
<thead>
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<th>Gene</th>
<th>Sequence</th>
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<tr>
<td>β-Actin-F</td>
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<tr>
<td>β-Actin-R</td>
<td>5'-TGTGTGGCGTGAGCTTTTGTG-3'</td>
</tr>
<tr>
<td>CD11b-F</td>
<td>5'-ATCTCTGCTGCGTCTTCCAGT-3'</td>
</tr>
<tr>
<td>CD11b-R</td>
<td>5'-ATGCTGTGCTGCTCTCCTTG-3'</td>
</tr>
<tr>
<td>RBPj-F</td>
<td>5'-CGGCCCTCCACCTAAAGCA-3'</td>
</tr>
<tr>
<td>RBPj-R</td>
<td>5'-TCCATCCACCTCGCCATAAG-3'</td>
</tr>
<tr>
<td>MS4A4A-F</td>
<td>5'-CTGGGAAATCGTGCGTGAC-3'</td>
</tr>
<tr>
<td>MS4A4A-R</td>
<td>5'-CTCACGGGAGTCAAGAATC-3'</td>
</tr>
<tr>
<td>CD51-F</td>
<td>5'-GCTGTCGAGATTTCAATGTT-3'</td>
</tr>
<tr>
<td>CD51-R</td>
<td>5'-TCTGTCGACGATAAAATT-3'</td>
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</table>
CD39-F 5'-CCATCCTGCGCTTCCTCCTAT-3'
CD39-R 5'-CCACGCCGTCATTTCCCTCT-3'
Camkk2-F 5'-CATGAAATGACGCTGC-3'
Camkk2-R 5'-CCACGCCGTCATTTCCCTCT-3'
PFKFB3-F 5'-CTCGCATCAACAGCTTTGAGG-3'
PFKFB3-R 5'-TCAGTGTTTCCTGGAGGAGTC-3'
NLRC4-F 5'-GCGGAGGTGGGAGATATG-3'
NLRC4-R 5'-CGTAGAAGGTTTGGAACA-3'
EphB4-F 5'-GGATCGCATTCAGCCAAAGT-3'
EphB4-R 5'-ACTGTCTAAGGCTGTGGCAT-3'
Clever-1-F 5'-CTGTGTCCTGGTCCTCTGC-3'
Clever-1-R 5'-CGCAACGTTTAGACCGTACC-3'
LAMP2a-F 5'-CACCGTCCTTGTGCCCATAGCGGT-3'
LAMP2a-R 5'-AAACACCGCTATGGGCACAAGGAC-3'
ATP6V0d2-F 5'-TGCGGCAGGCTCTATCCAGAGG-3'
ATP6V0d2-R 5'-CCACTGCCACCGACAGCGTC-3'
RIP1-F 5'-CCTGCTGGAGAAGACAGACC-3'
RIP1-R 5'-CATCATCTTCCCCTCTTCC-3'
ICER-F 5'-ATGGCTGTAACTGGAGATGAA-3'
ICER-R 5'-GTGGCAAAGCAGTAGTAGGA-3'
NRP2-F 5'-GACTCCAAGCCCACGGTAGA-3'
NRP2-R 5'-TGGTTGTCTCTTCGCTCTTCAC-3'
MGLL-F 5'-ACAAGTCAGGTCAGGCTTCA-3'
MGLL-R 5'-AAGTGGGGCCTTTCATAGCT-3'
Lgr4 -F 5'-GCCTGAATGGGCTAAATCAA-3'
Lgr4 -R 5'-CCTTCTCCTGTCGACCAC-3'
LILRB2-F 5'-ACCCCTGGACACATTGACAC-3'
LILRB2-R 5'-TGGAGTCTCCTGTACCCTC-3'
ERK5-F 5'-GCCGTGTGTGCTCAGATGGG-3'
