



# The negative effect of the PI3K inhibitor 3-methyladenine on planarian regeneration via the autophagy signalling pathway

Jing Kang <sup>1,2</sup> · Jinzi Chen<sup>1</sup> · Zimei Dong<sup>1</sup> · Guangwen Chen<sup>1</sup> · Dezeng Liu<sup>1</sup>

Accepted: 8 June 2021 / Published online: 17 August 2021

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

## Abstract

As an important PI3K (VPS34) inhibitor, 3-methyladenine (3-MA) can block the formation of autophagic vesicles in animals. Most toxicological studies using 3-MA have shown that 3-MA leads to serious disorders via autophagy suppression in mammals. However, no toxicological research on 3-MA has been performed on individuals undergoing regeneration. The freshwater planarian has powerful regenerative capability, and it can regenerate a new brain in 5 days and undergo complete adult individual remodelling in approximately 14 days. Moreover, it is also an excellent model organism for studies on environmental toxicology due to its high chemical sensitivity and extensive distribution. Here, *Dugesia japonica* planarians were treated with 3-MA, and the results showed that autophagy was inhibited and *Djvps34* expression levels were down-regulated. After exposure to 10 mM 3-MA for 18 h, all the controls showed normal phenotypes, while one-half of the planarians treated with 3-MA showed morphological defects. In most cases, an ulcer appeared in the middle of the body, and a normal phenotype was restored 7 days following 3-MA exposure. During regeneration, disproportionate blastemas with tissue regression were observed. Furthermore, 3-MA treatment suppressed stem cell proliferation in intact and regenerating worms. These findings demonstrate that autophagy is indispensable for tissue homeostasis and regeneration in planarians and that 3-MA treatment is detrimental to planarian regeneration via its effect on the autophagy pathway.

**Keywords** 3-MA · Autophagy inhibitor · Toxicological research · Planarian · PI3K signaling pathway

## Introduction

Autophagy is a lysosome-mediated degradation process by which aged proteins, misfolded proteins and damaged organelles are delivered to lysosomes for bulk degradation (Gonzalez-Estevez and Salo 2010; Kang et al. 2019a). Simultaneously, the function of autophagy is to control protein quality and maintain cellular homeostasis; the dysregulation of autophagy may lead to serious disorders in humans (Kang et al. 2019a; Zhang et al. 2020). In the heart, autophagy occurs constitutively at a basal level but is

enhanced under pathological conditions (Lavandro et al. 2015; Zhang et al. 2020). Many signalling pathways, including the phosphatidylinositol-3,4,5-triphosphate kinase (PI3K, VPS34) pathway, have been shown to be involved in the regulation of autophagy (Ding et al. 2018). The PI3K pathway has been found to regulate the formation of autophagosomes and autophagic vacuoles (Ding et al. 2018). As a PI3K inhibitor, 3-methyladenine (3-MA) has been reported to inhibit the activity of PI3K and block the formation of autophagosomes and autophagic vacuoles (Zhao et al. 2019). Christian et al. (2014) reported that autophagy is inhibited in the HTC11 human colon cancer cell line after 5 mM 3-MA exposure for 48 h. Autophagy is inhibited in the nerve cells of mouse embryos after treatment with 30 mM 3-MA for 6 h (Hou et al. 2016). In zebrafish, a large number of cells died after exposure to 5 mM 3-MA for 24 h due to autophagy inhibition (Pang et al. 2019).

Environmental exposure to autophagy inhibition has significantly increased in recent decades, mostly because of increased environmental pollution and antibiotic residue (Jiang et al. 2012; Zhang et al. 2019; Avila-Rojas et al. 2019). For example, tetracycline is a widely-used and well-tolerated

✉ Zimei Dong  
dzmhxx@163.com

✉ Guangwen Chen  
Chengw0183@sina.com

<sup>1</sup> College of Life Science, Henan Normal University, Xinxiang, China

<sup>2</sup> College of Life Science, Xingxiang Medical University, Xinxiang, China

antibiotic in the clinic that can inhibit autophagy in the ischaemic brain in rats that underwent a stroke (Jiang et al. 2012). Moreover, recent studies have shown that heavy metals also enhance cell damage by suppressing autophagic flux (Zhang et al. 2019). In addition, pesticide residues are important sources of environmental pollution, and their toxic effects are exerted via the mechanism of autophagy (Avila-Rojas et al., 2019). Simultaneously, autophagy is one of the key degradation systems in organisms, and the dysregulation of autophagy may lead to serious disorders in humans (Kang et al. 2019a; Zhang et al. 2020). Moreover, toxicological studies show that dysregulation of autophagy may lead to disorders in cell homeostasis and regenerative ability (Li et al. 2018; Zhang et al. 2020; Nieuwenhuis et al. 2020). Furthermore, the regenerative ability and homeostasis disruptions caused by dysregulated autophagy increase the risk of diseases, and the correlation between tissue regeneration and autophagy inhibition has been confirmed by many studies (Saera-Vila et al. 2016; Xu et al. 2020; Lee et al. 2019). Autophagy inhibition can effectively reduce muscle regeneration in a zebrafish model (Saera-Vila et al. 2016), and autophagy inhibition might suppress regeneration in ageing mouse livers (Xu et al. 2020). Additionally, autophagy plays critical roles in skeletal muscle homeostasis, regeneration and ageing in humans because muscle stem cell senescence is associated with suppression of autophagy during regeneration (Lee et al., 2019). However, the correlation between autophagy inhibition and individual regeneration is still not clear.

