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Hexosaminidase B-driven cancer cellmacrophage co-dependency promotes glycolysis addiction and tumorigenesis in glioblastoma

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Glycolytic metabolic reprogramming in cancer is regulated by both cancer intrinsic variations like isocitrate dehydrogenase 1 (IDH1) status and noncancerous microenvironment components like tumor associated macrophages (TAMs). However, the detailed mechanism remains elusive. Here, we identify hexosaminidase B (HEXB) as a key regulator for glycolysis in glioblastoma (GBM). HEXB intercellularly manipulates TAMs to promote glycolysis in GBM cells, while intrinsically enhancing cancer cell glycolysis. Mechanistically, HEXB elevation augments tumor HIF1 α protein stability through activating ITGB1/ILK/YAP1; Subsequently, HIF1α promotes HEXB and multiple glycolytic gene transcription in GBM cells. Genetic ablation and pharmacological inhibition of HEXB elicits substantial therapeutic effects in preclinical GBM models, while targeting HEXB doesn't induce significant reduction in IDH1 mutant glioma and inhibiting IDH1 mutation-derived 2hydroxyglutaric acid (2-HG) significantly restores HEXB expression in glioma cells. Our work highlights a HEXB driven TAMs-associated glycolysis-promoting network in GBM and provides clues for developing more effective therapies against it.

As a cancer hallmark, deregulating cellular metabolism contributes to the initiation and progression of malignancies¹. Increasing evidences suggest that cancer metabolic reprogramming is orchestrated by both intrinsic variations and the tumor microenvironment (TME)². Although several intrinsic mechanisms including proliferation-inducing oncogenes have been uncovered in metabolic reprograming³, the interplay between cancer cells and non-tumor TME components remains elusive.

Glioma is the most common primary malignant tumor in adult brain. According to molecular characteristics, gliomas are basically classified into IDH1 wild type and mutant form tumors. As the most common type of IDH1 wild type gliomas, glioblastoma (GBM) is notorious for its nature of invasive growth, which inevitably leads to recurrence and dismal prognosis even after aggressive therapeutic interventions, including extensive surgical resection, chemo- and radio-therapy⁴. The median survival of GBM patients is only 14.6 months, even worse than grade IV IDH1 mutant glioma⁵. Growing evidence suggests that GBM is accompanied by an enhanced energy production potentiality compared with grade IV IDH1 mutant tumor, as represented by the metabolic pattern towards glycolysis⁶. Previously, we identified that GBM featured a complex microenvironment and exhibited lower tumor purity with more abundant non-tumor cell

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infiltration, in which tumor-associated macrophages (TAMs) nearly account for 10-30%^{7,8}, regardless of tumor subtypes⁹. Recently, several pioneering studies discloses that TAMs, especially M2 (anti-inflammatory) TAMs, are involved in promoting aerobic glycolysis in cancer cells^{10,11}. The interplay between TAMs and cancer cells with enhanced glycolytic activity accounts for cancer resistance to current therapeutics. Immune cells like macrophages contribute to regulating the metabolism of glioma cells, while glioma cells drive non-tumor cells to establish tumor microenvironment conducive to their growth. There may be versatile regulators accelerating cancer cell glycolysis through intracellular mechanisms and simultaneously manipulating TAMs to establish a glycolysis-promoting network. However, the understanding on the key modulator with dual role of governing intrinsic and TAMs associated glycolysis promotion remains limited.

Therefore, in present study, we performed integrated analysis in seven GBM cohorts including bulk-tumor and single-cell RNAsequencing data to profile GBM intrinsic secretory protein in TME enhancing cancer cell glycolytic activity through eliciting the interplay between glucose metabolism of GBM cells and TAMs manipulation. The analysis highlights hexosaminidase B (HEXB) as a key regulator for promoting glycolysis in cancer cells via intrinsic and intercellular TAMs-associated mechanisms. Intracellularly, HEXB stabilizes tumor integrin beta-1 (ITGB1)/integrin-linked kinase (ILK) complex, which promotes the activity of yes-associated protein 1 (YAP1)/hypoxia inducible factor 1α (HIF1 α). HIF1 α reinforces transcription of HEXB and multiple glycolytic genes. Intercellularly, HEXB recruits M2-polarized TAMs, which supports the enhanced glycolytic activity in malignant cells and constitutes a TAMs-associated glycolysis-promoting network. Notably, HEXB activity can be ablated by mutant IDH1-derived hydroxyglutaric acid (2-HG) accumulation, indicating this mechanism predominantly exists in IDH1 wild type tumors. The application of HEXB inhibitor, Gal-PUGNAc (Gal-P), efficiently improves the survival of preclinical GBM models and the response to immune checkpoint blockade. Our findings propose that HEXB is a key modulator with promising therapeutic potential for achieving synergistic effects in GBM by inhibiting glycolytic fueling and remodeling TME.

Results

HEXB is an unfavorable indicator which facilitates the regulation of GBM cell glycolysis associated with TAMs

To characterize metabolic promoting signaling in grade IV glioma with divergent IDH1 status, we enrolled grade IV glioma samples from four glioma RNA-seq cohorts (CMU cohort n = 30, CGGA325 cohort n = 139, CGGA693 cohort n = 239, TCGA cohort n = 148; Supplementary Table 1) including 556 cases of grade IV gliomas (IDH1 wild type = 453; IDH1 mutant = 103) for analysis. The single-sample gene set enrichment analysis (ssGSEA) algorithms based on representative gene sets of four major metabolic modes, including glycolysis, oxidative phosphorylation, fatty acid and glutamine metabolism (Supplementary Data 1), were used for evaluating the signaling activation of these metabolic modes in grade IV glioma. Then, glycolysis was identified as the metabolic mode with most diverse activation status between IDH1 wild type glioma (Glioblastoma, GBM) and grade IV IDH1 mutant glioma compared to other three metabolic modes (Fig. 1a). We further validated the existed difference in glycolysis levels between grade IV IDH1 mutant and GBM in both male and female patient groups in CGGA325 and CGGA693 cohorts, which indicates the difference in glycolysis levels caused by IDH1 mutation status independent of sex (Supplementary Fig. 1a-d CMU and TCGA cohorts only include one and two female patients with IDH1 mutation, respectively. Thus, the metabolic capacity of different IDH1 mutation status cannot be statistically determined based on gender). Additionally, we evaluated the sex ratio of glioma patients in the above four glioma datasets and found that the ratio of males to females did not exceed 2:1 (Supplementary Fig. 1e). Meanwhile, we found that sex was not a key factor leading to poor prognosis in glioma patients through univariate Cox (Supplementary Fig. 1f-h) and Kaplan-Meier analysis (Supplementary Fig. 1i-k) (CGGA325, CGGA693 and TCGA cohorts; Since the survival information of CMU RNA-seq dataset in Fig. 1a isn't available currently, this analysis didn't include CMU RNA-seq dataset). Subsequently, we compared the correlation between glycolysis activation and the infiltration of macrophages represented by the above-calculated glycolysis score and macrophage scores based on Timer¹² and Xcell algorithms, respectively. We found that the upregulation of glycolysis score was significantly positively correlated with the elevation of macrophage scores. This indicates the increased infiltration of macrophages accompanying with enhanced glycolysis in GBM (Fig. 1b) and suggests the important role of tumor associated macrophages (TAMs) in promoting the glycolytic activity of GBM. Then, we investigated the function and regulatory process of glycolysis in the GBM microenvironment and compared the glycolysis activation status of malignant cells and TAMs in three single-cell RNA-sequencing GBM dataset (scRNA-seq datasets: GSE117891, GSE131928, and GSE139448) with the glycolysis score. We found that GBM cells had a significantly higher glycolysis score than TAMs (Supplementary Fig. 2a-c), which indicated that glycolysis may be more activated in malignant cells than TAMs in GBM.

Next, we sought to screen the crucial regulator in GBM facilitating cancer cell glycolysis associated with TAMs, which is not only expressed by both of GBM cells and TAMs, but also could potentially exhibit its function as secretory protein mediating the crosstalk between GBM cells and TAMs (Fig. 1c). Firstly, we examined differentially expressed genes (DEGs) between GBM and IDH1 mutant grade IV glioma in the above four glioma cohorts, respectively (CMU, CGGA325, CGGA693 and TCGA; Step 1). A total of 54 gene candidates (DEG1, Supplementary Data 2) were obtained from the intersection of these 4 datasets, which were significantly overexpressed in GBM. Secondly, we intersected DEG1 with a characteristic gene list of TAMs summarized from monocyte to macrophage transformation (Step 2) and M2 versus M1 macrophages (Step 3), both of which based on DEG analysis (DEG2, Supplementary Data 2) of a macrophage maturation and polarization dataset (GSE5099). The intersection of DEG1 (n = 54) and DEG2 (n = 381) resulted in 3 genes (SIPA1L1, GUSB and HEXB, Supplementary Data 2) (Step 4). Thirdly, given that the critical role of cancer cellmacrophage crosstalk in driving GBM cells glycolysis addiction and macrophages transformation, we sought to examine the regulator with elevation in both of GBM cells and TAMs and potentially involved in driving glycolysis addiction and tumorigenesis through inter- and intra-cellular mechanisms^{11,13}. We intersected these 3 genes with a glioma intrinsic gene list (Step 5 Trait 1)¹⁴ and a secretory protein related gene list (Step 5 Trait 2)¹⁵ (Supplementary Data 2). There were two candidates (GUSB and HEXB) meeting this criterion. Then, through prognostic analysis in CGGA and TCGA, we found that HEXB showed a stronger correlation with poor prognosis of GBM and glioma patients compared to CUSB (Step 5 Trait 3, Supplementary Table 2 and Supplementary Fig. 3), and its elevation served as a risk factor for GBM survival independent of sex (Supplementary Fig. 4). Finally, we examined three single-cell RNA-seq GBM datasets, which demonstrated HEXB highly-expressed in both of malignant cells and TAMs in GBM (GSE117891, GSE131928, and GSE139448; Step 5 Trait 4), while its expression was significantly upregulated in GBM compared to grade IV IDH1 mutant glioma (Supplementary Fig. 5). Therefore, we chose HEXB as the research target, which may serve as a potential determinant for TAMs-enhanced malignant cell glycolysis in GBM (Fig. 1c). Actually, HEXB was significantly overexpressed in IDH1 wild type glioma (Fig. 1d and Supplementary Fig. 5a-d), and the glioma cells transfected with IDH1 R132H mutant vector, instead of IDH1 D252G mutant vector, efficiently reduced the expression of HEXB (Fig. 1e). Given that IDH1 R132 mutation could lead to 2-HG accumulation in glioma TME¹⁶, we administered AGI, an inhibitor of 2-HG, to glioma cells transfected with



