Original Article

Tryptanthrin inhibits tumor angiogenesis via Notch/Dll4 signaling pathway in zebrafish

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Background: Anti-angiogenic pathways are important for inhibiting tumor growth and migration. Tryptanthrin has anticancer properties *in vivo* but its anti-angiogenesis activities and associated mechanisms remain unclear.

Methods: The effects of tryptanthrin were investigated *in vivo* using fluorescent labeling of blood vessels in zebrafish. Fluorescence quantitation was conducted to analyze the level of delta-like ligand 4 (*Dll4*) gene expression. Transcriptome sequencing and quantitative polymerase chain reaction (qPCR) analyses were performed to explore the molecular mechanisms of anti-tumor angiogenesis.

Results: Significant anti-tumor effects were observed in all 48-hpf (hours post-fertilization) zebrafish treated with tryptanthrin ($P<0.05$). The 6-hpf zebrafish were cultured to 48 and 72 hpf following tryptanthrin treatment. It was found that compared with the control groups, the fluorescence area and the number of complete internode vessels reduced significantly following treatment with medium and high concentrations of tryptanthrin ($P<0.05$). The relative expression of *Dll4* in the 48-hpf zebrafish was significantly inhibited only in the high concentration group ($P<0.05$). qPCR analysis revealed that the levels of *Krt18b*, *desma*, *Tnnt2c*, and *Krt4* gene expression were significantly up-regulated in zebrafish following *Dll4* overexpression. After *Dll4* knockdown, the level of *desma* and *Tnnt2c* gene expression was significantly up-regulated.

Conclusions: Tryptanthrin can inhibit tumor growth *in vivo* in a concentration-dependent manner by down-regulating *Dll4* protein expression, and at the same time up-regulating the level of *desma* and *Tnnt2c* gene expression.

Keywords: Tryptanthrin; angiogenesis; zebrafish; Notch; delta-like ligand 4 (*Dll4*)

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Introduction

The primary cause of death in cancer patients is the spread and metastasis of cancer cells (1). This study adopts a new approach for the development of novel anti-tumor medicines by exploring the mechanism of inhibiting tumor metastasis and recurrence. Angiogenesis, the formation of new blood vessels, is a key step in tumor growth, invasion and metastasis (2). Novel anti-tumor drugs that target the angiogenic pathway can clear the blood vessels necessary for tumor growth and metastasis, as well as re-organizing the tumor immune microenvironment (3). Recently, the anti-tumor activities of tryptanthrin have attracted great attention from researchers. Tryptanthrin has been shown to inhibit the proliferation, migration and angiogenesis of tumor vascular endothelial cells (4), and *in vivo* studies suggest that tryptanthrin exhibits low toxicity (5). Apoptosis, a form of programmed cell death, is closely related to tumor development and progress (6). Miao *et al.* (7) demonstrated that tryptanthrin could induce the apoptosis and differentiation of leukemia cells. Furthermore, tryptanthrin was also shown to be inhibit the production of drug-resistant genes in breast and colon cancer cells (8), inhibit the migration and metastasis of melanoma cells (9), inhibit the cell growth in neuroblastoma, and induce radiosensitization in non-small cell lung cancer cells (10). However, the anti-angiogenic effect of tryptanthrin *in vivo* and its associated molecular mechanism remain unclear.

DNA mutations of some molecules in the mammalian Notch signaling pathway are widely and closely related to the occurrence and development of malignant tumors; this is an important research and development target for anti-tumor drugs (11). In particular, there are five Notch ligands in the human body: Jagged 1, Jagged 2, Delta 1, Delta 3 and Delta 4. Among these, Delta 4 [delta-like ligand 4 (*Dll4*)] is the only ligand in the Notch pathway that is specifically expressed in epithelial cells. While *Dll4* is generally distributed in small amounts under normal conditions, it is abnormally expressed in tumor blood vessels to regulate tumor angiogenesis and control tumor vascular density (12). However, whether tryptanthrin can affect tumor angiogenesis via the Notch/Dll4 pathway has not been reported.

The transgenic zebrafish model has become a popular animal model for tumor angiogenesis research. The advantages of this model include ovulation *in vitro*, low cost, ease of reproduction and maintenance, rapid life cycle, and cellular transparency in which all internal organs and structures can be observed completely *in vivo*, even at advanced stages of development. This transparency is also helpful for observing gene transcription and protein expression (13). It has been found that compared with human umbilical vein endothelial cell and chicken embryo allantoic membrane models, the zebrafish model is more simplistic and practical, making it suitable for the screening of active ingredients in traditional Chinese medicine that exhibit antiangiogenic properties. In addition, the angiogenesis process of zebrafish embryos is very similar to that of malignant tumors (14,15). Therefore, in this study, we established a transgenic zebrafish model, investigated and observed the blood vessels of transgenic zebrafish using fluorescent labeling, and counted the intersegmental vessels (ISVs) to explore the inhibitory effect of tryptanthrin on angiogenesis. Real-time fluorescence quantitative polymerase chain reaction (qPCR) was used to study the expression of key genes involved in the vascular endothelial growth factor (VEGF)/Dll4-Notch signaling pathway. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-925/rc).

Highlight box

Key findings

- Tryptanthrin inhibits tumor angiogenesis by down-regulating Dll4 protein expression.

What is known and what is new?

- As reported, tryptanthrin could inhibit tumor angiogenesis *in vivo*.
- Tryptanthrin has an effect on anti-tumor angiogenesis through inhibition of the Notch/Dll4 signaling pathway and up-regulation of *desma* and *Tnnt2c* gene expression.

What is the implication, and what should change now?

