Dopamine receptor D2S gene transfer improves the sensitivity of GH3 rat pituitary adenoma cells to bromocriptine

Qiang Li, Zhipeng Su, Jie Liu, Lin Cai, Jiangleong Lu, Shaojian Lin, Zhengkun Xiong, Weiqiang Li, Weiming Zheng, Jinsen Wu, Qichuan Zhuge, Zhebao Wu

Abstract

Bromocriptine, a dopamine agonist (DA), has been used in the treatment of prolactinomas. Recent studies have indicated that dopamine 2 receptor short isoform (D2S) may play an important role in suppressing PRL synthesis and prolactinoma cell growth under DA treatment. In the current study, we investigated the role of D2S in the therapeutic action of bromocriptine in GH3 using both in vitro and in vivo approaches. Infection of adenovirus-D2S increased D2S expression in GH3 cells (P < 0.05). D2S expression significantly decreased the GH3 cell viability subjected to bromocriptine treatment in vitro (P < 0.05). In nude mice, adenovirus-D2S transfection sensitized GH3 xenograft to bromocriptine treatment evidenced by the significant inhibition of D2S expressed tumor growth as compared with vector control. Furthermore, decrease of Bcl-2 expression, increase of Bax, and active Caspase-3 were found in D2S expressed GH3 xenograft subjected to bromocriptine treatment. In summary, our study indicates that D2S expression plays a critical role in the therapeutic action of bromocriptine in pituitary adenomas and that adenovirus-mediated D2S gene transfer combined with bromocriptine may provide a novel treatment for DA-resistant prolactinomas.

Keywords: Pituitary adenoma; GH3 cell; Dopamine 2 receptor; Dopamine 2 receptor short isoform; Bromocriptine; Apoptosis

1. Introduction

Prolactinoma is the most predominant type of pituitary adenomas with dopamine receptor agonists (DAs) as the first-line treatment (Casaneueva et al., 2006; Colao and Savastano, 2011; Gillam et al., 2006; Wu et al., 2006). DAs are effective in suppressing prolactin (PRL) hypersecretion, reducing tumor size, and restoring gonadal function (Molitch, 2002; Wu et al., 2006). However, 10–20% of prolactinomas are resistant to DAs treatment (Melmed et al., 2011; Molitch, 2005). The resistance is mainly associated with a decrease in dopamine 2 receptor (D2R) expression (Caccavelli et al., 1994; Passos et al., 2009; Wu et al., 2010). The majority of dopamine-resistant prolactinomas present as large and/or invasive tumors, making surgical removal difficult (Wu et al., 2006). Thus, management of dopamine-resistant prolactinomas remains a therapeutic challenge.

D2R exists as two alternatively spliced subtypes, the long (D2L) and the short (D2S), which differs by an insertion of 29 amino acids in the third intracellular protein loop (Giros et al., 1989). The D2R subtypes have been shown to differentially activate intracellular signal transduction pathways due to this polypeptide region, which is involved in G protein coupling of the D2R (Missale et al., 1998; Wolfe and Morris, 1999). There is increasing evidence indicated that D2L and D2S may have different physiological functions. Iaccarino et al. (2002) demonstrated that D2S inhibited prolactin synthesis and lactotrope cell proliferation, while, D2L might facilitate PRL release. In addition, expression level of D2R subtypes appears to impact the response to DAs treatment in pituitary adenomas. Caccavelli et al. (1994) found that resistance to the bromocriptine therapy seemed to involve in the ratio of D2S to D2L and the D2S/D2L ratio was lower in resistant patients than that in responsive patients. Passos et al. (2009) reported that two patients with drug-resistant prolactinomas presented high D2R expression. They speculated that there could be a predominance of D2L over D2S receptor subtypes. Our previous study also demonstrated that the ratio of D2S/D2L was related to the responsiveness of prolactinomas to DA medication in which D2S mRNA played an important role in the therapeutic action of bromocriptine (Wu et al., 2010). Taken together, these data suggest that D2S may play a very significant role in the therapeutic action of bromocriptine in pituitary adenomas and that adenovirus-mediated D2S gene transfer combined with bromocriptine may provide a novel treatment for DA-resistant prolactinomas.
important role in suppressing PRL synthesis and prolactinoma cell growth under DA treatment.

GH3 cell line has comparative low expression of D2R, especially low expression of D2S (An et al., 2003). In the present study, GH3 cells were infected with adenovirus-mediated D2S gene, and then D2S expression was evaluated by RT–PCR and Western blot. We assessed the growth suppressive response of GH3 cells to bromocriptine combined with or without D2S infection in vitro and in vivo. In addition, we determined the role of D2S expression in the incidence of GH3 cell apoptosis and expression of apoptosis-related proteins after bromocriptine treatment.

2. Materials and methods

2.1. GH3 cells culture

GH3 cells purchased from the American Type Culture Collection were cultured in F12 medium (Hyclone, USA) supplemented with 2.5% fetal bovine serum, 15% horse serum (Gibco, USA), 100U/L penicillin and 100 mg/L streptomycin (Sigma, USA) on condition of 37°C and 5% CO2.

