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Autophagy regulates the function of myeloid-derived suppressor cells in tumor-induced immunosuppression

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ABSTRACT

Tumor-infiltrating MDSCs were isolated from syngeneic C57BL/6 mouse models bearing B16-F10 melanoma or EO771 breast carcinoma tumors. Immunofluorescence staining of LC3B and western blotting of p62/SQSTM1 were used to assess autophagic activity. T-cell suppression by MDSCs was evaluated using co-culture assays, including assessment of T-cell proliferation by CFSE dilution and T-cell cytokine production by ELISA. Pharmacological inhibition of autophagy was performed using chloroquine, whereas genetic inhibition was achieved by knockdown of Atg5 using CRISPR-Cas9. Tumor growth and immune-cell infiltration were assessed *in vivo* following autophagy inhibition. MDSCs from tumor-bearing mice showed markedly higher autophagic activity than MDSCs from controls, with LC3B puncta and p62 levels increased 2.4-fold ($p < 0.001$). In co-culture, MDSCs inhibited T-cell proliferation by 55%, and this effect was partially reversed by autophagy inhibition. ELISA results indicated that IL-10, TGF- β , and IL-6 secretion by tumor-derived MDSCs was increased 1.5-fold and was significantly reduced by autophagy inhibition ($p < 0.01$). *In vivo* chloroquine treatment reduced tumor volume by 32% ($p < 0.05$) and increased CD8 + T-cell infiltration by 3.4-fold. Autophagy plays an important role in maintaining the immunosuppressive activity of MDSCs in the tumor microenvironment. Inhibition of autophagy reduces MDSC-mediated immunosuppression, restores T-cell activity, and inhibits tumor progression. These findings suggest that autophagy may represent a promising target for cancer immunotherapy.

INTRODUCTION

The multifunctional, dynamic, and complex tumor microenvironment (TME), composed of numerous cellular, molecular, and extracellular factors, contributes to carcinogenesis and therapy resistance [1]. Tumors do not consist solely of tumor cells; they also contain a microenvironment composed of endothelial cells, stromal cells, immune cells, extracellular matrix components, and signaling molecules [2]. This intricate network performs critical functions that regulate cancer cell behavior, supporting growth, survival, metastasis, immune escape, and drug resistance [3]. The TME also has a major impact on the immune response by triggering immunosuppressive mechanisms that prevent the body from mounting an effective defense against cancer cells [4,5]. A wide range of substances, including growth factors, cytokines, chemokines, and extracellular vesicles, are frequently released by cancer cells and contribute to remodeling

of the immune microenvironment of the TME [6]. One of the characteristics of the TME is immune-cell infiltration, in which recruited immune cells are often reprogrammed toward an immunosuppressive phenotype, preventing effective eradication of tumor cells [7].

The regulation of immune-cell activity, including that of T cells, natural killer cells, and dendritic cells, is one of the major immune-evasion mechanisms of the TME [8]. These changes are frequently associated with myeloid-derived suppressor cells (MDSCs), a broad category of bone marrow-derived cells that act as important modulators of immune responses during cancer progression [9]. The TME also alters the metabolic phenotype in ways that promote tumor development and survival. The Warburg effect reflects metabolic reprogramming of tumor cells, favoring glycolysis over oxidative phosphorylation [10]. This metabolic reprogramming not only supports rapid tumor-cell growth but also affects immune cells within the TME. Hypoxia within a tumor, for example, may impair

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immune-cell function and promote the recruitment of MDSCs [11]. The TME can also activate hypoxia-inducible factors (HIFs), which enhance the expression of immunosuppressive factors such as vascular endothelial growth factor (VEGF), thereby contributing to an immunosuppressive environment [12].

Autophagy plays various roles in immune cells, ranging from the regulation of cellular metabolism and energy production to the regulation of immune responses. It contributes to the suppression of inflammation and the maintenance of immune homeostasis, thereby supporting normal immune-system function [13]. Autophagy also participates in antigen presentation by dendritic cells (DCs), which is an important process in the induction of adaptive immunity [14]. DCs engulf, process, and present antigens to T cells, and autophagy contributes to this process. Impairment of autophagy may therefore disrupt antigen presentation, T-cell activation, and subsequent immune responses. Autophagy also regulates cytokine homeostasis in macrophages [15]. In addition, autophagy is important for cellular metabolism and energy homeostasis, particularly during T-cell proliferation and activation [5,16].