- The Notch/Dll4 signaling pathway plays a crucial role in tumor angiogenesis. Tryptanthrin inhibits tumor growth and metastasis *in vivo* through inhibition of the Notch/Dll4 signaling pathway. These findings may open doors to potential new classes of anti-tumor medicine.

Methods

Chemicals

Tryptanthrin (Batch No. 20061211; *Figure 1*), 6,12-dihydro-6,12-dioxoindolo-(2,1-b)-quinazoline, was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). This compound was dissolved in dimethyl sulfoxide (DMSO)

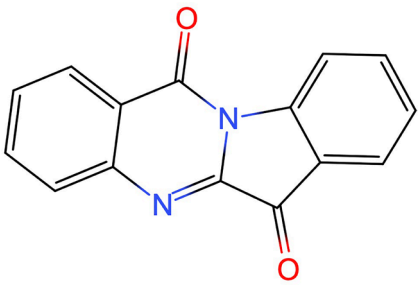

Figure 1 Chemical structure of tryptanthrin.

solution and stored at −70 ℃. This treated solution was continuously diluted in E3 medium, in which embryos and young fish were previously incubated (NaCl: 344 mg, KCl: 15.2 mg, $CaCl_2 \cdot 2H_2O$: 58 mg, $MgSO_4 \cdot 7H_2O$: 98 mg; constant volume: 20 mL). All chemicals and regents used in the study were of analytical grade.

Collection of zebrafish embryos

Fli1a-enhanced green fluorescent protein (EGFP) transgenic zebrafish purchased from the Chinese Zebrafish Resource Center (Wuhan, China) were adopted as a model, with strain and associated characteristics identified by referencing to the relevant literature (16). Zebrafish eggs were produced by mated adult zebrafish and incubated into embryos. Zebrafish embryos were staged by referencing the relevant literature (17). Each stage of embryo development was expressed in hours post-fertilization (hpf) or days post-fertilization (dpf). Fish were maintained in an automatic breeding facility (ESEN, Beijing, China) at 28±0.5 ℃ with a 14/10 h light/dark cycle. The embryos were obtained after the natural hybridization of adult fish. At 45 min post-fertilization, 20 eggs were randomly selected to identify fertilized eggs, and the fertilization rate of eggs produced by each group was calculated. At least three groups of eggs with a fertilization rate ≥70% were selected for mixing and reserve. This study was conducted according to the Guidelines for the Laboratory Animal Use and Care Committee of the Ministry of Health, China. Ethical approval was not required for this study in accordance with the national legislation and institutional requirements.

Chemical treatment

Tryptanthrin of different concentration was prepared. The 6- and 48-hpf zebrafish were treated with tryptanthrin of different concentration for 72 h continuously by water bath. The culture medium was changed every 24 h and the mortality rate was calculated. Embryos treated with 0.5% DMSO solution were used as vector controls. Each experiment was conducted three times, and each concentration was used in three replicates. The optimal tryptanthrin concentration was screened for the 6- and 48-hpf zebrafish, and low, middle, and high gradient concentration was established.

Establishment of a zebrafish tumor transplantation model

After the embryos grew to 48 hpf, HeLa cancer cells purchased from Fuzhou Zaiji Biotechnology Co., Ltd. (Fuzhou, China) were labeled with DII dye for 1 h at 37 ℃. The final concentration of the DII dye was 5 μM. The labeled HeLa cells were microinjected into the yolk sac of juvenile zebrafish. Each group included 15 zebrafish and each zebrafish was injected with tumor cells in range of 250 to 1,000. Following the injection, the surviving zebrafish that were successfully transplanted were screened and divided into four groups according to the different cell lines that had been injected. Three of the groups were treated with different concentration of tryptanthrin for 48 h and photographs were taken at 0 and 48 h under a fluorescence microscope (Nikon, SMZ800N, Nanjing, China) to observe the tumor growth in zebrafish. This experiment was divided into four groups: control group, low concentration of tryptanthrin treatment group (48 hpf), medium concentration of tryptanthrin treatment group (48 hpf), and high concentration of tryptanthrin treatment group (48 hpf).

Analysis of Dll4 gene expression in zebrafish

The 6-hpf zebrafish were divided into four groups, treated with tryptanthrin of different concentration, and cultured to 48 and 72 hpf to observe fluorescence intensity of the blood vessels in zebrafish and the number of total internode vessels *in vivo*. Four groups included a control group of 0.5% DMSO treatment, low concentration of the tryptanthrin treatment group (6 hpf), medium concentration of tryptanthrin treatment group (6 hpf), and high concentration of tryptanthrin treatment group (6 hpf). Next, 10 zebrafish in each group were randomly selected for RNA extraction and detection of *Dll4* gene expression by fluorescence quantitative methods.

Table 1 Primer sequences

Gene	Primer sequence (5'–3')
Elfa	F: CTTCTCAGGCTGACTGTGC
	R: CCGCTAGCATTACCCTCC
Tnnt2c	F: AAGAAGCTGACCGAACGTGA
	R: TTGAGCCACTTCCACAGGTC
Krt4	F: GCAACCTCCTCCACTCACTC
	R: TACTGCATAGGCTGACTGGC
Krt18b	F: TTGGTCGCCTGAATGTGGAG
	R: CATATCCTGGCCCTTGGGTG
Desma	F: CGCTGCCAAGAATATCAGCG
	R: TGTAGGACTGGAGCTGGTGA
Bfsp2	F: ATGTTCAGGCCAATGCAGAG
	R: ATCCTGGTCAGGTTTGCATC
Dll4	F: CCACGCAAGAATGGAGGTCT
	R: TCCATTGTCCTTCTCGTGGC

F, forward primer; R, reverse primer.