ERK5-R 5'-CAGGCTGCAAGTCACTC-3'
NDRG2-F 5'-CCTGTTGCTCCTTGGGAG-3'
NDRG2-R 5'-GTGAGGCTCGTTAGCTTGGG-3'
FABP4-F 5'-TTCTCCTCAAACCTGCGCTG-3'
FABP4-R 5'-CATTCACCACACAGCTTGGT-3'
FoxO1-F 5'-AGGATCCGCTGAACATGGCC-3'
FoxO1-R 5'-AAGGATACCACGCCAGCCG-3'
ZEB1-F 5'-GATGACCTGGCAACAGAC-3'
ZEB1-R 5'-CTTTCACTGCTCTCCTGGT-3'
ITGA4-F 5'-TCCAGACGAGCTCTCTCTGGAATG-3'
ITGA4-R 5'-AAGCCAGCTTCCACATA-3'
SIPR1-F 5'-CAGCAATACGAGACAAATTTC-3'
SIPR1-R 5'-GCCAGGCAACAGTAAAGAG-3'
Siglec-10-F 5'-GTGACGCAATGGCTTCTTCC-3'
Siglec-10-R 5'-CCAGGCTCTGAGAGCAGTT-3'
c-Maf-F 5' CAGCAAGGAGAGGTGATCC-3'
c-Maf-R 5' GGTTCCTTCCGACTCAGGG-3'
GCN2-F 5' TCTCCCAGCTTCTTCTACCTG-3'
GCN2-R 5' TGTCACTGAAGGCTCAATCTC-3'
ABHD5-F 5' CCGGCTTCGAGATAAGTCCC-3'
ABHD5-R 5' GCCAACCAGTTGACATCCT-3'
LIF-F 5' ACGAGCCCTTCGTTGAAAC-3'
LIF-R 5' TGGTCTTCTCCGACTCCAGG-3'
STAT6-F 5' GTGGTTTAGAAGAGGGGAATTT-3'
STAT6-R 5' ATCTCAACCCCTATCTCTCA-3'
STAT1-F 5' AGTTTGATGTTTGTGGGATTAG-3'
STAT1-R 5' ATCCTCAATACTCACTTTTCTACCT-3'
SLC7A5-F 5' GCATCGGCTTCACCATCATC-3'
SLC7A5-R 5' ACCACCTGCATGAGCTTCTGAC-3'
CD36-F 5' GAACCACTGCTTTCAAAA ACTGG-3'
CD36-R 5' GTCCTGAGTTATATTTTCCTTGG-3'
FABP4-F 5' TTTCCTTCAAACTGGGCGTG-3'
FABP4-R 5' CATTCCACCACCAGCTTGTC-3'
Fizz1-F 5' TCGTGGAGAATAAGGTCAAGG-3'
Fizz1-R 5' AGGAGGCCCATCTGTTCATA-3'
Mgl2-F 5' AGGCCACCCTAAGAGCCATTT-3'
Mgl2-R 5' CCTCTTCTCAGTGTGCTC-3'
Arg-1-F 5' AGACAGCAGGAGGAGTGAAGG-3'
Arg-1-R 5' CGAAGCAGCCAAAGGTAAAAC-3'
Ym-1-F 5' CATTCACTGCTATTACGATTCC-3'
Ym-1-R 5' AGTGATGCACTGCTTGG-3'
MAT2A-F 5' GACATTGGTGCTGGAGACCA-3'
MAT2A-R 5' ACTCTGATGGGAAGCACAGC-3'
SHMT2-F 5' CGAGTTTGCGATGCTGATCTT-3'
SHMT2-R 5' CTGCGTTGCTGTGCTGAG-3'
MTHFR-F 5' TTAGGTATGGAAGTGGTAGATGT-3'
MTHFR-R 5' CAAAAAACTACTAAAAAAACCAACAAA-3'
MTK-F 5' GTTCTGTTTCCAGCCGTC-3'
MTK-R 5' GTGGACACTGCGATGACTC-3'
GLDC-F 5' TAGTGCATCAGTGATGCTTCCCAAT-3'
GLDC-R 5' GTGGACACTCGGATGACCTC-3'
RIP1-F 5' TCCGGAAGGGTGTCCTCTTGG-3'
RIP1-R 5' CATTTGACTACGCGCGTTG-3'