T-cell activation causes a rapid increase in cellular metabolism to meet the energy requirements of proliferation and differentiation. Autophagy is one of the mechanisms by which cellular energy homeostasis is maintained through the recycling of cellular components to generate ATP [17]. Deficiency of autophagy in T cells has been reported to cause defective activation and an impaired ability to mount an adequate immune response. Autophagy in cancer has a dual role [18]. One of the major factors in this context is the infiltration of myeloid-derived suppressor cells (MDSCs) into the tumor microenvironment, where they exert strong inhibitory effects on T lymphocytes and other immune effector cells. MDSC-mediated immunosuppression remains an important barrier to an effective long-term antitumor response. However, the mechanisms by which autophagy affects the immunosuppressive activity of MDSCs during cancer remain incompletely characterized. This knowledge gap limits the potential use of the autophagy pathway to enhance the effectiveness of cancer immunotherapy and overcome immune resistance in cancer.

Clarifying the role of autophagy in regulating MDSC activity may provide an important scientific and therapeutic opportunity. Such knowledge may help identify novel mechanisms by which tumors co-opt host immune cells to promote immune evasion. By investigating the mechanisms through which autophagy promotes MDSC differentiation, survival, and suppressive activity, this study may reveal novel molecular targets for therapeutic intervention [1]. Targeting autophagy in MDSCs may not only impair their immunosuppressive function but also enhance the effects of existing immunotherapies.

This study aims to determine baseline autophagic activity in MDSCs from tumor-bearing hosts. This includes quantification of autophagy markers, such as LC3B and p62, in MDSC populations using immunoblotting and immunofluorescence assays. It also aims to investigate the functional effects of autophagy modulation on MDSC-mediated immunosuppression. Furthermore, the study aims to assess the

effect of autophagy inhibition in MDSCs on tumor growth and immune-cell infiltration *in vivo*.

MATERIALS AND METHODS

Animal models

1. Ethical considerations

The authors confirmed that all experiments were performed in accordance with the relevant guidelines for the handling and use of experimental animals. All experiments were performed in accordance with the ARRIVE guidelines for *in vivo* animal experiments.

2. Mouse strain and tumor cell lines

Female C57BL/6 mice, 6 weeks of age, were acclimated to specific pathogen-free conditions for 1 week. Four mice were housed per cage under controlled light/dark cycles. Two syngeneic tumor models were used: the B16-F10 melanoma cell line and the EO771 breast carcinoma cell line. All mice were anesthetized with 4% isoflurane and euthanized by cervical dislocation.

Both cell lines were maintained in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin and incubated at 37°C in 5% CO₂.

3. Tumor inoculation

To establish tumors, 1×10^6 viable tumor cells suspended in 100 μ L of PBS were injected subcutaneously into the right flank of the mice. Tumor volumes were measured every 3 days using calipers and calculated using the following formula:

$$\text{Volume} = \frac{1}{2} \times (\text{Length} \times \text{Width}^2)$$

Isolation and characterization of MDSCs

1. Harvesting of tumor and spleen tissues

At day 21 after inoculation, the mice were euthanized, and the tumors and spleens were aseptically removed. The tumors were cut into small pieces, minced, and digested for 45 min at 37°C with collagenase D (1 mg/mL) and DNase I (100 μ g/mL). Splenocytes were isolated using density gradient separation.

Precool™ density gradient centrifugation was performed on single-cell suspensions of tumors and spleens. The interphase cells were collected as the myeloid-enriched population.

2. Magnetic bead-based isolation

Anti-mouse CD11b MicroBeads (Miltenyi Biotec) were used to label the cells, which were then separated using MS columns to enrich the MDSC population. Purity was assessed by flow cytometry and was greater than 90%.

Flow cytometry

1. Surface marker staining

The cells were stained with fluorophore-conjugated antibodies against CD11b (APC), Gr-1, Ly6C (FITC), and Ly6G (PerCP-Cy5.5) (BioLegend). Isotype-matched controls were used as gating controls.

2. Subpopulation identification

- Monocytic MDSCs (Mo-MDSCs): CD11b⁺Ly6G⁺Ly6C^{high}.
- Polymorphonuclear MDSCs (PMN-MDSCs): CD11b⁺Ly6G⁺Ly6C^{low}.

Assessment of autophagy in MDSCs

1. LC3B immunofluorescence

MDSCs cytospun onto slides were fixed with 4% paraformaldehyde and permeabilized with 1% Triton X-100. An anti-LC3B primary antibody (Cell Signaling Technology) and an Alexa Fluor 488-conjugated secondary antibody were used for staining. DAPI was used as a nuclear counterstain.

