Exploration of the Effects of TGF-β Pathway-Based Pituitary Tumor of Rats on GH3 Cell Line after Intervention with Different Concentrations of TGZ

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The effect of the TGF-β pathway-based pituitary tumor of rats on the GH3 cell line after intervention with different concentrations of troglitazone (TGZ) is explored. The CH3 cell line of 24 clean male SD rats with pituitary adenoma is selected. The cells are divided into a blank contrast set and an experimental set. The experimental set is divided into different TGZ concentration sets, including 1 × 10^{-3} TGZ set, 1 × 10^{-4} TGZ set, and 1 × 10^{-5} TGZ set. The cell proliferation is detected by the CCK-8 method, the protein expressions of CD147, TGF-β1, and MMP-9 are detected by the western blot method, and the relative mRNA expressions of CD147, TGF-β1, and MMP-9 are detected by qRT-PCR method. The expression levels of CD147, TGF-β1, and MMP-9 in CH3 cells of pituitary adenoma rats are notoriously lower, while the expression of CD147, TGF-β1, and MMP-9 could be reduced by TGZ acting on the GH3 cell line. The specific mechanism of action of this effect on the invasive ability of GH3 cell lines is multifaceted, suggesting that peroxisome proliferator activator-receptor (PPAR-γ) agonists have good clinical application prospects in tumor therapy and can provide new targets and approaches for tumor drug therapy.

1. Introduction

Pituitary adenomas mostly occur in the adenopituitary tissue and are a relatively common clinical intracranial tumor with a relatively high clinical incidence. Among all intracranial tumors, the incidence is about 12% [1]. According to the classification of the World Health Organization, pituitary tumors can be divided into three major categories, namely, typical pituitary adenomas, atypical pituitary adenomas, and pituitary carcinomas. Pituitary adenomas, pituitary carcinomas, and benign pituitary adenomas, among which invasive pituitary adenoma (IPA) can compress the tissues around the pituitary gland [2]. For some sufferers with pituitary tumors, there may still be some biological characteristics of malignant tumors, but the concerning mechanism of the invasive growth of pituitary tumors is still unknown and there are few concerning studies [3]. Troglitazone (TGZ) is a peroxidase enzyme. It is a synthetic agonist of peroxisome proliferator activator receptor (PPAR-γ) and belongs to thiazolidinediones. Studies have found that the rat pituitary adenoma GH3 cell line highly expresses PPAR-γ, while CD147, TGF-β1, and MMP-9 are all factors concerning to the invasiveness of tumors, but there are few studies on the expression of TGZ [4–6].

In this paper, different concentrations of TGZ are applied to the GH3 cell line, and the changes of the protein and mRNA expression in GH3 cells are detected by western blot and fluorescence quantitative PCR, and the effect of TGZ on the invasiveness of GH3 cells is discussed. At the same time, the possible mechanism of action of PPAR-γ agonists to
reduce the invasiveness of pituitary adenomas is analyzed, in order to provide reliable experimental data and theoretical basis for further experimental research on the invasiveness of pituitary adenomas and the discovery of new clinical treatment methods.

The rest of this paper is organized as follows: Section 2 discusses related work, followed by the detection methods and statistical processing designed in Section 3. Section 4 shows the experimental results, and Section 5 concludes the paper with summary and future research directions.

2. Related Work

In the central nervous system, pituitary adenoma is one of the most common benign tumors, and its incidence is lower than that of glioma and meningioma, ranking third, accounting for 16.7% of all intracranial tumors [7]. The incidence rate has been increasing this year. Although the histological classification of pituitary adenomas is benign, some pituitary adenomas are biologically aggressive. In 1940, Akino first proposed the concept of IPA [8]. The main clinical manifestations of IPA are the invasion of the main structures of the parasellar region: the cavernous sinus can be invaded on both sides; the third ventricle can be affected upward; and the sphenoid sinus can be invaded downward. This type of tumor is difficult to treat, and it is difficult to completely cure it, even if it is completely removed under the microscope and is easy to relapse after surgery. To date, the mechanism of invasive pituitary gland development has not been fully understood. There are many studies on the mechanism of pituitary adenoma invasiveness and many factors have also been confirmed to be concerning to the invasiveness of pituitary adenomas. It is now generally believed that its mechanism is the result of the combined action of multiple factors [9] such as ras, Rb, and p53 16–31 are considered to be factors concerning to the invasiveness of pituitary adenomas. Pituitary tumor-transforming gene (PTTG) is a newly discovered protooncogene in recent years, which is closely concerns the occurrence of various tumors. An extensive number of studies in concerning fields have confirmed that there are many pathological factors involved in the occurrence and growth of invasive pituitary adenomas. However, the exact mechanism remains to be elucidated by further studies in order to treat invasive pituitary adenomas at the molecular level [10].

