Expression level comparison of marker genes related to early embryonic development and tumor growth

QIU-CHEN CAI[1*], DA-LUN LI[1*], YING ZHANG[1*], YUN-YI LIU[1*], PEI FANG[1], SI-QIN ZHENG[1], YUE-YAN ZHANG[1], YA-KUN YANG[1], CHUN HOU[2], CHENG-WEI GAO[3], QI-SHUN ZHU[1,2] and CHUAN-HAI CAO[4]

Schools of [1]Life Sciences, [2]Ecology and Environmental Science, and [3]Chemical Science and Technology, Yunnan University, Kunming, Yunnan 650500, P.R. China; [4]Taneja College of Pharmacy, University of South Florida, Tampa, FL 33612, USA

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Abstract. In tumor research, the occurrence and origin of tumors are the fundamental problems. In the 1970s, the basic discussion of the developmental biology problem of tumors was proposed, and it was believed that tumorigenesis is closely related to developmental biology. Tumors are abnormal biological structures in organisms, and their biological behavior is very similar to that of the early embryo. Many tumor-related genes also serve regulatory roles in the normal development and differentiation of embryos. However, it remains unclear whether gene expression in early embryos has any similarities with tumor cells. In this study, to compare the similarities and differences in gene expression between early embryos and tumor cells, reverse transcription-quantitative PCR was conducted to determine and compare the relative expression levels of nine tumor-related genes in the brain glioma cell line, T98G, and in the early embryo of *Spodoptera litura*, which is fast-growing, low-cost, easily accessible and easy to observe. The expression of tumor-related genes in early embryos and the similarity of regulatory mechanisms between early embryonic development and tumor growth were explored. In conclusion, tumor growth may be regarded as an abnormal embryogenic activation that happens in the organs of adult individuals.

Correspondence to: Dr Qi-Shun Zhu, School of Ecology and Environmental Science, Yunnan University, 3 South Outer Ring Road, East of University Town, Kunming, Yunnan 650500, P.R. China
E-mail: zhuqsfa@aliyun.com; qshzhu@ynu.edu.cn

Professor Chuan-Hai Cao, Taneja College of Pharmacy, University of South Florida, 12901 Bruce B. Downs Blvd, Tampa, FL 33612, USA
E-mail: ccao@usf.edu

[*]Contributed equally

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Introduction

The treatment and prognosis of tumors has been a major focus of research in tumor-related studies, and the study of tumor pathogenesis and mechanisms of tumorigenesis is even more difficult than the study of tumor treatment. In the 1970s, Pierce and colleagues proposed that tumors are a developmental biology problem, and he believed that tumorigenesis is closely related to developmental biology (1,2). In 1892, Lobstein and Recamier presented a fundamental discussion on whether tumors are embryonic physiological disorders and their origin (3-5). They argued that tumors are formed by the continuous proliferation of embryonic cells stored in the body for a long time and that there is a high degree of similarity between tumors and embryos (6). Moreover, a study has shown that genes related to tumors can affect the normal development and differentiation of cells (7). Tumors are the products of embryonic gene expression and the result of the activation and expression of numerous oncogenes in the body (7). Early studies have confirmed that interconversion between tumor cells and early embryonic cells is possible under specific conditions (8,9). In 2000, Hanahan and Weinberg (2) proposed six major characteristics of tumor cells, including unlimited replication, tissue invasion, insensitivity to growth resistance, self-sufficient growth, evasion of apoptosis and sustained angiogenesis. Subsequently, in 2011, they added four more features of tumor cells, including genomic instability, promotion of inflammation, avoidance of immune response and energy dysregulation (10). These characteristics are very similar to the biological features of early embryonic cells. For example, gene methylation and demethylation, cell implantation, functional gene expression, cellular immune evasion and other such aspects in the early embryonic growth and development are strikingly like the biological function and behavior of tumor cells (11-14). Both embryonic and tumor cells can be deprogrammed to achieve a proliferative stem cell state with potential for apoptosis and invasiveness. Therefore, it is hypothesized that the set of genes expressed in tumor cells may be the same as those expressed in embryonic cells, particularly those genes involved in deprogramming, proliferation and undifferentiation (4,15-21).

The present study focused on the similarity of gene expression related to early embryonic development and tumor growth. Nine factors highly associated with tumor regulation, including MYC, MYB, BCL-2, BCL-2-interacting protein 3 (BNIP3), p53, PTEN, PI3K, AKT and mTOR were selected as experimental research subjects. These nine factors occupy important positions in the large regulatory network of the body (22-27). Changing of their expression may lead to changes or even loss of control of the regulatory network (22,26-37). In addition, these factors are highly related to the development and growth, regulatory mechanisms and microenvironment maintenance of early embryos (4,6,17,30,38-42). MYC (29,31,43), MYB (26), BCL-2 (44-46), BNIP3 (47), p53 (27,48), PI3K (22,28), AKT (23,49) and mTOR (32,50-53) are regarded as proto-oncogenes that serve important roles in cell proliferation and differentiation, apoptosis, cell cycle regulation and metabolic processes. In previous studies of mouse embryonic development, high mRNA and protein expression levels of these proto-oncogenes were also detected in mouse embryos. The expression of these proto-oncogenes was highly associated with the successful implantation of fertilized eggs into the uterus, which could be determined by observing whether the mice became pregnant (6,30,38-42,45,54-56).