2.2. Construction of recombinant adenoviruses and cell infection

The D2S gene was introduced by using an adenoviral vector. The Adenoviral vector containing either enhanced green fluorescent protein (EGFP) gene alone (subsequently referred to as “Ad-EGFP”) or both EGFP and D2S gene (subsequently referred to as “Ad-D2S”) was synthesized by Changsha Yingrun Biological Engineering Company Ltd (Changsha, China). In brief, the coding sequence of D2S (NM_016574) was amplified by RT–PCR. The PCR fragments and the pYr-adshuttle-6 plasmid were digested with two endonucleases Nhe I and Sal I and then ligated with T4 DNA ligase to construct plasmid pYr-adshuttle-6-D2S. The plasmid was transformed to competent DH5α Escherichia coli for identification. The correct recombinant pYr-adshuttle-6-D2S was then recombined using the LR reaction (Invitrogen, CA, USA) into the pΔd/BL-DEST to form pAd-D2S. After being linearized by Pac I, the homologous recombinant pΔd-D2S was transfected into the HEK293 cell line to package the recombinant adenovirus vector containing the D2S gene (Ad-D2S). High titer adenovirus was obtained by repeated infection in HEK293 cells.

In all experiments, Ad-D2S infected cells were compared with Ad-EGFP infected cells. Viral infections were done by using a multiplicity of infection of 10 (10MOI), a viral dose that shows high-level transgenic expression and has no effect on the viability of GH3 cells in the preparing experiment. The viral suspension was replaced by complete F12 culture medium 6 h after the infection.

2.3. Evaluation of infection efficiency

The infection efficiency was evaluated by immunofluorescent microscopy. EGFP positive cells were counted under fluorescence microscope 48 h after infection. The efficiency of infection was calculated as EGFP positive cells divided by total cells. After being infected by virus, the cells were incubated overnight at 4°C with a rabbit anti-mouse D2S monoclonal antibody (Abnova Company, USA, at a 1:1000 dilution). The membranes were then washed three times with TBST (tris-buffered saline with tween-20) for 10 min and probed with the horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG polyclonal antibody at 37°C for 2 h. After three washes, the membranes were developed by an enhanced chemiluminescence system. The band intensity was measured by densitometry using the Quantity One software (Bio-Rad Laboratories, Inc. USA) and the amount of D2S protein present was further measured by densitometry and height. The protein levels were normalized to that of β-actin.

2.4. In vitro cell viability assay

Cell viability assay was performed using a Cell Counting Kit-8 (CCK-8; Donjindo, Japan) according to the manufacturer’s instructions. GH3 cells (5 × 10^4 /well) transfected with Ad-D2S or Ad-EGFP were cultured in flat-bottom 96-well microtiter plates and treated with bromocriptine (2 μmol/L, Sigma, USA), then incubated for 24 h. After adding 10 μl CCK-8 in each well, incubation continued for additional 4 h. The cells in each well were measured as absorbance at 450 nm in spectruptic. The percentage of GH3 cell survival was calculated as follows: cell survival (%) = ([At−Ab]/[Ac−Ab]) × 100%, where At, Ab and Ac were absorption at 450 nm for the treated, blank and control group, respectively.

2.5. GH3 cell xenograft

All xenograft studies were performed according to protocol approved by the Institutional Animal Care and Use Committee (IACUC). Thirty Homozygous athymic nude (nu/nu) female mice at 4–6 weeks of age were purchased from the Animal Experimental Center of Chinese Academy of Sciences (Shanghai, China). The nude mice were injected with 5 × 10^6 GH3 cells suspended in 200 μL of phosphate-buffered saline (PBS) subcutaneously into their flanks. About two weeks after GH3 cells injection, the nude mice bearing xenograft tumor were used for pharmacological studies when the tumors increased to about 5 mm in diameter.

2.6. Pharmacological studies in vivo

2.6.1. D2S infection and bromocriptine treatment

Thirty nude mice bearing xenograft tumor were randomly and averagely divided into five groups: (1) the blank group with only PBS treatment (subsequently referred to as “PBS group”); (2) the control group with only Ad-EGFP infection (subsequently referred to as “Ad-EGFP group”); (3) the group with only Ad-D2S infection (subsequently referred to as “Ad-D2S group”); (4) the group with combination treatment of Ad-EGFP infection and bromocriptine (subsequently referred to as “Ad-EGFP + BRC group”); (5) the group with combination therapy of Ad-D2S infection and bromocriptine (subsequently referred to as “Ad-D2S + BRC group”).

