β -Arrestin2 Inhibits the Apoptosis and Facilitates the Proliferation of Fibroblast-like Synoviocytes in Diffuse-type Tenosynovial Giant Cell Tumor

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Abstract. *Background/Aim:* Diffuse-type tenosynovial giant cell tumor (TGCT) is a rare benign proliferative synovial neoplasm of uncertain etiology, and the efficacy of surgical resection is not satisfactory. Therefore, there is an urgent need to explore the pathogenesis and identify novel therapeutic targets for TGCT. *Materials and Methods:* Synovial tissues were collected from patients with TGCT and osteoarthritis (OA). Differences of mRNA expression between TGCT and OA were explored using mRNA-seq. In addition, fibroblast-like synoviocytes (FLS) were treated with small interfering RNA (siRNA) or adenovirus in order to knockdown or overexpress β -arrestin2 (Arrb2), respectively. FLS proliferation and apoptosis were evaluated using the MTT assay and the caspase 3 activity assay, respectively. *Results:* The expression of Arrb2 in TGCT was significantly higher than that in OA. The overexpression of Arrb2 promoted the proliferation of FLS and inhibited its apoptosis, while knocking down Arrb2 had the opposite effect. Further

studies showed that Arrb2 can activate the PI3K-Akt signaling pathway, leading to increased proliferation of TGCT. *Conclusion:* Arrb2 facilitates the proliferation and inhibits the apoptosis of TGCT FLS through activating the PI3K-Akt cell survival pathway, providing new insight into the molecular mechanism of TGCT.

Diffuse-type tenosynovial giant cell tumor (TGCT) is a rare benign proliferative synovial neoplasm characterized by villous and nodular lesions of an uncertain etiology (1). The main clinical features of TGCT are synovial hyperplasia and recurrence. The hyperplastic synovial tissue will produce a large amount of bloody synovial fluid and cause excessive swelling of the joints, further causing joint pain and dysfunction (2). Uncontrollable excessive synovial proliferation can even cause malignant transformation of TGCT and result in amputation (3). Fibroblast-like synoviocytes (FLS), as the main constituent cell type of TGCT, play an important role in its proliferation, recurrence, and malignant transformation (4). Because its pathogenesis is currently unclear, the therapeutic mainstay of TGCT is limited to removing the entire pathological synovial tissue (5). However, surgical resection alone often cannot completely remove the diseased synovium; the residual synovium will rapidly proliferate and cause postoperative recurrence, at a rate of approximately 21-50% (6). Postoperative radiotherapy is often needed to reduce recurrence, but radiotherapy can lead to a series of complications such as joint adhesion (7). Therefore, there is an urgent need to explore the pathogenesis of TGCT and identify novel therapeutic targets, especially focusing on the inhibition of its proliferation and recurrence.

β -Arrestin 2 (Arrb2) is widely expressed in various tissues and involved in mediating G protein coupled receptor (GPCR) desensitization, internalization, degradation and

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recycling (8). Recent studies have shown that Arrb2 is not only a signal terminator, but also a scaffold protein for GPCRs to mediate pathway signaling (9). It can enter the nucleus and, directly or indirectly, regulate the transcription factors and gene expression (10). Studies have reported that it plays a regulatory role in tumor occurrence, invasion and metastasis, in lung (11), ovarian (12), and liver cancer (13). However, it remains unclear whether Arrb2 is involved in the pathogenesis of TGCT and related to the high proliferation and recurrence ability of TGCT FLS. In this regard, we used mRNA-seq to compare TGCT and osteoarthritis (OA) synovial tissue by high-throughput sequencing, aiming to explore the molecular mechanisms of TGCT high proliferation and recurrence.

Materials and Methods

Patient samples. A total of 21 and 20 synovial tissues were collected from TGCT and OA patients, respectively, under protocols approved by the Ethics Committee of Peking University Third Hospital. All patients had signed an informed consent form. TGCT synovial tissues were derived from patients who had undergone an arthroscopic synovectomy, while OA synovial tissues were obtained at the time of total knee replacement. All synovial tissues were used for further FLS culture and histological testing. The clinical characteristics of these patients are listed in Table I.