control or IDH1 R132H mutant vector to further clarify whether HEXB downregulation was caused by 2-HG accumulation induced by IDH1 R132H mutation. We found that inhibiting IDH1 mutation-derived 2-hydroxyglutaric acid (2-HG) significantly restored HEXB expression in IDH1 mutant glioma cells (Fig. 1e). This indicates a potential HEXB reduction mechanism associated with metabolites induced by IDH1

mutation. Meanwhile, molecular subgroup analysis showed that HEXB was significantly overexpressed in mesenchymal subtype of glioma, which mostly exhibited low purity of tumor tissues and enriched with macrophages (Supplementary Fig. 5g,h). Additionally, according to integrated analysis in single-cell RNA-seq cohorts, HEXB was mainly expressed in GBM cells as well as macrophages (Supplementary

Fig. 1 | Comprehensive profiling discloses HEXB as a key intrinsic secretary protein-coding gene in IDH1 wild type glioma associated with cancer cell gly-colytic activity and TAMs manipulation. a The analysis of metabolic difference between IDH1 wild type and IDH1 mutant grade IV glioma (CMU n = 30; CGGA325 n = 139; CGGA693 n = 239; TCGA n = 148; circle marked black indicates statistical difference). b The correlation analysis between macrophage ratio calculated by TIMER2.0 (https://timer.cistrome.org) and Xcell (https://aran-lab.com/software/xcell/) algorithms, respectively, and glycolysis score established by ssGSEA method and corresponding gene set downloaded from Gene Set Enrichment Analysis website (GSEA, http://www.broadinstitute.org/gsea/index.jsp) in indicated GBM cohorts. c The filtering flow diagram showing the profiling of potential micro-environment related glycolytic targets in indicated GBM cohorts, which identified HEXB as the potential secretory target and malignant and macrophage co-expressed gene in IDH1 wild type glioma. Figure 1c were created with BioR-ender.com released under a Creative Commons Attribution-NonCommercial-

Fig. 5i-k). This was supported by multiple-color immunofluorescence analysis in GBM samples, in which HEXB staining was overlapped with glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (IBA1). Additionally, the co-expression ratios of HEXB with CCR2 (specific marker of peripheral blood macrophages) and CD163 (M2 marker) were higher than CX3CR1 (microglia marker) and CD86 (M1 marker), respectively (Fig. 1f). These data indicate HEXB as a common protein expressed by both of GBM cells and TAMs, especially M2 TAMs, while GBM cells demonstrate a higher HEXB positive cell ratio than TAMs (Fig. 1f).

Then, qPCR and ELISA analysis in cell line samples demonstrated an elevation of HEXB mRNA and secretion in GBM cells and macrophages than other cell components (Fig. 1g). Besides, according to previous reports^{17,18}, the *HEXA* and *HEXB* gene separately encoded the α and β subunit of hexosaminidase and HEXA protease was heterodimer complex composed of α - β subunits, in contrast to HEXB protease, which was homodimer composed of β - β subunits. The immunohistochemical detection of HEXA and HEXB protein in glioma tissue samples disclosed that the expression of HEXB protein in GBM was significantly higher than grade IV IDH1 mutant glioma (Fig. 1d and Supplementary Data 3), while the expression of HEXA protein had no significant difference between GBM and grade IV IDH1 mutant glioma (Supplementary Fig. 6). This supported HEXB homodimer as the main form of hexosaminidases in GBM. In addition, we conducted the intervention and phenotypic detection of HEXA with the application of recombinant HEXA protein and HEXA knockdown. We observed that HEXA knockdown and the administration of recombinant HEXA protein had no significant regulatory effect on the lactate production and proliferation of GBM cells (Supplementary Fig. 7). Therefore, it is reasonable to infer that hexosaminidase mainly exerts its tumor promoting effect in GBM through its homodimer form, HEXB. Together, these findings suggest that HEXB is a secretion protein expressed by both of GBM cells and TAMs, and its overexpression is an unfavorable indicator for GBM survival and may contribute to GBM cells glycolysis regulation associated with TAMs.

HEXB is necessary for cancer cell glycolysis and tumorigenicity in GBM

Considering the driving role of malignant cells in constituting TME and the proportion of HEXB positive cells in GBM cells than macrophages, we next investigated the effect of cancer cell intrinsic HEXB elevation in glycolytic activity and tumorigenicity with three primary GBM sphere cell lines (GSC1, GSC21 and GSC63). Indeed, glucose consumption and lactate production in GBM cells were severely decreased in HEXBsilenced cells (Fig. 2a, b and Supplementary Fig. 8a–d). Meanwhile, according to Seahorse analysis, genetic ablation of HEXB and its pharmacological inhibition by Gal·P¹⁹ (An inhibitor of HEXB, suppressing its enzyme activity, Supplementary Fig. 8e–g) significantly attenuated the glycolytic rate of GBM cells (Fig. 2c–e and Supplementary NoDerivs 4.0 International license. **d** Representative IHC images (Left) and analysis (Right) of HEXB staining in clinical grade IV IDH1 wild type (GBM) and mutant glioma tissue samples (n = 32, including IDH wild type n = 16 and IDH mutant n = 16). **e** Western blotting analysis of HEXB in indicated GSC1 cells (Upper panel: GSC1 transfected with IDH1(WT), negative control, IDH1(R132H) or IDH1(R132H/D252G) mutant vectors, respectively; lower panel: GSC1 with or without the transfection of R132H vectors, and then treated with indicated concentration of 2-HG inhibitor AGI. **f** Representative multi-color immunofluorescence co-staining images (Left and middle) and analysis (Right) of HEXB, IBA1, CCR2, CX3CR1, CD86, CD163, and GFAP in clinical GBM tissue samples (n = 3 independent samples). **g** The analysis of HEXB expression and secretion in indicated cell samples by qPCR (Left) and ELISA (Right) (n = 3 independent samples). Data are from 3 (**f**) or representative of 3 independent experiments with similar results (**d**, **e**, **g**). Data were presented as mean ± SEM. Statistical analyzes were determined by Pearson's correlation test (b), two-tailed Student's t-test (**d**, **f**). Source data are provided as a Source Data file.

Fig. 9a-d). Conversely, treatment with recombinant human HEXB (rhHEXB) enhanced the reduced glycolytic activity in GSCs induced by HEXB disruption (Supplementary Fig. 9e-h). Considering the important role of glycolysis in malignant cell tumorigenicity, we examined cell proliferation in the above primary GBM cells after HEXB modulation. Silencing HEXB by shRNA significantly decreased the proliferation of GBM cells in vitro (Supplementary Fig. 10a) and their growth in vivo (Fig. 2f, g), and HEXB knockdown did not significantly affect their apoptosis (Supplementary Fig. 10b,c), indicating this effect of HEXB on tumor growth potentially dependent on regulating glucose metabolism in GBM cells, rather than by inducing cell apoptosis. The supplement of pyruvate to mimic the glycolytic effects reversed the growthinhibiting effects of silencing HEXB in GBM cells (Supplementary Fig. 10d). Then, we used homologous and heterologous orthotopic xenograft mice model to evaluate the role of cancer cell intrinsic HEXB in promoting the tumorigenicity of GBM cells. The mice transplanted with HEXB-silenced GBM cells showed delayed tumor formation and prolonged survivals compared to control groups (Fig. 2f, g). The administration of Gal-P efficiently reduced the in vivo tumorigenicity of GSCs obtained from human GBM samples and mGSCs derived from mice spontaneous GBM tumor tissues established by sleeping beauty (SB) transposon techniques (Supplementary Fig. 10e). Altogether, these results indicate that HEXB is a key modulator for GBM cell glycolysis and is involved in the regulation of GBM cell proliferation by enhancing their glycolytic activity.