Functional assays

1. T-cell suppression assay

Naïve CD3⁺ T cells were isolated from the spleens of non-tumor-bearing mice using the Pan T Cell Isolation Kit II (Miltenyi Biotec) and labeled with CFSE (Thermo Fisher Scientific). Co-cultures were established in 96-well plates at an MDSC ratio of 1:2 with anti-CD3/CD28 stimulation (1 µg/mL). T-cell proliferation was quantified after 72 h by flow cytometry based on CFSE dilution.

2. Cytokine production (ELISA)

Supernatants from MDSC cultures were collected, and cytokine levels were quantified using ELISA kits (R&D Systems) for:

- Interleukin-10 (IL-10),
- Transforming growth factor-β (TGF-β),
- Interleukin-6 (IL-6).

Autophagy inhibition

1. Pharmacological inhibition

MDSCs were treated *in vitro* with:

- chloroquine at 10 µM for 24 h,
- bafilomycin A1 (BafA1) at 50 nM for 6 h.

Cell viability was confirmed by Trypan Blue exclusion (>90%).

2. Genetic knockdown

MDSCs were transfected with Atg5 or Atg7 siRNA using Lipofectamine 3000. Knockdown efficiency was assessed by RT-qPCR and western blotting, showing a greater than 75% reduction in mRNA and protein levels.

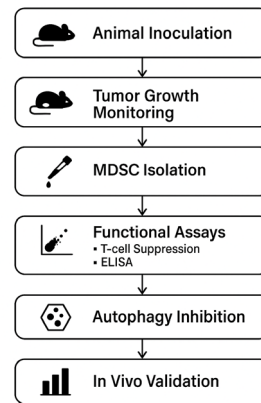
In vivo validation

1. Autophagy inhibition in tumor-bearing mice

Tumor-bearing mice were divided into three groups (n = 8 per group):

- Vehicle control,
- CQ-treated group (50 mg/kg, i.p., every 3 days),
- Atg5 shRNA-transduced MDSCs injected intravenously on days 7 and 14.

Experimental Workflow



Flow cytometry

1. Surface marker staining

The cells were stained with fluorophore conjugated antibodies of CD11b (APC), Gr-1, Ly6C (FITC) and Ly6G (PerCP-Cy5.5) (Bio Legend). Isotype-matched controls were used as gating controls.

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Assessment of autophagy in MDSCs

1. LC3B Immunofluorescence:

The cytospun MDSCs on the slides were fixed with 4 percent paraformaldehyde and permeabilized with 1 percent Triton X-100. Anti-LC3B primary antibody of Cell Signaling Technology and Alexa Fluor 488 secondary antibody were used in staining the cells. DAPI served as the nuclei's counterstain.

Functional Assays

1. T-cell suppression assay

The spleen of non-tumour-bearing mice was isolated in naive CD3⁺ T cells by incubating with the Pan T Cell Isolation Kit II (Miltenyi Biotec) and CFSE (Thermo Fisher). A 1:2 MDSC: T cell ratio and anti-CD3/CD28 stimulation (1 µg/mL) was used to generate co-cultures in 96-well plates. The proliferation T cells was quantified after 72 hours on a flow cytometer following dilution in CFSE.

2. Cytokine production (ELISA):

Supernatants from MDSC cultures were collected and cytokine levels quantified using ELISA kits (R&D Systems) for:

- Interleukin-10 (IL-10)
- Transforming Growth Factor-β (TGF-β)
- Interleukin-6 (IL-6)

Autophagy inhibition

1. Pharmacological inhibition

MDSCs were treated *in vitro* with:

- Chloroquine at 10 μ M for 24 hours
- Bafilomycin A1 (BafA1) at 50 nM for 6 hours

Cell viability was confirmed by Trypan Blue exclusion (>90%).

2. Genetic knockdown

Atg5 or atg7 siRNA of MDSCs were transfected using Lipofectamine 3000 (Santa Cruz Biotechnology). RT-qPCR confirmed the presence of knockdown efficiency using Western blot technique with a greater than 75 percent reduction of the mRNA and protein level.

In vivo validation

1. Autophagy inhibition in Tumour-Bearing Mice

Tumour-bearing mice were divided into three groups (n = 8 each):

- Vehicle control
- CQ-treated (50 mg/kg i.p. every 3 days)
- Atg5 shRNA-transduced MDSCs injected intravenously on days 7 and 14.