The regulatory mechanisms of PPAR-γ ligand agonists are mainly PPAR-γ-dependent and -independent. After PPAR-γ binds and activates its ligand agonist, it can combine with the retinoic X receptor (RXR) to form a heterodimer PPAR-γ/RXR, which can interact with the peroxisome growth factor response element, and it interacts and regulates the expression of target genes. This pathway is a trans-transcriptional activation mechanism after the activation of PPAR-γ ligands [11]. In addition, the mechanism of action of PPAR-γ also has a trans-transcriptional inhibition mechanism, which can inhibit the signal transduction pathway activated by transcriptional factors. The heterodimeric PPAR-γ/RXR can bind to transcriptional factors, thereby inhibiting the gene transcription induced by the factor. Moreover, with the deepening of research, it has been found that PPAR-γ is very closely concerned with the occurrence and development of tumors [12]. An extensive number of studies have found that PPAR-γ agonists have obvious antitumor effects and their mechanisms include inhibiting cell proliferation, promoting cell apoptosis and differentiation, inhibiting angiogenesis, and reducing tumor invasiveness. However, there are few reports that PPAR-γ reduces the invasiveness of pituitary adenomas, and the mechanism of action is not yet clear. PPAR-γ is involved in the differentiation and apoptosis of tumor cells and its relationship with tumors has attracted increasing attention [13]. PPAR-γ agonists (ligands) have obvious antitumor effects, can inhibit cell proliferation and promote cell apoptosis and differentiation, and also have certain inhibitory effects on angiogenesis. In the theory of tumor cell invasion and metastasis step hypothesis, it is believed that the first step of tumor invasion and metastasis is de-adhesion between tumor cells and between the tumor and extracellular matrix. It plays an important role in the process of invasion and metastasis [14]. In contrast with the blank contrast set, after TGZ acted on GH3 cells for 48h, the protein and mRNA expressions of CD147, TGF-β1, and MMP-9 in the TGZ experimental set with different concentrations showed a downward trend (all P < 0.05), and the higher the drug concentration, the higher the protein expression, and the lower the protein expression (all P < 0.05).