Fluorescence quantitative validation of the Dll4 gene pathway transcriptome sequencing

Elfa was selected as the internal reference gene, and fluorescent qPCR primers for each gene were designed using Primer 5 software (*Table 1*).

Statistical analysis

Tumor fluorescence areas of zebrafish were measured using Capture software and photographed under a fluorescence microscope. The shot was parameterized with Adobe Photoshop 7.0 software. All diagrams were created using GraphPad Prism 5. The tumor fluorescence area was measured using Image J software. Microsoft Excel software was used to calculate the tumor fluorescence area proliferation rate of the same zebrafish. The proliferation rate was calculated using the following formula:

$$\text{Proliferation rate} = \left(\frac{\text{Tumor fluorescence area of 48 h zebrafish} - \text{Tumor fluorescence area of 0-hpf zebrafish}}{\text{Tumor fluorescence area of 0 h zebrafish}} \right) \times 100\% \quad [1]$$

The inhibition rate was calculated using the following formula:

$$\text{Inhibition rate} = \left(\frac{\text{ISVamount of experimental group}}{\text{ISVamount of vehicle control}} \right) \times 100\% \quad [2]$$

A measuring ruler was placed into the ruler setting area of the measuring plate in advance. Data presentation: mean ± standard deviation (SD). A Mann Whitney test was used to analyze significant differences between the groups.

Results

Screening of the optimal concentration for treatment of tryptanthrin in zebrafish

Zebrafish (48 hpf) were treated with tryptanthrin of different concentration and cultured to 96 hpf. The results showed that 0.78 μg/mL was not lethal to the zebrafish. Similarly, the 6-hpf zebrafish were treated with tryptanthrin of different concentration and cultured to 72 hpf. The results showed that 0.39 μg/mL was not lethal to the zebrafish. Hence, low-, medium-, and high-concentration tryptanthrin treatment groups were selected for subsequent intervention of blood fluorescence strain on juvenile fish (*Tables 2,3*).

Anti-tumor effects of tryptanthrin

There was a significant reduction of tumor fluorescence area in the low concentration treatment (48 hpf), medium concentration treatment (48 hpf) and high concentration treatment (48 hpf) compared with the control group ($P<0.05$; *Figure 2*).

Table 2 Zebrafish treated with different concentrations of tryptanthrin

Concentration (μg/mL)	96-hpf mortality (%)	72-hpf mortality (%)
0	0	0
0.1	0	0
0.2	0	0
0.39	0	1.67±2.89
0.78	0	46.67±2.89
1	37.77±7.73	–
1.56	100	98.33±2.89
3.12	100	100

Data are shown as mean ± standard deviation; hpf, hours post-fertilization.

Table 3 Screening of tryptanthrin concentration gradient in zebrafish

Concentration gradient	Concentration (μg/mL)
Low concentration treatment (48 hpf)	0.078
Medium concentration treatment (48 hpf)	0.39
High concentration treatment (48 hpf)	0.78
Low concentration treatment (6 hpf)	0.039
Medium concentration treatment (6 hpf)	0.2
High concentration treatment (6 hpf)	0.39

hpf, hours post-fertilization.

Effect of tryptanthrin on angiogenesis in zebrafish

The 6-hpf zebrafish were treated with tryptanthrin of different concentration and cultured to 48 and 72 hpf. The vascular fluorescence intensity analysis showed that the fluorescence areas of the 48-hpf zebrafish treated with the medium or high concentration of tryptanthrin were significantly different compared with the control group ($P<0.05$). Moreover, the fluorescence areas of the 72-hpf zebrafish treated with a low (0.039 μg/mL), medium (0.2 μg/mL), and high (0.39 μg/mL) concentration of tryptanthrin differed significantly from that of the control group ($P<0.05$; *Figure 3A-3D*).

After analyzing the number of intact ISVs *in vivo*, we found intact ISV counts in the 48-hpf zebrafish treated with tryptanthrin of low (0.039 μg/mL), medium (0.2 μg/mL), and high (0.39 μg/mL) concentration were significantly lower than that in the control group ($P<0.05$; *Figure 3E*). Moreover, compared with the control group, the intact ISVs counts were significantly lower in the 72-hpf zebrafish treated with tryptanthrin of medium (0.2 μg/mL) and high (0.39 μg/mL) concentration ($P<0.05$; *Figure 3F*).

Dll4 gene expression in 48- and 72-hpf zebrafish cultured from 6-hpf zebrafish treated with tryptanthrin of different concentration

Compared with the control group, the relative level of *Dll4* gene expression in the 48-hpf zebrafish treated with low-concentration tryptanthrin (0.039 μg/mL) was not