Freshwater planarians, belonging to the phyla Platyhelminthes, order Tricladida and class Turbellaria, are among the most abundant predators in aquatic ecosystems (Gonzalez-Estevéz 2009). Moreover, they have powerful regenerative capability and can regenerate a new brain in 5 days and undergo complete adult individual remodelling in 14 days. Therefore, it is an excellent model animal for study in the field of regenerative medicine and toxicity (Zhang et al. 2017; Zhang et al. 2018; Kang et al. 2019b). The freshwater planarian *Dugesia japonica* is widely distributed in East Asia and is an excellent freshwater species for studying regenerative medicine and assessing toxicity (Ross et al. 2017; Kang et al. 2019b; Danielle et al. 2020). Therefore, to elucidate the role of autophagy inhibition in planarian regeneration, our study focused on the regulatory effects of 3-MA and investigated the relationship between 3-MA effects and individual *D. japonica* regeneration.

## Materials and methods

### Animals and experimental design

The animals used in these experiments were the freshwater planarian *D. japonica* collected from Shilaogong, Henan

Province, China. They were cultured in autoclaved tap water and incubated in the dark at 20 °C. Planarians were fed beef liver once a week until sexual maturity was reached. The worms (0.8–1 cm in length and weighing 18–20 mg) were selected and starved for 1 week in the dark at 20 °C before all experiments (Dong et al. 2017; Kang et al. 2019b).

A P13K inhibitor, 3-MA (Sigma-Aldrich, St. Louis, MO, USA) is widely used as an inhibitor of autophagy. Therefore, this study aimed to determine whether autophagy was inhibited in planarians after 3-MA exposure for different concentrations and for different lengths of time; to this end TEM was performed. On the basis of the literature (Christian et al. 2014; Hou et al. 2016; Pang et al. 2019), 5 mM and 10 mM concentrations were tested. Several different exposure times (6 and 18 h) were also selected. The preliminary experimental results revealed no autophagic vesicles in *D. japonica* after 10 mM 3-MA exposure for 18 h but autophagic vesicles were observed in the controls. Therefore, it was decided that an 18-hour exposure of *D. japonica* to a 10 mM 3-MA dose was appropriate for this study.

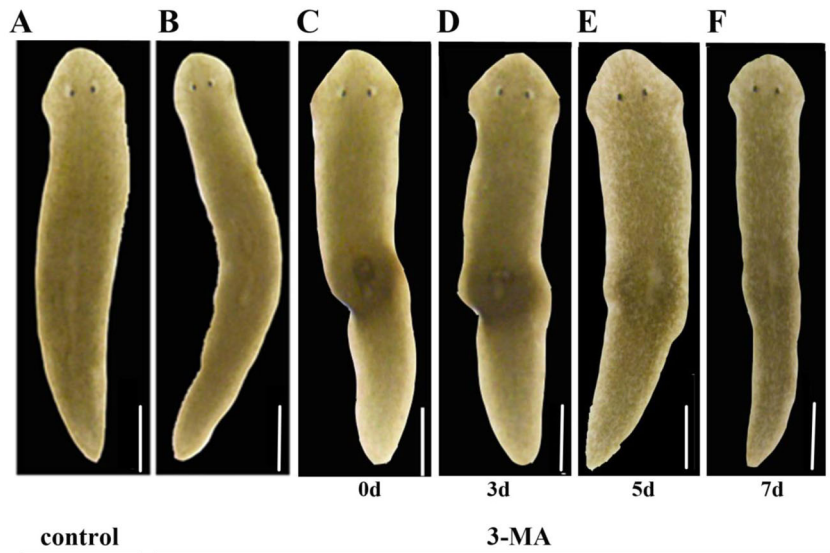
### 3-MA treatment

To produce a 0.5 M 3-MA solvent solution, 7.5 mg of 3-MA was added to 0.1 ml of sterilized double steaming water and mixed. Then, the solution was placed in a refrigerator set at 4 °C. The animals were exposed to 10 mM 3-MA for 18 h. Subsequently, they were rinsed three times and cultured in autoclaved tap water for 7 days. Images were captured with a Leica digital camera attached to a compound stereomicroscope (M165C, Germany). To analyse the effects of 3-MA during *D. japonica* regeneration, 20 normal worms (Fig. 1B) were selected after 10 mM 3-MA exposure for 18 h and compared with the control worms (Fig. 1A). Then, the worms were cut at the pre-pharyngeal and post-auricle levels to produce two fragments (Fig. 2B). The head and tail fragments were separately incubated in autoclaved tap water for 14 days, and images were captured following their amputation and separation.

### Transmission electron microscopy (TEM)

Planarian individuals were placed in on the inside wall of a 100-ml glass beaker and sacrificed with 2% dilute hydrochloric acid (HCl). Then, they were fixed with 2.5% glutaraldehyde at room temperature for 2 h. Then, the fixed samples were sent to the TEM Laboratory Center of Xinxiang Medical College, where they were washed repeatedly with buffer solution, fixed with osmium acid, dehydrated, embedded with epoxy resin, sliced into ultrathin sections, stained with uranium acetate and lead citrate, and observed and imaged by a professional technician (Abdel et al. 2014; Kang et al. 2019b).