The main function of the anti-oncogenic biomarker, PTEN, is to promote apoptosis and hinder cell proliferation, migration and local adhesion (57). Downregulation or loss of PTEN expression was found in a variety of tumors, such as non-small cell lung cancer and glioma (37,57). A previous study showed that PTEN has low expression levels in the zygote and blastocyst stages in early embryonic development of mice (58).

Since the early embryos used in the present study were those before gastrulation, it was impossible to obtain human or large primate early embryos due to ethical restrictions. Furthermore, retrieving the early embryos of mice could be painful for the mice (59). Therefore, the insect model was chosen in the present study. *Drosophila* could be a good model as much of the research on humans has been conducted with *Drosophila* (60). However, the eggs of *Drosophila* are too small to clearly distinguish the embryonic development stage within them (61).

Spodoptera litura is an omnivorous and gluttonous lepidopteran pest and is closely related to *Drosophila* (62). Their eggs are ideal for early embryonic studies, as they are flat and hemispherical, with a diameter of about 0.4-0.5 mm; they are yellow and white when they are newly laid, turning black before hatching, and the eggs are neatly stacked together (63,64). *S. litura* early embryonic development occurs between 1 and 8 h after egg laying, with the earliest divisions generally occurring at 2 h after egg laying (63,64). In the present study, the newly laid eggs of *S. litura* were used to analyze the expression of genes related to tumor metabolism, to understand the similarities and differences between these early embryos and tumors.

Materials and methods

Materials. Experiments involved two different cell lines, T98G human glioma (65) and human astrocyte (HA) (66), which were provided by the Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China). The original T98G cells were purchased from American Type Culture Collection (CRL-1690) and the HA cells were purchased from ScienCell Research Laboratories, Inc. (#1800). *S. litura* was from the Key Laboratory of the University in Yunnan Province for International Cooperation in Intercellular Communications and Regulations, Yunnan University (Kunming, China).

S. litura breeding. *S. litura* were maintained in an artificial climate incubator in the following conditions: Temperature, 27±1˚C; humidity, 60-80%; and 12-h light/dark cycle (67). Larvae were feed with artificial synthetic diet and adult moths were feed with 10% honey solution. The formulation of the artificial diet was the same as that used by the Key Laboratory of the University in Yunnan Province for International Cooperation in Intercellular Communications and Regulations (67). Provision of food and water was *ad libitum*. Both the larvae and adult moths were fed with a diet containing a large amount of water, so additional drinking water was not provided. The feed and honey solution were refreshed every 2 days. Larvae between first and sixth instar were kept in breathable boxes until pupation began. Larvae were transferred to a fine sand box for pupation. The pupae were separated from the male and female according to the position of the cloaca on the pupae. After emergence, the adult *S. litura* were placed in glass boxes with a 1:1 sex ratio to mate and lay eggs; they were fed with honey solution at the bottom of the boxes. The mating and spawning of the eggs were recorded. Two hours after oviposition, the eggs for RNA extraction were harvested and put into liquid nitrogen for preservation. The hemocytes of 3rd instar larvae were extracted for use as a control. The hemolymph was allowed to escape by puncturing the hematopoietic cavity from the larval gastropods and collecting it in a 1.5-ml Eppendorf tube containing 5 μl 5% reduced glutathione. After obtaining 1 ml hemolymph, it was gently pipetted and centrifuged at 10,000 x g for 5 min at 4˚C. The precipitated fraction was hemocytes.

Cell culture. T98G and HA cells were thawed at 37˚C, centrifuged at 500 x g for 1 min at room temperature to remove DMSO, and cultured as follows: The T98G cells were cultured at 37˚C in a 5% CO_2 atmosphere in Eagle's Minimum Essential Medium containing L-glutamine (Gibco; Thermo Fisher Scientific, Inc.) with the addition of 10% (v/v) fetal bovine serum (FBS) (Lonza Group Ltd.) and 1% (v/v) solution of penicillin and streptomycin (Gibco; Thermo Fisher Scientific, Inc.) (65). HA cells were cultured in HA medium containing DMEM/F12 (Gibco; Thermo Fisher Scientific, Inc.), 10% (v/v) FBS (Gibco; Thermo Fisher Scientific, Inc.), 2% B27 supplements (Gibco; Thermo Fisher Scientific, Inc), 3.5 mM glucose (Sigma-Aldrich), 10 ng/ml fibroblast growth factor 2 (Alomone Labs), 10 ng/ml epidermal growth factor (Alomone Labs), and 1% penicillin/streptomycin (Gibco; Thermo Fisher Scientific, Inc.) (66).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA samples for qPCR were extracted with RNA extraction kit (R6934-01; Omega Bio-Tek, Inc.), reverse transcription was carried out using a PrimeScript™ RT reagent Kit (RR047Q; Takara Bio, Inc.) according to the manufacturer's protocol and the concentration was determined. The relative expression

