ARTICLE

ACUTE MYELOID LEUKEMIA

Loss of bisecting GlcNAcylation on MCAM of bone marrow stoma determined pro-tumoral niche in MDS/AML

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Bone marrow (BM) stroma plays key roles in supporting hematopoietic stem cell (HSC) growth. Glycosylation contributes to the interactions between HSC and surrounding microenvironment. We observed that bisecting N-acetylglucosamine (GlcNAc) structures, in BM stromal cells were significantly lower for MDS/AML patients than for healthy subjects. Malignant clonal cells delivered exosomal miR-188-5p to recipient stromal cells, where it suppressed bisecting GlcNAc by targeting MGAT3 gene. Proteomic analysis revealed reduced GlcNAc structures and enhanced expression of MCAM, a marker of BM niche. We characterized MCAM as a bisecting GlcNAc-bearing target protein, and identified Asn 56 as bisecting GlcNAc modification site on MCAM. MCAM on stromal cell surface with reduced bisecting GlcNAc bound strongly to CD13 on myeloid cells, activated responding ERK signaling, and thereby promoted myeloid cell growth. Our findings, taken together, suggest a novel mechanism whereby MDS/AML clonal cells generate a self-permissive niche by modifying glycosylation level of stromal cells.

Leukemia (2023) 37:113–121; https://doi.org/10.1038/s41375-022-01748-1

INTRODUCTION

The bone marrow (BM) niche is part of a physiological microenvironment in which hematopoietic stem cells (HSCs) are maintained and respond to regulatory signals under various physiological conditions [1]. BM niche dysfunction strongly affects development of hematological malignancies, and vice versa. BM stroma plays major roles in regulation of a variety of cellular processes, particularly quiescence, differentiation, proliferation, maturation, and apoptosis of HSCs [2, 3]. Conversely, the BM niche can be remodeled by hematopoietic malignant cells to generate a pro-tumoral niche surrounding [4, 5]. Crosstalk between BM stromal cells and malignant cells is thus strongly involved in disease initiation and progression.

Glycosylation is the most common post-translational modification of proteins, and specific glycan patterns frequently serve as stem cell markers [6, 7]. Numerous types of glycoconjugates have been shown to interfere with neoplastic cell processes or microenvironments of these cells, leading to malignant progression [8, 9]. We demonstrated in 2013 that human BM stromal cells HS27a (but not HS5) facilitated engraftment of clonal cells from myelodysplastic syndrome (MDS) patients, a process mediated by highly expressed melanoma cell adhesion molecule MCAM/CD146 [10]. Our follow-up 2015 study showed that HS5, in comparison with HS27a, have higher expression of bisecting N-acetylglucosamine (GlcNAc) (β1,4-linked GlcNAc attached to core β-mannose residue, catalyzed by MGAT3) [11]. Bisecting GlcNAc modification regulates physicochemical properties of numerous cell surface glycoproteins, notably integrins, growth factor receptors, and adhesion molecules [12–14]. Our analyses demonstrated that levels of MGAT3 and responding bisecting GlcNAc on BM stroma were significantly lower for MDS and acute myeloid leukemia (AML) patients than for healthy donors (HD). We evaluated the underlying mechanism whereby bisecting GlcNAc remodels the BM niche by modulating MCAM on stromal cells, and thereby affects proliferation of MDS/AML clonal cells.

MATERIALS AND METHODS

Cell culture

Myeloid leukemia cell line KG1a, and BM-derived stromal cell lines HS5 and HS27a, were kindly donated by Prof. H. Joachim Deeg (Fred Hutchinson Cancer Center; Seattle, WA, USA). SKM-1, a cell line established from MDS progressing to AML, was donated by Prof. Xiao Li (Shanghai Jiao Tong University). These cells were all cultured as described previously [15].

Generation of conditional *MGAT3*$^{loxP/loxP}$ mice

MGAT3$^{fl/fl}$ transgenic mice were generated using a homologous combination knockout strategy, in which exon 2 of MAGT3 gene was flanked with two loxP sites. Conditional deletion of *MGAT3* gene was accomplished by crossbreeding leptin receptor (*LepR*)-*Cre* transgenic mice (donated by Prof.

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Received: 12 June 2022 Revised: 21 October 2022 Accepted: 26 October 2022
Published online: 5 November 2022