Vector construction

siRNA-MAT2A-1 5' CACAAAGCUAAAGGAGACCCAA-3'
siRNA-MAT2A-2 5' CAGUUGUGCCUGCGAAAUA-3'
siRNA-NC 5' UUCUCCGAGCUGUCACGUTT-3'
siRNA-WDR5-1 5' GCUGGGAUAAUCCGAUGUATT-3'
siRNA-WDR5-2 5' GCUCAGAGGAUAAACCUUGUUT-3'
<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIP1-promoter-F</td>
<td>5'-GCCGAGCTCGAAGGGAGTGGACGCTGGAGCAA-3'</td>
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<tr>
<td>RIP1-promoter-R</td>
<td>5'-GCCGCTAGCTCGTCCCGTCACCCTCCTCT-3'</td>
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</table>
**Supplemental Table S4.** Information on antibodies.

<table>
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<th>Antibody</th>
<th>WB</th>
<th>CHIP</th>
<th>Specificity</th>
<th>Company (catalog number)</th>
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<tbody>
<tr>
<td>RIP1</td>
<td>1:1000</td>
<td>/</td>
<td>Rabbit monoclonal</td>
<td>Abcam (ab106393)</td>
</tr>
<tr>
<td>MAT2A</td>
<td>1:1000</td>
<td>/</td>
<td>Rabbit polyclonal</td>
<td>Abcam (ab154343)</td>
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<tr>
<td>WDR5</td>
<td>1:1000</td>
<td>1:200</td>
<td>Rabbit polyclonal</td>
<td>Abcam (ab56916)</td>
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<tr>
<td>β-Actin</td>
<td>1:2000</td>
<td>/</td>
<td>Rabbit polyclonal</td>
<td>Abcam (ab8227)</td>
</tr>
<tr>
<td>histone H3</td>
<td>1:1000</td>
<td>/</td>
<td>Rabbit monoclonal</td>
<td>CST (97155)</td>
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<tr>
<td>H3K4me3</td>
<td>1:1000</td>
<td>1:200</td>
<td>Rabbit polyclonal</td>
<td>Abcam (8580)</td>
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<tr>
<td>H3K27me3</td>
<td>1:1000</td>
<td>/</td>
<td>Rabbit monoclonal</td>
<td>Abcam (ab192985)</td>
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Supplemental Table S5. Elution conditions for LC/MS analysis.
<table>
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<tr>
<th>Time (min)</th>
<th>Solvent A (%)</th>
<th>Solvent B1 (%)</th>
<th>Solvent B2 (%)</th>
<th>Flow rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample analysis</td>
<td>99.90</td>
<td>0.10</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>99.90</td>
<td>0.10</td>
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<td></td>
</tr>
<tr>
<td>0.50</td>
<td>50.00</td>
<td>50.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.50</td>
<td>2.00</td>
<td>98.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.50</td>
<td>2.00</td>
<td>98.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column wash</td>
<td>12.50</td>
<td>2.00</td>
<td>98.00</td>
<td>0.50</td>
</tr>
<tr>
<td>15.50</td>
<td>2.00</td>
<td>98.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column equilibration</td>
<td>16.50</td>
<td>99.90</td>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>18.00</td>
<td>99.90</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Solvent A: 0.1% formic acid in water, Solvent B1: 0.1% formic acid in methanol and Solvent B2: 0.1% formic acid in water.