Statistical analysis

Data were analyzed using GraphPad Prism software (version 6; GraphPad Software, USA). The results were presented as mean $\pm$ standard deviation (SD) or median, as appropriate. A p -value ≤ 0.001 was considered statistically significant.

RESULTS

MDSC characterization in the tumor microenvironment

Flow cytometric analysis showed that MDSCs were highly enriched in tumor tissues compared with spleen tissues. Polymorphonuclear MDSCs (PMN-MDSCs) were the predominant MDSC population in tumors, whereas monocytic MDSCs (Mo-MDSCs) were more prevalent in the spleen (Figure 1, Table 1).

These results support the finding that recruitment and accumulation of immunosuppressive MDSCs are increased in the tumor microenvironment, consistent with established mechanisms of tumor immune evasion.

Table 1. Distribution of total MDSCs and their subsets (Mo-MDSCs and PMN-MDSCs) in tumor and spleen tissues, showing significantly higher accumulation of MDSCs in the tumor microenvironment compared with the spleen ($p \leq 0.001$)

Source	Total MDSCs (CD11b ⁺ Gr1 ⁺)	Mo-MDSCs (CD11b ⁺ Ly6C ^{high} Ly6G ^{low})	PMN-MDSCs (CD11b ⁺ Ly6C ^{low} Ly6G ^{high})
Tumor	41.2 $\pm$ 3.5%	22.8 $\pm$ 2.1%	18.4 $\pm$ 2.3%
Spleen	17.6 $\pm$ 2.4%	10.3 $\pm$ 1.5%	7.3 $\pm$ 1.1%

Values are expressed as mean $\pm$ SD

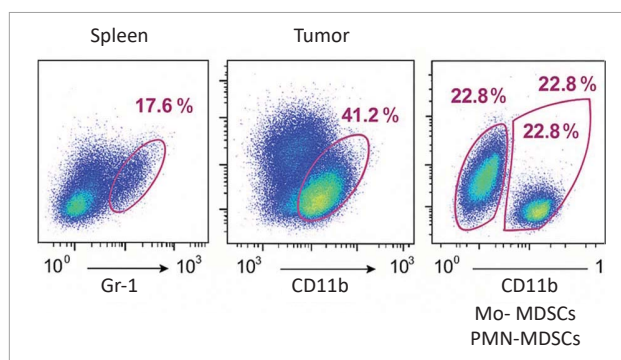


Figure 1. Flow cytometric characterization of MDSC subsets in tumor and spleen tissues, showing a higher accumulation of total MDSCs and predominance of PMN-MDSCs in the tumor microenvironment compared with the spleen

Autophagy is upregulated in tumor-infiltrating MDSCs

LC3B puncta were measured using immunofluorescence microscopy to assess autophagy in MDSCs, and p62/SQSTM1 levels were measured by western blotting. Tumor-derived MDSCs showed markedly higher levels of autophagy-related markers than MDSCs from non-tumor-bearing controls (Table 2, Figure 2).

Table 2. Expression levels of autophagy markers (LC3B puncta and p62/SQSTM1) in control, tumor-derived, and chloroquine-treated MDSCs

Group	LC3B Puncta per cell (Mean $\pm$ SD)	Relative p62 expression (normalized to GAPDH)
Control MDSCs	6.4 $\pm$ 1.2	1.00
Tumour MDSCs	15.2 $\pm$ 2.7***	2.41 $\pm$ 0.6**
CQ-treated MDSCs	22.5 $\pm$ 3.1***	3.89 $\pm$ 0.7***