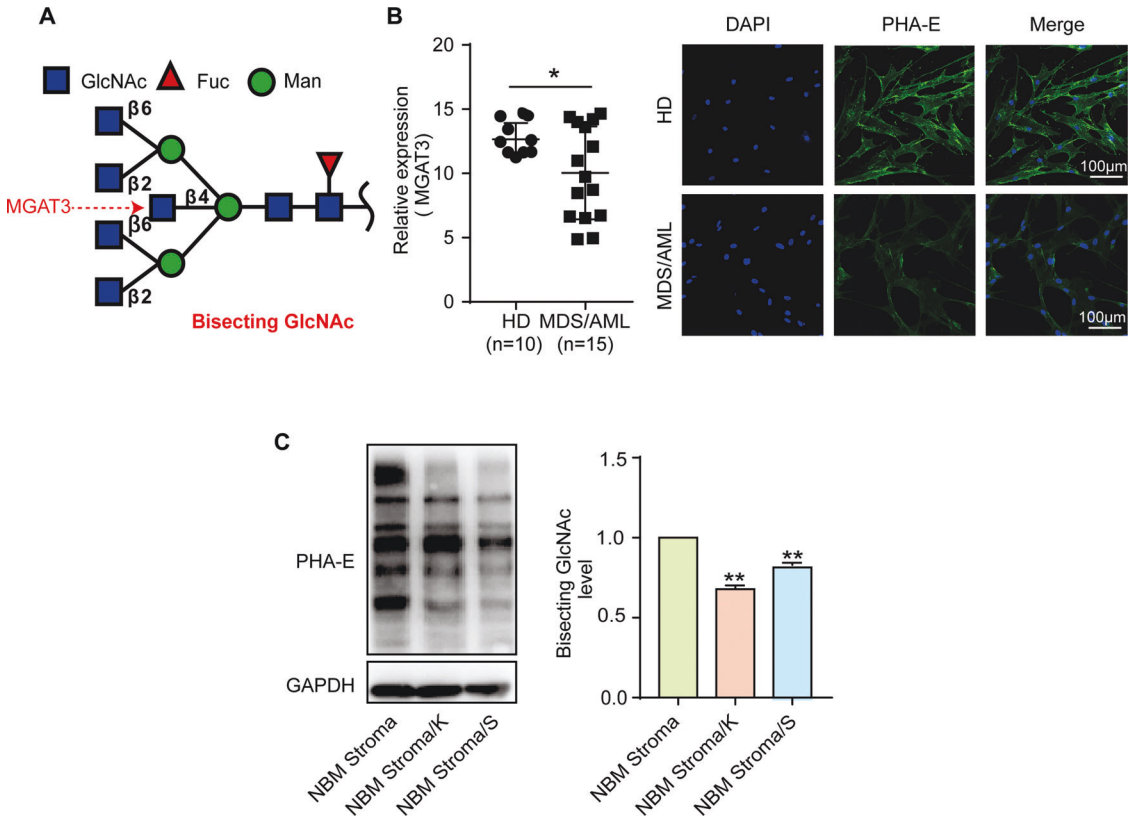

Fig. 1 Expression of bisecting GlcNAc in BM stromal cells. A Bisecting GlcNAc structures. **B** MGAT3 gene expression at mRNA level in BM stroma from HD or MDS/AML patients. Confocal images of bisecting GlcNAc in BM stroma from HD and MDS/AML patients. **C** Lectin blotting analysis of bisecting GlcNAc in NBM stroma co-cultured with KG1a ("K") or SKM-1 ("S").

Caiwen Duan, Shanghai Jiao Tong University) with *MGAT3*$^{fl/fl}$ mice. Animal experiments were approved by the Animal Care and Use Committee of Northwest University.

In vivo mouse experiment

LepR-Cre; *MGAT3*$^{fl/fl}$ or *MGAT3*$^{fl/fl}$ mice (age 6–8 wk) were i.p. injected with busulfan (20 mg/kg) for two days to suppress BM activity [16]. 5×10^6 of KG1a/SKM-1 cells were injected via tail vein. Nod.cg-Prkdc^{scid}Il2rg^{tm1wjll} (NSG) mice (age 6–8 wk) were i.p. injected with busulfan (30 mg/kg) for 24 h [17]. 2×10^6 of HS5, HS5-MCAM, or HS5-MCAM-Mu cells together with 6×10^6 of KG1a/SKM-1 were co-transplanted via tail vein. Peripheral blood was assayed on days 10, 17, and 24, and mononuclear cells were stained with anti-CD45 Ab for FACS analysis. Mice were then euthanized, and spleen and BM were collected.

RESULTS

Levels of bisecting GlcNAc and MGAT3 in BM stroma

Expression of bisecting GlcNAc and its glycosyltransferase MGAT3 were examined in primary MDS/AML BM stroma (Fig. 1A). MGAT3 mRNA expression and bisecting GlcNAc levels were significantly lower for MDS/AML patients than HD (Fig. 1B). And bisecting GlcNAc in stromal cells was significantly downregulated by co-culture with KG1a or SKM-1 cells (Figs. 1C, S1A–C). These findings demonstrate the aberrant expression of bisecting GlcNAc in MDS/AML stromal cells, and the ability of myeloid cells to alter N-glycosylation levels of niche cells.

Effect of bisecting GlcNAc in stroma on myeloid cell proliferation

By introducing MGAT3 into HS27a, we established a stable transfectant (termed HS27a-M3) that expressed high bisecting GlcNAc level (Fig. S2A). Co-culture of primary CD34 + cells from MDS/AML patients (termed primary CD34+ cells), KG1a or SKM-1 with HS27a-M3 resulted in reduced proliferation (Fig. S2A). Conversely, co-culture of primary CD34+ cells, KG1a or SKM-1 with a MGAT3-knockdown version of HS5 (termed HS5-shM3) resulted in enhanced proliferation (Fig. S2B). Normal BM stroma was treated with forskolin, an adenylyl cyclase activator that stimulates MGAT3 expression [18]. This treatment resulted in clear enhancement of bisecting GlcNAc level (Fig. 2A). Proliferation of primary CD34+ cells, KG1a or SKM-1 co-cultured with forskolin-treated NBM stroma vs. nontreated stroma was reduced (Figs. 2B, S2C, D). These findings demonstrate that upregulation of bisecting GlcNAc level in stroma induced by MGAT3 overexpression inhibited myeloid cell proliferation, and conversely myeloid cell proliferation was enhanced by downregulation of bisecting GlcNAc level.

We generated *LepR-Cre; MGAT3*$^{fl/fl}$ mice in order to investigate the functional role of bisecting GlcNAc in the BM niche in vivo (Fig. S3A). These mice showed strongly reduced expression of MGAT3 and stromal bisecting GlcNAc (Fig. S3B), retarded growth relative to *MGAT3*$^{fl/fl}$ (Fig. S3C), and development of severe anemia and thrombocytopenia (Fig. S3D). KG1a/SKM-1 cells grew well when transplanted into *L*epR-Cre; *MGAT3*$^{fl/fl}$ (Figs. 2C, S3E). These findings illustrate substantial effects on the hematopoietic microenvironment of MGAT3 expression and bisecting GlcNAc production in BM stroma.