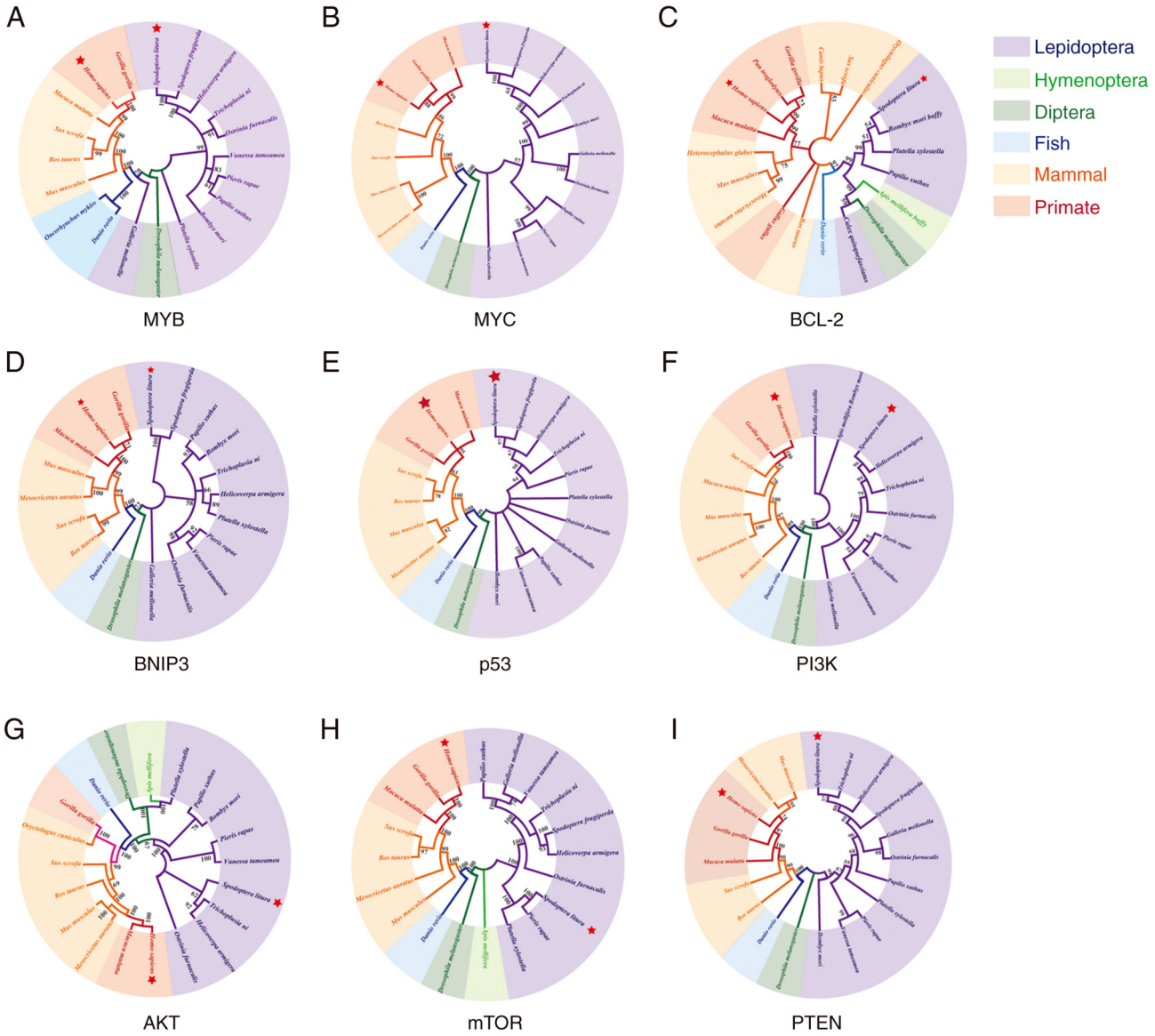

Figure 1. NJ phylogenetic tree of oncogenes and anti-oncogenes in 20 species. NJ phylogenetic trees of the oncogenes (A) MYB, (B) MYC, (C) BCL-2, (D) BNIP3, (E) p53, (F) PI3K, (G) AKT and (H) mTOR as well as for (I) the anti-oncogene PTEN in 20 species, including *Spodoptera litura* and *Homo sapiens*. BNIP3, BCL-2-interacting protein 3; NJ, neighbor joining.

levels of nine tumor-related genes and β-actin in early embryos and hemocytes of *S. litura*, the T98G and HA cell lines were measured by qPCR using an Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Inc.). The qPCR reaction procedure was as follows: 2 min at 50˚C and 10 min at 95˚C to activate the enzyme; 5 sec at 95˚C and 35 sec at 60˚C for 40 PCR cycles; 15 sec at 95˚C, 1 min at 60˚C, 30 sec at 95˚C and 15 sec at 60˚C to determine the melt curve. mRNA levels were quantified using the $2^{-\Delta\Delta Cq}$ method (68) and normalized to the internal quantitative reference gene β-actin. The primers used for T98G and HA cells are listed in Table SI, and those used for *S. litura* are listed in Table SII.

Artemether (ART) treatment. Hatching rate measurement, head width measurement and ART treatment were conducted under *S. litura* breeding conditions. Newly laid eggs of *S. litura* were soaked for 10 sec at 27˚C in 1 ml ART solution [300 ng/μl dissolved in 0.1% (v/v)] three times each day until eggs started hatching. As a blank control, newly laid eggs were soaked for 10 sec at 27˚C in 1 ml H_2O, and as a negative control, newly laid eggs were soaked for 10 sec at 27˚C in in 1 ml 0.1% DMSO solution. ART itself is slightly soluble in water and 0.1% DMSO was added to the solution to increase the solubility of ART, so a 0.1% DMSO negative control group was set up in this experiment. The hatching rate of eggs was counted after 2 days of treatment. For the experiment on larvae ART treatment, 270 healthy newly hatched larvae were reselected and were divided into three groups. The groups of larvae were fed with a normal diet, a diet containing 0.1% DMSO and a diet containing 300 ng/μl ART, respectively, for 14 days until they pupated. During the feeding period, the width of the larvae's head capsule was measured once a day. All ART treatment experiments included three independent replicates.