The TGF-β signal transduction pathway is a very important intracellular signal pathway, which is involved in the regulation of cell proliferation, migration, programmed death, and other biological behaviors of cells and plays an important role in the occurrence and development of many tumors [15]. TGF-β1 is closely concerned with tumors. In the process of tumor progression, TGF-β1 can provide a suitable microenvironment for tumor growth, infiltration and metastasis by stimulating angiogenesis, immunosuppression, and synthesis of the extracellular matrix [16]. The role of TGF-β1 in tumors is worthy of study, and it plays almost diametrically opposite roles in different stages of tumor development. During the initial stages of tumorigenesis, TGF-β1 was observed to act as a tumor growth suppressor to inhibit tumor growth. When the tumor developed to the mid-late stage, the inhibitory effect of TGF-β1 on the tumor disappeared completely, and instead, it promoted the formation of abnormal blood vessels in the tumor. Moreover, to promote the tumor invasive ability through this pathway, it is generally believed that this transition is caused by the change of components of the TGF-β1 signaling pathway. Moreover, changes in the components of the TGF-β1 transduction pathway have been found in malignant tumors of many systems. The expression of CD147 is enhanced in a variety of tumors. It is widely expressed in tumor cells and cells around the tumor. It has an important regulatory role in the occurrence, growth, and metastasis of tumors; tumor cells can detect the increased expression of CD147 [17]. Studies have shown that the pathological grade of various malignant tumors and the
prognosis of sufferers are concerned with the expression of CD147. Both CD147-expressing tumor cells and the culture medium of the tumor cells can induce fibroblasts to produce MMPs. In the absence of CD147-expressing tumor cells, the CD147 gene was transfected into fibroblasts by inactivated adenovirus, and the experimental results showed that the expression of MMPs was notoriously increased. They also found that even the recombinant CD147 protein also had this effect [18]. The expression level of CD147 closely concerns the expression level of various tumor MMPs in vivo. Recent studies have shown that reverse transcription of cysteine-rich proteins closely concerns the mechanism by which MMP-9 regulates tumor local invasion and distant metastasis. In gastric cancer and cervical cancer, MMP-9 has also been found to play an important role in cell metastasis and infiltration. The occurrence, development, invasion, and migration of central nervous system tumors also closely concern MMP-9 [19]. The relationship between glioma and MMP-9 has been deeply studied, and an extensive number of experimental data show that MMP-9 promotes the proliferation of glioma-infiltrating tissue cells. Takano found an extensive positive correlation between MMP-9 and MVD in glioblastoma. This indicates that MMP-9 can affect the proliferation of glioma by participating in the formation of tumor angiogenesis [20]. Basic research on pituitary adenomas showed that the expression of MMP-9 was notoriously higher in invasive pituitary adenomas than in noninvasive pituitary adenomas.

3. Detection Methods and Statistical Processing

3.1. Experimental Animal Cells. The CH3 cell line of 24 clean-grade male SD rats with pituitary adenomas is selected and was provided by Tianqin Biological Company.

3.2. Detection Methods

3.2.1. Cell Culture. The CH3 cells are grown in a DMEM medium containing 15% horse serum, 2.5% fetal bovine serum, and 100 μ/ml penicillin, then the constant temperature incubator is set to 37°C, at 5% CO₂, and the cells are put into conventional culture. After the cells are put in, it is necessary to observe and record the growth of the cells and pay special attention to the degree of confluence. When the value reaches 85%, it is digested and subcultured. Log-phase CH3 cells are taken and seeded in 6-well cell plates at a density of 1 × 10⁵ cells per well.

3.2.2. Drug Reagent Configuration. In this experiment, cells are divided into a blank contrast set and an experimental set. The experimental set is divided into different TGZ concentration sets, including the 1 × 10⁻³ TGZ set, 1 × 10⁻² TGZ set, and 1 × 10⁻¹ TGZ set.

TGZ is a white powder with a total amount of 10 mg and a relative molecular mass of 441.54. It is dissolved in 0.5 ml DMSO to prepare a 20 mg/ml solution, and a serum-free F12 medium is added to obtain an experiment with a concentration of 1 × 10⁻³ mol/L liquid medicine. After filter sterilization, it is stored in a refrigerator at 4°C. We use the serum-free F12 medium for dilution and prepare experimental liquids with concentrations of 1 × 10⁻³ mol/L, 1 × 10⁻⁴ mol/L, and 1 × 10⁻⁵ mol/L for temporary use.

3.2.3. CCK-8 Assay to Detect Cell Proliferation. The change process of cell proliferation is detected by the CCK-8 method. First, cervical cancer cells are taken and placed in a 96-well plate, and the cell culture solution is placed in the well plate. 104 cells are inserted into the cells for culture, and after 24 hours, they are treated according to the methods of the blank contrast set at 1 × 10⁻³ mol/L set, 1 × 10⁻⁴ mol/L set, and 1 × 10⁻⁵ mol/L set. After culturing for 12 h, 10 μL of the CCK-8 solution is added to each well, and the cells are placed in an incubator for 4 h. The OD value at 490 nm is measured by a microplate reader as a measure of cell viability. After 1 h, the absorbance A value is measured at a wavelength of 450 nm by the microplate reader to indicate cell viability. Cell viability = (OD value of the experimental set ÷ OD value of the blank contrast set) × 100%.