Exosomal miR-188-5p of myeloid cells suppresses stromal bisecting GlcNAc level

Exosomes are packaged with bioactive proteins, lipids, or nucleic acids, and mediate cell-cell communication [19]. We purified exosomes from KG1a to investigate regulation by myeloid cells on stromal bisecting GlcNAc expression (Fig. S4A–C). Co-culture

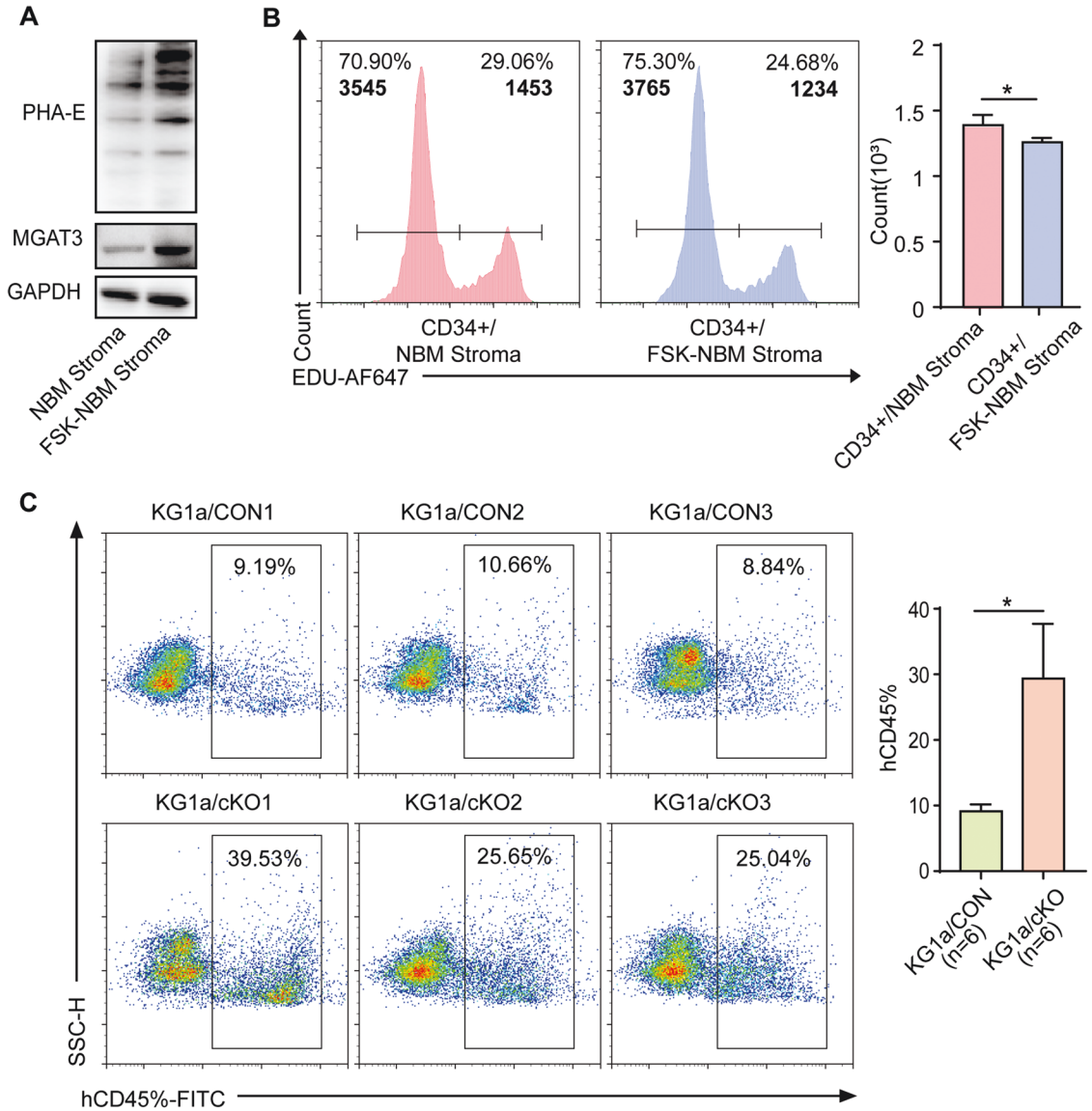

Fig. 2 Bisecting GlcNAc in NBM stroma affects myeloid cell proliferation. A NBM stroma were treated with 20 μM forskolin for 12 h, and expression of MGAT3 and bisecting GlcNAc were evaluated. **B** Primary CD34+ cells were co-cultured with forskolin-treated ("FSK-NBM Stroma") or nontreated NBM stroma for 48 h, and their proliferation was assayed by FACS. **C** FACS analysis of percentage of KG1a in peripheral blood of CON and cKO mice.

of HS5, HS27a, or primary NBM stroma with KG1a cells, or treatment with KG1a-derived exosomes, resulted in significant reduction of MGAT3 expression and of bisecting GlcNAc levels (Fig. 3A). Using miRNA-seq analysis in combination with TargetScan and miRBD database, we identified miR-188-5p as MGAT3-targeting miRs (Fig. S4D). Kaplan-Meier analysis showed significant correlation of overall AML patient survival with miR-188-5p expression (Fig. 3B). miR-188-5p expression was notably higher in exosomes derived from plasma of MDS/AML patients, vs. HD (Fig. 3B). MGAT3 and bisecting GlcNAc expression in HS27a or primary stroma were downregulated by co-culture with KG1a, but restored by treatment with miR-188-5p inhibitor (Fig. S4E, F). Conversely, transient transfection of miR-188-5p mimic into HS5, HS27a, or primary stroma led to downregulation of MGAT3 expression (Fig. 3C). Luciferase reporter assay revealed direct binding of miR-188-5p to 3′-UTR of MGAT3 (Figs. 3C, S4G). These findings indicate that exosomal miR-188-5p from myeloid cells reduced stromal bisecting GlcNAc levels by targeting and silencing MGAT3 gene.