HEXB facilitates glycolysis by activating YAP1 nuclear translocation

Next, to explore the mechanism of HEXB regulating GBM cell glycolysis, we transcriptionally profiled endogenous HEXB-silenced GSC1, and performed enrichment analysis based on down-regulated genes positively related to HEXB knockdown. As shown in Fig. 3a, the top gene terms enriched with HEXB knockdown included response to glucose, integrin mediated signaling pathway, leukocyte migration (Fig. 3a, Supplementary Data 4) and YAP1-conserved signature (Fig. 3b). Due to the well-established role of YAP1 in cancer glucose metabolism and integrins signaling^{20,21}, we sought to examine whether cancer intrinsic HEXB regulated GBM cell glycolysis through activating YAP1 signaling. IHC analysis disclosed that HEXB was positively correlated with YAP1 expression in GBM tissues (Fig. 3c). HEXB knockdown resulted in a significant reduction of YAP1 expression (Fig. 3d), while the supplement of exogenous HEXB facilitated the nuclear translocation of YAP1 in shHEXB GSC1 (Fig. 3e). It has been reported that phosphorylation of YAP1 by its major upstream signaling Hippo pathway leads to its sequestration in the cytoplasm and ubiquitination-dependent proteasomal degradation, while nonphosphorylated YAP1 is able to enter the nucleus to serve as a coactivator facilitating gene transcription. Consistent with this observation, inhibition of HEXB with Gal-P treatment in GBM cells induced



enhanced LATS1 phosphorylation, a core kinase of Hippo signaling pathway, while reduced total YAP1 and elevated p-YAP1 (Fig. 3f). In contrast, the supplement of recombinant human HEXB (rhHEXB) efficiently decreased phosphorylation of LATS1 and YAP1, while elevating total YAP1 (Fig. 3g). Then, we used a YAP-5SA mutant plasmid to mimic constitutively active YAP1 and found that YAP-5SA transduction efficiently counteracted the inhibition of GBM cell glycolytic activity and proliferation induced by Gal-P (Fig. 3h and Supplementary Fig. 11a–d). In contrast, a selective YAP1 inhibitor, verteporfin, remarkably abolished the enhancement of GBM glycolysis and proliferation induced by rhHEXB treatment (Fig. 3i and Supplementary Fig. 11e–h). Together, these findings suggested YAP1 Fig. 2 | HEXB serves as a key regulator enhancing glycolytic activity in

GBM cells. a, **b** The analysis of glucose consumption (**a**, n = 3 independent samples) and lactate production (**b**, n = 3 independent samples) in GSC1 (Left), GSC21 (Middle) and GSC63 (Right) samples transduced with indicated HEXB-silencing or control vectors, respectively. **c**-**e** The analysis of glycolytic rate represented by extracellular acidification rate (ECAR) of GSC1 (**c**), GSC21 (**d**) and GSC63 (**e**) samples transfected with indicated HEXB knockdown or control vector (Left), or treated with Gal-P or DMSO (Right) (n = 3-5 independent samples as indicated in the source

as the major downstream mediator of HEXB to accelerate GBM cell glycolysis and proliferation.

HEXB promotes YAP1 activation in GBM cells by stabilizing ITGB1/ILK complex

HEXB could promote the transformation of GM2 ganglioside to GM3 ganglioside. Previously, it has been reported that GM3 ganglioside serves as a protective factor for glioma and inhibits proliferation and invasion of glioma cells²². Similarly, we observed that the treatment of sodium ganglioside GM3 (50 µg/ml and 100 µg/ml) on GBM cells didn't significantly promote the proliferation of GSCs (Supplementary Fig. 12a). Meanwhile, a previous report²³ demonstrated that GM2 activator (GM2A) was the cofactor of HEXB and directly involved in the transformation and metabolism of ganglioside GM2 to GM3. Thus, we knocked down GM2A with siRNA in GBM cells and observed that the proliferation and lactate production of GSCs were not significantly affected by siGM2A (Supplementary Fig. 12b-d). These observations support that HEXB facilitate the development of GBM independent of its end-product, GM3. Moreover, the tumor promoting effect of HEXB was significantly restrained by the application of HEXB inhibitor Gal-P, and the activation effect of HEXB on glycolysis process and downstream signal axis was significantly weakened after the inhibition of its enzymatic activity by Gal-P (Fig. 2c-e and Fig. 3f). The above results support that the tumor promoting effect and downstream pathway activation of HEXB depends on its enzyme activity and independent of its end-products GM3.

Next, to further illustrate the mechanism of HEXB mediated YAP1 activation in GBM cells, immunoprecipitation-mass spectrometry (IP-MS) was employed to identify potential binding partners of HEXB. Then, membrane receptors integrin beta-1 (ITGB1) and integrin-linked kinase (ILK) was revealed to bind with HEXB (Fig. 4a, Supplementary Data 5). Additionally, after treating GSCs such as GSC1 and GSC21 with GST-tagged HEXB recombinant protein for 48 h, we fixed the cells and performed immunofluorescence detection, presenting that GST-HEXB was mainly localized on the cell membrane (Supplementary Fig. 13a). Substantial evidences highlight ILK as an integrin-bound signaling transmitter promoting YAP1 nuclear accumulation²⁴⁻²⁶. Therefore, it was reasonable to assume that ITGB1 and ILK formed a transmembrane protein complex involved in HEXB-derived YAP1 activation. We confirmed the direct binding of HEXB with ITGB1 and ILK and forming a protein complex by performing co-IP and glutathione S-transferase (GST) pull-down assays (Fig. 4b-d). Then, according to structural informatics simulations to identify potential binding sites of HEXB to ITGB1 (Fig. 4c), we constructed five truncated mutant plasmids and one wild-type GST-tagged plasmid (Fig. 4e). HEXB A2 mutant (81-200aa deletion) protein was found to lose the binding ability with ITGB1 (Fig. 4e). As the enzyme active center (asp196) of HEXB located in 81-200aa fragment and considering the obvious inhibition effect of Gal-P on HEXB enzyme activity, we hypothesized that the enzyme domain (asp196) of HEXB was involved in its interaction with ITGB1. Thus, we constructed a GST-tagged HEXB plasmid with asp196 deletion. GST pull-down assay disclosed a significantly decreased binding ability of HEXB with ITGB1 induced by asp196 mutation (Fig. 4f), indicating that the enzyme domain of HEXB was crucial to maintain the binding relationship between HEXB and ITGB1.

Interfering with HEXB did not affect ITGB1 or ILK transcription (Fig. 4g), and 100 ng/ml cycloheximide treatment significantly

data file). **f** Survival curves of indicated GSC1 (Left), GSC21 (Middle) and GSC63 (Right) orthotopic models (n = 6, log-rank analysis). **g** Representative in vivo bioluminescence imaging (Upper) and H&E staining (Lower) images demonstrating the tumor size in indicated mice orthotopic models. Data are representative of 3 independent experiments with similar results (a-g). Data were presented as mean ± SEM. Statistical analyzes were determined by two-tailed Student's t-test (**a**, **b**) and log-rank test (**f**). Source data are provided as a Source Data file.

accelerated the degradation of ITGB1/ILK in HEXB-knockdown cells (Fig. 4h). These observations indicate the key role of HEXB in maintaining ITGB1/ILK complex stability. In addition, the supplement of rhHEXB to HEXB-knockdown cells significantly restored the accelerated degradation of ITGB1/ILK complex induced by HEXB knockdown (Fig. 4i). In contrast, supplementation of purified recombinant HEXB protein with asp196 mutation or deletion of 81-200aa did not restore ITGB1/ILK degradation (Supplementary Fig. 13b). HEXB silencing induced ITGB1/ ILK protein degradation was remarkably reduced by treatment with chloroquine (CQ) or 3-methyladenine (3-MA), instead of MG132 (Fig. 4j), and HEXB mainly influenced K63-dependent ubiquitination of ITGB1 (Supplementary Fig 13c,d). Additionally, since HEXB functioned through interacting with ITGB1, we applied ITGB1 neutralizing antibody on the basis of administering HEXB recombinant protein. Indeed, with ITGB1 blockade, the effect of HEXB recombinant protein on the downstream YAP1-HIF1 α signaling axis activation in GBM cells also disappeared (Fig. 4k). Meanwhile, the lactate production and glycolytic phenotype of GBM cells were significantly attenuated (Fig. 4l, m). Moreover, to verify the important role of ITGB1/ILK complex in the HEXB-mediated aggressive GBM phenotype, we conducted a series of rescue experiments. As expected, treatment with HEXB exerted marginal influence on GBM glycolysis and proliferation without abundant ITGB1 or ILK expression (Supplementary Fig. 13e-h). Additionally, the enhanced YAP1 activity induced by HEXB was abolished after silencing ITGB1 and ILK, respectively (Supplementary Fig. 13i,i). Altogether, these results indicate that ITGB1/ILK is required for HEXB to enhance YAP1 activity in GBM cells, and HEXB binds and protects the ITGB1/ILK complex from lysosome-dependent degradation.