Neighbor joining (NJ) tree construction. The gene sequences of MYB, MYC, BCL-2, BNIP3, p53, PI3K, AKT, mTOR and

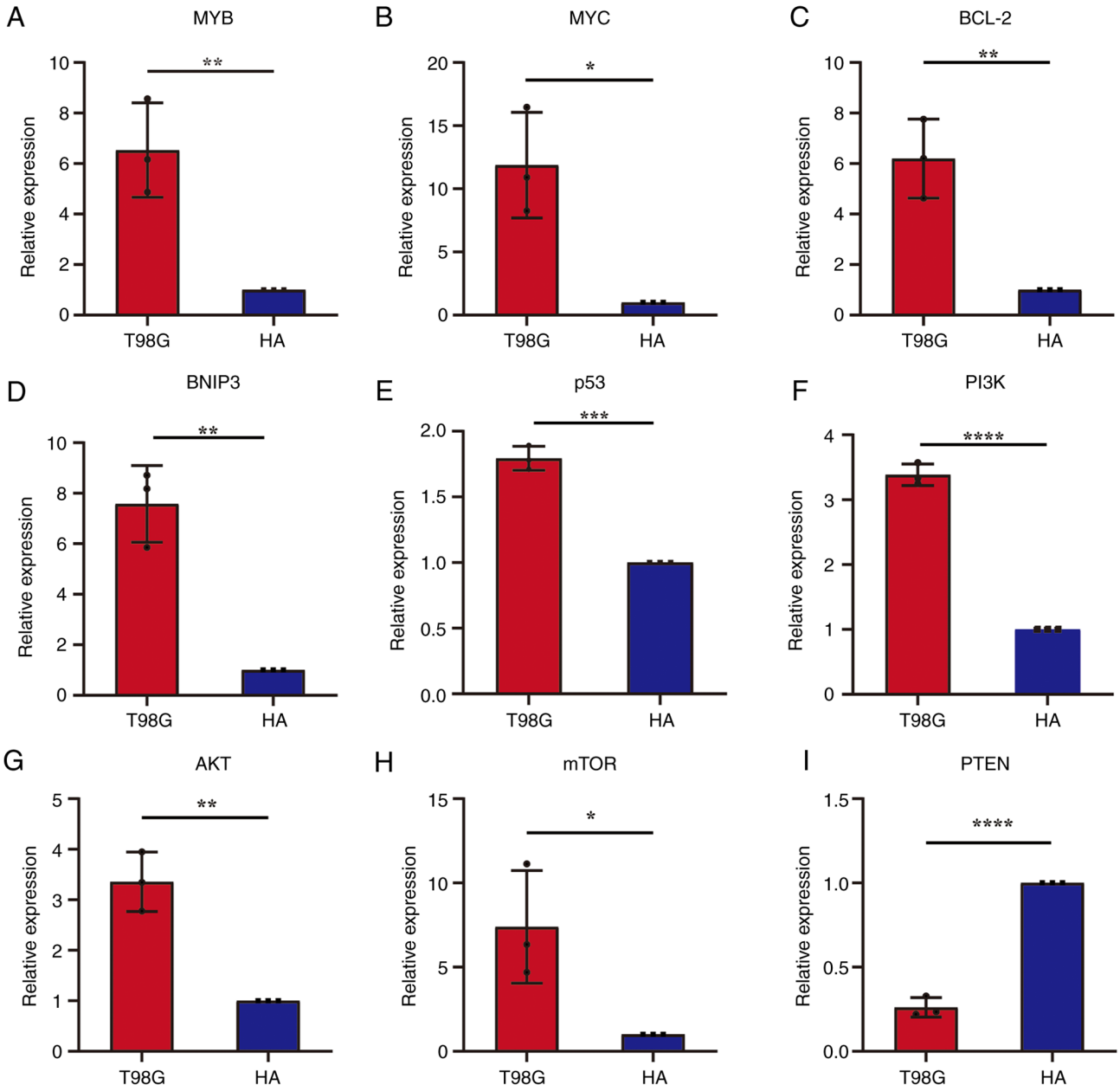

Figure 2. Relative mRNA expression levels of eight oncogenes and an anti-oncogene in T98G and HA cells. mRNA expression levels of the oncogenes (A) MYB, (B) MYC, (C) BCL-2, (D) BNIP3, (E) p53, (F) pI3K, (G) AKT and (H) mTOR and (I) the anti-oncogene PTEN in T98G human glioma and HA human astrocyte cells. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. BNIP3, BCL-2-interacting protein 3; ns, not significant.

PTEN of *S. litura*, *Homo sapiens* and 18 other invertebrates and vertebrates were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/gene/?term=). The accession numbers of all downloaded sequences are listed in Table SIII. The FASTA file was analyzed with MEGA7.0 (69), and the systematic cluster tree was constructed by NJ.