Identification and function of bisecting GlcNAcylated MCAM in BM stroma

With quantitative proteomic analysis (Fig. S5A), the differentially expressed proteins were identified in stroma from *MGAT3*$^{fl/fl}$ (CON) and *LepR-Cre; MGAT3*$^{fl/fl}$ conditional knockout (cKO) mice (Fig. 4A). 380 differentially expressed proteins (fold change >1.5 or <0.67; $p < 0.05$) were identified; they consisted of 190 upregulated and 190 downregulated proteins (Fig. 4A). Expression of MCAM, a typical adhesion molecule that supports hematopoietic cell growth, was significantly increased in *LepR-Cre; MGAT3*$^{fl/fl}$ (Fig. S5B). Western blotting revealed downregulated bisecting GlcNAc and upregulated MCAM expression in stroma from cKO mice (Fig. S5C). Consistently, MCAM expression in BM stroma was higher for MDS/AML patients than for HD (Figs. 4A, S5D).

We hypothesized, in view of these findings, that MCAM expression is affected by stromal bisecting GlcNAc level. MCAM expression was upregulated in HS5-shM3 but downregulated in HS27a-M3, consistently with this hypothesis (Fig. S5E). We cloned MGAT3 gene into a tetracycline-inducible gene expression system

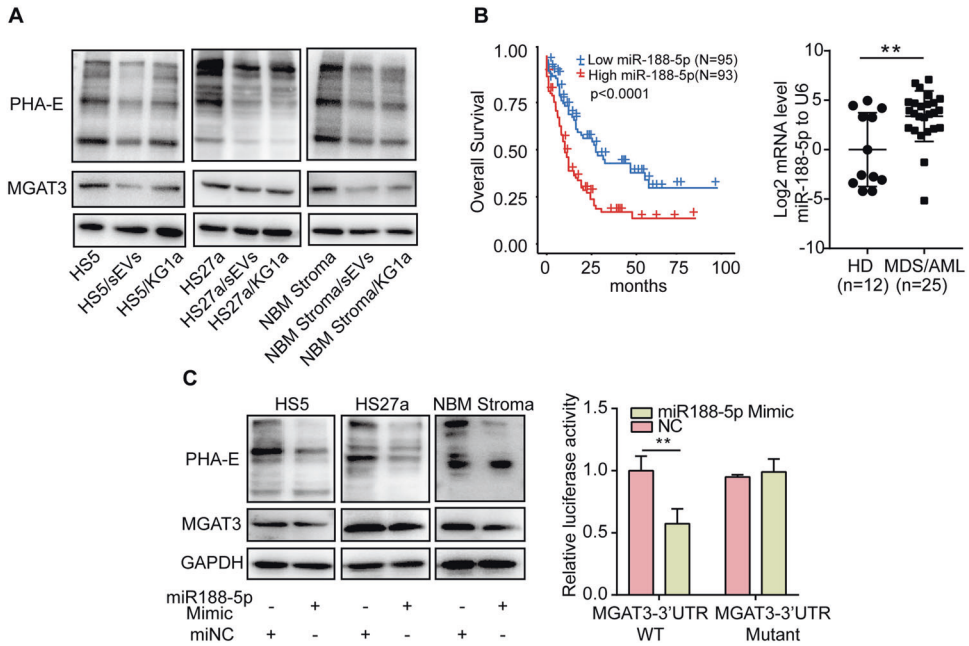

Fig. 3 Exosomal miR188-5p inhibits bisecting GlcNAc by targeting MGAT3. A MGAT3 expression and bisecting GlcNAc level in HS5, HS27a, and NBM stroma treated with 50 μg/ml KG1a cell exosomes or co-cultured with KG1a. **B** Overall survival of miR188-5p expression in AML patients, from TCGA database. miR-188-5p expression at mRNA level in exosomes from plasma of HD ($n = 12$) or MDS/AML patients ($n = 25$). **C** Lectin blotting analysis of MGAT3 and bisecting GlcNAc levels of HS5, HS27a, and NBM stroma treated with miR188-5p mimic. HEK293T cells were co-transfected with miR188-5p mimic or miR188-5p mimics negative control ("miNC") and two reporter plasmids psiCHECK2 (wild-type or mutant MGAT3 3′-UTR sequence), and luciferase activities of transfectant cells were assayed.

and transfected it into HS27a. Treatment of these cells with doxycycline (dox) resulted in time-dependent increased MGAT3 and bisecting GlcNAc expression, and decreased MCAM expression (Fig. S5F). Introduction or silencing of MGAT3 in primary stroma led respectively to down- and upregulation of MCAM expression (Fig. 4B). These findings demonstrate that MCAM expression in stromal cells was regulated by bisecting GlcNAc modification.

Increase of bisecting GlcNAc levels did not alter MCAM expression at the mRNA level (Fig. S6A), suggesting that modulation of MCAM expression by bisecting GlcNAc modification is a post-translational event. MCAM is a typical transmembrane glycoprotein, and we accordingly used Sulfo-NHS-LC-Biotin to label MCAM on cell membrane (Fig. S6B). Total MCAM content was much lower in HS27a-M3 than in HS27a cells. In HS27a-M3, relative to HS27a, biotin-labeled MCAM level on cell membrane was lower, whereas MCAM level in cytoplasm was higher (Fig. S6C). Blocking of cytosolic protein synthesis by cycloheximide accelerated MCAM degradation (Fig. S6D). In most cases, intracellular proteins are degraded via ubiquitin-proteasome pathways, whereas extracellular proteins and cell surface proteins enter cells by endocytosis and are degraded via lysosomal pathways [20–22]. MCAM expression in HS27a-M3 was enhanced by treatment with lysosomal inhibitor chloroquine, but unaffected by treatment with proteasome inhibitor MG132 (Fig. S6E). MCAM in HS27a-M3 was shown to be localized mainly in lysosomes (Fig. S6F). These findings indicate that bisecting GlcNAc modification affects MCAM stability, and causes MCAM degradation via a lysosomal pathway.

We identified unique peptide GLSQSQGN#LSHVDWFSVHK of bisecting GlcNAc-modified MCAM, characterized by pep+HexNAc3Hex1 at Asn 56 (Fig. S7A). Forced expression of either wild-type MCAM or N56D mutated MCAM in HS5, termed HS5-MCAM and HS5-MCAM-Mu, resulted in nearly identical MCAM levels (Fig. S7B). MCAM was rarely modified with bisecting GlcNAc in HS5-MCAM-Mu indicating that Asn 56 is the major bisecting GlcNAc modification site (Fig. 4C).