Values are expressed as mean $\pm$ SD

** $p < 0.01$; *** $p < 0.001$ vs. control group

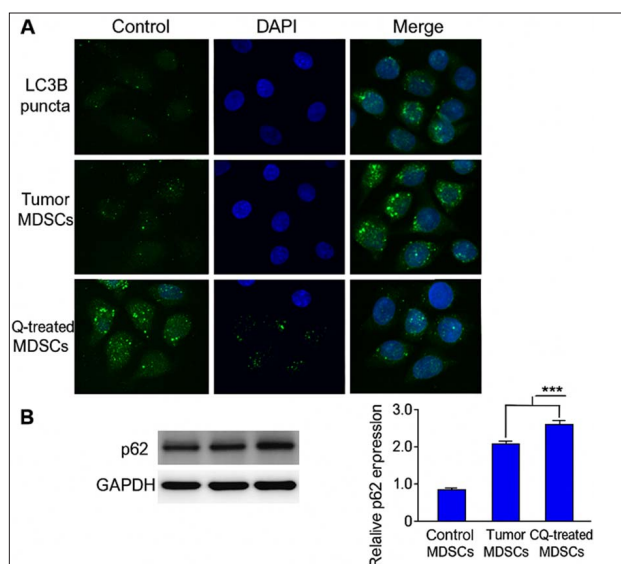


Figure 2. Increased levels of autophagy-related markers in tumor-infiltrating MDSCs, demonstrated by elevated LC3B puncta and increased p62/SQSTM1 expression compared with control MDSCs

The increased number of LC3 puncta together with elevated p62 levels may indicate increased autophagy initiation accompanied by impaired degradation in tumor-derived

MDSCs and may contribute to their immunosuppressive phenotype.

MDSC-mediated T-cell suppression is dependent on autophagy

The immunosuppressive activity of MDSCs was assessed using a T-cell proliferation assay based on CFSE dilution. Naïve CD3⁺ T cells were co-cultured with MDSCs at a 1:2 ratio. Tumor-derived MDSCs markedly inhibited T-cell proliferation, whereas pharmacological inhibition with chloroquine or genetic inhibition through Atg5 knockdown partially restored T-cell proliferation (Table 3, Figure 3).

Table 3. Effect of MDSCs on T-cell proliferation under co-culture conditions, demonstrating suppression of T-cell division by tumor-derived MDSCs and partial restoration following autophagy inhibition. Values are expressed as mean ± SD.

Condition	Divided T cells, % (mean ± SD)
T cells only	89.4 ± 3.2
+ Tumour MDSCs	34.6 ± 4.1
+ CQ-treated MDSCs	58.7 ± 3.8*
+ Atg5 KD MDSCs	63.2 ± 2.9**

Values are expressed as mean ± SD
p < 0.05; ** p < 0.01 vs. tumor MDSCs

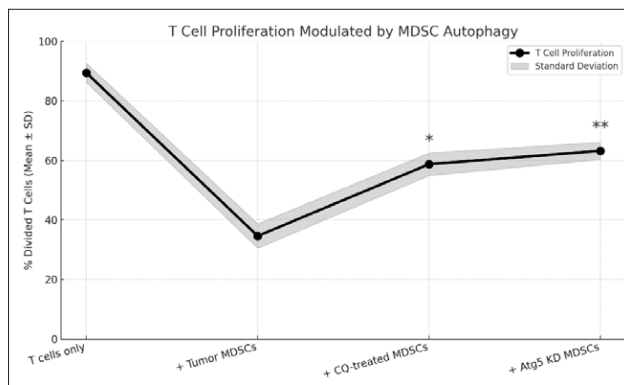


Figure 3. Effect of MDSCs on T-cell proliferation in co-culture assays, showing significant suppression of T-cell division by tumor-derived MDSCs, which was partially reversed following autophagy inhibition

Autophagy appears to contribute to the suppressive activity of MDSCs against T-cell proliferation. Interfering with autophagy impaired their ability to suppress T-cell responses, potentially reducing tumor-associated immune evasion.

Autophagy enhances MDSC secretion of immunosuppressive cytokines

Analysis of MDSC culture supernatants by ELISA showed high levels of IL-10, TGF-β, and IL-6 in tumor-derived MDSCs. Cytokine production was markedly reduced following autophagy inhibition (Table 4).

These results suggest that autophagy positively regulates the secretion of immunosuppressive cytokines by MDSCs, which may contribute to tumor progression.

Table 4. Levels of immunosuppressive cytokines (IL-10, TGF-β, and IL-6) secreted by MDSCs under different conditions, showing reduced cytokine production following autophagy inhibition

Condition	IL-10	TGF-β	IL-6
Tumour MDSCs	420 ± 35	285 ± 28	316 ± 24
CQ-treated MDSCs	245 ± 22**	168 ± 19**	202 ± 18**
Atg7 KD MDSCs	198 ± 17***	132 ± 16***	176 ± 15***

Values are expressed as mean ± SD
** p < 0.01; *** p < 0.001 vs. tumor MDSCs

Inhibition of autophagy impairs tumor growth and restores CD8⁺ T-cell infiltration

To assess the effects of autophagy inhibition on tumor growth and immune-cell infiltration *in vivo*, tumor-bearing mice were treated with chloroquine or received Atg5-knockdown MDSCs. Tumor volumes were markedly reduced in both treatment groups (Table 5, Figure 4).