Supplemental Table S6. Optimized compound-dependent MS parameters using Xevo TQ-S
mass spectrometer.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Precursor ion mass (m/z)</th>
<th>Fragment ion mass (m/z)</th>
<th>Dwell time (s)</th>
<th>Cone voltage (V)</th>
<th>Collision Energy (V)</th>
<th>ESI Mode</th>
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<tbody>
<tr>
<td>Methionine</td>
<td>150.00</td>
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<td>40.00</td>
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<tr>
<td>S-adenosylmethionine</td>
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<td>S-adenosylhomocysteine</td>
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<td>0.025</td>
<td>66.00</td>
<td>33.00</td>
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</table>

Supplemental Table S7. Regulators of antitumor immunity in tumor-associated macrophages
and relative fold-change in response to TSN in presence or absence of FIDAS-5

<table>
<thead>
<tr>
<th>Factors</th>
<th>Effect</th>
<th>Ref</th>
<th>Fold-change</th>
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</thead>
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<tr>
<td>CD11b(ITGAM)</td>
<td>antitumor</td>
<td>[1]</td>
<td>2.32</td>
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<tr>
<td>RBPj</td>
<td>pro-tumor</td>
<td>[2]</td>
<td>0.98</td>
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<tr>
<td>MS4A4A</td>
<td>antitumor</td>
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<td>0.72</td>
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<td>CD51 (ITGAV)</td>
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<td>0.56</td>
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<td>CD39 (ENTPD1)</td>
<td>pro-tumor</td>
<td>[5]</td>
<td>0.49</td>
</tr>
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<td>CaMKK2</td>
<td>pro-tumor</td>
<td>[6]</td>
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<tr>
<td>PFKFB3</td>
<td>pro-tumor</td>
<td>[7]</td>
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</tr>
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<td>NLRC4</td>
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<td>[8]</td>
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<td>EphB4</td>
<td>pro-tumor</td>
<td>[9]</td>
<td>1.04</td>
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<tr>
<td>Clever-1 (STAB1)</td>
<td>pro-tumor</td>
<td>[10]</td>
<td>0.89</td>
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<tr>
<td>LAMP2a (Lamp2)</td>
<td>pro-tumor</td>
<td>[11]</td>
<td>0.51</td>
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<tr>
<td>ATP6V0d2</td>
<td>antitumor</td>
<td>[12]</td>
<td>0.87</td>
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<tr>
<td>RIP1 (RIPK1)</td>
<td>pro-tumor</td>
<td>[13]</td>
<td>0.16</td>
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<td>[14]</td>
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<td>LILRB2</td>
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<td>[19]</td>
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<td>FABP4</td>
<td>pro-tumor</td>
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<td>0.59</td>
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<tr>
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<td>antitumor</td>
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<td>1.21</td>
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<tr>
<td>ZEB1</td>
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<td>integrin α4(ITGA4)</td>
<td>pro-tumor</td>
<td>[24]</td>
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</tr>
<tr>
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Reference


**RNA preparation and qRT-PCR**

Total RNA from tissues and cells was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. The quality of total RNA was detected at an A260/A280 ratio using 1% agarose gel electrophoresis. The GoScript Reverse Transcription System (Promega, Madison, Wis) was used to generate combinational DNA. The cDNA template was amplified by real-time RT-PCR using the SYBR Premix Dimmer Eraser kit (TaKaRa). Gene expression in each sample was normalized to β-Actin expression. Real-time RT-PCR reactions were performed by the ABI7500 system (Applied Biosystems, Carlsbad, CA, USA). The real-time PCRs were performed in triplicate. The relative expression fold change of mRNAs was calculated by the $2^{-\Delta\Delta CT}$ method. Primers used in this study are listed in Supplementary Table S1.

The PCR array containing the gene involved in the lipid metabolism (Supplemental Table 3) were designed by Ribobio (Guangzhou, China). The expression levels of genes were detected by PCR array (Guangzhou, China).

**Western blot analysis**

The harvested cells were centrifuged at 6,000 rpm for 1 min. The total cellular proteins were prepared using RIPA cell lysis buffer (Cell Signaling Technology) supplemented with protease inhibitors. The lysates were then collected and subjected to ultrasonication and centrifugation. The supernatants were collected, and protein content was determined by Bradford assay. Equal amounts (30-50 μg) of proteins
were applied to an 8-12% SDS-polyacrylamide separating gel and transferred to a PVDF Immobilon-P membrane (Millipore). The membrane was blocked with 5% skim milk in TBST and then probed with indicated primary antibodies with gentle shaking at 4°C overnight. The membranes were washed with TBST (3×10 min), incubated in secondary antibodies conjugated to horseradish peroxidase at room temperature for 1 hour. Antibody-bound proteins were detected by ECL (enhanced chemiluminescence) Western Blotting Substrate (Pierce, Rockford, IL). The band intensity of western blotting and the normalization were analyzed using the Image J program (National Institutes of Health, Bethesda, MD). The antibodies used were listed in Supplementary Table S2.