The YAP1/HIF1 α axis transcriptionally regulates HEXB to form a positive feedback loop

Feedback response²⁷ is a common mechanism for cross-talk among effector pathways utilized by cancer cells to promote their malignant behaviors during cancer progression. Subsequently, we investigated whether feedback mechanism was involved in the regulation of GBM cell glycolysis and tumorigenicity induced by HEXB mediated YAP1 activation. Exogenous administration of HEXB recombinant protein to HEXB-knockdown GBM cells up-regulated both of HEXB mRNA and protein level, and Gal-P treatment efficiently reduced it (Fig. 5a-d and Supplementary Fig. 14a). It was reported that YAP1 could bind with HIF1a to maintain its stability and further activate transcription of downstream oncogenic glycolytic genes (like PKM2) to enhance glycolytic activity²⁸. Since YAP1 served as a co-transcription factor for the classical driver of cancer glycolysis-HIF1 α , we then sought to examine whether exogenous HEXB could activate YAP1/HIF1 α to facilitate endogenous HEXB transcription. Genetic inhibition of HEXB in GBM cells induced significant reduction of HIF1α and YAP1 (Fig. 5e). Co-IP assay in GSC1 confirmed the binding between YAP1 and HIF1 α (Fig. 5f). The stability of HIF1 α protein was weakened when the cells were treated with YAP1 inhibitor, verteporfin (Fig. 5g). The expression and stability of HIF1 α were significantly reduced in HEXB knockdown GSC1 sample (Fig. 5h). To determine whether HIF1 α mediated the oncogenic effects of HEXB, we performed rescue experiments. The pro-glycolytic effects of exogenous HEXB stimulation were significantly weakened in HIF1α knockdown GBM cells (Supplementary Fig. 14b–f). This supported HIF1 α as the main downstream effector for



HEXB mediated glycolytic activity regulation in GBM cells. Exogenous administration of HEXB recombinant protein significantly upregulated the transcriptional activity of HIF1 α . Administration of a HEXB inhibitor or endogenous knockdown of HEXB significantly attenuated the transcriptional activity of HIF1 α (Fig. 5i, j). In addition, knockdown of ITGB1 or ILK alone efficiently attenuated the transcriptional activity of HIF1 α .

and administration of HEXB recombinant protein in this situation failed to activate HIF1 α transcriptional activity (Supplementary Fig. 14g,h). This supported that HEXB activated YAP1 through ITGB1/ILK complex. Then we knocked down the individual nodes of the ITGB1/ILK/YAP1 axis in GSC1 cells. The suppression of each node significantly decreased HEXB transcription, while HIF1 α overexpression or transfection of YAP-

Fig. 3 | HEXB facilitates glycolysis by promoting YAP1 nuclear localization. a Enrichment analysis (Upper: Bubble diagram of HEXB related functional enrichment analysis: Lower: the description of GO term information) of downregulated genes associated with HEXB-knockdown according to RNA sequencing data from shHEXB GSC1 sample in comparison to the data obtained from control GSC1 sample (n = 3, hypergeometric test). **b** Heatmap showing the relative expression pattern of YAP1-conserved signature genes after HEXB knockdown (GSC1 shNC vs. shHEXB samples). c Representative IHC images (Left) and correlation analysis (Right, n = 16, Pearson's correlation analysis) of HEXB and YAP1 staining in GBM tissue samples. d Western blotting analysis of YAP1 in indicated HEXB silenced and control GBM cell samples (The samples derive from the same experiment but different gels for HEXB, YAP1, and β-actin were processed in parallel). e Immunocytochemical analysis showing the translocation of YAP1 after exogenous supplement of HEXB recombinant protein in shHEXB GSC1 cells. f Western blotting analysis of indicated proteins related to Hippo signaling pathway in GBM cells treated with indicated doses of HEXB inhibitor Gal-P (Left: GSC1 middle: GSC21, and right: GSC63). (The

SSA plasmid in GBM cells significantly increased their HEXB expression (Fig. 5k and Supplementary Fig. 14i–l). Moreover, the analysis by JASPAR and PROMO confirmed there were multiple potential binding motifs of HIF1 α in the promoter region of *HEXB* gene. Therefore, we hypothesized that HIF1 α may activate *HEXB* transcription. The subsequent chromatin immunoprecipitation (ChIP) experiments in GSC1 and HEK293T cells showed that HIF1 α could bind the 215–236 base region of HEXB promoter (Fig. 5I and Supplementary Fig. 14m).

Previously, the accumulation of 2-HG caused by IDH1 mutation has been reported²⁹ to weaken the activity of HIF prolyl hydroxylases and then cause the accumulation of HIF1 α protein. This indicates a potential more activated status of HIF1 α in IDH1 mutant glioma. In contrast, there are studies revealing that the activation of HIF1 α signaling is not principally regulated by mutant IDH1, but more likely via other recognized mechanisms like hypoxia, transcriptional regulation, and major genetic and epigenetic alterations induced by gain-offunction or loss-of-function mutations³⁰. In addition, 2-HG may cause the degradation of HIF1 α through stimulating Prolyl-hydroxylase domain (PHD) activity³¹. Considering the critical role of HIF1 α on inducing HEXB expression in GBM, we sought to examine the activation status of HIF1 α in glioma with different IDH1 status. We firstly collected the downstream target genes of HIF1a and established a HIF1α score through ssGSEA method to evaluate the activation status of HIF1α signaling pathway in glioma, according to CMU, CGGA and TCGA glioma datasets (Supplementary Fig. 14n). The results demonstrate a consistent significant elevation of HIF1α score in IDH1 wild type glioma than IDH1 mutant tumor, which implicates a higher activation of HIF1α signaling pathway in IDH1 wild type glioma. Secondly, for detecting the HIF transcriptional activity, a dedicated HIF1a transcriptional activity assay kit (Cayman, 10006910) was used for measuring the HIF transcriptional activity, which demonstrated that the transcriptional activity of HIF1a in IDH1 wild type GSC1 and mGSCs were significantly higher than that in GSC1 transfected with IDH1 mutant vector and IDH1 mutant mGSCs, respectively (Supplementary Fig. 14o). Thirdly, disrupting HEXB in IDH1 wild type GBM cells efficiently restrained the expression of PKM2 and HK2, two HIF1a downstream targets $^{\rm 32-36}$, which could be rescued by the supplement of HIF1 α activator, CoCL2 (Supplementary Fig. 14p). These data support a higher activation of HIF1 α in IDH1 wild type GBM cells than IDH1 mutant glioma cells. Collectively, the above results suggested that HIF1α could activate HEXB transcription, while HEXB promoted HIF1α stability via the ITGB1/ILK/YAP1 axis, thus forming a positive feedback regulatory loop in GBM cells (Fig. 5m).

HEXB promotes GBM malignancy through facilitating the reeducation of macrophages

Due to the prominence of M2 macrophages to regulate cancer cell glycolysis and to validate the pro-glycolytic effect of M2-polarized

samples derive from the same experiment but different gels for LATS1, P-LATS1, YAP1, P-YAP1, and β-actin were processed in parallel). g Western blotting analysis of indicated proteins related to Hippo signaling pathway in indicated GBM Cell samples transduced with shHEXB or sgHEXB vector, and then treated with indicated doses of HEXB recombinant protein (Left: GSC1 middle: GSC21, and right: GSC63). (The samples derive from the same experiment but different gels for LATS1, P-LATS1, YAP1, P-YAP1, and β -actin were processed in parallel). **h** The analysis of glycolytic rate represented by extracellular acidification rate (ECAR) in indicated GBM cells transduced with YAP-5SA or control vector, and then with Gal-P administration (n = 3-5 independent samples as indicated in the source data file). i The analysis of glycolytic rate represented by ECAR in indicated GBM cells with YAP1 inhibitor verteporfin pre-treatment, and then with HEXB recombinant protein administration (n = 3-5 independent samples as indicated in the source data file). Data are representative of 3 independent experiments with similar results (c-i). Data were presented as mean ± SEM. Statistical analyzes were determined by Pearson's correlation test (c). Source data are provided as a Source Data file.

macrophages on GBM cells, we employed THP1-derived monocytes to establish TAMs model and co-cultured with GBM cells as previously described³⁷. After 48 h co-cultured with THP1-derived M2 macrophages, GBM cells exhibited significantly enhanced glycolytic activity compared to other co-cultured groups (Supplementary Fig. 15a-d). HEXB, which is regarded as a marker of monocytes^{38,39}, has not been reported to be involved in the function and phenotype regulation of macrophages. Therefore, we further investigated whether HEXB was involved in regulating the functional phenotype of macrophages. Firstly, our inhouse cohort demonstrated that HEXB elevation was accompanied with TAMs, especially M2 TAMs (Fig. 6a). Similarly, the data obtained from TCGA indicated that the glioma samples with HEXB elevation exhibited a distinct immune cell composition compared to tumor samples with reduced HEXB expression, especially M2 macrophages showing the most significant differences (Supplementary Fig. 15e). This was supported by IHC analysis in GBM samples, which revealed the positive correlation and co-localization of HEXB with M2 macrophage marker, CD163 (Fig. 6b and Supplementary Data 3). In contrast, the expression of M1 macrophage marker CD86 in IDH1 wild type glioma (GBM) was low and didn't show significant correlation with the expression of HEXB (Supplementary Fig. 15f). Furthermore, considering the close relation of HEXB elevation to upregulated TAMs ratio in GBM (Fig. 1b, Fig. 6a and Supplementary Fig. 15e), we next sought to test whether HEXB was involved in promoting the infiltration and phenotype transition of macrophages in GBM TME and whether HEXB-targeted strategy could effectively restrain the recruitment of M2 TAMs. Exogenous HEXB could significantly promote the chemotaxis of THP1-derived macrophages, and adding Gal-P under exogenous HEXB stimulation significantly weakened the chemotaxis ability of macrophages (Fig. 6c). Exogenous HEXB significantly reversed the induced M1 polarization process (Fig. 6d and Supplementary Fig. 15g), while treatment with Gal-P robustly inhibited M2 polarization of macrophages (Fig. 6e and Supplementary Fig. 15g). Additionally, analysis in an expression cohort from macrophage maturation and polarization experiment (GSE5099) disclosed that the endogenous expression of HEXB increased during the process of macrophage polarization to the M2 phenotype (Supplementary Fig. 15h). Genetic ablation of HEXB significantly attenuated the infiltration of IBA1⁺ macrophages in orthotopic xenograft derived from immunecompetent (transplanted with SB mGSCs and GL261 mice GBM cells) and immune-incompetent mice models (transplanted with human primary GSCs) (Fig. 6f). After Gal-P treatment, we observed more MHCII and lower CD206 positive cells in mice tumor samples (Fig. 6g and Supplementary Fig. 15i). Moreover, we explored the potential molecular mechanism by which HEXB recruited macrophages to tumor tissues. IP experiment disclosed that ITGB1 could also be used as the membrane receptor of HEXB in macrophages (Supplementary Fig. 15i), and the application of ITGB1 neutralizing antibody could