Histological assessment. TGCT and OA patient's synovial tissues were excised and fixed in 4% paraformaldehyde for 48 h, dehydrated in a series of ethanol, and embedded in paraffin. The samples were cut into 6- μ m thick sections and stained with hematoxylin-eosin (H&E). For immunohistochemistry (IHC), the paraffin sections were incubated with 3% H₂O₂ for 15 min following by incubation with pepsin for 30 min at 37°C for non-specific antigen blocking. The sections were then incubated with antibodies against Ki-67 (Abcam, Cambridge, MA, USA; 1:500) and CD163 (Abcam, Cambridge, MA, USA; 1:200) overnight at 4°C. Samples were then incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies (Zsjiangbio, Beijing, PR China) at room temperature for 1 h before adding 3,3'-diaminobenzidine (DAB) for visualization.

mRNA-seq array analysis. Total RNA was extracted from 3 TGCT and 3 OA synovial tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Microarray analysis was performed and analyzed by a commercial company (NovelBio Bio-Pharm Technology Co., Ltd, Beijing, China) using the VAHTSTM Total RNA-seq (H/M/R) Library Prep Kit. Differential gene and transcript expression analysis of RNA-seq were examined using the TopHat and Cufflinks algorithms (14). Significant analysis was performed using the *p*-value and false discovery rate (FDR) analysis (15). At the same time, differentially expressed genes were identified with: fold change >2 or fold change <0.5, FDR <0.05. Pathway analysis was used to identify the significantly affected pathways of the differentially expressed genes, according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (16). The threshold of significance was defined by *p*-value (17). Pathway activity network was constructed using Cytoscape (18) for graphical representations

Table I. Clinical characteristics of TGCT and OA patients.

Characteristics	TGCT (n)	OA (n)
Patients	21	20
Gender		
Male	8	17
Female	13	3
Age*	41.7 $\pm$ 9.2	58.5.2 $\pm$ 7.5
Location		
Knee	18	20
Elbow	2	0
Ankle	1	0

TGCT, Tenosynovial giant cell tumor; OA, osteoarthritis. *Data presented as mean $\pm$ standard deviation.

of enriched biological pathways with significance (*p*<0.05), including up-regulated and down-regulated ones.

Cell isolation and culture. FLS were isolated using enzymatic dispersion of the synovial tissue from TGCT and OA patients. Briefly, the synovial tissues were minced and incubated with 1 mg/ml type I collagenase (Invitrogen, Carlsbad, CA, USA) in low-glucose Dulbecco's modified Eagle's medium (DMEM Gibco, Carlsbad, CA, USA) for 2-3 h at 37°C. Then, the cell suspensions were placed in culture dishes with DMEM supplemented with 10% (volume/volume, v/v) fetal bovine serum (Invitrogen), 100 U/ml penicillin, and 100 μ g/ml streptomycin, in a 37°C, 5% CO₂ incubator. After the third passage, non-FLS cells were completely depleted from these culture systems and the remaining cells were primarily FLS.

Cell transfection. Arrb2 small interfering RNA (siRNA) and scrambled negative control siRNA were obtained from Genechem Co. Ltd. (Shanghai, PR China). FLS were transfected with Arrb2 siRNA and scrambled negative control siRNA via Lipofectamine Max (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The final concentration of siRNA was 20 nM. The Arrb2 overexpression adenovirus (Ad-Arrb2) and negative control adenovirus (Ad- β -gal) were also obtained from Genechem Co. Ltd. FLS were transfected with control adenovirus (Ad- β -gal) and Ad-Arrb2 designed to overexpress Arrb2. The final multiplicity of infection (MOI) was 20.

Western blot. TGCT and OA FLS were lysed in radioimmuno-precipitation assay (RIPA) lysis buffer, separated by SDS polyacrylamide gel electrophoresis (PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were then incubated with corresponding primary antibodies overnight at 4°C, followed by the corresponding secondary antibodies (1 h at room temperature), and visualized using the BIO-RAD ChemiDoc XRS + system (Bio-Rad Laboratories, Hercules, CA, USA). The antibody against Arrb2 (Cat#: sc13140; 1:1,000 dilution) was from Santa Cruz (Delaware, CA, USA); antibodies against phospho-ERK (Cat#: cst. 4370; 1:1,000 dilution), total-ERK (Cat#: cst 4695; 1:1,000 dilution), phospho-Akt (Cat#: cst 4060s; 1:1,000 dilution) and total-Akt (Cat#: cst 9272s; 1:1,000 dilution) were from Cell Signaling Technology (Boston, MA, USA); antibody against Arrb1 (Cat#: ab32099-100; 1:2,000 dilution) was from Abcam (Cambridge, UK) and to

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from EasyBio. (Cat#: BE0023; 1:2,500 dilution, Beijing, PR China). The cells lines used in each test were obtained from five different patients, and the experiment used five biological replicates; each biological sample tests was performed in triplicate.