Mutation at Asn 56 had no effect on MCAM expression at protein or mRNA levels (Figs. 4C, S7B). However, MCAM level on cell membrane was significantly lower in HS5-MCAM-Mu than in HS5-MCAM (Fig. S7C). Similarly, when MCAM was pulled down by Sulfo-NHS-LC-Biotin strategy as described above, MCAM level on cell membrane was significantly lower in HS5-MCAM-Mu than in HS5-MCAM (Fig. 4C). For the two cell types, MCAM expression was assayed in total cell lysates, on cell membrane, and in cytoplasm. In HS5-MCAM-Mu, relative to HS5-MCAM, MCAM level was lower on cell membrane, and higher in cytoplasm (Fig. S7D). For HS5-MCAM-Mu, cycloheximide treatment to block novel cytosolic protein synthesis accelerated MCAM degradation (Fig. S7E), while MCAM expression was enhanced by chloroquine treatment and unaffected by MG132 (Fig. S7F). MCAM in HS5-MCAM-Mu was shown to be localized in lysosomes (Fig. S7G). Thus, localization of MCAM on cell membrane could be reduced by either removal of N-glycan structures at Asn 56 by mutation, or enhanced bisecting GlcNAc modification at this site.

Effect of MCAM on myeloid cell proliferation

In vivo and in vitro experiments showed that proliferation of myeloid cells co-cultured with HS5-MCAM increased, whereas that of cells co-cultured with HS5-MCAM-Mu declined to the level of cells co-cultured with HS5 (Figs. 5A, S8A, B). MCAM on cell membrane evidently supports myeloid cell proliferation.

Based on the above finding, transplantation experiments were performed to clarify the in vivo role of bisecting GlcNAc modification of MCAM in myeloid cell proliferation (Fig. S8C). Proportion of KG1a/SKM-1 cells in peripheral blood of NSG mice was significantly higher when KG1a/SKM-1 were co-injected with HS5-MCAM cells, relative to co-injection with HS5 or HS5-MCAM-Mu (Figs. 5B, S8D, E). Expression in BM and spleen of $CD45^+$ signal, a myeloid cell marker, was significantly lower for

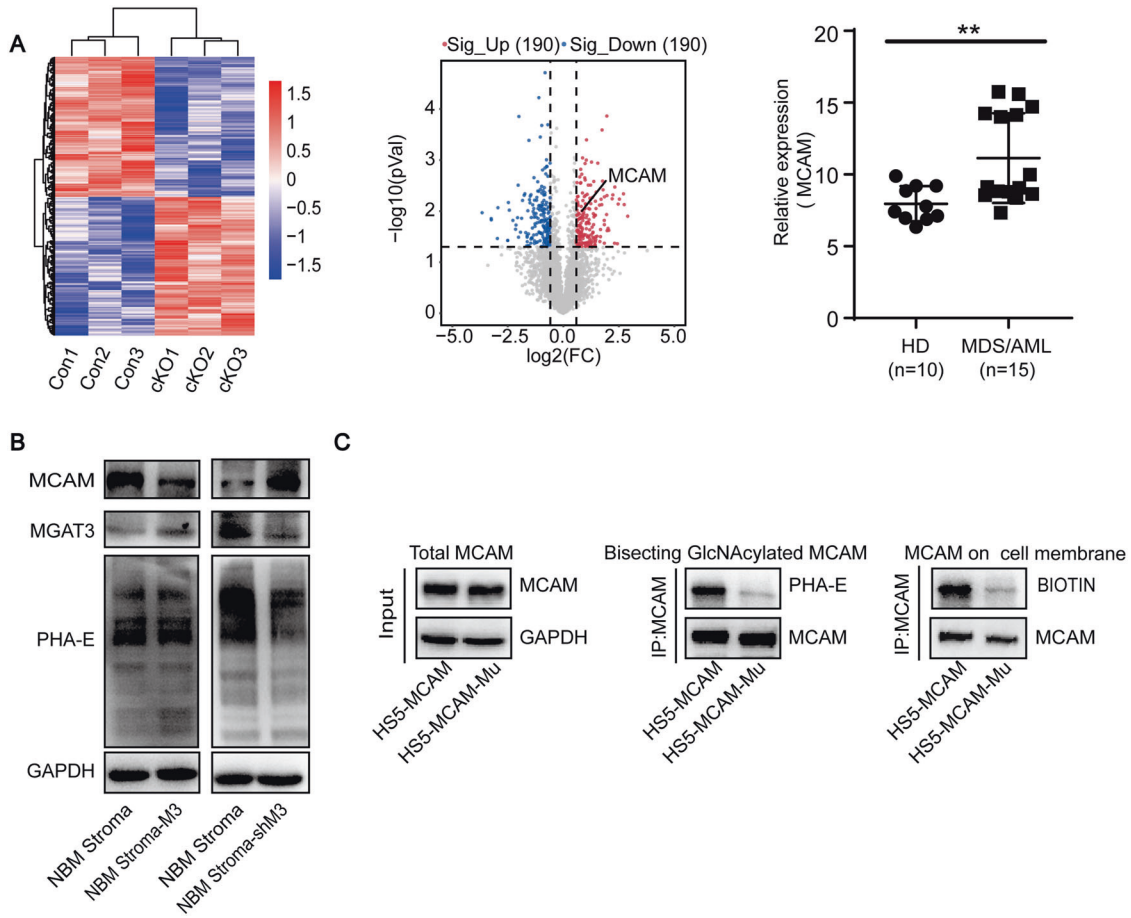

Fig. 4 Identification and function of bisecting GlcNAcylated MCAM in BM stroma. A Heatmap of differentially expressed proteins in BM stroma derived from *MGAT3*$^{fl/fl}$ *or LepR-cre; MGAT3*$^{fl/fl}$ mice. Volcano plot of identified proteins in BM stromal cells. MCAM expression at mRNA level in BM stroma from HD or MDS/AML patients. **B** Expression of bisecting GlcNAc, MCAM, and MGAT3 in NBM stroma-MGAT3 and NBM stroma-shMGAT3. **C** Bisecting GlcNAcylated MCAM was assayed by IP/western blotting. Sulfo-NHS-LC-Biotin-labeled MCAM was IP'd by streptavidin-conjugated agarose. Total MCAM and MCAM on cell membrane were assayed by western blotting.