**Fig. 1** Effects of 3-MA treatment on intact planarians ( $n = 20$  animals for each treatment). **A** The control planarian is normal; **B** Half of planarians are normal after 3-MA treatment; **C–E** Planarians show ulcer in the middle of the body for 0, 3, 5 d after 3-MA treatment; **F** Planarians are normal for 7 d after 3-MA treatment. Scale bar, 500  $\mu\text{m}$



### Real-time PCR (RT-PCR)

After exposure to 10 mM 3-MA for 18 h, worm RNA was extracted from 6 worms using RNAiso plus (TaKaRa, China) and then subjected to agar electrophoresis. In addition, the concentration of RNA was measured with a spectrophotometer (Thermo, United States), and the amount of RNA template was calculated according to the concentration. Then, reagents were added according to the instructions of a cDNA reverse transcription kit (TaKaRa, China) and mixed. The mixture was placed in a thermal cycler for reverse transcription of the mRNA to cDNA. Real-time PCR primers were mixed with the cDNA template and SYBR Green Master Mix reagent for real-time PCR detection (Dong et al. 2017). The following *Djvps34* RT-PCR primers were used: F-5' CGTAGTTTGGCTGGTTA TTGTGTC 3' and R-5' GTAAAGGTTTAGGATCATT GCCC 3'. The *Djβ-actin* gene (accession number: AB292462) was used as the housekeeping gene in the experiment (Dong et al. 2017; Dong et al. 2015; Kang et al. 2019a, 2019b). The expression ratios were determined using the  $2^{-\Delta\Delta\text{CT}}$  method, which was described by Kang et al. (2019a, 2019b). The statistical analyses were performed with one-way analysis of variance (ANOVA) using SPSS 13.0 software (Dong et al. 2017).  $P < 0.05$  was considered significant, and  $P < 0.01$  was considered extremely significant.

### Whole-mount immunofluorescence (WIF)

After 10 mM 3-MA exposure for 18 h, 40 normal worms were selected (Fig. 1B). Then, 20 worms were cultured in autoclaved tap water for the intact immunofluorescence experiment. In addition, other worms were cut as described, and the head and tail fragments were separately incubated in autoclaved tap water for observation in the regenerative

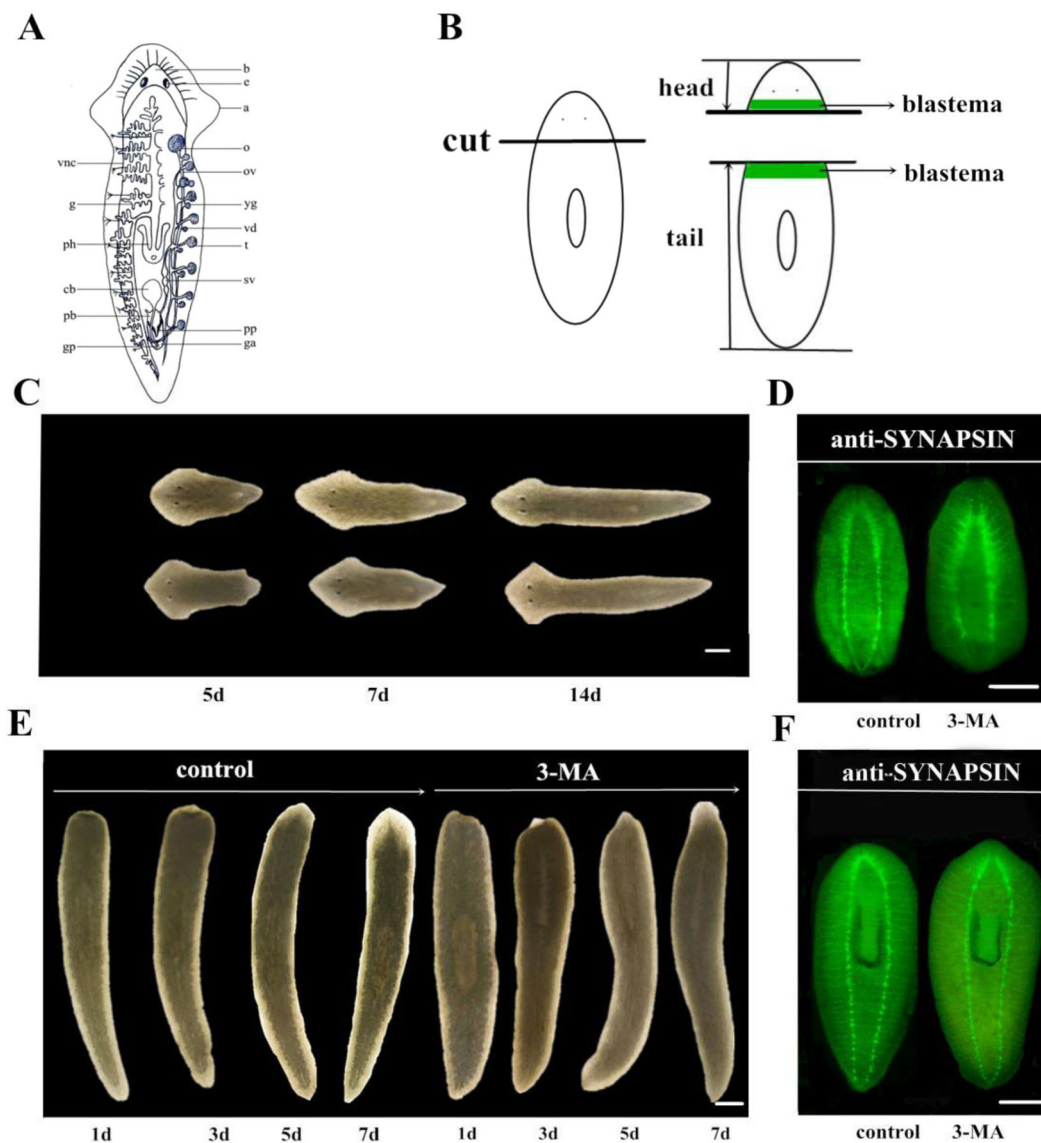
immunofluorescence experiment. Cell proliferation and nervous system phenotypes of the intact and regenerating worms were examined by whole-mount immunofluorescence as described previously (Dong et al. 2019).

