Dear Editor,

Immune-mediated tumor elimination depends on the production of cytokines and the recruitment of immune cells in the tumor microenvironment. Cell death-related signals such as ATP release from tumor cells are crucial for the activation of downstream immune responses. GOLM1, also known as GOLPH2 and GP73 as a Golgi transmembrane protein involved in the transport of protein cargo through the Golgi apparatus has been extensively studied in various cancers for its multifunctional roles in promoting cancer proliferation and metastasis through the AKT/mTOR pathway. Its secreted form has been used as a serum biomarker in patients along with hepatocellular carcinoma (HCC) tumorigenesis and progression. However, its functional role in anticancer immunity is still unclear.

Our immunohistochemical results demonstrated that GOLM1 expression levels were significantly higher in malignant HCC tissues than in benign liver tissues (Fig. 1a, b). Kaplan–Meier survival analysis indicated that HCC patients with high GOLM1 expression (cutoff value = 96.5) showed a worse prognostic factor (Fig. 1c). We further evaluated the inverse correlation between CD8 and GOLM1 expression levels in HCC tissues, which suggested that GOLM1 might be involved in immune regulation in HCC (Fig. 1d, supplementary Fig. S1a). Importantly, the tumors with a high expression level of GOLM1 were poorly differentiated, indicating that GOLM1 may predict the malignant progression of HCC (Supplementary Tables 1–3). Collectively, GOLM1 expression is elevated in tumor cells and inversely correlated with CD8+ T cell infiltration and clinical outcome in the tumor microenvironment of human HCC.

To further determine the role of GOLM1 in anti-tumor immunity, we firstly generated several independent clones of H22 hepatoma and MCA205 fibrosarcoma cell lines that lacked GOLM1 expression (Fig. 1e, supplementary Fig. S1b). Both GOLM1−/− H22 hepatoma and MCA205 fibrosarcoma exhibited a vigorous reduction in tumor growth in immune-competent mice as compared with their WT parental cells (Fig. 1f, g). Interestingly, both GOLM1−/− H22 and MCA205 cells grew into tumors at similar sizes as their corresponding WT cells in T-cell-deficient nu/nu mice (Supplementary Fig. S1c). Although abdominal massive malignant ascites produced in both groups at an early stage, the lack of GOLM1 expression in H22 tumor cells prolonged mice median survival significantly from 21 days to 29.5 days. In addition, 40% of mice have totally recovered at day 40 manifested by abdominal malignant ascites disappearance (Fig. 1h). These findings demonstrated that tumor regression associated with GOLM1 deficiency occurs in an immune-dependent fashion.

Indeed, the knockout of GOLM1 increased the number of tumor-infiltrating CD4+ or CD8+ T cells, IFNγ-production cytotoxic T lymphocytes, higher proportions of F4/80+ MHCII+ macrophages and CD11c+ MHCII+ dendritic cells including CD11b+Ly6G+ cell subtype (Fig. 1i–l, supplementary Fig. S1d). Moreover, IFNγ secreted by TILs isolated from H22 and MCA205 GOLM1−/− tumors environment presented more than that of the corresponding GOLM1+/+ tumors (Fig. 1m, n). Particularly, GOLM1−/− tumor displayed a greater proportion of apoptotic cells staining with the noteworthy expression of activated caspase-3 than in GOLM1-sufficient controls (Fig. 1k, l), implying that increased cell death may trigger the recognition by antigen-presenting cell and elicitation of the specific antitumor immune response. Altogether, GOLM1 deficiency likely promotes T cells and APCs recruitment into tumors leading to increased production of cytokines like IFNγ.

Chemotherapy-induced immunogenic cell death (ICD) is expected to influence the composition and the architecture of tumor immune infiltration, which contributes to the elimination of residual tumor cells. In our study, Annexin V+ DAPI− subpopulations of GOLM1−/− cells were increased as compared to WT cells, suggesting that GOLM1 deficiency promoted the early stage of apoptosis (Fig. 1o, supplementary Fig. S1e). Consistently, the western blotting analysis indicated that the intracellular levels of cleaved-PARP and cleaved-Caspase8 (p43/41) were increased along with the reduction of cleaved-FLIP, after MTX treatment in GOLM1−/− H22 cells (Supplementary Fig. S1f).

Importantly, GOLM1 significantly increased the secretion of ATP in response to MTX treatment supporting that ATP might play a critical role in antitumor immunity in GOLM1−/− tumor (Fig. 1p). To abolish extracellular ATP in the tumor microenvironment, the ecto-ATPase CD39 was overexpressed on the surface of tumor cells. The presence of CD39 on GOLM1−/− MCA205 cells significantly restored tumor growth to a similar rate as GOLM1+/+ MCA205 (Fig. 1q). Thus, GOLM1 deficiency may promote antitumor immunity through an increased extracellular ATP release.