HS5-MCAM-Mu co-injected relative to HS5-MCAM co-injected group (Figs. 5C, S8F–H). These findings indicate that the supportive effect of stromal cells on myeloid cell proliferation was disrupted in glycosylation-deficient N56D mutant of stroma because of reduced MCAM expression on stromal cell membrane.

Membrane proteins involved in myeloid cell interaction with MCAM

To clarify the molecular mechanism underlying the effect of MCAM Asn 56 glycosylation on myeloid cell proliferation, we performed IP-MS to identify membrane proteins on KG1a that interact with MCAM on stromal cells (Fig. S9A). 163 differentially expressed proteins were identified (Fig. 6A). Among the 29 overlapping proteins that interacted with MCAM in HS5-MCAM, in HS5-MCAM-Mu, and in interactome from string database [23], CD13, a human myeloid plasma membrane glycoprotein was identified (Fig. 6A). CD13 was shown to bind more strongly to wild-type MCAM than to mutant MCAM (Figs. 6B, S9B). To confirm the possibility that MCAM-bound CD13 is derived mainly from myeloid cells rather than stromal cells, we knocked down CD13 expression in HS5-MCAM and HS5-MCAM-Mu (Fig. S9C). Inhibition of CD13 in stroma, MCAM can still pull down CD13 from the co-cultured lysate, suggesting these CD13 are mainly originated from myeloid cells (Figs. 6B, S9D).

Activated CD13 has been reported to facilitate cell proliferation by inducing extracellular signal-regulated kinase (ERK) phosphorylation [24]. We therefore pretreated KG1a and SKM-1 cells with the aminopeptidase inhibitor bestatin, a specific inhibitor of CD13, to block CD13 on cell membrane (Fig. S9E–G). ERK signaling was activated in KG1a co-cultured with HS5-MCAM, but clearly suppressed in KG1a co-cultured with HS5-MCAM-Mu, and in bestatin-pretreated KG1a co-cultured with HS5-MCAM (Fig. S9H). However, ERK signaling showed unaffected expression in KG1a after co-cultured with bestatin-pretreated HS5-MCAM (Fig. S9I). Similarly, cell proliferation was significantly enhanced in KG1a after co-culture with HS5-MCAM, but suppressed in KG1a co-cultured with HS5-MCAM-Mu or in bestatin-pretreated KG1a co-cultured with HS5-MCAM. While cell proliferation of KG1a was unaffected after co-cultured with bestatin-pretreated HS5-MCAM (Fig. 6C). The similar results were found using SKM-1 cells as cell model (Fig. S9J–L).

These findings, taken together, demonstrate that MCAM on stromal cells binds to CD13 on myeloid cells, activates ERK signaling, and promotes myeloid cell growth.

DISCUSSION

Abnormal hematopoiesis may result from genetic dysregulation, or from a dysfunctional structure acting by itself or affecting crosstalk with hematopoietic stem cells [25]. Many factors are involved in crosstalk between stroma and hematopoietic cells; among these, glycoconjugates play key roles in modulating functions of HSPCs in BM [26–28].