Statistical analysis. mRNA expression levels between T98G cells and HA cells, and between early embryos and larval hemocytes were compared by unpaired Student's t-test. mRNA expression of H_2O-treated, DMSO-treated and artemether (ART)-treated eggs was compared using one-way ANOVA and Tukey. The hatch rate of H_2O-treated, DMSO-treated and ART-treated eggs was compared using Fisher's test. The 14-day measurements of larvae head capsule width in the H_2O-treated, DMSO-treated and ART-treated larvae were compared using two-way ANOVA and Tukey. The 14-day measurements of larvae head capsule width were also compared within all three groups using two-way ANOVA and Tukey to confirm normal larval growth within the group (70). GraphPad Prism 9.0 (GraphPad Software, Inc.) was used for data analysis and graph plotting. P<0.05 was considered to indicate a statistically significant difference.

Results

Gene building sequence alignment of S. litura. As shown in Fig. 1, MYB, MYC, BCL-2, BNIP3, p53, PI3K, AKT, mTOR and PTEN genes are related between the 20 species, indicating

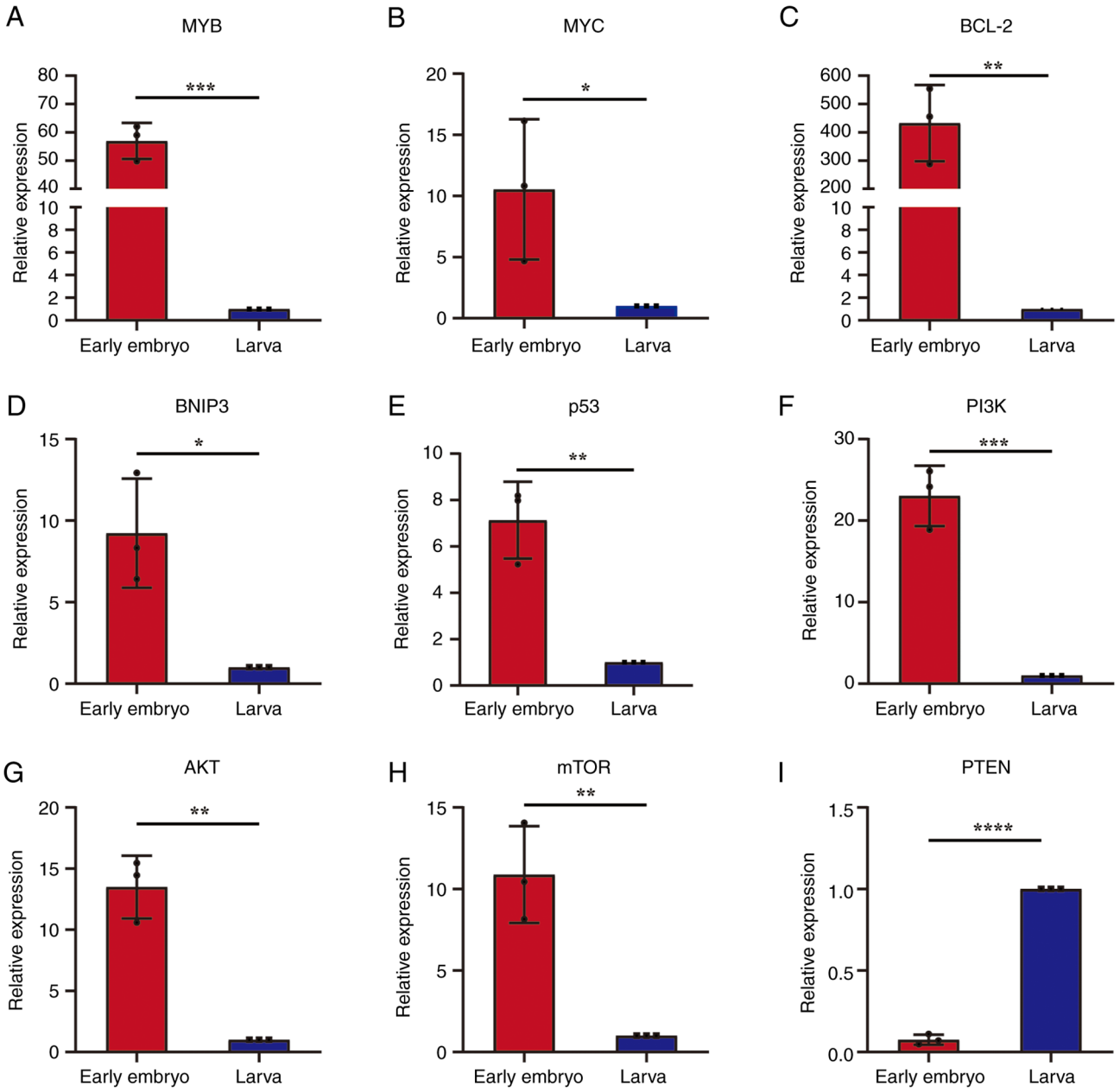

Figure 3. Relative mRNA expression of eight oncogenes and one anti-oncogene in early embryos and larval hemocytes of *Spodoptera litura*. mRNA expression levels of the oncogenes (A) MYB, (B) MYC, (C) BCL-2, (D) BNIP3, (E) p53, (F) pI3K, (G) AKT and (H) mTOR, as well as (I) the anti-oncogene, PTEN, in early embryonic and larval hemocyte cells. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. BNIP3, BCL-2-interacting protein 3.

the evolutionary developmental conservation of the nine tumor-related factors.