We evaluated nerve development levels by immunofluorescence (antibody: anti-SYNAPSIN, diluted 1:200) on the 5th day of regeneration (Gonzalez-Estevéz et al. 2012). Fluorescence signals were detected with a stereo fluorescence microscope (Axio Zoom. V16, Germany). For each experiment, we selected 20 animals and repeated the observations three times. More than 80% of the changes were statistically significant.

To analyse the relationship between cell proliferation and 3-MA treatment in tissue turnover, we performed immunofluorescence using phospho-H3P antibody (diluted 1:2000, Gonzalez-Estevéz et al. 2007). Fluorescence signals of intact and regenerating worms were detected with a stereo fluorescence microscope (Axio Zoom. V16, Germany). The numbers of H3P + cells/ $\text{mm}^2$  were counted and quantified in all groups. The statistical analyses were performed with one-way analysis of variance (ANOVA) using SPSS 13.0 software (Dong et al. 2017).  $P < 0.05$  was considered significant, and  $P < 0.01$  was considered extremely significant.

### Statistical analysis

All experiments were biologically repeated three times. The data are the means  $\pm$  standard deviation (SD), and statistical analyses were performed by Student's t-test for group pairs and one-way analysis of variance (ANOVA) for multiple groups using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).  $P < 0.05$  was considered significant, while  $P < 0.01$  was considered extremely significant (Dong et al. 2015).



**Fig. 2** Effects of 3-MA treatment in regeneration planarians ( $n = 20$  animals for each treatment). **A** Schematic draw in sexual maturity individuals of *D. japonica* (Dong et al. 2015) (vnc ventral nerve cords, g gut, ph pharynx, cb copulatory bursa, pb penis bulb, Gp genital pore, b brain, e eye, a auricle, o ovary, ov oviduct, yg yolk gland, Vd vas deferens, t testis, sv seminal vesicle, pp penis papilla, ga genital atrium, Ca common atrium); **B** Schematic illustration of amputation

(Green areas: the blastemas); **C** Head regeneration for 5, 7 and 14 d after 3-MA treatment; **D** Head regeneration of the CNS for 5 d by whole-mount immunofluorescence (antibody: anti-SYNAPSIN) after 3-MA treatment; **E** Tail regeneration for 1, 3, 5 and 7 d with disproportionate blastemas after 3-MA treatment; **F** Tail regeneration of the CNS for 5 d by whole-mount immunofluorescence (antibody: anti-SYNAPSIN) after 3-MA treatment. Scale bar, 500  $\mu\text{m}$

## Results

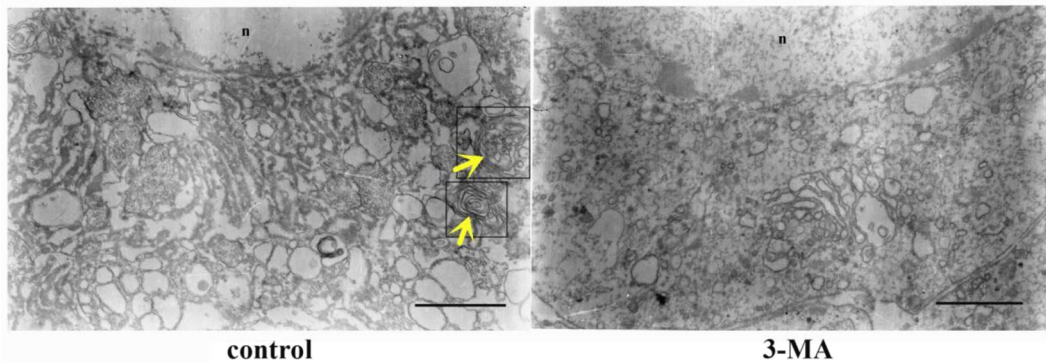
### 3-MA treatment induced ulcers, which returned to normal in intact worms by the 7th day of treatment

The intact worms were exposed to 3-MA for 18 h, and the results showed that 50% of the worms presented ulcers in the middle of the body (Fig. 1C). The ulcers had stopped getting larger by the 3rd day (Fig. 1D), and gradually, healthy tissue was growing on the 5th day of treatment

(Fig. 1E). Finally, the worms recovered and were normal intact worms by the 7th day of treatment (Fig. 1F).