significantly weaken the macrophage chemotactic effect of HEXB recombinant protein (Supplementary Fig. 15k). These results support that HEXB recruit macrophages through ITGB1 receptor on macrophages. Then, assays in THP1 and PBMC-derived macrophages revealed that the expression and secretion of HEXB in M2 macrophages were significantly upregulated (Fig. 6h–j). Besides, the analysis of glycolytic rate (ECAR) in HEXB ablation GBM cells with treatment of conditioned medium (CM) obtained from THP1-derived M2 macrophages combined with or without HEXB inhibitor Gal-P treatment

implicated that the enhanced glycolytic activities of GBM cells associated with the incubation of CM from M2 macrophages could be disrupted by the supplement of Gal-P (Supplementary Fig. 15l). Thus, we speculate that HEXB facilitates the establishment of feedback between malignant cells and M2 macrophages in GBM TME. Next, we used intracranial co-transplantation model to verify this intercellular positive feedback between cancer cells and macrophages. Indeed, tumor growth was accelerated in GSC1-implanted and macrophage coinjection group compared with the control group. Meanwhile, mice Fig. 4 | HEXB promotes YAP1 activation by stabilizing the ITGB1/ILK complex in GBM cells. a The flow diagram of IP-MS using GSC1 cell lysates precipitated by HEXB antibody. Figure 4a were created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license. b Co-IP analysis of the binding relationship between HEXB and ITGB1/ILK. (The samples derive from the same experiment but different gels for ITGB1, ILK, and HEXB were processed in parallel). c 3D molecular simulation docking between HEXB and ITGB1 based on Swiss-model and HDOCK software. d In vitro GST pull down experiment conducted to validate the direct binding relationship between HEXB and ITGB1. e In vitro GST pull down assay showing the binding relationship between ITGB1 with indicated HEXB fragments (Δ 1 mutant: 2-80aa deletion, Δ 2 mutant: 81-200aa deletion, Δ3 mutant: 201-350aa deletion, Δ4 mutant: 351-500aa deletion. $\Delta 5$ mutant: 501-556aa deletion). **f** In vitro GST pull down assay of showing the binding relationship between ITGB1 and GST-tagged HEXB (wild type or mutant HEXB with asp196 deletion, respectively). g The qPCR analysis of ITGB1 mRNA expression in GSC1 transduced with indicated HEXB knockdown or control vector (n = 3 independent samples). **h**-**j** Western blotting analysis of the degradation rates of ITGB1/ILK in indicated GBM cell samples with CHX treatment (100 ng/ml) (h: GSC1 transduced with HEXB knockdown or control vector, respectively; i: GSC1

transduced with HEXB knockdown vector and then treated with exogenous HEXB recombinant protein or BSA, respectively; j: GSC1 transduced with HEXB knockdown or control vector, respectively, and pretreated with MG132, CQ or 3-MA, respectively; n = 3 independent samples). (The samples derive from the same experiment but different gels for ITGB1, ILK, and β -actin were processed in parallel). k Western blotting analysis of indicated proteins related to Hippo signaling pathway in indicated GBM cell samples transduced with shHEXB or sgHEXB vector, and then treated with HEXB recombinant protein or ITGB1 neutralizing antibody. (The samples derive from the same experiment but different gels for LATS1, P-LATS1, YAP1, P-YAP1, and β -actin were processed in parallel). I The analysis of lactate production in indicated GBM cell samples transduced with shHEXB or sgHEXB vector, and then treated with HEXB recombinant protein or ITGB1 neutralizing antibody (n = 3 independent samples). **m** The analysis of glycolytic rate (ECAR) of indicated GBM cell samples (GSC63) transduced with sgHEXB vector and then treated with HEXB recombinant protein or ITGB1 neutralizing antibody (n = 3)independent samples). Data are from 3 (j, right) or representative of 3 independent experiments with similar results (b, d, e-m). Data were presented as mean ± SEM. Statistical analyzes were determined by two-tailed Student's t-test (g, j, l). Source data are provided as a Source Data file.

implanted with GSC1 cells and co-injected with HEXB pre-treated macrophages demonstrated the fastest tumor growth compared with other groups, while Gal-P significantly impeded tumor progression (Fig. 6k). Then, we performed lenti-viral mediated Hexb knockdown in mGSCs. mGSCs with Hexb knockdown or negative control were intracerebrally implanted in C57BL/6 mice. Survival analysis revealed that Hexb knockdown (Supplementary Fig. 16a) significantly prolonged mice survival (Supplementary Fig. 16b). Immunohistochemical staining also showed that the ratio of CD163 and IBA1 positive cells were significantly reduced by genetic inhibition of Hexb in mGSCs, which indicated macrophage infiltration, especially M2 macrophage infiltration, was restrained with Hexb knockdown in mGSCs (Supplementary Fig. 16c,d). Together, these results support the promoting role of HEXB in the progression of GBM through regulating the chemotaxis of macrophages and M2 polarization in immunocompetent mice.

Targeted intervention of HEXB is more effective in IDH1 wild type patients

Finally, we sought to clarify the reason why IDH1 mutant glioma show reduced HEXB expression. The promoter methylation analysis disclosed a significant lower methylation level of the HEXB promoter in IDH1 wild type patients than IDH1 mutant tumors (Supplementary Fig. 17a). In addition, we conducted western blotting analysis with SB mGSC samples with different IDH1 status and found IDH1 R132H mutant cells showing a lower HEXB expression compared to IDH1 wild type mGSCs (Supplementary Fig. 17b). Moreover, we found that HEXB elevation exhibited prognostic value only in IDH1 wild type gliomas in TCGA cohort (Supplementary Fig. 17c,d). Based on the above results, we hypothesized that Gal-P inhibitor therapy for HEXB may be more effective in IDH1 wild type gliomas with HEXB elevation. Indeed, through in vitro and in vivo experiments including seahorse metabolic analysis, cell viability test and intracranial mice model assays, we confirmed that HEXB-targeted therapy with Gal-P was more effective in IDH1 wild type glioma cells and showed more efficient growth inhibition on the tumors derived from these cells (Fig. 7a-d and Supplementary Fig. 17e-h). We also observed that Gal-P treatment not only significantly restrained the staining intensities of ITGB1, ILK, YAP1 and HIF1 α in IDH1 wild type tumor samples derived from GSC1, but also efficiently reduced the staining intensities of macrophage (CCR2 and CX3CR1) and glycolytic markers (GLUT3 and HK2) in these samples (Supplementary Fig. 17i,j). Meanwhile, HEXB up-regulation exhibited a robust survival predictor value only in IDH1 wild type glioma (Fig. 7e, f and supplementary Fig. 18). Moreover, we found that targeting HEXB could significantly improve the effectiveness of anti-PD1 or CTLA4 immunotherapy (Fig. 7g, h). These results suggested a potential clinical application of HEXB-targeted therapy in IDH1 wild type glioma. Together, our study illuminates the role of HEXB in aberrant glucose metabolism and immunosuppressive microenvironment in GBM and brings a new target for developing an effective combination therapy (Fig. 8).

Discussion

Reprogrammed metabolism and a tumor-promoting microenvironment are common hallmarks and driving forces across solid and systematic malignancies. However, current knowledge about how tumor cells utilize both of these processes to promote malignant progression remains limited¹¹. Here, we identified a previous poorly characterized secretory protein, HEXB, as a critical regulator facilitating the interplay between cancer cell glycolysis and TAMs manipulation in GBM. HEXB accelerated cancer cell aerobic glycolysis and activated TAMs-associated glycolytic promotion in cancer cells. In mechanism, HEXB activated cancer intrinsic YAP1 by forming a protein complex with ILK and ITGB1 in GBM cells, thereby enabling YAP1/HIF1 α -dependent HEXB transcription and enhancing their glycolytic activity. HEXB enhanced the recruitment of M2 TAMs, which further augmented HEXB secretion in TAMs and thus constituted an intercellular positive feedback network between malignant cells and TAMs.