mRNA expression levels of tumor marker genes in T98G cells. The mRNA expression levels of eight oncogenes including MYB, MYC, BCL-2, BNIP3, p53, PI3K, AKT and mTOR in T98G and HA cells were detected (Fig. 2A-H). The results demonstrated that the mRNA expression levels of the eight oncogenes in T98G cells were significantly higher compared with that in the control group. The mRNA expression level of the anti-oncogene, PTEN, in T98G cells was significantly lower compared with that in the HA cell control group (Fig. 2I). These results demonstrated that seven of these nine genes may be excellent indicators of tumor cells. As soon as the expression of these marker genes is detected, it is possible to distinguish cells which are more like tumor cells, and which are not.

mRNA expression of tumor marker genes in early embryos and larval hemocytes of S. litura. The mRNA expression levels of the eight oncogenes (MYB, MYC, BCL-2, BNIP3, p53, PI3K, AKT and mTOR) and the anti-oncogene (PTEN) were determined in *S. litura* early embryos and larval hemocytes (Fig. 3). The results demonstrated that the mRNA expressions of the eight oncogenes in early embryos were significantly higher compared with that in the larval control group (Fig. 3A-H). mRNA expression of the anti-oncogene, PTEN, in early embryos was significantly lower compared with that in the larval hemocytes control group (Fig. 3I). The expression levels of these oncogenes in early embryos of *S. litura* showed the