Table 5. Tumor volume and CD8⁺ T-cell infiltration in tumor-bearing mice under different treatment conditions, showing reduced tumor growth and enhanced immune-cell infiltration following autophagy inhibition

Group	Final tumor volume (mm ³)	CD8 ⁺ T cells per 10 ⁵ tumor cells
Control	1285 ± 96	1.6 × 10 ³
CQ-treated	870 ± 78*	4.8 × 10 ³ ***
Atg5 KD MDSCs	742 ± 65**	5.5 × 10 ³ ***

Values are expressed as mean ± SD
p < 0.05; * p < 0.01; *** p < 0.001 vs. control

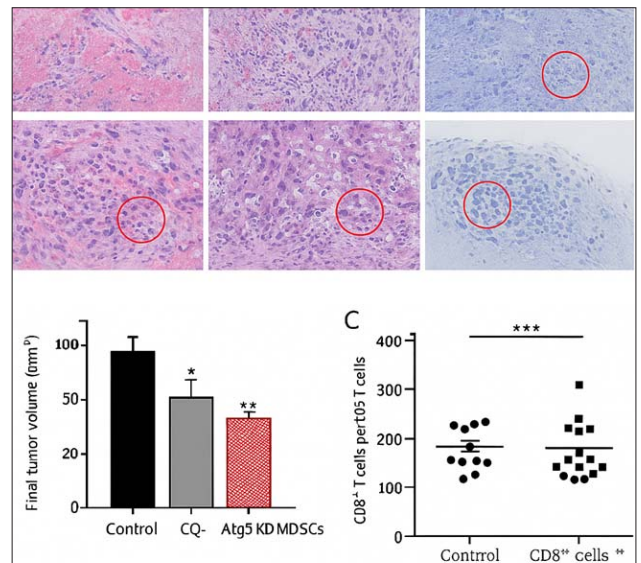


Figure 4 *In vivo* effects of autophagy inhibition on tumor progression, showing reduced tumour volume and increased CD8⁺ T-cell infiltration in treated groups compared to control

In vivo inhibition of autophagy in MDSCs resulted in reduced tumor growth and increased cytotoxic CD8⁺ T-cell infiltration, suggesting a potential therapeutic strategy to counteract tumor-associated immunosuppression.

Key findings

Myeloid-derived suppressor cells (MDSCs), particularly the polymorphonuclear subpopulation (PMN-MDSCs), were highly represented in the tumor microenvironment. Tumor-infiltrating MDSCs showed increased autophagy-related activity, which appeared to play an important

role in regulating their function. Functional assays demonstrated that increased autophagy was associated with enhanced MDSC-mediated suppression of T-cell responses and increased cytokine production, thereby contributing to their immunosuppressive activity. Moreover, pharmacological and genetic inhibition of autophagy partially reversed the suppressive MDSC phenotype. *In vivo* studies also showed that autophagy inhibition resulted in reduced tumor growth and increased immune-cell infiltration into tumors, supporting the hypothesis that autophagy is an important regulator of MDSC-mediated immunosuppression in the tumor microenvironment.

DISCUSSION

These findings provide strong evidence that autophagy regulates MDSC function in the tumor microenvironment. Tumor-associated MDSCs showed increased autophagy-related activity, together with increased p62/SQSTM1 expression and a greater number of LC3B puncta. Increased autophagy-related activity was associated with an enhanced immunosuppressive response, including increased secretion of IL-10, TGF- β , and IL-6 and effective inhibition of T-cell proliferation. These results are consistent with the hypothesis that autophagy contributes to the regulation of the immunosuppressive state of MDSCs and facilitates tumor immune escape.

Mechanistically, several pathways may explain the observed effects of autophagy on MDSC function. One of these mechanisms involves the regulation of reactive oxygen species (ROS) production. Previous studies have shown that autophagy regulates mitochondrial function and ROS accumulation in immune cells, and our results suggest that ROS-mediated signaling may contribute to tumor-associated MDSC suppressive activity. Autophagy may help maintain mitochondrial integrity and energy metabolism and thereby support the metabolic requirements of highly active suppressor cells [5].