MTT assay. The cell proliferation of TGCT or OA FLS was evaluated by MTT assay. FLS were maintained in 96-well plates with 3,000 cells/well. MTT solution (Sigma, Saint Louis, MO, USA) was added to each well at day 0, 2, 4 and 6 of the culture periods. After 4 h of incubation at 37°C, the supernatant was removed, and 200 μ l dimethyl sulfoxide was added to dissolve the precipitate. Absorbance was examined at 450 nm using a Multiskan plate reader (Thermo Fisher Scientific, Boston, MA, USA). Each measurement was repeated three times.

Relative caspase 3 activity assay. Caspase 3 activity detection of TGCT and OA FLS was performed using the caspase 3 Activity Kit (Solarbio, Beijing, PR China). After 6 days of culture treated with Ad-Arrb2, Arrb2 siRNA, or Akt inhibitor (LY294002, Cat#: 9901, CST, Boston, MA, USA), total protein was extracted from FLS and incubated with 90 μ l of prepared reaction buffer and 10 μ l caspase-3 substrate (Ac-DEVD-pNA, 2 mM), in 96-well plates at 37°C for 4 h in the dark. Then, the reaction mixtures were quantified at a wavelength of 405 nm using a Multiskan plate reader (Thermo Fisher Scientific). Each measurement was performed in triplicate.

Statistical analysis. All statistical analyses were performed using SPSS 20.0 (IBM Corp, Chicago, USA). Data were presented as mean $\pm$ standard deviation. Statistical comparisons of two independent groups were performed using the Student's *t*-test. Multiple comparisons were performed using one-way ANOVA. Statistically significant data are indicated by **p*<0.05, ***p*<0.01, and ****p*<0.001.

Results

Arrb2 is highly expressed in TGCT FLS. In the present study, we first confirmed the general pathological features of TGCT via arthroscopy and MRI and compared them with OA. Under arthroscopy, the TGCT synovium was significantly hyperplastic compared to the OA synovium, and it was reddish brown due to hemosiderin. MRI showed that the synovial tissue of TGCT was widely diffused in the joint cavity, and caused severe joint edema (Figure 1A). Furthermore, H&E staining showed that the TGCT synovial lining layer was thickened. The immunohistochemical analysis revealed that the expression of Ki-67 and CD 163 was significantly up-regulated compared to OA (Figure 1B). To determine whether there are differences in the levels of mRNA expression between TGCT and OA, we used mRNA-seq array and identified 1,314 mRNAs that were significantly different; 780 mRNAs were down-regulated and 534 mRNAs were up-regulated. Among them, we found that Arrb2 was significantly up-regulated in TGCT, compared to the OA (Figure 1C), and the expression of Arrb2 was closely related to the regulation of cell proliferation (Figure 1D). Next, we used western bolt to verify the protein levels of

Arrb2 and Arrb1 in tissue samples and cell samples from TGCT and OA patients. The results showed that Arrb2 was highly expressed in both TGCT tissue (Figure 1E) and FLS (Figure 1F), compared to OA-derived samples, while there was no significant difference in Arrb1 expression; thus, confirming the mRNA-seq array results. Hence, we focused on Arrb2 for further study.

Arrb2 facilitates the proliferation and inhibits the apoptosis of TGCT FLS. We first examined the proliferation ability of TGCT and OA FLS by MTT assay. The results showed that the proliferation ability of TGCT FLS was significantly stronger than that of OA FLS. At the same time, the caspase 3 activity assay showed that, TGCT FLS apoptosis was lower than that of OA FLS (Figure 2A). Overexpression of FLS Arrb2 by Ad-Arrb2 at MOI 20 showed significant up-regulation of Arrb2 (Figure 2B) compared to the negative control (Ad- β -gal), while overexpression of Arrb2 did not affect the expression of Arrb1 (Figure 2C). Subsequently, we tested the proliferation and apoptosis of transfected TGCT and OA FLS to overexpress Arrb2. The results demonstrated that following overexpression, regardless of TGCT or OA, the proliferation ability of FLS was enhanced and apoptosis was inhibited, compared to the negative control cells (Figure 2D and E).