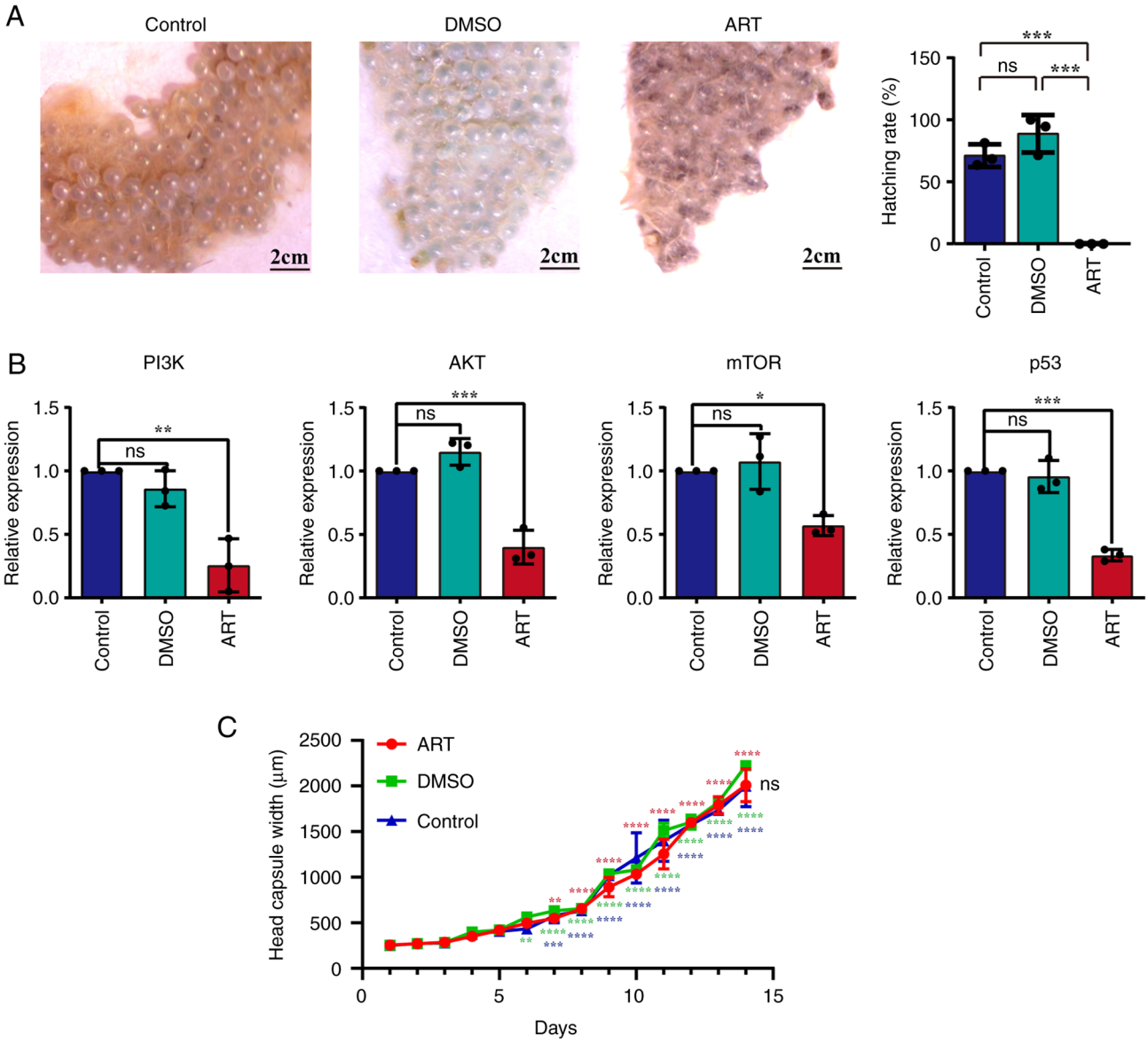

Figure 4. Viability of *Spodoptera litura* eggs and embryos following treatment with the anti-tumor compound, ART. (A) Eggs looked still healthy and glossy after treatment with H2O and DMSO, but the eggs became no longer glossy and gradually turned black after treatment with ART, indicating that the eggs had started to die. The hatch rates of *S. litura* eggs were significantly decreased after ART treatment. ***P<0.001. (B) The mRNA expression levels of PI3K, AKT, mTOR and p53 in *S. litura* embryos treated with ART were reduced. *P<0.05; **P<0.01; ***P<0.001. (C) Head capsule width was measured and analyzed to evaluate the growth and the development of *S. litura* larvae in H_2O-treated, DMSO-treated and ART-treated larvae. To indicate normal larval growth within the group, the larval head capsule widths within a single group were also compared with those of newly hatched 1st instar larvae on day 1. All three groups of larvae showed the same growth trend. **P<0.01, ***P<0.001 and ****P<0.0001 vs. day 1 (same group). No significant differences in growth trends were observed among the larvae of the ART, DMSO and control groups. ART, artemether; ns, not significant.

same trend in T98G cells, suggesting that the metabolisms of tumor cells are more like *S. litura* early embryos than differentiated cells such as hemocyte.

Anti-cancer drugs cause the death of eggs but not fully developed larvae. In our previous studies, ART was demonstrated to exhibit excellent antitumor effects *in vitro* and *in vivo* via targeting several oncogenes and anti-tumor genes (36,71-75). The results demonstrated that the hatching rate of eggs was significantly decreased after ART treatment compared with H_2O treatment and DMSO treatment (Fig. 4A). Furthermore, following ART treatment, the mRNA expression levels of PI3K, AKT, mTOR and p53 of early embryos were also significantly downregulated (Fig. 4B). However, when *S. litura* 1st instar larvae, which had completed their embryonic development and emerged from eggs, were fed the same concentration of ART solution for 14 days, the growth and development of larvae were not significantly affected (Fig. 4C). These results suggested that embryonic development may share some similarities with tumor cells in terms of gene expression, which can be altered by ART.

Discussion

Malignant glioma is one of the most serious tumors, yet little is known about the pathogenesis of malignant glioma and other tumors (36). The causes of tumor formation are a matter of developmental biology. In our perspective of view, oncogenic

factors in the environment and oncogenes in cells may initiate the rapid cell division, an ability obtained by cells after they became embryonic stem cells to ensure survival (76). In the absence of oncogenic stimulation, the rate of cell division would be reduced. Therefore, simply looking for carcinogenic factors and cancer suppressing drugs is not a solution to the cancer treatment problems such as side effects and drug resistance. From this perspective, the present study focused mainly on the similarity of the regulatory mechanisms between early embryonic development and tumor growth.

A number of *in vitro* systems have been established for the study of malignant gliomas, including the well-known U87, U251 and T98G cell lines. The glioma cell line used in this study was T98G because, morphologically, it is a fibroblast and is more likely to form cell clusters, which are more similar to the cellular division and proliferation of early embryos (77). In addition, this study detected expression levels of p53. Therefore, T98G cells, which could stably express p53, was an excellent candidate (78). Since p53 in *S. litura* has two opposite functions of both promoting and inhibiting apoptosis (79,80), we hypothesized that it could be determined whether similar mutations had occurred in p53 in early embryos and hemocytes of *S. litura* using the sequences of wild-type and mutant p53 in T98G cells as a reference. However, as the mutation sites could not be accurately identified by Sanger sequencing, transcriptome, proteome and SNP analyses will be performed in future studies.

Since the early embryos used in this study were those before gastrula, it was not possible to obtain human early embryos, as it is contrary to ethics and the original intention of treating diseases. The most commonly used human embryonic cell lines are not at this stage. Therefore, human embryonic cell lines were not selected. Retrieving the early embryos of mice could also be extremely painful for mice, which should be avoided. Other vertebrates such as chicken were also considered; however, due to the lack of suitable husbandry facilities and larger breeding sites, and the difficulty and expense of obtaining other vertebrate materials, an insect model organism was finally chosen for the present research. Therefore, we chose the insect *S. litura*, which is closely related to *Drosophila*, as it grows fast, is inexpensive and is easy to obtain. Besides, *S. litura* lay larger eggs and it is easy to observe the embryonic stage in the eggs with a low magnification dissecting microscope (62,67). However, *S. litura* is mainly used for specific innate immunity studies (67,70,79,81-83), and, to the best of our knowledge, this is the first time that *S. litura* is being used in oncology research. Therefore, organisms, such as *Drosophila*, are also included in the NJ tree to show that *S. litura* is related to *Drosophila* and could potentially be used in oncology research.

Nine factors highly associated with tumor regulation, including MYC, MYB, BCL-2, BNIP3, p53, PTEN, PI3K, AKT and mTOR, were selected for investigation. These nine factors occupy very important positions in the large regulatory network as changes in their expression and function may lead to changes or even loss of control of the regulatory network (22-27). There is an obvious difference between the present and previous studies-the use of invertebrates as experimental materials (23,25-27,29,37,45,84) According to the NJ phylogenetic trees of 20 species presented in this study, these nine tumor-related key regulators are evolutionarily conserved in these species, suggesting that their functions may also be conserved.

The results of the present study revealed high expression levels of MYC, MYB, BCL-2 and BNIP3 mRNA in T98G cells, which was also observed in early embryos. The oncogenes MYC and MYB may serve similar roles in the growth-promoting regulation mechanisms of early embryogenesis and tumor growth (25,26,29), and the oncogenes BCL-2 and BNIP3 may serve anti-apoptotic and microenvironmental roles in both early embryonic stage and tumor growth (25,46,85,86). This conclusion supports the embryogenic concept of a tumor and indicated that functional genes that serve a dominant role in the tumor and early embryos may be identical.