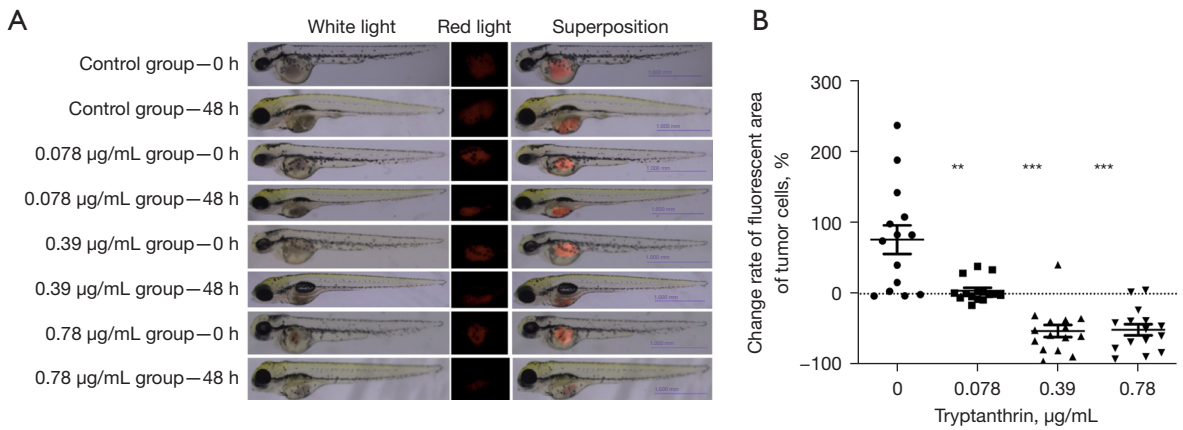

Figure 2 Inhibition of tumor growth in 48-hpf zebrafish treated with tryptanthrin. (A) Changes in tumor fluorescence in zebrafish; (B) change rate of the tumor fluorescence area in zebrafish. Images in (A) were recorded on a Nikon SMZ800N stereo fluorescence (white light and red light) microscope. The scale bar in (A) represents 1,000 μm; magnification: 4×. **, $P<0.01$; ***, $P<0.001$ were considered statistically significant. hpf, hours post-fertilization.

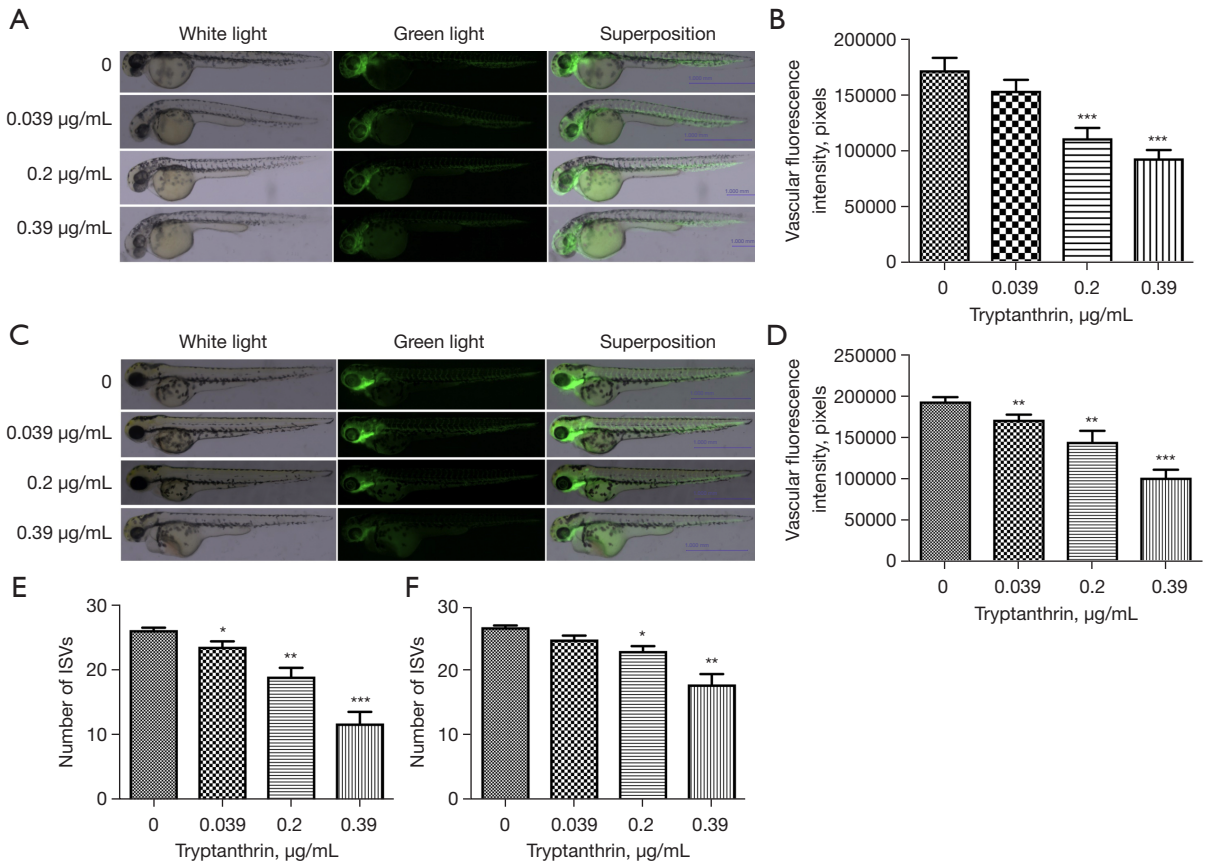

Figure 3 Inhibition of angiogenesis in zebrafish treated with tryptanthrin. (A) Vascular fluorescence phenotypes in 48-hpf zebrafish; (B) statistical diagram of vascular fluorescence intensity in 48-hpf zebrafish; (C) vascular fluorescence phenotypes in 72-hpf zebrafish; (D) statistical diagram of vascular fluorescence intensity in 72-hpf zebrafish; (E) statistical diagram of intact ISVs in 48-hpf zebrafish; (F) statistical diagram of intact ISVs in 72-hpf zebrafish. Images in (A,C) were recorded on a Nikon SMZ800N stereo fluorescence (white light and green light) microscope; The scale bar in (A,C) represents 1,000 μm; magnification: 4×. *, P<0.05; **, P<0.01; ***, P<0.001 were considered statistically significant. ISVs, intersegmental vessels; hpf, hours post-fertilization.

significantly different; however, there was a significant decrease in expression following treatment with the medium (0.2 μg/mL) and high (0.39 μg/mL) concentrations (P<0.05). However, compared with control group, the relative levels of *Dll4* gene expression in the 72-hpf zebrafish treated with low (0.039 μg/mL) and medium (0.2 μg/mL) concentrations were not significantly different. There was a significant decrease in *Dll4* gene expression following treatment with high-concentration tryptanthrin (0.39 μg/mL) (P<0.01; *Figure 4*).