Arrb2 knockdown inhibits the proliferation and increases apoptosis of TGCT FLS. We then constructed Arrb2 siRNA to transfect FLS. Western blot analysis confirmed that Arrb2 siRNA effectively knocked down the expression of Arrb2, without affecting Arrb1 expression (Figure 3A and B). Moreover, we analyzed the effects on cell proliferation ability and apoptosis after silencing Arrb2, using the MTT and caspase-3 assays. We confirmed that silenced Arrb2 inhibited the proliferation and induced the apoptosis of both TGCT and OA FLS, compared to the negative control (Figure 3C and D).

Arrb2 regulates the TGCT FLS proliferation and apoptosis by regulating the PI3K-Akt cell survival pathway. We next investigated the mechanism underlying the effects of Arrb2 on the cell proliferation ability and apoptosis. The KEGG pathway analysis results showed that, when compared to OA, the activation of multiple pathways in TGCT had changed, including TGF- β signaling pathway, Rap 1 signaling pathway, PPAR signaling pathway and PI3K-Akt signaling pathway. Among them, PI3K-Akt, as a pathway related to cell survival, was significantly activated (Figure 4A). In order to verify the KEGG results, western bolt analysis was performed. The results confirmed that the phosphorylation levels of Akt and Erk1/2 were significantly higher in TGCT synovial tissue or FLS than in OA (Figure 4B).

To further examine the role of Arrb2 in the PI3K-Akt pathway in FLS, we treated the cultured TGCT and OA FLS

Figure 1. *Arrb2* is up-regulated in TGCT patients compared with OA. (A) Comparison of the characteristics of TGCT and OA synovitis under arthroscopy and MRI. (B) Comparison of the pathological features of TGCT and OA synovitis using H&E and IHC; Scale bar: 100 μ m. (C) Heatmap of the differentially expressed mRNA patterns in TGCT versus OA synovitis, the relative expression is indicated by color scale (red: high expression). (D) Gene ontology analysis between TGCT and OA synovitis (green: up regulation, red: down regulation). (E-F) Western blot verifies the expression of *Arrb2* and *Arrb1* in TGCT and OA synovitis tissues (E) and FLS (F). TGCT, Tenosynovial giant cell tumor; OA, osteoarthritis; FLS, fibroblast-like synoviocytes; OSag FS PD, oblique sagittal fat suppressed-proton density imaging; H&E, hematoxylin-eosin staining; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

with Ad-*Arrb2*. After overexpressing *Arrb2*, the phosphorylation levels of Akt and Erk1/2 in both TGCT and OA FLS were significantly higher than that of the negative controls, indicating that *Arrb2* can effectively activate this pathway (Figure 4C). Furthermore, inhibition of the PI3K-Akt pathway with Akt inhibitor in Ad-*Arrb2*-transfected TGCT FLS inverted the effects of *Arrb2* overexpression on cell proliferation and apoptosis (Figure 4D). Next, using western blot, we analyzed the phosphorylation levels of PI3K-Akt pathway after *Arrb2* silencing. The results showed that after treatment of TGCT and OA FLS with *Arrb2* siRNA, the phosphorylation levels of Akt and Erk1/2 were

significantly down-regulated, compared to the negative controls (Figure 4E and F). Taken together, although the PI3K-Akt pathway may not be the only pathway regulated by *Arrb2* in TGCT (other pathways could also be involved), we conclude that *Arrb2* positively regulates survival and negatively regulates apoptosis of TGCT and OA FLS, at least partially, by activating the PI3K-Akt pathway.