3.2.4. Western Blot Detection of CD147, TGF-β1, and MMP-9 Protein Expressions. The CH3 cell line cells of each set are collected and added to radioimmunoprecipitation (RIPA) lysate to extract total protein. After the extraction is completed, the quality of the protein needs to be detected. The used method is the BCA method. At the same time, it is necessary to formulate the separation gel and set the concentration of the separation gel to 10%. Then, 35 μg of the sample is taken for separation, the protein is transferred to the membrane, 5% nonfat milk powder is closed and cultured for 1.5 hours, and CD147 and TGF-β1 are added. MMP-9 antibody is diluted to 1:800, incubated overnight at 4°C, then added to the secondary antibody and placed at room temperature, and developed after 1.5 h. It is processed by photographing, and the purpose of photographing is to analyze the gray value of its band. The analytical substance is a gel imager, and β-actin is used as a contrast to analyze the gray value of the protein band.

3.2.5. qRT-PCR Method to Detect the Relative Expression of CD147, TGF-β1, and MMP-9 mRNA. First, we collect all CH3 cells, add TRizol reagent to tissue cells, mix them completely, and extract total cell RNA. Then, we select a suitable UV spectrometer and use it to detect the total RNA quality at A260/A280. We dilute 1 μl total RNA by 50 times, add RNase free dH2O to synthesize cDNA template, then take 10 μl SYBR green, 0.8 μl forward and reverse primers, 2 μl cDNA template, and 0.4 μl ROX reference, and add sterilized water to make it up to 20 μl. The PCR instrument performs real-time PCR reactions. Table 1 shows the PCR primer sequences. The relative expression levels of CD147, TGF-β1, and MMP-9 mRNA are calculated by the 2^ΔΔCt method.


4. Experiment Results

4.1. Contrast of Proliferation of CH3 Cells in Different Sets of Rats. Table 2 shows the contrast of the proliferation of CH3 cells in different sets of rats. In Table 2, the contrast with the blank contrast set is represented as *P < 0.05, the contrast with the 1 × 10⁻³ TGZ set is represented as #P < 0.05, and the contrast with the 1 × 10⁻⁴ TGZ set is represented as †P < 0.05. It is obvious from Table 2 that the viability and survival rate of CH3 cells in the 1 × 10⁻³ TGZ set, 1 × 10⁻⁴ TGZ set, and 1 × 10⁻⁵ TGZ set are notoriously lower than those in the blank contrast set (P < 0.05); 1 × 10⁻⁴ TGZ set and 1 × 10⁻⁵ TGZ set are notoriously lower than those in the 1 × 10⁻³ TGZ set (P < 0.05); the viability and viability of CH3 cells in the 1 × 10⁻⁵ TGZ set are notoriously lower than those in the 1 × 10⁻⁴ TGZ set (P < 0.05).

4.2. Expression of the CD147 Protein in CH3 Cells of Different Sets of Rats. Table 3 shows the expression of the CD147 protein in CH3 cells of different sets of rats. In Table 3, the contrast with the blank contrast set is represented as *P < 0.05; the contrast with the 1 × 10⁻³ TGZ set is represented as #P < 0.05; and the contrast with the 1 × 10⁻⁴ TGZ set is represented as †P < 0.05. Figure 1 shows the images of CD147 protein in CH3 cells of different sets of rats. From the above experimental results, it can be observed that when TGZ is applied to GH3 cells for 48 h, the protein expression of CD147 in the experimental sets with different concentrations of TGZ decreased in contrast with the blank contrast set (all P < 0.05), and the higher the drug concentration, the higher the protein expression.

4.3. Expression of the TGF-β1 Protein in CH3 Cells of Different Sets of Rats. Table 4 shows the expression of TGF-β1 protein in CH3 cells of rats in different sets. In Table 4, the contrast with the blank contrast set is represented as *P < 0.05, the contrast with the 1 × 10⁻³ TGZ set is represented as #P < 0.05, and the contrast with the 1 × 10⁻⁴ TGZ set is represented as †P < 0.05. Figure 2 shows the images of the TGF-β1 protein in CH3 cells of different sets of rats. It can be seen from Table 4 and Figure 2 that in contrast with the blank contrast set, the protein expression of TGF-β1 in the experimental sets with different concentrations of TGZ decreased (all P < 0.055) after TGZ acted on GH3 cells for 48 h, and the higher the drug concentration, the higher the protein expression.
Table 5: MMP-9 protein expression in CH3 cells of rats in different sets (x ± s).