Dll4 gene regulation

Compared with wild-type group, the level of *Dll4* of zebrafish (*zDll4*) gene expression in the overexpression group was significantly increased, indicating that the target gene was successfully overexpressed (*Figure 5A*). Compared with wild-type group, the number of intersegment vessels in the overexpression group was significantly decreased (*Figure 5B-5D*). Furthermore, compared with the clustered regularly interspaced short palindromic repeats associated protein (CasRx) control group, the level of *zDll4* gene expression in the experimental group [*zDll4*-single-guide RNA (sgRNA) + CasRx] was significantly decreased. This finding indicated that target gene was successfully knocked down (*Figure 5E*). Compared with the CasRx control group, the number of intersegment vessels was significantly decreased in the knockdown group (*Figure 5F-5H*).

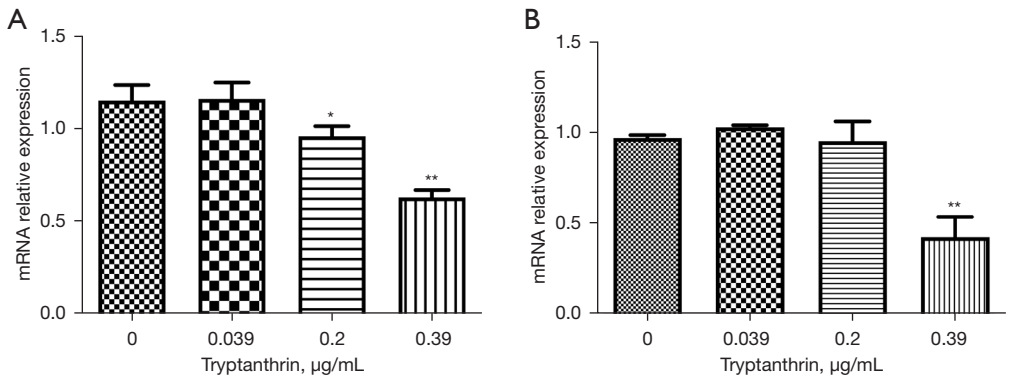

Figure 4 Effect of tryptanthrin on *Dll4* gene expression of zebrafish. (A) Relative level of *Dll4* gene expression in 48-hpf zebrafish; (B) relative level of *Dll4* gene expression in 72-hpf zebrafish. *, P<0.05; **, P<0.01 were considered statistically significant. *Dll4*, delta-like ligand 4; hpf, hours post-fertilization.

Transcriptome sequencing and fluorescence quantitative verification of the Dll4 gene pathway

We quantitatively detected the expression of the *Dll4* gene related pathway. Analysis of Gene Ontology (GO) revealed the differential expression of intermediate-filament related GO. *Krt18b*, *Krt4*, *Bfsp2*, *desma*, and *Tnnt2c* were expressed following the knock down of the *Dll4* gene (*Figure 6*).

Detection results of *in-situ* PCR and qPCR analyses are presented in *Figure 7*. Fluorescence quantitation of *Dll4* gene overexpression showed that compared with the overexpression control group, there were significant differences in the level of *Dll4*, *Krt18b*, *desma*, *Tnnt2c*, and *Krt4* gene expression in the overexpression group (P<0.05). However, no significant differences were observed in the expression of the *Bfsp2* gene between the overexpression group and overexpression control group (P>0.05).

In addition, fluorescence quantitation of the *Dll4* gene knockdown showed that compared with the knockdown control group, there were significant differences in the level of *Dll4*, *desma*, and *Tnnt2c* gene expression in the knockdown group (P<0.05). However, no significant difference was found in the level of *Krt18b*, *Krt4*, and *Bfsp2* gene expression between the knockdown group and knockdown control group (P>0.05).

Discussion

Our study reported that tryptanthrin could inhibit angiogenesis *in vivo* by regulating the VEGF/Notch-Dll4 signaling pathway. This study was the first to use transgenic zebrafish to explore molecular mechanism by which tryptanthrin regulates angiogenesis. We found that tryptanthrin could significantly inhibit tumor angiogenesis, the level of *Dll4* gene expression, and up-regulate *desma* and *Tnnt2c* gene expression to mediate tumor angiogenesis.

Multiple natural phytochemicals have been shown to have anti-tumor properties. The primary mechanism is by blocking the proliferation of tumor cells and inducing the differentiation or apoptosis of tumor cells (18). Indigo Naturalis, a traditional Chinese medicine with the active ingredient, tryptanthrin, has been used to treat psoriasis. In addition, the formation of pathological blood vessels in psoriasis has been found to promote and maintain inflammation, which is also an angiogenesis-inducing factor (19). As this mechanism is similar to that of tumor angiogenesis, we speculate that tryptanthrin also has an effect on anti-tumor angiogenesis. It has previously been reported that tryptanthrin can inhibit the expression of vascular cell adhesion molecule-1 induced by tumor necrosis factor-α in human vascular endothelial cells, but experiments have only been conducted *in vitro* (20). Therefore, fluorescent vascular labeled zebrafish were used as an *in vivo* model in this study, and low, medium and high concentration of tryptanthrin was found to be able to significantly reduce the tumor fluorescence area in zebrafish. Although only medium and high concentration of tryptanthrin could significantly inhibit angiogenesis in 48-hpf zebrafish, the angiogenesis of 72-hpf zebrafish was significantly inhibited with any concentration of tryptanthrin.