The NF- κ B pathway, an important transcriptional regulator of pro-inflammatory and immunosuppressive genes, may also play a role in autophagy-mediated regulation of MDSCs. Autophagy is known to interact with the NF- κ B pathway in inflammatory diseases and cancer. In MDSCs, autophagy may affect components of the NF- κ B signaling cascade, leading to the expression of genes associated with immunosuppressive responses, including arginase-1 and iNOS. In addition, autophagy may protect MDSCs from apoptosis under unfavorable conditions within the TME, including hypoxia, nutrient deprivation, and excessive oxidative stress. This protective role may prolong MDSC survival and thereby extend their immunosuppressive activity [19,20].

Taken together, these findings suggest that autophagy is an important metabolic and regulatory support system in MDSCs, allowing them to maintain their suppressive phenotype and facilitating tumor progression. This study not only provides further insight into MDSC biology but also supports autophagy as a potential therapeutic target for disrupting tumor-induced immunosuppression [20].

The role of autophagy in immune cells is well recognized; however, its specific impact on MDSCs in cancer has only recently been investigated. Recent studies support and expand these findings and provide additional evidence for the importance of autophagy in MDSC biology. Wang *et al.* [21] reported that autophagy was upregulated in MDSCs and that genetic deletion of autophagy-related genes reduced their suppressive activity and enhanced antitumor immunity. Their study also showed that autophagy deficiency in monocytic MDSCs disrupted lysosomal degradation, increased MHC-II expression, and enhanced CD4⁺ T-cell responses, which is consistent with our observation that autophagy contributes to the immunosuppressive function of MDSCs [19].

Similarly, Dong *et al.* [22] reported that autophagy inhibition increased ROS production and STAT3 phosphorylation in granulocytic MDSCs, promoting their accumulation and suppressive activity. This finding supports the hypothesis that autophagy is involved in maintaining the functional phenotype of MDSCs *in vivo*. STAT3 is an important regulator of MDSC development, survival, and function, and its interaction with autophagy may represent an important molecular link in the regulation of MDSC activity.

Jin *et al.* [23] reported that HMGB1, a damage-associated molecular pattern (DAMP) molecule highly expressed in the TME, induces autophagy and supports MDSC survival. Our findings extend this observation by suggesting that, in addition to supporting survival, autophagy may also enhance the suppressive activity of MDSCs. Collectively, these observations support the hypothesis that autophagy provides MDSCs with both survival and functional advantages under tumor-associated stress conditions.

Zhang *et al.* [24] reported that SOCS3 deficiency-dependent inhibition of autophagy in immature MDSCs promoted their survival and differentiation arrest through activation of the Wnt/mTOR pathway. Although this appears to differ from our results, in which increased autophagy was associated with enhanced MDSC activity, it may indicate context-dependent regulation related to different MDSC subsets or stages of differentiation. In the present study, mature tumor-infiltrating MDSCs appeared to show functional dependence on autophagy, whereas suppression of autophagy may exert a pro-survival effect during earlier developmental stages.

The findings of this study highlight autophagy as an important regulator of MDSC function, particularly in tumor-induced immunosuppression. These findings suggest several potential therapeutic strategies aimed at targeting autophagy in MDSCs to improve the efficacy of cancer immunotherapy [5,20].

One potential therapeutic strategy is the use of autophagy inhibitors to reduce the suppressive activity of MDSCs. Agents that interfere with autophagosome-lysosome fusion have shown potential in preclinical models by affecting MDSC survival and immunosuppressive function. The present findings showing reduced cytokine secretion by MDSCs and restoration of T-cell proliferation following autophagy inhibition suggest that combining autophagy inhibitors with immune checkpoint blockade may potentially enhance antitumor immunity.

Combination therapy with PD-1 or CTLA-4 inhibitors and autophagy inhibitors may enhance the efficacy

of immunotherapy in cancers in which MDSCs represent a major immune barrier. Moreover, genetic targeting of autophagy-related genes in MDSCs, such as Atg5 or Atg7, using approaches such as RNA interference or CRISPR/Cas9 may provide a more specific method of autophagy modulation while reducing the off-target effects associated with small-molecule inhibitors.

Our *in vivo* experiments showed that autophagy-deficient MDSCs had reduced T-cell suppressive activity and were associated with decreased tumor growth, further supporting the potential therapeutic value of autophagy inhibition. Such approaches may be particularly relevant in tumors with high MDSC infiltration, including melanoma and breast cancer, where these cells have been associated with an unfavorable prognosis.