### 3-MA treatment induced disproportionate blastemas with tissue regression during planarian regeneration

After 3-MA treatment for 18 h, the worms were amputated. In the morphological observation experiments, more than 50% of the changes were statistically significant. Then,

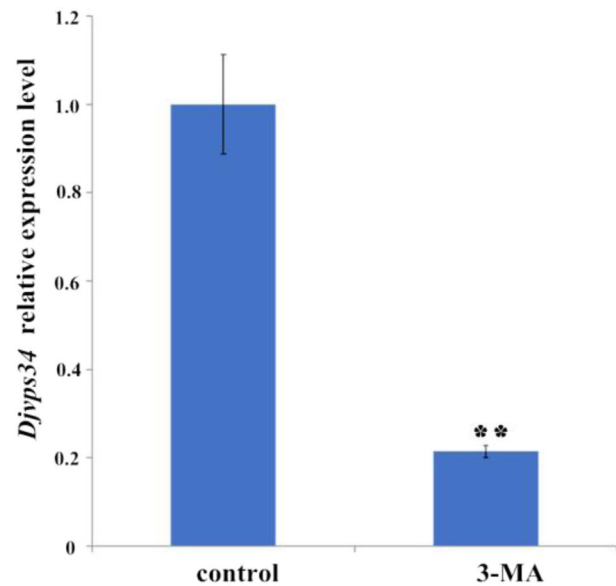


**Fig. 3** Ultrastructural changes in *D. japonica* induced by 3-MA treatment ( $n = 3$  animals for each treatment). Yellow arrows, autophagic vesicles (AVs); scale bars, 1  $\mu\text{m}$

regenerating fragments were cultured in autoclaved tap water and incubated in the dark at 20 °C. The fragments displayed slower regeneration speed compared with the control worms and exhibited normal phenotypes after 14 days of regeneration (Fig. 2C). In the head fragments, a new pharynx (Fig. 2C) and full ventral nerve cords (Fig. 2D) were not observed on day 5 of regeneration. Smaller tails were found between 5 and 7 days of regeneration compared with the controls (Fig. 2C). Correspondingly, 70% of the tail fragments regenerated more slowly and had smaller blastemas from day 1 to day 5 (Fig. 2E) than the controls. On the 7th day, a disproportionate head had taken shape (Fig. 2E). Fluorescence microscopy showed that there was an immature central nervous system (CNS) (Fig. 2F) and ventral nerve cord (VNC) (Fig. 2D) on the 5th day of worm regeneration. More than 80% of the changes were statistically significant.

### 3-MA treatment inhibited autophagy vesicle formation and *Djvps34* expression during planarian tissue regeneration

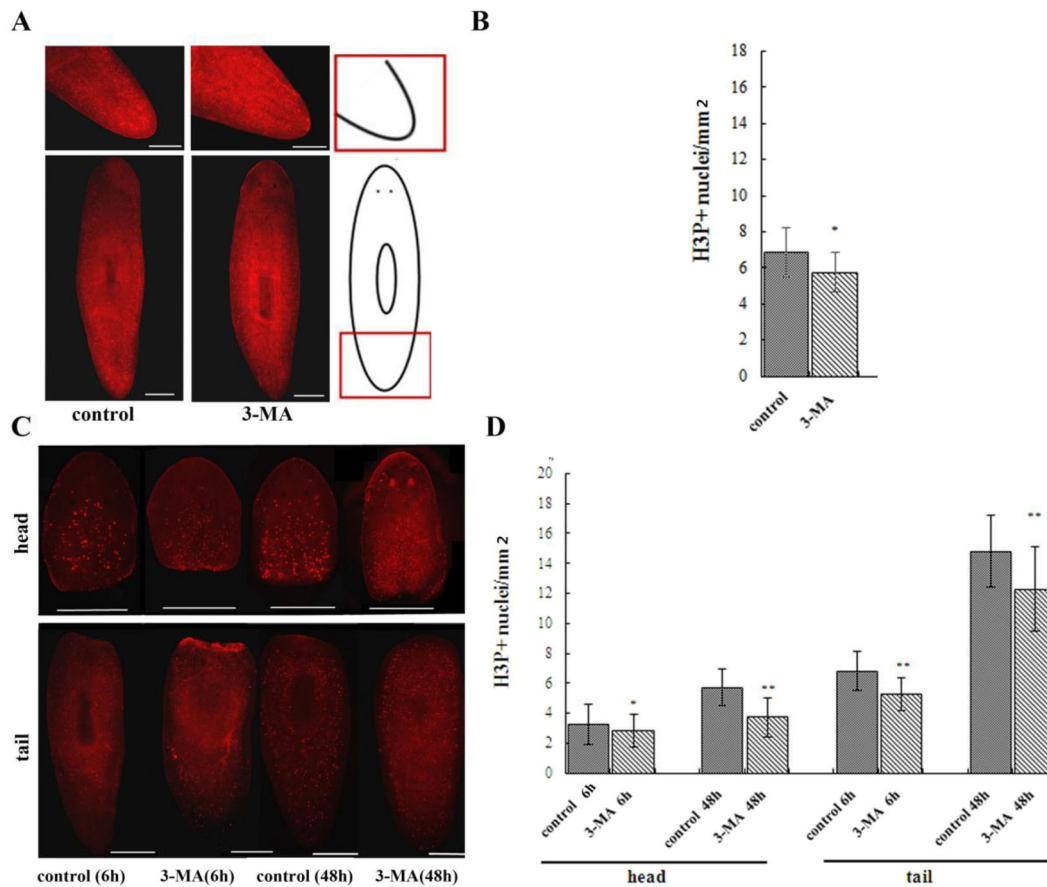
TEM is an effective method of detecting autophagic vesicles (Gonzalez-Estevez et al. 2007). Therefore, TEM was used to detect whether autophagy was inhibited in planarians after 3-MA exposure at different concentrations and exposure times. The results showed that the number of autophagic vesicles (yellow arrows) decreased in the treated animals after 10 mM 3-MA exposure for 18 h (Fig. 3). In these images, more than 80% of the changes were statistically significant. As an inhibitor of autophagy, 3-MA has the ability to suppress *Vps34* expression in mammals (Ding et al. 2018). Here, RT-PCR was performed in *D. japonica* after 3-MA treatment. We found that *Djvps34* expression was significantly down-regulated in parallel with autophagy being inhibited in the planarians (Fig. 4). This result indicated that 3-MA may inhibit the formation of autophagy in *D. japonica* through the PI3K pathway.