Glycolysis is a building block for cancer cell survival and proliferation. Tumor cells exhibit energy-dependency on high consumption of glucose and its conversion into lactate through glycolysis^{40,41}. Although the role of HEXB in recessive disorders has been well investigated, few studies have explored its role in cancer⁴². The conduction of comprehensive profiling and functional assays indicated that malignant effects of HEXB were driven by the YAP1-dependent metabolic tendency towards glycolysis. YAP1 has been well established as a fundamental transcriptional activator boosting tumor glycolysis^{20,43}. Aerobic glycolysis-derived lactate accumulation increases the transcriptional activity of YAP1 complexes⁴⁴. In turn, YAP1 accelerates cellular glucose uptake and the glycolytic rate by promoting transcription of GLUT3, PKM2 and HK2²⁸. Previous studies reported that different subunits of integrin bidirectionally regulated the transcriptional activity of YAP145. We found that HEXB directly bound to ITGB1 and its signaling transmitter ILK. This transmembrane protein complex stabilizes ILK and thus suppresses the Hippo pathway to increase nuclear trans-localization of YAP1⁴⁶. Under physiological conditions, hyperactivation of YAP1 results in expression of its negative regulators, LATS1/2 kinases, to establish negative feedback control. This mechanism buffers the YAP-activating signal, thus preventing tumorigenesis⁴⁷. However, tumor cells reconstruct the feedback



network during malignant transformation and progression. The existence of negative feedback loop helps cancer cell evading growth supervision, avoiding replicative senescence and resisting cell death⁴⁸, while positive feedback loops develop to enhance oncogenic signaling. Consistent with this observation, exogenous HEXB significantly upregulated expression of endogenous HEXB in cancer cells. Considering that YAP1 binds to HIF1 α and maintain its stability²⁸, which increases HEXB transcription, our findings depict an HEXB-driven positive feedback loop dependent on YAP1/HIF1 α transcription activity. Since hypoxia significantly elevates expression of HIF1 α , it is reasonable to assume that the malignant influences of HEXB positive feedback would be further magnified in a hypoxic environment. These findings support

Fig. 5 | **YAP1/HIF1α** contributes to the regulation of HEXB transcription in GBM cells. **a**-**d** qPCR analysis of HEXB mRNA in shHEXB and control GSC1 (**a**, **b**) and mGSCs (**c**, **d**) under administration of indicated exogenous HEXB recombinant protein (**a**, **c**) or Gal-P (**b**, **d**), respectively (*n* = 3 independent samples). **e** Western blotting analysis of HIF1α and YAP1 protein in Hexb silenced and control mGSCs. (The samples derive from the same experiment but different gels for HEXB, HIF1α, YAP1, P-YAP1, and β-actin were processed in parallel). **f** Co-IP assays of YAP1 and HIF1α interaction in indicated GSC1 samples. **g** Western blotting analysis of HIF1α protein stability in GSC1 samples with indicated verteporfin or DMSO treatment (*n* = 3 independent samples). **h** Western blotting analysis of HIF1α protein stability in GSC1 transduced with shNC or shHEXB vector, respectively (*n* = 3 independent

HEXB as a crucial regulator in cancer cell glycolysis and clarify the mechanism by which HEXB enhances the energetic favoring to it. Further studies will be required to explore this feedback axis under various TME conditions to clarify its applicable scope.

Over the past decade, the tumor has increasingly been recognized as a type of aberrant organ. From this perspective, the riddle of tumor growth can only be clarified by exploring tumor cell biology under the context of TME. We previously found that the proportion of tumor cells within GBM tissue accounted for only 73% of total cells⁹, and GBM cells selectively recruited immune cells represented by macrophages to establish an appropriate TME conductive to their survival⁴⁹. In current study, we found that GBM-derived HEXB promoted TAM recruitment and M2 polarization. The polarized M2 TAMs secreted more HEXB to accelerate tumor cell glycolysis and activated the YAP1/ HIF1α-dependent HEXB transcriptional loop. Therefore, HEXB promoted glycolysis by forming an intercellular positive feedback network with TAMs. Given the malignant promoting role of TAMs, a TAM depletion strategy may provide a survival advantage. However, a phase II clinical trial targeting TAMs with CSF1R inhibitor PLX3397 did not show any significant therapeutic improvement in recurrent GBM⁵⁰. This dilemma suggested that despite TAMs is important to the malignancy progression of GBM, depleting TAMs alone only achieves marginal therapeutic benefits. Therefore, we sought that blocking versatile targets with synergic influence in tumor cells and tumorsupporting cells may achieve a more desirable outcome. Indeed, inhibition of HEXB significantly prolonged the survival of GBM-bearing mice with remarkably suppression on tumor cell malignancy and M2 TAM enrichment. This demonstrates the potential of HEXB targeted therapy for achieving synergistic effects in GBM by inhibiting cancer glycolytic fueling and TAMs associated TME remodeling. The blockade of HEXB impedes malignant cell glycolysis addiction in GBM, and we believe that HEXB may be a potential target for combined metabolic and immunotherapy strategy to reverse the dilemma of immunotherapy in glioma. Further investigation of the mechanism by which HEXB regulates TAMs should be encouraged to consolidate the theoretical basis for initiating HEXB-targeted treatment.

The mutational status of IDH1 is a dominant molecular biomarker for glioma classification. Our previous investigation on large samplesize glioma cohorts revealed distinct immunosuppressive status of patients under different IDH1 status⁵¹, and the immunosuppressive TME of IDH1 wild type gliomas was characterized by abundant TAMs enrichment. Profiling of metabolic atlas in IDH1 wild type GBM showed the glycolysis pathway was indispensable⁵². Considering dual roles of HEXB in maintaining glycolytic activity of glioma cells and regulating macrophage behaviors, we conducted the analysis between IDH1 backgrounds with HEXB. IDH1 mutation significantly suppressed HEXB expression and its positive feedback loop via 2-HG accumulation. Gal-P, an HEXB inhibitor, elicited substantial effect in IDH1 wild type tumors. However, it did not work well in IDH1 mutant tumors. These observations suggested that clinical implications of HEXB mainly depended on IDH1 mutational status of tumors.

In conclusion, HEXB is a critical mediator regulating the interplay between cancer cell glycolysis and infiltrating TAMs in IDH1 wild type samples). **i**, **j** Effects of indicated endogenous or exogenous intervention of HEXB on HIF1 α transcriptional activity in GSC1(**i**) and mGSCs (**j**) (n = 3 independent samples). **k** qPCR analysis of HEXB mRNA in indicated GSC1 samples with HIF1 α overexpression or transfected with YAP-5SA plasmid n = 3 independent samples). **I** ChIP-PCR assay of GSC1 sample precipitated with HIF1 α antibody showing the relative enrichment fold among indicated fragments of HEXB promoters (n = 3 independent samples). **m** Schematic graphic showing intrinsic regulatory mechanism of HEXB in GBM cells. Data are from 3 (**g**, right and **h**, lower) or representative of 3 independent experiments with similar results (**a**–**l**). Data were presented as mean ± SEM and analyzed by two-tailed Student's t-test (**a**–**d**,**g**–**l**). Source data are provided as a Source Data file.

glioma. HEXB intrinsically activates YAP1/HIF1 α and intercellularly manipulates TAMs to promote GBM cell glycolysis and progression. HEXB inhibition elicits substantial therapeutic effects in preclinical glioma models, which provides foundation for the future clinical translation of HEXB targeting strategy in GBM.

Methods

Ethics statement

The experimental protocol was approved by the ethics committee of Hospital of China Medical University. Animal experiments were conducted in accordance with the China Medical University Animal Care and Use Committee guidelines and approved by the Institutional Review Board of Hospital of China Medical University.

Cell culture

Human cell lines including THP-1, SH-SY5Y, NHA and Jurkat were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). The glioma spheres (GSC1, GSC21, GSC24, GSC63) used in this study was generated from primary GBM tumors. The human peripheral blood mononuclear cells (PBMC) were extracted from venous blood of donors with brain diseases collected in EDTA tubes (BD Vacutainer), using Ficoll density gradient centrifugal separation method (GE Healthcare, 17-1440-02)³⁷. Glioma spheres were cultured in DMEM/F-12 medium (10565018, Gibco) containing 2% B27 supplement (17504044, Gibco), epidermal growth factor (EGF, 20 ng/ml), basic fibroblast growth factor (bFGF, 20 ng/ml) and heparin (2.5 µg/ml). Only early passage GSC cells were used for the study. Macrophages were induced from THP-1 cells and PBMCs using 10 nM phorbol-12-myristate-13-acetate (PMA), then induced into M1-like macrophages with lipopolysaccharide (1µg/mL) and IFN-y (20 ng/ mL) for 48 h, or induced into M2-like macrophages with IL-4 (20 ng/ mL) for 48 h. Macrophages were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/ streptomycin. All cells were cultured at 37 °C with 5% CO2. mGSCs were extracted from murine spontaneous GBM established by sleeping beauty transposon method described before^{53,54}. Briefly, SB is a twopart system consisting of transposon DNA and transposase. SB transposase can cut off the transposon DNA and paste it into the TA dinucleotide of the target genome. We separated the transposase and transposon on different plasmids. The transposon plasmid with oncogenic genes (SV40-LgT and NRAS-G12V) was mixed with the plasmid encoding transposase and injected into the brain of fetal mouse (within 24 h after born) to induce spontaneous glioma. After tumor formation, the tumor specimen in the brain of mice were collected and cut into small pieces, digested into single cells with Accutase. After red blood cells were lysed by Red Blood Cell Lysis Buffer, cell suspensions were passed through a 70 µm stainless steel mesh and re-cultured in the serum-free stem cell medium.

Mice and intracranial xenograft tumor models

Six-to-eight-week-old female BALB/c nude mice and male C57BL/6 N mice were purchased from Beijing Vital River Laboratory Animal Technology. All mice were resided in specific pathogen-free (SPF)



conditions. Mice lived in a cycle of 12 h of light and 12 h of darkness. The temperature and humidity of the mouse feeding environment are 18-22 °C and 50–60%, respectively. The maximal tumor burden is 1500 mm³ permitted by ethics committee and the weight of the tumor must not exceed 10% of the body weight of the mouse. None of the tumor volumes of mice in this study exceeded the regulations.