| Set                | MMP-9 protein value |
|--------------------|---------------------|
| Blank contrast set | 0.323 ± 0.023       |
| 1 × 10⁻³ TGZ set   | 0.192 ± 0.013*      |
| 1 × 10⁻⁴ TGZ set   | 0.236 ± 0.021*      |
| 1 × 10⁻⁵ TGZ set   | 0.287 ± 0.024*      |

The contrast of CD147, TGF-β1, and MMP-9 mRNA expressions in CH3 cells of different sets of rats.

Table 6: MMP-9 protein expression in CH3 cells of different sets of rats.

| Set                | CD147 mRNA | TGF-β1 mRNA | MMP-9 mRNA |
|--------------------|------------|-------------|------------|
| Blank contrast set | 1.000 ± 0.000 | 1.000 ± 0.000 | 1.000 ± 0.000 |
| 1 × 10⁻³ TGZ set   | 0.499 ± 0.023* | 0.281 ± 0.018* | 0.526 ± 0.019* |
| 1 × 10⁻⁴ TGZ set   | 0.579 ± 0.021* | 0.762 ± 0.035* | 0.629 ± 0.012* |
| 1 × 10⁻⁵ TGZ set   | 0.689 ± 0.035* | 0.953 ± 0.022* | 0.896 ± 0.024* |

4.4. MMP-9 Protein Expression of CH3 Cells in Different Sets.

Table 5 shows the MMP-9 protein expression in CH3 cells of rats in different sets. In Table 5, the contrast with the blank contrast set is represented as †P < 0.05; the contrast with the 1 × 10⁻³ TGZ set is represented as ‡P < 0.05; and the contrast with the 1 × 10⁻⁴ TGZ set is represented as §P < 0.05. Figure 3 shows the images of the MMP-9 protein in different sets of CH3 cells of rats. From the above experimental results, it can be observed that in contrast with the blank contrast set, the expression of the MMP-9 protein in the experimental sets of TGZ with different concentrations decreased (all P < 0.05) after TGZ acted on GH3 cells for 48 h, and the higher the drug concentration, the higher the protein expression.

4.5. Contrast of CD147, TGF-β1, and MMP-9 mRNA Expressions in CH3 Cells of Different Sets of Rats.

Table 6 shows the MMP-9 protein expression in CH3 cells of different sets of rats. In Table 6, the contrast with the blank contrast set is represented as †P < 0.05; the contrast with the 1 × 10⁻³ TGZ set is represented as ‡P < 0.05; and the contrast with the 1 × 10⁻⁴ TGZ set is represented as §P < 0.05. Figure 4 shows the contrast of CD147, TGF-β1 and MMP-9 mRNA expressions in CH3 cells of different sets of rats. It is clearly evident from Table 6 and Figure 4 that in contrast with the blank contrast set, the mRNA expressions of CD147, TGF-β1, and MMP-9 in the TGZ experimental sets with different concentrations all showed a downward trend (all P < 0.05) after TGZ acted on GH3 cells for 48 h, and the higher the drug concentration, the lower the protein expression level (all P < 0.05).

5. Conclusion

Pituitary adenomas mostly occur in the adenopituitary tissue and are a relatively common clinical intracranial tumor with a relatively high clinical incidence. The effect of TGF-β pathway-based pituitary tumor rats on the GH3 cell line after intervention with different concentrations of TGZ is explored. The expression levels of CD147, TGF-β1, and MMP-9 in pituitary tumors are notoriously lower, and the expression of CD147, TGF-31, and MMP-9 could be reduced by TGZ acting on the GH3 cell line. The specific mechanism of action of low-invasion GH3 cell lines is multifaceted, which suggests that PPAR-receptor agonists have good clinical application prospects in tumor therapy and can provide new targets and approaches for the tumor drug therapy.

Data Availability

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Chunmei Hu is the first author.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.
Authors’ Contributions

Chunmei Hu mainly contributed to the study. All authors have read and approved the final manuscript.

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