Discussion

TGCT, also known as pigmented villonodular synovitis (PVNS), is characterized by rapid proliferation and high

Figure 2. *Arrb2* facilitates the proliferation and inhibits the apoptosis of TGCT FLS. (A) MTT and caspase 3 activity assay of TGCT and OA. (B) Western blot showing the overexpression ability of *Arrb2* adenovirus vector. (C) Western blot showing the effect of *Arrb2* adenovirus vector on *Arrb1* expression. (D) MTT and caspase 3 activity assay of OA FLS treated with or without *Arrb2* adenovirus vector. (E) MTT and caspase 3 activity assay of TGCT FLS treated with or without *Arrb2* adenovirus vector. TGCT, Tenosynovial giant cell tumor; OA, osteoarthritis; FLS, fibroblast-like synoviocytes; MTT, 3-(4,5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide; MOI, multiplicity of infection; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

recurrence rate and its pathogenesis is currently unclear to clinicians (19). Due to the incomplete research on the pathogenesis of TGCT, a complete surgical synovectomy remains the principle treatment; however there is no generally accepted target about the surgical procedure and the postoperative adjunctive therapy (20). Therefore, further understanding of TGCT pathogenesis and an exploration of novel therapeutic targets for patients is urgently required. TGCT as well as other types of synovitis are very different in terms of pathological characteristics. Herein, we showed that under arthroscopy, the TGCT synovial tissue was obviously hyperplastic and was reddish brown due to the deposition of hemoglobin. Immunohistochemical analysis demonstrated that the expression of the proliferation marker Ki-67 and the macrophage marker CD163 was higher in TGCT than in OA synovial tissue.

In order to explore the pathogenesis of TGCT and find new therapeutic targets, we used an mRNA-seq high-throughput assay to screen the differentially expressed genes between TGCT and OA synovial tissue. The results showed that 780 mRNAs were down-regulated and 534 were up-regulated. Among them, *Arrb2* was significantly up-regulated in TGCT compared to OA, and the expression of *Arrb2* was

closely related to the regulation of cell proliferation. *Arrb2* is the main member of the arrestin family in non-retinal expression (21). It is generally known that β -arrestin modulates GPCR signaling through receptor desensitization and internalization (22). Many recent studies have shown that β -arrestin unexpectedly acts as scaffold protein for many signal molecules in the cytoplasm and nucleus, thereby regulating gene expression and cell responses (23-25). There is increasing evidence that abnormal β -arrestin expression is involved in several types of tumors (26) as well as being related to cell survival (27), apoptosis (28), migration (29) and proliferation (26). Jude Masannat *et al.* found that knockout of *Arrb2* decreases the proliferation and migration rates of renal cell carcinoma, *in vitro*, and xenograft tumor growth in animals (30). Similarly, Fanling Hong *et al.* showed that *Arrb2* overexpression promoted the growth of human endometrial carcinoma heterotransplants and inhibited the endometrial cell apoptosis (31). On the other hand, Yimei Wang *et al.* indicated that *Arrb2* promotes cell death and inhibits cell pro-survival signaling (32). These discrepancies may be due to the distinct *Arrb2* conformational states triggered by different upstream stimuli and different interacting proteins in different tissues.

Figure 3. Knockdown the Arrb2 inhibits the proliferation and increases the apoptosis of TGCT FLS. (A) Western blot showing the knockdown ability of Arrb2 siRNA. (B) Western blot showing the effect of Arrb2 siRNA on Arrb1 expression. (C) MTT and caspase 3 activity assay of OA FLS treated with or without Arrb2 siRNA. (D) MTT and caspase 3 activity assay of TGCT FLS treated with or without Arrb2 siRNA. TGCT, Tenosynovial giant cell tumor; OA, osteoarthritis; MTT: 3-(4,5)-dimethylthiazazo (-z-y1)-3,5-di-phenytetrazoliumromide; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

However, there is no report on its role in TGCT. Our western bolt results confirmed that whether it is synovial tissue or FLS, the expression of TGCT Arrb2 was higher than that of OA. However, some studies have shown that the expression of Arrb1 is also essential for maintaining the malignant phenotype of tumors (33-35). Therefore, we also tested the expression of Arrb1 and found that there was no difference between TGCT and OA.

Subsequently, we tested the proliferation and apoptosis of TGCT and OA FLS and found that the proliferation ability was higher and apoptosis was lower in TGCT than in OA FLS. To further explore the effect of Arrb2 on FLS proliferation and apoptosis, we constructed an Arrb2 overexpression adenovirus vector and Arrb2 siRNA to interfere with the expression Arrb2 in FLS. The results confirmed that when Arrb2 overexpression in FLS

significantly promoted cell proliferation and inhibited cell apoptosis. On the contrary, when Arrb2 was knocked down, cell proliferation was inhibited and apoptosis returned to normal levels. Interestingly, this phenomenon not only occurred in the TGCT FLS, but also in the OA FLS, indicating that Arrb2 can not only regulate cells with high proliferation characteristics, but also cells with relatively weak proliferative ability. A previous study has also shown that Arrb2 is positively correlated with human FLS proliferation (36), which is consistent with our research.