For intracranial tumor xenograft transplantations, the indicated glioma cells were transplanted into the right cerebral cortex at a depth of 3.0 mm using intracranial injection at a density of 5×10^5 cells in a volume of 3μ l. Gal-P was administrated intraperitoneally every two days after cells were inserted for 5 days. Mice were maintained until manifestation of neurological signs. For immunohistochemical (IHC)

Fig. 6 | **HEXB contributes to establishing the feedback between malignant cells and M2 macrophages in GBM. a** CIBERSORT analysis showing microenvironmental components in indicated GBM sample groups stratified by HEXB expression in CMU cohort (High n = 15 patients; Low n = 15 patients). **b** Representative IHC images (Left) and analysis (Right) of CD163 staining in GBM samples (n = 16 patients, High n = 8 patients; Low n = 8 patients). **c** Transwell assays detecting the chemotactic effect of Gal-P and rhHEXB stimulation on THP1-derived macrophages (n = 3 independent samples). **d**, **e** FACS analysis of MHCII, CD206 and CD11b positive cells in PBMC-derived macrophages with indicated treatment. **f** IHC analysis of IBA1 staining in indicated GBM tumor tissues (n = 3 independent samples). **g** IHC analysis of MHCII and CD206 positive cells in mouse tumor derived from mGSCs and then with Gal-P intraperitoneal treatment (n = 3 independent samples). **h**, **i** The expression of HEXB in indicated polarized THP-1 and PBMC derived

analysis, mice were sacrificed at day 21. The section with the largest tumor cross-sectional area was selected for tumor size measurement in the intracranial glioma models. Female BALB/c nude mice were applied for the xenograft experiments of human GBM cells (GSC derived from primary tumor resection samples), and male C57BL/6 N mice were employed for the intracranial transplantation assays of mouse GBM cells (mGSCs). Mouse sex was not considered in the design of these experiments.

In vivo GBM cells and macrophages co-implantation experiments were performed as previously described^{8,55-59}. Briefly, a 3 μ l cell suspension (2 × 10⁵ GBM cells with or without 1 × 10⁴ THP-1 derived macrophages) were intracranially implanted into BALB/c nude mice.

Human Samples

Informed consent was obtained from the patients for the use of their samples. The histological diagnoses of these samples were confirmed by two neuropathologists according to the 2021 World Health Organization (WHO) classification guidelines. Overall survival (OS) was calculated from the date of diagnosis to death or the end of follow-up. The point of death was defined by death certification, which could be acquired by local hospitals or police stations. The IDHI mutation status was detected by a combination of molecular pathology and immuno-histochemical staining. This study was approved by the Ethics Committee of Hospital of China Medical University.

IHC Staining

The expression of indicated markers such as HEXB, IBA1, PKM2, YAP1 and CD163 in clinical patients' tissues was detected by IHC. Paraffin sections immunohistochemistry was conducted as mentioned previously⁶⁰. Through a series of graded alcohol dewaxing and rehydration steps, antigen retrieval was performed in a citrate buffer at pH 6 for 2 min. Subsequently, endogenous peroxidase was blocked using 3% H₂O₂ for 12 min at room temperature. Primary antibodies were then added and incubated overnight at 4 °C, followed by incubation with secondary antibodies. The results were independently analyzed by 2 investigators. These expression intensities were calculated by German Immunohistochemical Score (GIS)⁶¹. Percentage of positive cells was graded as 0 (negative), 1 (up to 10%), 2 (11-50%), 3 (51-80%), or 4 (>80% positive cells) and staining intensity as 0 (no staining), 1 (weak), 2 (moderate), or 3 (strong). The final immunoreactive GIS was defined as the multiplication of both grading results (percentage of positive cells * staining intensity).

Immunofluorescence staining

For clinical specimens, 4-µm thick section slides from frozen human tissue were washed three times in PBS. Then, the sections were permeabilized with 0.5% Triton X-100 for 20 min. After 5% BSA incubation for 1 h, sections were incubated with primary antibodies like HEXB, IBA1 or GFAP antibodies at 4 °C overnight. Following incubation with fluorescein (FITC) or rhodamine (TRITC) secondary antibody and 4',6diamidino-2-phenylindole (DAPI), the samples were detected using macrophages (n = 3 independent samples). **j** The ELISA detection of secretory HEXB protein in the culture supernatant obtained from indicated polarized THP-1 derived macrophages (n = 3 independent samples). **k** Survival curves (Left) and representative tumor images (Right: in vivo bioluminescence (Upper) and H&E images (Lower) at 21 days after the mice intracranial co-implantation of GSC1 and THP-1 derived macrophages) of indicated GSC1 orthotopic models (Group a: GSC1; Group b: GSC1 + M0 macrophage; Group c: GSC1+rhHEXB pretreated macrophage; Group d: GSC1+rhHEXB pretreated macrophage with administration of Gal-P). Data are representative of 3 independent experiments with similar results (**b**–**k**). Data were presented as mean ± SEM. Statistical analyzes were determined by two-tailed Student's t-test (**a**–**c**,**f**–**j**) and log-rank test (**k**). Source data are provided as a Source Data file.

fluorescence microscope. The images were merged digitally to monitor the co-localization condition.

For the staining of cell samples, the indicated cell samples were dissociated into single cells with accutase and then seeded in confocal dishes coated with 0.5% laminin preparedly in advance. The plates were fixed by methanol for 15 min, and then were permeabilized with 0.5% Triton X-100 for 20 min. After 5% BSA incubation for 1 h, sections were incubated in YAP1 antibodies at 4 °C overnight. Following incubation with FITC secondary antibody and DAPI, the samples were detected using confocal fluorescence microscope. The images were merged digitally to detect the co-localization of indicated markers.

Multiplexed immunofluorescence staining and analysis

Samples were cut into 5-µm thick sections and loaded onto adhesion microscope slides. The slides were preprocessed with deparaffinization, rehydration, and antigen retrieval for multiplexed immunofluorescence staining. Multiplexed immunofluorescence staining of tissue was performed using TG TSA Multiplex IHC Assay Kits (TissueGnostics Asia-Pacific Ltd.). Visualization of the different fluorophores were achieved on the TissueFAXS Spectra Systems (TissueGnostics GmbH, Vienna Austria) and StrataQuest analysis software (Version 7.1.129, TissueGnostics GmbH, Vienna, Austria).

Cell proliferation assay

After the preparation of single cell solution, glioma spheres cells were seeded into 96-well plates at a density of 1×10^3 cells in 100 µl medium per well and cultured for indicated days. Then, 20 µl MTS solution was added into each well and incubated for 3 h at 37 °C. Optical density (OD) values at the absorbance of 490 nm of each well were detected with a microplate reader.

Cell migration assay

Transwell inserts with pore size of $8 \,\mu\text{m}$ were used for in vitro cell migration assays. $2 \times 10^4 / 200 \,\mu\text{l}$ macrophages were resuspended in RMPI-1640 containing 0.2% FBS and then seeded into the upper chambers of the Transwell inserts. The lower chamber contained 600 μ l of RMPI-1640 medium containing 20% FBS. The transwells were collected after 22 h culture. The membrane was fixed with methanol and stained with 1% crystal violet solution. The cells on the lower side of the membrane were observed and photographed.

Flow cytometry

Flow cytometry was performed as previously described⁶². For M1 and M2 macrophage marker measurement, macrophages were collected, digested into mononuclear cell suspension and stained with anti-CD11b followed by incubation with the indicated antibodies (MHCII or CD206) for 30 min and analyzed by flow cytometry. For cell apoptosis analysis, the cells were collected and suspended in PBS containing Annexin V and PI for 15 min and then directly run on a flow cytometry. FACS data were analyzed using FlowJo software (version 10.4).



Detection of glycolytic level in cells

Glucose assays and lactate assays were performed to measure the glycolytic level in cells. Glucose assays were performed according to product instruction of high sensitivity glucose assay kits (Sigma). Lactate assays were performed following product instruction of lactate assay kit II (Sigma). Glycolysis capacities of indicated cell samples were analyzed through the Seahorse XFe 96 Extracellular Flux Analyzer (Seahorse Bioscience)²⁸. Consecutive measurements were detected under basal conditions and after the sequential addition of 10 mM glucose (inhibiting mitochondrial ATP synthase), 2 μ M oligomycin (revealing cellular maximum glycolytic capacity), and 50 mM 2-deoxy-glucose (2-DG, a glucose analog inhibiting glycolysis through

Fig. 7 | **IDH1 mutation restrains the efficiency of HEXB-targeted intervention in glioma. a** MTS assay of cell viability in GSC1 transduced with indicated vector (control or IDH1 RG132H vector), and then treated with Gal-P or DMSO, respectively (n = 3 independent samples). **b** ECAR assay detecting the glycolytic activity in GSC1 transduced with indicated vector (control or IDH1 RG132H vector), and then treated with Gal-P (100 μ M) or DMSO, respectively (n = 3 independent samples). **c**, **d** Representative H&E images and survival analysis of indicated GSC1 (**c**) and mGSCs (**d**) orthotopic models with Gal-P or DMSO treatment (Left), respectively (Middle, H&E; Right, survival analysis, n = 6). **e**, **f** Overall survival curves stratified by HEXB expression level in CMU IDH1 wild type and mutant glioma samples (IDH1

wild type n = 72, HEXB low n = 36 and high n = 36; IDH1 mutant n = 32, HEXB low n = 16 and high n = 16). **g** Representative H&E images (Middle) and survival analysis (Lower) of indicated mGSCs orthotopic models with Gal-P or PD-1 antibody or combination (Upper), respectively. **h** Representative H&E images (Middle) and survival analysis (Lower) of indicated mGSCs orthotopic models with Gal-P or CTLA4 antibody or combination (Upper), respectively. Data are representative of 3 independent experiments with similar results (a-h). Data were presented as mean ± SEM and analyzed by two-tailed Student's t-test (**a**, **b**) and log-rank test (**c**-**g**). Source data are provided as a Source Data file.



Fig. 8 | Graphical abstract of the effects of HEXB in glioma. Figure 8 were created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license.

competitive bind to glucose hexokinase). The evaluation of glycolysis was established through the measurement of extracellular acidification rate (ECAR).