The high mRNA expression level of the BNIP3 in early embryos and tumor cells suggested that the microenvironment of the early embryo may be highly similar to that of a tumor. BNIP3 is a downstream target protein of hypoxia-inducible factor, HIF-1α (24). High expression of HIF-1α in hypoxic environments can directly promote the high expression of BNIP3. HIF-1α and BNIP3 serve important roles in the maintenance of the hypoxic microenvironment in early embryos and tumors (24,47,84). In a previous study, it was demonstrated that embryonic development and embryonic cell growth require a good healthy microenvironment rather than a hypoxic tumor-like microenvironment (87). Therefore, the high similarity of the microenvironment between early embryos and tumors suggests that there may be a high degree of similarity between the gene expression in early embryos and tumor cells, as the similar microenvironments are regulated by the similar expression of genes.

p53 mRNA expression in the early embryos of *S. litura* was also examined. Previous studies have reported that bracovirus could upregulate p53 in *S. litura* larval hemocytes to induce apoptosis, suggesting that p53 in hemocytes might function similarly to human wild-type p53 (67,80). However, p53 mRNA expression in early embryos of *S. litura* and T98G cells were higher compared with larval hemocytes and HA cells, respectively, suggesting that p53 in early embryos, consistent with tumors, lost its apoptotic function and serves a role as growth promoter (48). The high expression of p53 in early embryos suggested similarities in the expression and function of p53 between early embryos and tumors.

The expression of the anti-oncogene, PTEN mRNA in T98G cells and early embryos of *S. litura* exhibited the same trend of low expression, which suggested that PTEN is expressed at a very low level or not expressed at all in the early embryonic stage or in tumors, and the proapoptotic effect is inhibited. Previous studies demonstrated that PTEN expression was low in tumor cells compared with normal cells (37,57). The present results suggested that PTEN expression was also low in *S. litura* early embryos. The similarity of the early embryo and the tumor cell was further demonstrated by the low expression of PTEN in both compared with that in normal somatic cells.

The high expression levels of the PI3K, AKT and mTOR in early embryos and T98G cells suggested that this signaling pathway may serve an important regulatory role in both, confirming the high similarity of signaling pathway regulation between early embryos and tumors. Previous studies have

demonstrated that PI3K, AKT and mTOR expression was high in tumor cells compared with normal cells (23). The present results suggested that these genes were also highly expressed in *S. litura* early embryos. mTOR is an important node at which multiple signaling pathways intersect and is therefore an important link in the regulatory network (23,88,89).

In the present study, the similarity in the expression trends of functionally important genes between the early embryos and tumor cells was discussed by comparing the mRNA expression levels of nine evolutionarily conserved tumor-associated regulatory factors in early embryos and T98G cell lines. In addition, when early embryos and developed larvae of *S. litura* were treated with artemether [It was considered a typical antitumor compound in our previous studies, which could cause apoptosis by inhibiting the expression of oncogenes such as mTOR and BCL-2 and increasing the expression of oncogenes such as PTEN in cancer cells; however, artemether has no effect on normal cells (36,72-75)], our results demonstrated that artemether killed the early embryos but not the larvae. In addition, the present study revealed that the expression of oncogenes was reduced, and the expression of the anti-tumor gene was increased in *S. litura* early embryos after treatment with artemether, and the hatching rate of eggs was reduced, and the mortality rate increased after treatment with artemether, which was similar to the increase in apoptosis of tumor cells after treatment with artemether (36). These data suggested that gene expression and metabolism of early embryos and glioma cells are extremely similar.

The results of the present experiments preliminarily confirm the concept of the embryonic origin of tumors, and place tumors from a cancer-based perspective into the perspective of individual development and evolution, that is, tumor-related regulatory factors are used to protect and promote early embryonic development and ensure the normal growth of living individuals in the early stages of their development (4,5). However, towards the end of an individual's life, tumor-associated regulatory factors are reactivated to create an embryonic-like mechanism, which competes strongly with the host and eventually outcompetes the host (90,91). The tumor is a life-regulating mechanism that has evolved over a long period of time and has been selected by natural selection and is both the beginning and the end of life (91,92).

The present study will help to reveal the gene expression regulating early embryonic development, expand the study of tumorigenesis, enrich the discourse that tumor-associated regulators are products of individual development and population evolution, and further contribute to the exploration of the nature of life and tumors and the complex relationship between them.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

QCC, QSZ and CHC conceived and designed the experiments. DLL performed the qPCR of *Spodoptera litura* samples and tumor cell samples. PF and YYZ bred *Spodoptera litura* for all experiments, harvested hemocytes and performed mRNA extraction of hemocytes. YKY and CH performed head capsule width measurement and collected all measurement data. CWG and SQZ prepared the ART solution for ART treatment of newly laid eggs and larvae and performed ART treatment of newly laid eggs. YZ and YYL analyzed all sequences, calculated genetic distances, variability and conservation between species, and constructed NJ trees in the article. QCC, DLL, YZ and YYL analyzed the data. QCC and DLL drafted the manuscript. CWG, QSZ and CHC and revised the manuscript. CWG, QSZ and CHC reviewed the manuscript. CWG, QSZ and CHC confirmed the authenticity of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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