We found both the *zDll4* gene overexpression group and knockdown group (*zDll4*-sgRNA + CasRx) exhibited a significantly reduced number of ISVs. Moreover, the

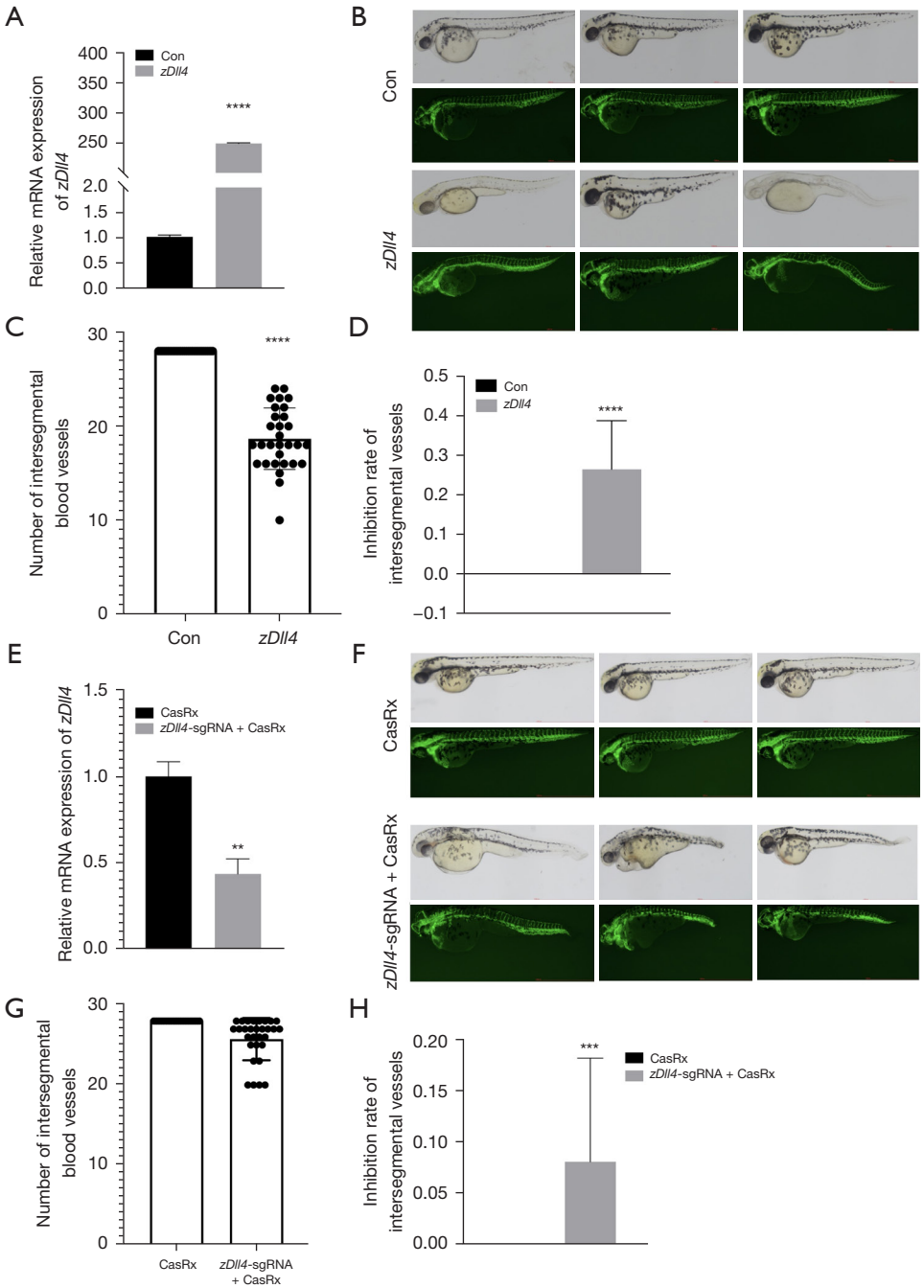

Figure 5 Regulation of *Dll4* genes on the number of intersegment vessels in zebrafish. (A) Detection of *zDll4* gene overexpression; (B) observation of the number of intersegment vessels after 48 h of *zDll4* gene overexpression; (C) intersegment vessel count after 48 h of *zDll4* gene overexpression; (D) inhibition rate of the number of intersegment vessels after 48 h of *zDll4* gene overexpression; (E) detection of *zDll4* gene knockdown; (F) observation of the number of intersegment vessels after 48 h of *zDll4* gene knockdown; (G) intersegment vessel count after 48 h of *zDll4* gene knockdown; (H) inhibition rate of the number of intersegment vessels after 48 h of *zDll4*gene knockdown. Images in (B,F) were recorded on a Nikon SMZ800N stereo fluorescence (white light and green light) microscope. The scale bar in (B,F) represents 1,000 μm; magnification: 4×. **, P<0.01; ***, P<0.001; ****, P<0.0001 were considered statistically significant. Con, control; *zDll4*, delta-like ligand 4 of zebrafish; CasRx, clustered regularly interspaced short palindromic repeats associated protein; sgRNA, single-guide RNA.