The molecular mechanism by which Arrb2 regulates the proliferation and apoptosis of TGCT FLS was also explored in this study. The KEGG pathway analysis results showed that, compared with to OA, multiple pathways were significantly activated in TGCT. Among them, the PI3K-Akt pathway is widely reported to be related to cell survival and

Figure 4. *Arrb2* regulates the TGCT FLS proliferation and apoptosis by regulating the PI3K-Akt cell survival pathway. (A) The KEGG pathway analysis of mRNA-seq results between TGCT and OA synovitis. (B) Western blot showing the phosphorylation levels of Akt and Erk1/2 in TGCT and OA synovitis tissues and FLS. (C) Western blot showing the phosphorylation levels of Akt and Erk1/2 in TGCT and OA FLS treated with or without *Arrb2* adenovirus vector. (D) MTT and caspase 3 activity assay of TGCT FLS treated with or without *Arrb2* adenovirus vector and Akt inhibitor. (E-F) Western blot showing the phosphorylation levels of Akt and Erk1/2 in TGCT and OA FLS treated with or without *Arrb2* siRNA. TGCT, Tenosynovial giant cell tumor; OA, osteoarthritis; MTT, 3-(4,5)-dimethylthiazolium (-z-y1)-3,5-di-phenyltetrazolium bromide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

proliferation (37, 38), especially in tumors (39, 40). Furthermore, the PI3K-Akt pathway plays an important role in synovitis; our previous research has confirmed that inflammatory factors can regulate the invasion and migration of OA FLS through the PI3K-Akt pathway (41). At the same time, Arrb2 can participate in several types of cancers by acting as a scaffold protein in various signaling pathways, including extracellular regulatory protein kinase (ERK1/2), Wnt/ β -catenin and c-Src (9). Previous research has also shown that β -arrestins are involved in cell proliferation, apoptosis, and differentiation by mediating the Akt phosphorylation response to extensive stimulation in different cell types (42, 43). Furthermore, PI3K-Akt signaling has been shown to play a central role in Arrb2-mediated cell protection (44, 45). Therefore, we tested whether PI3K-Akt pathway is the downstream signaling mediator of the Arrb2-induced anti-apoptotic and pro-proliferation effects in TGCT. We first compared the phosphorylation levels of Akt and ERK1/2 in TGCT and OA, demonstrating that whether it is synovial tissue or FLS, phosphorylation levels of these proteins were significantly higher in TGCT than in OA. When Arrb2 was overexpressed or knocked down, the phosphorylation levels of Akt and ERK1/2 in FLS were significantly increased or decreased, respectively (Figure 4C, E and F), suggesting that Arrb2 can effectively regulate the activation of this pathway. Most importantly, Akt inhibitor inverted the effects of Arrb2 overexpression on cell proliferation and apoptosis of TGCT FLS. The above results strongly suggest that Arrb2 regulates the TGCT FLS proliferation and apoptosis mainly through the PI3K-Akt cell survival pathway.

In conclusion, this is the first detailed study to explore whether and how Arrb2 affects TGCT FLS proliferation and apoptosis. Our findings demonstrated that Arrb2 facilitates the proliferation and inhibits the apoptosis of TGCT FLS mainly through PI3K-Akt activation, providing new insights into the molecular mechanism of TGCT. In this regard, Arrb2 may have the potential to be a molecular target for TGCT therapy.

Conflicts of Interests

The Authors declare that they have no competing interests.

Authors' Contributions

CXC conducted the majority of the experiments and completed the manuscript; YZ participated in the experiment and helped with manuscript preparation; YFA and XZ conceived the project and designed the experiments; JC and FW collected the clinical specimens; XYN and XQH revised the manuscript; XF, XND and JYZ analyzed the data, prepared the draft figures; YFA and XZ supervised the project; CXC and YZ contributed equally in this work. All Authors approved the final article.

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