Selective autophagy helps to regulate the clearance of dying cells by the generation of energy-dependent engulfment signals including ‘eat me’ and ‘find me’ signals. We found that GOLM1 knockout increased the abundance of LC3 puncta and the expression levels of the key proteins of autophagy including LC3II/LC3I and ATG7 (Fig. 1r, s, and supplementary Fig. S2a). We observed that the autophagy upstream suppressors such as phospho-AKT (Thr308), phospho-AKT (Ser473), and phospho-mTOR (Ser2481) were reduced to lower levels in GOLM1−/− cells than GOLM1+/+ cells after starvation. On the other hand, ULK1 complex proteins including ULK1, ATG13, and FIP200, which are essential to initiate autophagy, were maintained at high levels in GOLM1−/− cells instead of a strong reduction in GOLM1−/− cells under the starvation condition (Fig. 1s). To further verify the contribution of GOLM1 regulated autophagy formation to ATP release, we generated GOLM1−/− Atg5−/− cells and observed that GOLM1−/− Atg5−/− cells abolished the increased ATP release in GOLM1−/− cells (Fig. 1t). As TSC2 is an important autophagy activator in the AKT-mTOR signaling pathway, we have also generated GOLM1−/− Tsc2−/− cells and observed that GOLM1−/− Tsc2−/− tumors grew at similar rates as GOLM1+/+ tumors, much faster than GOLM1−/− tumors (Fig. 1u). In addition, our HCC tissue microarray analysis indicated that the expression of GOLM1 is a negative correlation with...
the expression of LC3 (Fig. 1v). Together, the results further support that GOLM1 promotes tumor growth by suppressing autophagy formation and ATP release via the AKT/mTOR pathway.

RNA sequencing was performed to further explore the potential downstream molecules that may contribute to GOLM1 promoting tumor growth. Interestingly, the top three enriched Gene Ontology subsets were "immune system process", "response to stimulus" and "response to stress" (Fig. 1w). Clustering analysis data indicated that the following categories of genes were upregulated in Golm1 deficient cells as compared to Golm1 wild-type cells.
WT cells: (1) chemokines and chemokine receptors like Ccl12, Cxc110, Ccr5, and Cxcr3, which may be involved in the recruitment of T cells; (2) early myeloid genes like H2-d1 and Tap1 which were linked to antigen processing and presentation; (3) CD molecules like Cd4, Cd8a, and Cd68; and (4) IFN signaling or ISGs such as Ifng, Stat1, Ikar, Oas1a, Irf2, and Irf8 (Fig. 1x). We next compared the tumor growth rates between Golm1−/− and Golm1+/+ cells in Ikar−/− mice. As shown in Fig. 1y, the sizes of Golm1−/− and Golm1+/+ tumors in Ikar−/− mice were similar at multiple different time points. These results indicate the indispensable roles of the IFN pathway on Golm1−/− tumor growth.

In summary, we found elevated GOLM1 in tumor cells correlated with reduced CD8 T cell infiltration into the tumor microenvironment and reduced prognosis of HCC in Chinese cohorts. More importantly, we have demonstrated that Golm1−/− cancer cells grew at a much faster rate than Golm1+/− cancer cells in immune-competent mice but at a similar rate in immunodeficient mice, suggesting GOLM1 has a novel role in suppressing anti-cancer immunity.

Our studies have also provided evidence indicating that GOLM1 may inhibit immune response in the tumor microenvironment and promote tumor growth through potential mechanisms dependent upon the AKT/mTOR-mediated regulation of autophagy formation and extracellular ATP release (Supplementary Fig. S3). Interestingly, another paper found that GP73 upregulates PD-L1 expression by enhancing the level of EGFP and promoting the phosphorylation of STAT3, indicating that GP73 participates in anti-tumor immunity through kinds of pathways. So, future studies are required to further determine the molecular connections among these multiple different players. Nevertheless, our studies identified GOLM1 as a profound checkpoint blocker target for HCC immunotherapy.

**DATA AVAILABILITY**

Data are available upon reasonable request.

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**AUTHOR CONTRIBUTIONS**

Study design: G.C. and H.Y.; performing experiments concerning murine tumor models: T.S., X.W., and J.L.; FACS and RNA-seq analysis: T.S., X.W., and L.L.; ELISA and ELISPOT assay: T.S., X.W.; cell culture, qRT-PCR and immunohistochemistry experiments: T.S., X.W., and N.Q.; collecting patient samples: M.S.; HCC tissue array staining and analysis: J.H.; writing manuscript: H.Y., G.C., L.L., and T.S.

**ADDITIONAL INFORMATION**

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