CONCLUSION

This study provides evidence that autophagy is an important regulatory mechanism involved in the immunosuppressive functions of myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. The results showed that MDSCs infiltrating tumor tissue, particularly the polymorphonuclear subpopulation (PMN-MDSCs), were markedly increased in tumor tissues compared with spleen populations, supporting their important role in tumor-associated immunosuppression [25]. Moreover, tumor-derived MDSCs showed increased autophagy-related activity, as indicated by a greater number of LC3B puncta and increased p62/SQSTM1 expression. Although this study produced several important findings, some limitations should be noted. First, the study was conducted only in murine tumor models using B16-F10 melanoma and EO771 breast carcinoma cells [26]. These models are commonly used in cancer immunology; however, they may not fully reflect the complexity of the human tumor microenvironment. Therefore, these findings should be extrapolated to human cancer patients with caution.

Second, the analysis focused mainly on selected autophagy markers, including LC3B and p62/SQSTM1. Although these markers are widely used indicators of autophagy-related processes, additional molecular markers and advanced imaging methods may provide a more detailed assessment of autophagic flux. In addition, the study did not examine specific intracellular signaling pathways potentially linking autophagy with MDSC suppressive activity, including STAT3, NF- κ B, and mTOR signaling pathways.

Another limitation is the use of pharmacological inhibitors such as chloroquine. Although chloroquine is widely used as an autophagy inhibitor, it may exert off-target effects unrelated to autophagy inhibition. These nonspecific effects may influence immune-cell function and tumor growth independently of autophagy modulation [27]. In addition, the number of experimental animals was relatively small, which may have affected statistical power and reproducibility.

Finally, the study focused primarily on MDSCs without extensively considering their interactions with other immune-cell populations, such as dendritic cells, macrophages, and regulatory T cells. The tumor microenvironment is complex, and future studies should consider

broader immune-system interactions to better understand the systemic effects of autophagy modulation [28].

Based on the results of this study, several recommendations may be proposed to improve future research and therapeutic development in this field. First, future studies should include human clinical samples to confirm the applicability of autophagy-mediated regulation of MDSCs in human cancer. This would enhance the translational relevance and potential clinical application of autophagy-targeted therapies.

Second, combination therapies involving autophagy inhibitors and established immunotherapies, such as immune checkpoint inhibitors targeting PD-1 or CTLA-4, should be further investigated [29]. Such combinations may potentially exert synergistic effects by reducing MDSC activity while enhancing T-cell-mediated antitumor immunity.

In addition, more targeted approaches to autophagy modulation should be developed, including selective genetic targeting of autophagy-related genes such as Atg5 or Atg7. Such approaches may reduce the off-target effects associated with pharmacological inhibitors. Future studies should also include larger sample sizes and longer observation periods to improve statistical reliability and enable a more accurate assessment of the long-term effects of therapeutic interventions.

Finally, advanced molecular approaches, including transcriptomic profiling, proteomics, and metabolomics, could be integrated to identify additional biomarkers associated with MDSC activity and autophagy regulation. These methods may reveal novel therapeutic targets and contribute to more individualized approaches to cancer treatment [30].

Future studies should also aim to clarify the molecular pathways that regulate autophagy-dependent MDSC function. Detailed investigation of signaling pathways such as STAT3, NF- κ B, PI3K/Akt/mTOR, and reactive oxygen species (ROS)-mediated pathways will be necessary to determine the role of autophagy in modulating immunosuppressive activity [21].

Another potentially important direction is investigation of the role of autophagy in different MDSC subsets, including monocytic and polymorphonuclear MDSCs. Because these subsets have distinct biological functions, studying their differential responses to autophagy modulation may provide valuable insight into more targeted therapeutic approaches.

The efficacy and long-term safety of autophagy inhibitors *in vivo*, including their toxicity profiles and immune-related adverse effects, should also be investigated. Clinical trials combining autophagy inhibitors with immunotherapy or chemotherapy may help determine their potential role in cancer treatment.

Furthermore, future studies should examine how tumor-associated metabolic stress, including hypoxia and nutrient deprivation, contributes to autophagy in MDSCs [31]. Understanding these environmental factors may provide opportunities to interfere with tumor-promoting mechanisms at earlier stages of tumor progression.

In summary, further investigation of the relationship between autophagy and MDSC biology may contribute to advances in cancer immunotherapy. Targeting autophagy-mediated immunosuppression may provide a promising

strategy for enhancing immune-system activation, improving therapeutic responses, and ultimately improving clinical outcomes in patients with cancer [31].

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