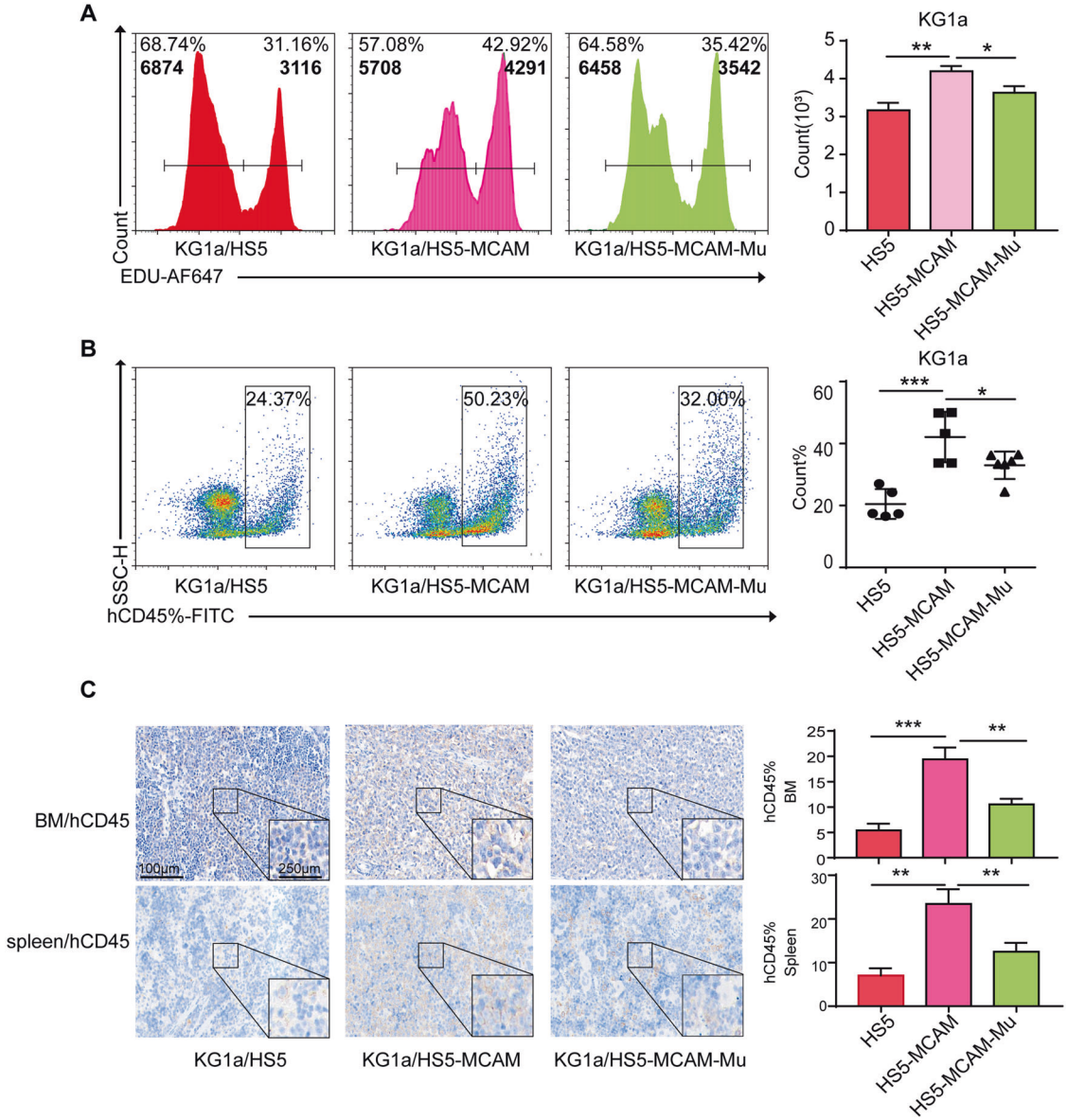

Fig. 5 Effect of bisecting GlcNAc modification of MCAM on myeloid cell proliferation. A Proliferation of KG1a co-cultured with HS5, HS5-MCAM, and HS5-MCAM-Mu. **B** FACS analysis of KG1a percentages in peripheral blood of NSG mice co-injected with HS5, HS5-MCAM, or HS5-MCAM-Mu. **C** Immunohistochemical staining and statistical analysis of CD45 in BM and spleen of NSG mice.

Glycoconjugates on cell membranes affect nearly all interactions between cells and their surrounding environment. Several glycoengineering strategies have been developed to improve homing and engraftment of cells following hematopoietic stem cell transplantation [29–31]. In this study, we observed downregulated levels of bisecting GlcNAc and MGAT3 in BM stroma of MDS/AML patients. Bisecting GlcNAcylation is a specific type of N-glycosylation that affects adhesion, migration, and other cellular functions by modifying adhesion molecules and receptors (notably E-cadherin, integrins, tetraspanins, and EGFR) [14, 32, 33]. Bisecting GlcNAc is also involved in organ growth and development [34]. Low bisecting GlcNAc levels in stromal cells promote proliferation of hematopoietic clonal cells. Malignant clonal cells packaged miR-188-5p into exosomes for delivery to recipient stromal cells, where it reduced MGAT3 expression and bisecting GlcNAc level. These and similar findings demonstrate the ability of malignant cells to reprogram their microenvironment [35].

We identified MCAM as a bisecting GlcNAc-bearing target protein in stromal cells. MCAM was originally identified in human melanoma as an adhesion molecule glycoprotein [36]. It was subsequently shown to be highly expressed in other tumors and in endothelial cells. MCAM plays functional roles in a variety of cellular processes, including transendothelial migration, proliferation, and cancer metastasis [37–39]. The previous findings show that MCAM is a useful marker associated with BM niche, together with LepR [40–42]. MCAM overexpression in mesenchymal stromal cells (MSCs) enhanced adhesion of HSPCs to MSCs, and supported HSPC growth. In contrast, silencing of MCAM in MSCs suppressed HSPC proliferation, and strongly reduced formation of long-term culture-initiating cells [43]. Our 2013 study indicated that high MCAM expression in stromal cells facilitated engraftment of cloned MDS patient cells in a mouse xenotransplantation model [10]. Consistently with the finding by P. Bianco's group of high MCAM expression in MDS/AML patients [44], we observed that high MCAM expression in stromal cells supported myeloid cell proliferation in vitro and in vivo.