**Fig. 4** Quantitative PCR (qPCR) showing the relative expression level of *Djvps34* in 3-MA treatment animals ( $n = 6$  animals). Asterisks indicate statistical significance (\*\* $P < 0.01$ ). Samples were collected at 8th d after feeding

### 3-MA treatment suppressed stem cell proliferation in planarians

Neoblasts (stem cells) are the only proliferative cells in planarians, and their mitotic activities were evaluated by H3P immunofluorescence (Dong et al. 2019). To analyse the relationship between stem cell proliferation and 3-MA treatment, we performed immunofluorescence using a phospho-H3P antibody in intact and regenerating worms following 3-MA treatment. The results showed decreased mitotic activity in the intact worms after 3-MA treatment compared to the controls (Fig. 5A, B). Following planarian amputation, 3-MA treatment suppressed stem cell proliferation as evidenced by observations made at the 6th hour and 48th hour of regeneration (Fig. 5C, D).



**Fig. 5** Whole-mount immunofluorescence using anti-phospho histone3 after 3-MA treatment ( $n = 20$  animals for each treatment) **A** Immunofluorescence with anti-H3P antibody following 3-MA treatment in intact worms; **B** Mitotic density in intact planarian ( $*P < 0.05$ );

**C** Immunofluorescence with an anti-H3P antibody following 3-MA treatment (Scale bar, 500  $\mu\text{m}$ ) in regeneration worms; **D** Mitotic density in regeneration planarians ( $**P < 0.01$ ,  $*P < 0.05$ )

## Discussion

Autophagy is regulated by multiple pathways, among which the PI3K pathway is the most important pathway in the initial stage of autophagosome formation (Zhao et al. 2019). Furthermore, the synthesis of autophagosomes can proceed smoothly when the pI3K-III complex is successfully activated in yeast and mammals (Zhang et al. 2020). VPS34 is a catalytic unit of the class III phosphatidylinositol 3-kinase complex (PI3K-III) and is the progenitor of the phosphoinositide 3-kinase (PI3K) family (Xia et al. 2014). VPS34 can phosphorylate phosphatidylinositol to produce PtdIns3P, which is important in autophagy and endocytic pathways (Ohashi et al. 2018). 3-MA has been established as an autophagy inhibitor with effects realized via the PI3K pathway (Ding et al. 2018). In *D. japonica*, 3-MA efficiently suppressed the formation of autophagosomes and inhibited *Djvps34* expression. These results indicate that 3-MA treatment caused autophagy inhibition via the PI3K pathway in freshwater planarians, similar to that previously observed in vertebrates (Zhang et al. 2020).

Autophagy is an evolutionarily conserved mechanism that continuously maintains the homeostasis of all tissues (Kang et al. 2019a; Gonzalez-Estevez and Salo 2010). Further, it has been reported that autophagy also plays a key role in the antioxidant repair system (Kang et al. 2019a; Gonzalez-Estevez and Salo 2010). In the present study, we treated planarians with 3-MA to assess its toxicity. 3-MA exposure led to serious disorders in *D. japonica*, and most worms formed ulcers in the middle of the body and healthy tissue was restored by the 7th day of treatment. Moreover, 3-MA treatment induced disproportionate blastemas with tissue regression during planarian regeneration, and the number of blastemas was restored by the 14th day of regeneration. These findings suggested that 3-MA treatment caused tissue homeostasis disorder in both regenerating and intact worms by inhibiting autophagy. However, autophagy slowly returned to normal levels following exposure, and homeostasis also slowly returned to normal levels in *D. japonica*.