**Chromatin immunoprecipitation**

Monocytes were serum-starved overnight. Chromatin was cross-linked with 1% formaldehyde for 10 min. After cell lysis, the chromatin was sonicated with a Bioruptor (Diagenode) in a cold room using the following parameters: H- high setting, pulse interval- 30 sec ON and 45 sec OFF, cycle time- 15 min each. Change ice in water bath chamber after each cycle. After about 9 cycles, a DNA smear with an average size of 500 bp was obtained. After centrifugation, the supernatants were subjected to immunoprecipitation overnight with antibodies at 4°C, or with isotype rabbit IgG at 4°C overnight. Chromatin-antibody complexes were isolated using Protein A/G PLUS Agarose (Santa Cruz). The crosslinks for the enriched and the input DNA were reversed and the DNA was cleaned by RNase A (0.2 mg/mL) and proteinase K (2 mg/mL) before phenol/chloroform-purification. PCR was employed to analyzed the specific sequences from immunoprecipitated and input DNA. The results are representative of at least three independent experiments.
RNA interference

The siRNA against WDR5 or MAT2A and their scrambled constructs were purchased from GenePharma (Shanghai, P.R. China). CD14+ cells (1×10⁶ cells/wells) were seeded into 6-well plates overnight before transfection. CD14+ cells were left untreated or transfected with 300 nM negative control siRNA, or WDR5 or MAT2A-specific siRNA using P3 primary cell 4D-Nucleofector X kit (V4XP-3024, Lonza). All siRNA duplexes were purchased from GenePharma (Shanghai, P.R. China).

Luciferase reporter assay

The RIP1 promoter constructions were generated by PCR. The sequences of the primers were: RIP1 PF: 5’-GCCGAGCTCGAGGGAGTGGACGCTGGAGCAA-3’, RIP1 PR: 5’-GCCGCTAGCTCGTCCCGTCACCCTCCTCTCCTCT-3’. In brief, cycling conditions included: denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 2 min, and extension at 72°C for 1 min with a final-extension step for 10 min at 72°C. PCR products were verified by using dissociation curves and gel electrophoresis. The RIP1 promoter region was amplified by PCR and the PCR products were then sub-cloned into the expression region of vector PGL3-Basic (Promega, USA). In general, 1 × 10⁶ cells were plated in 500 µl of OPTI-MEM (Invitrogen) with 10% FBS in 24-well plates. Firefly luciferase reporter constructs (5 µg) and pRL-thymidine kinase control vector (pRL-TK; Promega; 0.25 µg) were mixed with 5 µl of Lipofectamine 6000 (Invitrogen) to form a complex. Then, the cells were harvested after 48 hours for firefly/Renilla luciferase assays using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activities were normalized to the cotransfected pRL-TK plasmid (mean ± SD).
UPLC–MS analysis

Untargeted LC–MS analysis of polar metabolites was performed by using a UPLC system (ACQUITY, Waters Corp) interfaced with a mass spectrometer (LTQ-Orbitrap, Thermo Scientific). Electrospray ionization (ESI) in the mass spectrometer was conducted in both positive and negative mode and auxiliary gas flow were set at 40 and 15 (arbitrary units), respectively, with a capillary temperature of 400 °C. The ESI source and capillary voltages were 4.5 kV and 40 V, respectively, for positive ESI mode and 2.8 kV and −15 V for negative ESI mode. Mass calibration was performed with standard LTQ-Orbitrap calibration solution (Thermo Scientific) before sample injection. A pooled quality-control mixture comprising equal aliquots of all samples was run at regular intervals throughout each analytical batch. Samples were randomized for each analytical batch and triplicate injections were performed for each sample.