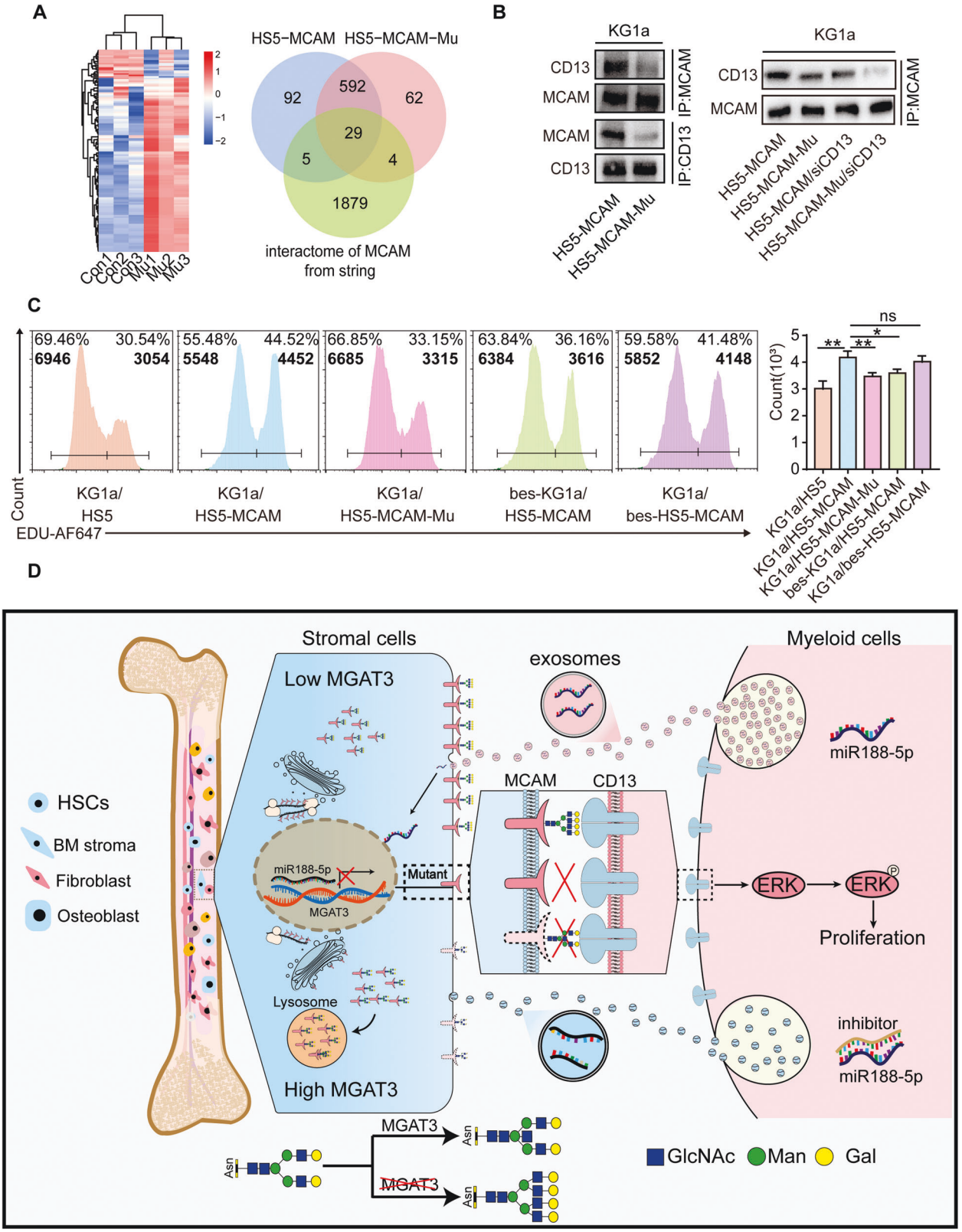

Fig. 6 Interaction of KG1a membrane proteins with stromal cell MCAM. A Interaction of differentially expressed proteins with MCAM was analyzed by IP-MS and presented as heatmap. Proteomic analysis of membrane protein interaction with MCAM on KG1a. **B** Western blotting analysis of MCAM/ CD13 interaction. IP/western blotting analysis of MCAM/ CD13 interaction in KG1a and CD13 silenced HS5-MCAM or HS5-MCAM-Mu cells. **C** Proliferation of KG1a co-cultured with HS5, HS5-MCAM, HS5-MCAM-Mu or bestatin-pretreated HS5-MCAM and of bestatin-pretreated KG1a ("bes-KG1a") co-cultured with HS5-MCAM. **D** Bisecting GlcNAc on MCAM of BM stroma generates a pro-tumoral niche by exosomal miR-188-5p in malignant clonal cells (conceptual model).

N-glycosylation plays essential roles in protein folding and trafficking, and whole N-glycosylation knockdown or mutation at certain N-glycosylation sites resulted in misfolding, mis-distribution, instability, and/or degradation of such glycoproteins as glucose transporter GLUT4, human tripeptidyl-peptidase I, and dopamine transporter [45–47]. When bisecting GlcNAc level was high, MCAM content in membranes was significantly decreased and localization of MCAM in lysosomes was increased. Bisecting GlcNAc appeared to modulate MCAM translocation to cell membrane, and to induce degradation of MCAM via lysosomal pathways. IP-MS analysis identified Asn 56 on MCAM as the key bisecting GlcNAc-bearing site. Promoting effect on malignant clonal cell proliferation in vitro and in vivo was weaker for glycosylation-deficient N56D mutant of HS5 (HS5-MCAM-Mu) than for HS5-MCAM. Knockdown of N-glycosylation at Asn 56 reduced MCAM localization on cell membrane, indicating that such localization depends on other N-glycan structures not yet identified.

In regard to functional mechanism, we found that MCAM on stromal cell membrane supported malignant clonal cell growth through interaction with the transmembrane aminopeptidase CD13. CD13 is widely expressed in myeloid cells, stromal cells and other cells [48, 49]. It is overexpressed in many tumor cells and plays a key role in tumor angiogenesis, invasion, and metastasis, and also has been associated with multidrug resistance [50]. $CD13^+$ myeloid BM-derived cells (BMDCs) promote angiogenesis and vascular maturation by regulating pericyte recruitment through increased production of MCP-1 and MMP-9 [51]. It was documented that specific CAR-T cells have been developed to target CD13 to eradicate AML cells [49]. We observed that elimination of bisecting GlcNAc on MCAM by Asn 56 mutation, or by bestatin treatment, notably reduced CD13 binding, inactivated ERK signaling, and suppressed myeloid cell growth. The supportive function of stroma on clonal cell growth therefore appears to depend on interaction between MCAM on stromal cells and CD13 on clonal cells.

In conclusion, MDS/AML clonal cells can modify bisecting GlcNAc levels of BM stroma through exosome secretion, thereby promoting malignant clonal cell proliferation and survival, and suppressing normal hematopoiesis (Fig. 6D). Novel therapeutic strategies that restore a healthy BM niche, based on glycosylation modification, may show improved effectiveness against MDS/AML and similar disorders.

DATA AVAILABILITY

The data supporting the conclusions of this article have been given in this article and its additional files.

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AUTHOR CONTRIBUTIONS

XL and FG conceived the study. JF, YW, BL, XY, LL, JW, XZ, QC, YZ, JG, HL, ZT and ZD performed the experiments and data analysis. FG and XL supervised research and wrote the paper. All authors read and approved the final paper.

FUNDING

This study was supported by the National Science Foundation of China (No. 32071274, 82100148, 31971211), Science Foundation for Distinguished Young Scholars of Shaanxi Province (2021JC-39), the Natural Science Foundation of Shaanxi Province (2021SF-294), and the Youth Innovation Team of Shaanxi Universities.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41375-022-01748-1.

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