Planarians continuously adapt their body (morphallaxis) to different environmental stresses, which involves a cell

proliferation process (Gonzalez-Estevez and Salo 2010). Moreover, they are equipped with cellular mechanisms that enable them to modulate the balance between cell proliferation and autophagy during tissue turnover (Kimberly et al. 2012). In addition, autophagy is tightly coupled to cell proliferation during stress-induced events (Gonzalez-Estevez and Salo 2010). To analyse the relationship between cell proliferation and autophagy suppression in *D. japonica*, we performed immunofluorescence using an anti-H3P antibody to detect the cell proliferation rate after 3-MA treatment. The results showed a reduction in mitotic activity in planarians after 3-MA treatment. We suspected that the planarians showed a reduced cell proliferation rate in response to the inhibition of autophagy. Because of cell proliferation, much energy was required, and autophagy was activated in the control group. However, autophagy in the experimental group was inhibited after treatment with 3-MA, and the proliferation of the cells was also inhibited, and the energy supply and demand reached a relative balance. In mammals, cells can survive through autophagy when tissues need energy to respond to stress (Sahani et al. 2014; Dong et al. 2019). We speculated that autophagy was also strictly controlled and regulated at the energy base line in planarians.

In this work, exposure to 10 mM 3-MA for 18 h led to serious disorders in *D. japonica*. Moreover, the number of autophagic vesicles and *Djvps34* expression decreased after 3-MA treatment. After amputation, disproportionate blastemas with tissue regression were observed during regeneration. Furthermore, the immunofluorescence results indicated that 3-MA treatment suppressed cell proliferation during tissue regeneration. These results revealed that treatment with the autophagy inhibitor 3-MA might induce negative effects on planarian homeostasis via the PI3K pathway.

### Data availability

All data, models, and code generated or used during the study appear in the submitted article.

**Acknowledgements** We especially thank Dr. Xin Yan from Max-Delbrueck Center in the Helmholtz Association and Dr. Yanli Liu for their critical reviews of this manuscript.

**Author contributions** JK performed TEM, 3-MA treatment, morphological observation and quantitative real-time PCR. JZC performed immunofluorescence. GWC and ZMD conceived of the study, participated in its design and coordination and helped to draft the manuscript. DZL had been involved in revising the manuscript. All authors read and approved the final manuscript.

**Funding** This work is supported by grants from the National Natural Science Foundation of China (32070427, 31572267, 31471965 and U1604173), the Puyang Field Scientific Observation and Research Station for Yellow River Wetland Ecosystem project of Henan

Province, Key Technologies and Program of Henan Province (202102110261 and 202102110383), as well as Major public welfare project of Henan Province (201300311700) and the Science and technology project of Xinxiang City (GG2020012).

### Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Research involving human participants and/or animals' note** This study do not involve endangered or protected species, and the collection of specimens is approved by the Forestry Department of Wild Animal Protection, Henan Province, China.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

### References

- Abdel HH, Abdelhabib S, Lamjed M, Mukhtar A, Alexander V, Sirotkin, Suliman Y, AI O, Maha A, Mohamed S, AI A, Ibrahim M, Alhazza JR, Nyengaard SA (2014) Infertility in the hyperplastic ovary of freshwater planarians: the role of programmed cell death. *Cell Tissue Res* 358:607–620
- Avila-Rojas SH, Lira-León A, Aparicio-Trejo OE, Reyes-Fermin LM, Pedraza-Chaverri J (2019) Role of autophagy on heavy metal-induced renal damage and the protective effects of curcumin in autophagy and kidney preservation. *Medicina (Kaunas, Lithuania)* 55(7):360
- Christian R, Celine L, Caroline A, Carole BL, Damien G, Stephane M, Annie L, Soumeiya B, Bruno JG (2014) 3-MA inhibits autophagy and favors long-term integration of grafted Gad67-GFP GABAergic precursors in the developing neocortex by preventing apoptosis [J]. *Cell Transplant* 23(11):1425–1450
- Danielle I, Veronica B, Daniel C, Christina R, Sumi O, Ameet S, Eva-Maria SC (2020) *Dugesia japonica* is the best suited of three planarian species for high-throughput toxicology screening. *Chemosphere* 253:126718
- Ding DF, Xu SY, Zhang OF, Zhao W, Zhang XP, Jiang YX, Wang GP, Dai ZL, Zhang JZ (2018) 3-Methyladenine and dexamethasone reverse lipopolysaccharide-induced acute lung injury through the inhibition of inflammation and autophagy. *Exp Therap Med* 15:3516–3522
- Dong Z, Cheng FF, Yuwen YQ (2015) Identification and expression analysis of a *Spsb* gene in planarian *Dugesia japonica*. *Gene* 564:168–175
- Dong ZM, Yang T, Yang YB, Dou H, Chen GW (2017) *DjhnRNPA2/B1*-like gene is required for planarian regeneration and tissue homeostasis. *Gene* 10(633):9–16
- Dong ZM, Cheng FF, Yang YB, Chen GW, Liu DZ (2019) Expression and functional analysis of flotillins in *Dugesia japonica*. *Exp Cell Res* 374(1):76–84
- Gonzalez-Estevez C (2009) Autophagy meets planarians. *Autophagy* 5(3):290–297
- Gonzalez-Estevez C, Salo E (2010) Autophagy and apoptosis in planarians. *Autophagy* 15(3):279–292
- Gonzalez-Estevez C, Felix DA, Aboobaker AA, Salo E (2007) Gtdap-1 promotes autophagy and is required for planarian remodeling during regeneration and starvation. *PNAS* 104(33):13373–13378