Protein Extraction and Immunoblotting

Cells were lysed using a mixture of RIPA buffer and PMSF at a 10:1 ratio. Each sample with same micrograms of protein was loaded onto a lane and electrophoresed. Then, the protein was transferred to a polyvinylidene difluoride (PVDF) membrane (0.45 μ m). The PVDF membranes were blocked with 5% skimmed milk and incubated with the primary antibody overnight at 4 °C. Next day, the membranes were incubated with secondary antibodies at room temperature for 1 h. Protein expression was visualized with a chemiluminescence ECL reagent. The uncropped and unprocessed scans of the most important blots have been deposited in Source Data file.

Immunoprecipitation mass spectrometry (IP-MS) analysis

HEXB immunoprecipitant (n = 1) and related control IgG immunoprecipitant (n = 1) prepared from GSC1 whole-cell lysates were resolved on SDS-PAGE gels, and protein bands were excised. The samples were digested with trypsin, and then subject to LC-MS/MS analysis. Swissprot_Human mass spectra were used as the standard reference. Trypsin/P was used for cleavage. MS data were captured and analyzed by Shanghai Bioprofile.

Real-time quantitative PCR (RT-PCR)

Total RNA was isolated by MiniBEST Universal RNA Extraction Kit (TaKaRa) according to the manufacturer's instructions. Total RNA was reversely transcribed into cDNA with Prime-Script RT Master Mix (TaKaRa). qPCR was performed by SYBR Green Master Mix (TaKaRa) for triplicate. The mRNA expression of target genes was calculated by the $2^{\Delta\Delta Ct}$ method with normalizing to 18 s mRNA expression. The sequence information of primers was provided in Supplementary Table 3.

Enzyme linked immunosorbent assay (ELISA)

We performed ELISA analysis to test the secretion of HEXB according to the instructions of the HEXB ELISA kit (Cloud-Clone Corp). 100 μ l of standard and test samples were added into the plate and incubated at 37 °C for 1 h. After removing the supernatant, 100 ul detection solution A were added into each well and incubated at 37 °C for 1 h. Then washed for 3 times, 100 μ l detection solution B were added into each well and incubated at 37 °C for 30 min. The plates were washed for another 5 times and 90 μ l TMB solution was added and incubated at 37 °C for 15 min. 50 μ l terminated solution was added and then the OD values at the absorbance of 450 nm were detected.

RNA sequencing analysis

The libraries were sequenced on the Illumina HiSeq 2000 platform using the 101-bp pair-end sequencing strategy. The original image data

generated by the sequencing machine were converted into sequence data via base calling (Illumina pipeline CASAVA v1.8.2). Hg 19 Ref Seq (RNA sequences, GRCh37) was downloaded from the UCSC Genome Browser. Gene expression was calculated using the FPKM method.

Co-immunoprecipitation (Co-IP) and GST pull-down assays

For Co-IP assays, cell samples were collected and then incubated with 400 µl lysis buffer with protease inhibitors for 30 min on ice. The supernatant was collected and 2 µg of target protein antibody (HEXB, YAP1, HIF1α) and IgG antibody were added and incubated at 4 °C overnight. Next, 50 µl of protein A/G magnetic beads were added and incubated for 2 h at room temperature. The binding complex was collected and washed three times with washing buffer. Finally, the precipitate was boiled with loading buffer and analyzed by western blotting. For GST pull-down, GST-labeled HEXB plasmids with full length and truncated mutation as well as ITGB1 overexpression plasmids were constructed from OBiO Technology (Shanghai). GST-HEXB with full length and the truncated mutation as well as ITGB1 protein were extracted from a mix based on TNTT7 in vitro transcription and translation coupling system based on corresponding plasmids. Then, GST-labeled HEXB recombinant protein was mixed with ITGB1 protein and incubated at 4 °C overnight. The binding complex was captured by GST magnetic beads. Finally, the precipitate was boiled with loading buffer and analyzed by immunoblotting.

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was performed using the Simple ChIP Enzymatic Chromatin IP Kit (Cell Signaling Technology). In brief, 1% formaldehyde fixation, nuclear preparation and chromatin digestion, ChIP, elution, reverse cross-linking, and purification of DNA were performed according to the manufacturer instructions. And 2% lysate was used as an input control, and the remaining lysate was immunoprecipitated with either IgG (control) or HIF1 α antibody. The immunoprecipitated DNA was amplified by qPCR.

Detection of the HIF1 α transcriptional activity

The HIF1 α transcriptional activities were detected by a dedicated HIF1 α transcriptional activity assay kit (Cayman, 10006910), following the manufacturer's instructions. Briefly, after nuclear protein extraction and quantification, 50 µg nuclear protein samples were added to each well and followed by supplementing a mixed and uniform reagent kit working solution. Then, the absorbance values were measured with an enzyme-linked immunosorbent assay (ELISA) reader at 450 nm, which reflects the relatively high or low transcription activities of HIF1 α in indicated groups.

Computational Analysis

The present study employed the expression matrix and clinical information of glioma samples from four RNA-seq datasets: CMU RNA-seq cohort (https://ngdc.cncb.ac.cn/gsa-human/s/3qyKeG8n), the Chinese Glioma Genome Atlas (CGGA) RNA-seq cohorts, including CGGA325 and CGGA693 cohorts (http://www.cgga.org.cn), and the Cancer Genome Atlas RNA-seq cohort (TCGA cohort, https://tcga-data.nci.nih. gov/tcga/) (Supplementary Table 1). The ssGSEA R package was used to calculate four metabolic mode scores of each patient in indicated datasets based on corresponding metabolic gene-sets (Glycolysis, oxidative phosphorylation, fatty acid, and glutamine metabolism) downloaded from Gene Set Enrichment Analysis website (GSEA, http:// www.broadinstitute.org/gsea/index.jsp) (Supplementary Data 1).

GSEA was performed to explore whether the identified sets of genes showed statistical differences between indicated groups. Gene sets were submitted to the DAVID website (https://david.ncifcrf.gov/) to perform gene ontology and pathway annotation. GSE131928 GBM, GSE139448 GBM, and GSE171891 glioma datasets were applied for detecting the expression of HEXB in GBM and non-malignant cell populations. There are 8 GBM samples included in GSE117891 glioma dataset, which were subjected for the analysis, GSE5099 dataset was used for detecting the expression of HEXB in different status of macrophages or monocytes. The t-distributed stochastic neighbor embedding (TSNE) analysis with R programming language was employed to access expression patterns of grouped cells in GBM. Limma R package was performed to identified the DEGs between two different groups (LogFC > 0.5 and P value < 0.05). Macrophage score reflecting relative macrophage proportions was calculated based on the TIMER2.0 (https://timer.cistrome.org)¹² and Xcell (https://aran-lab. com/software/xcell/) algorithm, respectively. Pearson correlation analysis was used to compare the correlation between glycolysis score and macrophage ratio calculated by two algorithms. A HIF1α score was established with the downstream target genes of HIF1α (ELVIDGE HI-F1A_TARGETS_DN from GSEA; https://www.gsea-msigdb.org/gsea/ msigdb/cards/ELVIDGE_HIF1A_TARGETS_DN.html) and ssGSEA method to evaluate the activation status of HIF1α signaling pathway in glioma, according to CMU, CGGA and TCGA glioma datasets.

Statistics & reproducibility

No statistical method was used to predetermine sample size. No data were excluded from the analyzes. After establishment of tumors, mice were allocated randomly into different treatment groups. In vivo experiments, the investigators were not blinded to groups since the data was acquired and analyzed by the same person. Image J, SPSS 20.0, GraphPad Prism 8 and R language were used for statistical analysis. Differences in the characteristics between groups were tested using unpaired two-tailed Student's t test or one-way ANOVA test. The results are presented as means \pm standard error of mean (SEM). Prognosis differences between groups were assessed by Kaplan–Meier curves and log-rank tests. Pearson's test was used to evaluate the correlation between variables.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw RNA-sequencing data of GBM clinical samples reported in CMU cohort (Fig. 1a-c) generated in this study has been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences under accession code HRA006353. The raw data of HRA006353 are available from the corresponding author (Dr. A.H. Wu, ahwu@cmu.edu.cn) upon request, which will be released within one week after receiving the requirement and available for one month. The raw RNA-sequencing data of GSC shHEXB and control samples (Fig. 3a) has been deposited in Gene Expression Omnibus (GEO) database under accession code GSE242231. The raw data of MS to identify the potential interacting proteins (Fig. 4a) has been deposited in the Proteomics IDEntifications database via the PRIDE partner repository with the dataset identifier PXD045019. All data are available in the main text or in the supplementary materials upon request, access can be obtained by contacting the corresponding author via email. The reagent and resource presented in present study was provided in Supplementary Data 6. The related data generated in this study are provided in the Supplementary Information and Source Data file. Source data are provided with this paper.

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Author contributions

C. Zhu, W. Cheng, P. Cheng and A.H. Wu conceived and designed the study. W. Cheng, P. Cheng, and A.H. Wu supervised the study. C. Zhu, X. Chen, and T. Q. Liu develop methodologies and performed in vitro and in vivo experiments; C. Zhu, X. Chen, L. Cheng, and T. Q. Liu contributed to data acquisition and analyzes. C. Zhu, X. Chen, T. Q. Liu, L. Cheng, W. Cheng, P. Cheng, and A.H. Wu drafted and edited the manuscript. C. Zhu, W. Cheng, P. Cheng, and A. H. Wu obtained fundings. All authors reviewed and approved the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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