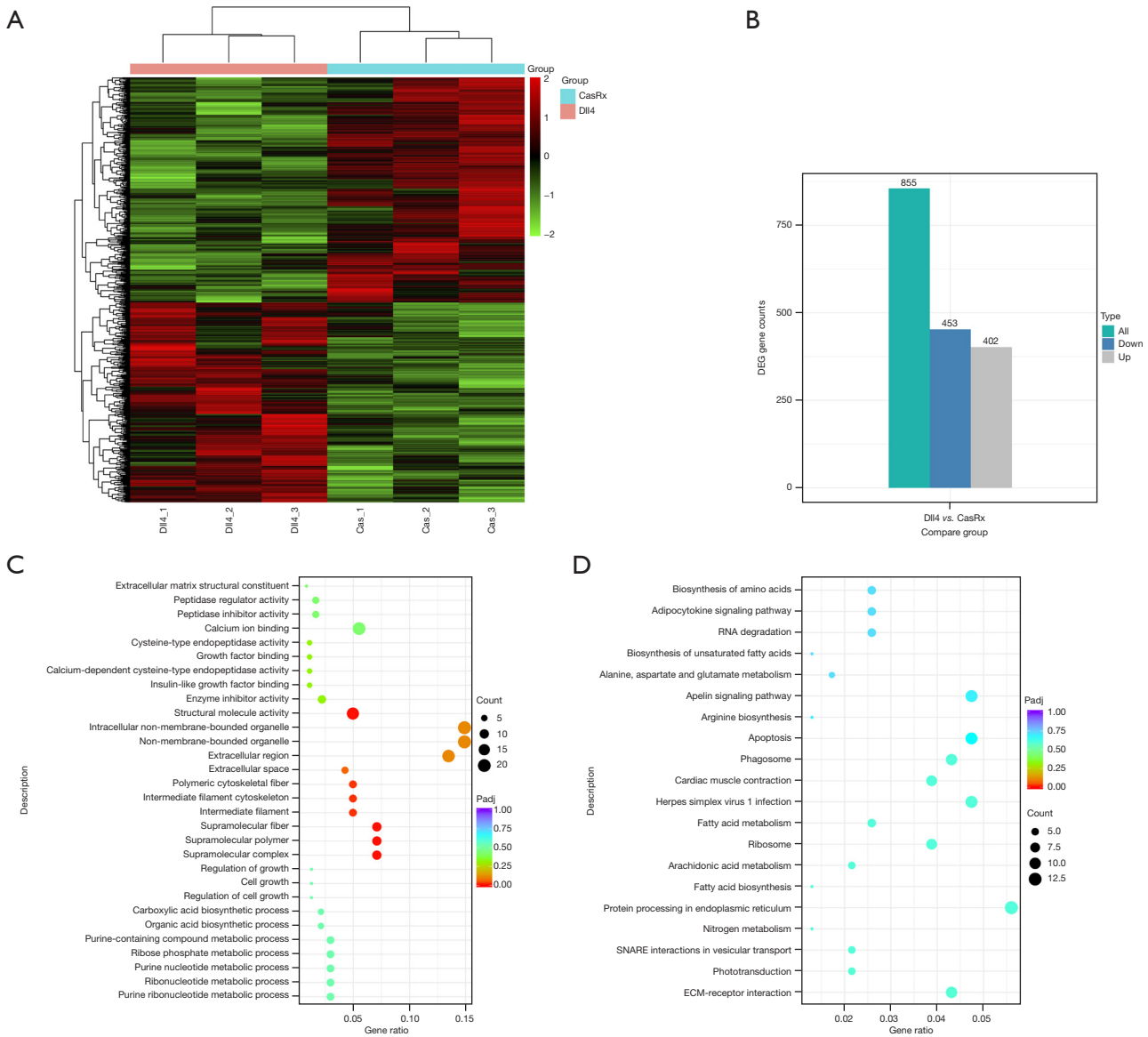

Figure 6 Transcriptomics of *Dll4* gene overexpression and knockdown. (A) Analysis diagram of transcriptomics; (B) bar chart of transcriptomics; (C) GO analysis; (D) KEGG analysis. CasRx, clustered regularly interspaced short palindromic repeats associated protein; Dll4, delta-like ligand 4; DEG, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Dll4 gene played a key role in angiogenesis and regulation during embryonic development, which is consistent with previous studies. It was also reported that in transgenic mice, the *Dll4* gene was overexpressed, making the aortas enlarged in double transgenic embryos and the mice died 10.5 days before the embryonic stage, which may be caused by pre-embryonic arteriovenous fusion (21). Moreover, it was demonstrated that *Dll4* gene expression was low in adult blood vessels and up-regulated in tumor blood vessels (22-24). For example, the level of *Dll4* gene expression in the vessels of clear cell renal cell carcinoma was found to be up-regulated by nearly nine times than that of normal renal tissue and was closely related to VEGF levels. In addition, high *Dll4* gene expression in endothelial cells was found to be a significant adverse prognostic factor. For instance, vascular progression occurred more rapidly in breast tumors with higher *Dll4* gene expression (25). Therefore, reducing the level of *Dll4* gene expression represents an important