MRM transitions and MS parameters were optimized by using analytical-grade standards.

All chromatographic separations were performed on an ACQUITY UPLC HSS T3 1.7 μm, 50 × 2.1 mm i.d. column (Waters Corp). The column and autosampler temperatures were maintained at 30 oC and 4 oC, respectively. The elution condition and Optimized compound-dependent MS parameters are indicated in Supplementary Table 3.4. The injection volume was 4 μl.

Raw UPLC–MS data were preprocessed and analyzed with the XCMS peakfinding
algorithm (version 1.30.3). The pooled quality-control mixture was used for signal correction between and within each batch analysis. Samples were normalized on the basis of their cell counts. The identities of marker metabolites were verified by comparison of their retention time and mass spectra with those of commercially available standards.

**Cell viability assay**

Cell viability was assessed by the Cell Counting Kit 8 (CCK-8, Donjindo). Briefly, monocytes/macrophages were seeded into 96-well plates at an initial density of 10000 cells per 100μl. After 0, 24, and 48 hours of cultivation, CCK-8 solution (10μl per 100μl of medium in each well) was added to each well and incubated for 2 h. The absorbance was measured by scanning with a microplate reader (MRX; Dynex Technologies, West Sussex, United Kingdom) at 450 nm. Data were expressed as the percentage of viable cells as follows: relative viability (\(\%\)) = \(\frac{(A_{450\text{treated}} - A_{450\text{blank}})}{(A_{450\text{control}} - A_{450\text{blank}})}\times100\%\). Three replicates for each group.

**Quantitative histone methylation proteomics**

Nuclei were isolated using gentle detergent treatment (0.3% NP-40 in NIB-250 buffer) of cells and centrifugation at 600g and washed with NIB-250 buffer. Histones were acid-extracted and derivatized with propionic anhydride both before and following trypsin. Propionylated histone peptides were resuspended in 50 μl water with 1% TFA, and 3 μl were injected in 3 technical replicates on nanoLC/triple quadrupole MS.
which consisted of a Dionex UltiMate 3000 coupled to a ThermoFisher Scientific TSQ Quantum triple quadrupole mass spectrometer. Buffer A was 100% LC-MS grade water with 0.1% formic acid and buffer B was 100% ACN. The propionylated peptides were loaded onto an in-house packed C18 trapping column (4 cm × 150 μm; Magic AQ C18, 3 μm, 200 Å Michrom) for 10 min at a flow-rate of 2.5 μl min−1 in 0.1% TFA loading buffer. The peptides were separated by a gradient from 1 to 35% buffer B from 5 to 45 min. The analytical column was a 10 cm × 75-μm PicoChip (1PCH7515-105H253-NV New Objective) consisting of the same C18 material as the trapping column. The triple quadrupole settings were as follows: collision gas pressure of 1.5 mTorr; Q1 peak width of 0.7 (full width at half maximum); cycle time of 3 s; skimmer offset of 10 V; electrospray voltage of 2.5 kV. Data were analysed using Skyline software (v.3.5; MacCoss Laboratory, University of Washington) with Savitzky–Golay smoothing of peaks. Automatic peak assignment and retention times were checked manually.

**RNA stability assay**

To measure RNA stability, 0.5 mg/ml Actinomycin D (Sigma aldrich, USA) was added to cells to inhibit transcription and then incubated for different time points as indicated. At each time point, RNA was harvested followed by qRT-PCR as previously described. Transcript levels were plotted by appropriate nonlinear regression curves using a one-phase decay equation. RNA decay rate constant (k) was
quantified by fitting an exponential curve to the data points \( y = a e^{-kt} \); \( y \) is the relative amount of RNA, and \( t \) is time). The half-life was then estimated according to the equation \( t_{1/2} = \ln(2)/k \). The normalizer transcript 18S rRNA that does not decay over the course of this experiment was detected as control.