- Gonzalez-Estevez C, Felix DA, Smith MD, Paps J, Morley SJ, James V (2012) SMG-1 and mTORC1 act antagonistically to regulate response to injury and growth in planarians. *PLoS Genet* 8(3): e1002619
- Hou N, Liu N, Han J, Li J (2016) 3-MA enhances chemotherapeutic effect of 5-FU by inhibiting autophagy in colon cancer [J]. *J Xian Jiaotong Univ* 37(1):49–53
- Jiang Y, Zhu J, Wu L, Xu G, Dai J, Liu X (2012) Tetracycline inhibits local inflammation induced by cerebral ischemia via modulating autophagy. *PLOS ONE* 7(11):e48672
- Kang J, Dong Z, Wang J, Chen G, Liu D (2019a) Autophagy-related *Djatg8* is required for remodeling in planarians *Dugesia japonica*. *Biol Open* 8(12):bio45013
- Kang J, Dong Z, Hao Q, Wang J, Chen G, Liu D (2019b) The regulation of rapamycin in planarian *Dugesia japonica* Ichikawa & Kawakatsu, 1964 regeneration according to TOR signaling pathway. *Ecotoxicol Environ Saf* 10(185):109680
- Kimberly C, Bret T, Alejandro JP, Alvarado S (2012) TORC1 is required to balance cell proliferation and cell death in planarians. *Dev Biol* 65(2):458–469
- Lavandero S, Chiong M, Rothermel BA, Hill JA (2015) Autophagy in cardiovascular biology. *J Clin Invest* 125:55–64
- Lee D, Bareja A, Bartlett D, White J (2019) Autophagy as a therapeutic target to enhance aged muscle regeneration. *Cells* 8(2):183
- Li CX, Cui LH, Zhuo YZ, Hu JG, Cui NQ, Zhang SK (2018) Inhibiting autophagy promotes collagen degradation by regulating matrix metalloproteinases in pancreatic stellate cells. *Life Sci* 1(208):276–283
- Nieuwenhuis B, Barber AC, Evans RS, Pearson CS, Eva R (2020) PI 3-kinase delta enhances axonal p13 to support axon regeneration in the adult CNS. *EMBO Mol Med* 12(8):e11674
- Ohashi Y, Tremel S, Williams RL (2019) VPS34 complexes from a structural perspective. *J Lipid Res* 60(2):229–241
- Pang J, Xiong H, Zhan T, Cheng G, Jia H, Ye Y, et al. (2018) Sirtuin 1 and autophagy attenuate cisplatin-induced hair cell death in the mouse cochlea and zebrafish lateral line. *Front Cell Neurosci* 12:515
- Pang J, Xiong H, Zhan T, Cheng G, Jia H, Ye Y, et al. (2019) Sirtuin 1 and autophagy attenuate cisplatin-induced hair cell death in the mouse cochlea and zebrafish lateral line. *Front Cell Neurosci* 12:515
- Ross KG, Currie KW, Pearson BJ, Zayas RM (2017) Nervous system development and regeneration in freshwater planarians. *Wiley Interdisciplinary Reviews. Dev Biol* 6(3):e266
- Saera-Vila A, Kish PE, Louie KW, Grzegorski SJ, Klionsky DJ, Kahana A (2016) Autophagy regulates cytoplasmic remodeling during cell reprogramming in a zebrafish model of muscle regeneration. *Autophagy* 12(10):1864–1875
- Sahani MH, Itakura E, Mizushima N (2014) Expression of the autophagy substrate SQSTM1/p62 is restored during prolonged starvation depending on transcriptional upregulation and autophagy-derived amino acids [J]. *Autophagy* 10:431–441
- Xia P, Wang S, Huang G, Du Y, Zhu P, Li M et al. (2014) Rnf2 is recruited by wash to ubiquitinate ambr1 leading to down-regulation of autophagy. *Cell Res* 24(8):943–958
- Xu F, Hua C, Tautenhahn HM, Dirsch O, Dahmen U (2020) The role of autophagy for the regeneration of the aging liver. *Int J Mol Sci* 21(10):3606
- Zhang HC, Liu TY, Shi CY, Chen GW, Liu DZ (2017) Genotoxicity Evaluation of an Urban River on Freshwater Planarian by RAPD Assay. *Bull Environ Contam Toxicol* 98(4):484–488
- Zhang HC, Ma KX, Yang YJ, Shi CY, Chen GW, Liu DZ (2018) Molecular cloning, characterization, expression and enzyme activity of catalase from planarian *Dugesia japonica* in response to environmental pollutants. *Ecotoxicol Environ Saf*. 165:88–95
- Zhang P, Li Y, Fu Y, Huang L, Liu B, Zhang L, Shao XM, Xiao D (2020) Inhibition of Autophagy signaling via 3-methyladenine rescued nicotine-mediated cardiac pathological effects and heart dysfunctions. *Int J Biol Sci* 16(8):1349–1362
- Zhang Y, Chen H, Fan Y, Yang Y, Gao J, Xu W et al. (2019) Cytotoxic effects of bio-pesticide spinosad on human lung A549 cells. *Chemosphere* 230(SEP.):182–189
- Zhao L, Zhang B, Cui Y, Hou C, Zeng Q, Gao T, Zhang Z, Yu J, Wang Y, Wang A, Liu H (2019) 3-Methyladenine alleviates excessive iodine-induced cognitive impairment via suppression of autophagy in rat hippocampus. *Environ Toxicol* 4(14):912–920