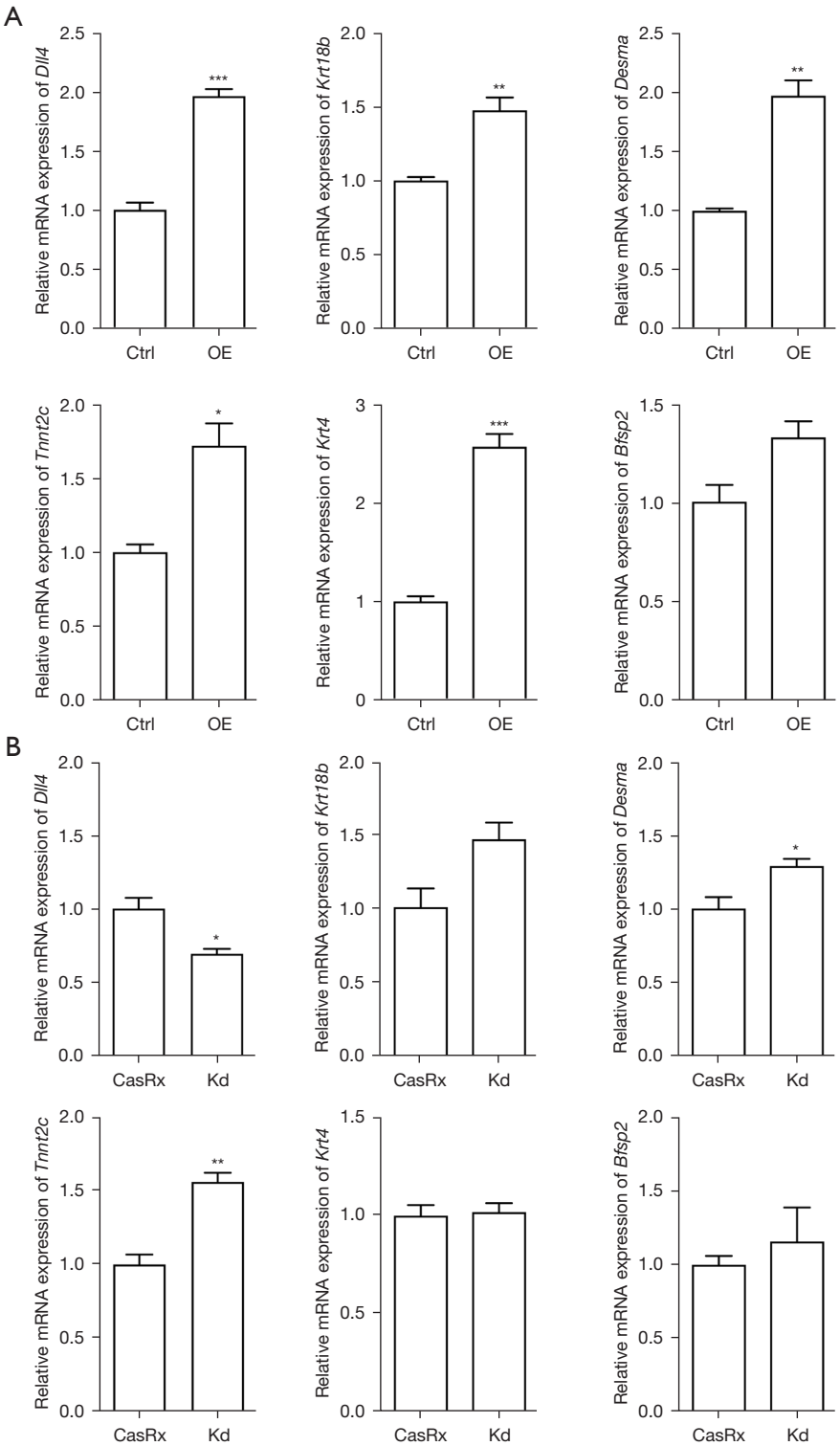

Figure 7 Significance analysis of fluorescence quantitation of the *Dll4* gene. (A) Significance analysis of fluorescence quantitation of *Dll4* overexpression; (B) significance analysis of fluorescence quantitation of *Dll4* knockdown. *, P<0.05; **, P<0.01; ***, P<0.001 were considered statistically significant. *Dll4*, delta-like ligand 4; Ctrl, overexpression control group; OE, overexpression group; clustered regularly interspaced short palindromic repeats associated protein; Kd, knockdown group.

approach to inhibit tumor growth and reproduction. In our study, tryptanthrin of different concentration was used to treat 6-hpf zebrafish, which were then cultured to 48 and 72 hpf. Our findings revealed that only treatment with a high concentration of tryptanthrin could significantly reduce *Dll4* gene expression.

To the best of our knowledge, our study is the first to investigate gene expression in the Dll4 signaling pathway via gene overexpression and knockdown, as well as real-time PCR detection. We found that the level of *Krt18b*, *Krt4*, *Bfsp2*, *desma*, and *Tnnt2c* gene expression was up-regulated in both the overexpression and knockdown groups; however, only the expression of *desma* and *Tnnt2c* was significant (P>0.05). The human *desmin* gene is encoded by a single gene, while zebrafish contain two *desmin* gene homologues (*desma* and *desmb*) (26). Desmin is a smooth muscle intermediate filament protein expressed in fibrotic tissue associated with tumor "adhesion hyperplasia" wound healing and matrix. Additionally, desmin was described as a pericyte marker, which was associated with blood vessels from early stages of capillary germination throughout the entire angiogenesis process (27,28). Therefore, desmin may represent a biomarker for predicting the efficacy of anti-angiogenic therapy for cancer in our future study. Moreover, cardiac troponin T isoform 2 (Tnnt2) has been shown to play an important role in the myofibrils of zebrafish and humans (29). It has been demonstrated that a loss of *Tnnt2c* in developing zebrafish embryos can lead to heart malformations (30). However, few studies have been conducted on the anti-tumor effect of *Tnnt2c*, and further basic studies are required to reveal its role in tumor angiogenesis.

Conclusions

In summary, our study found that tryptanthrin had an effect on anti-tumor angiogenesis *in vivo*. The associated anti-tumor mechanism may be the inhibition of the Notch/Dll4 signaling pathway and the up-regulation of *desma* and *Tnnt2c* gene expression. Therefore, tryptanthrin may represent a potential candidate drug or lead compound for anti-tumor angiogenesis therapy in the future.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-925/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted according to the Guidelines for the Laboratory Animal Use and Care Committee of the Ministry of Health, China. Ethical approval was not required for this study in accordance with the national legislation and institutional requirements.

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