The respective groups were injected with 30 μl of the Ad-EGFP suspension, Ad-D2S suspension or PBS, into their xenograft tumors every three days for four weeks. Then nude mice of groups Ad-EGFP + BRC and Ad-D2S + BRC received intraperitoneal injection of bromocriptine (0.1 μg/g body weight, Sigma, USA) every 94°C for 30s, 55°C for 30 s and 72°C for 1 min; and finally 72°C for 7 min. The size of the amplified product was 450 bp. The total tissue protein extracts were prepared by lysing cells with RIPA-buffer at 48 h after infection, and quantitatively analyzed by Bradford method. Equal amount of protein samples were separated by electrophoresis in a 12% sodium dodecyl sulfate polyacrylamide gel (SDS–PAGE) and transferred onto polyvinylidene fluoride membranes. After blocking the non-specific binding sites for 60 min with 5% non-fat milk, the membranes were incubated overnight at 4°C with a rabbit anti-mouse D2S monoclonal antibody (Abnova Company, USA, at a 1:1000 dilution). The membranes were then washed three times with TBST (tris-buffered saline with tween-20) for 10 min and probed with the horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG polyclonal antibody at 37°C for 2 h. After three washes, the membranes were developed by an enhanced chemiluminescence system. The band intensity was measured by densitometry using the Quantity One software (Bio-Rad Laboratories, Inc. USA) and the amount of D2S protein present was further measured by densitometry and height. The protein levels were normalized to that of β-actin.
day for four weeks. The tumor volume was monitored by digital calipers every three days and calculated by using the modified ellipsoidal formula: tumor volume (mm$^3$) = (width)$^2$ x length/2.

2.6.2. Ultra structural observation

The xenograft tumor specimens collected from each group were processed as follows: fixed initially by 2.5% glutaraldehyde and 1% osmium tetroxide, then dehydrated by alcohol with gradient concentration and acetone. After resin embedment, the tumor specimens were sliced and stained with 3% uranyl acetate and lead citrate. The sections were observed under the electron microscope.

2.6.3. Evaluation of apoptosis

The terminal deoxynucleotidyl transferase-mediated UTP end-labeling (TUNEL) assay was performed to measure the cell apoptosis according to the manufacturer’s instructions (Roche, Switzerland). Briefly, after deparaffinization and rehydration, the slides were incubated in 3% H$_2$O$_2$ in methanol at room temperature for 10 min and then digested with 0.02% protease/PBS at 37 °C for 30 min. The slides were then incubated with reaction buffer containing 0.17 units/ml Tdt, 0.2 nmol biotin-16-dUTP, and 2.5 mM cobalt chloride at 37 °C for 1 h. The reaction was terminated by transferring the slides into TB buffer (300 mM sodium chloride and 30 mM sodium citrate) and incubating for 15 min at room temperature. Then, after a thorough washing in PBS, the slides were incubated with an avidin–horseradish peroxidase conjugation. The specific labeling of free 3'-OH ends of DNA was visualized by staining the sections with a fast 3,3-diaminobenzidine tetrahydrochloride tablet dissolved in PBS. The results were analyzed by the software Image-Pro Plus 6.0 and calculated as integrated optical density (IOD), which represented the number of apoptotic cells.

2.6.4. Western blot of apoptosis-related protein

The apoptosis-related proteins, including B cell leukemia 2 (Bcl-2), Bcl-2 associated X protein (Bax) and active caspase-3 were...
measured by Western blot as described above. The rabbit anti-mouse Bcl-2 (or Bax) polyclonal antibody (Abnova Company, USA, at a 1:1000 dilution) and the rabbit anti-mouse active caspase-3 polyclonal antibody (Abnova Company, USA, at a 1:100 dilution) were used as the primary antibody. The secondary antibody was HRP-conjugated goat anti-rabbit IgG polyclonal antibody. Rat β-actin was used as endogenous control.

2.7. Statistical analysis

Values represented as means ± SD, and statistics were performed by analysis of variance (one way or two way ANOVA) by using SPSS 13.0 for Windows (SPSS, Inc.). The number of apoptotic cells identified by TUNEL was determined in duplicate slides from independent experiments. P values less than 0.05 were considered significant.

3. Results

3.1. Adenoviral transduction efficiency and D2S expression

First, we determined the efficiency of D2S infection in GH3 cells. The cells grew well 48 h after the infection and the percentage of EGFP positive cells was from 80.3% to 92.8% (Fig. 1A), indicating a high infection efficiency of in GH3 cells. We extracted total RNA from the infected GH3 cells, the transcription of D2S was evaluated by RT–PCR. DNA slices of D2S gene was detected with the size of about 450 bp. On the other hand, the corresponding slices of expansion were not found in either the control PBS group or vector group (Fig. 1B). Western Blot results further confirmed that D2S expression at the Ad-D2S group was significantly higher than that of other groups (Supplementary Fig. 1). These findings indicate that D2S expression increase the sensitivity of GH3 cells to bromocriptine treatment in vitro.

3.2. Cell survival rate after bromocriptine treatment in GH3 cell infected with Ad-D2S-EGFP

We determined the role of D2S on the viability of GH3 cells subjected to bromocriptine treatment using CCK-8 assay. As shown in Fig. 2, GH3 cell survival rate with Ad-D2S infection was significantly reduced by 51.8%, 66.1%, 65.5% (P < 0.01) at 48 h, 72 h, 96 h, respectively, as compared to the control PBS group. There was no significant difference in the cell survival rate between the Ad-EGFP group and the control PBS group (Fig. 2). These findings indicated that D2S expression increase the sensitivity of GH3 cells to bromocriptine treatment.

3.3. D2S transfection and bromocriptine treatment in GH3 xenograft model

All mice developed visible tumors at one week after the GH3 cells implantation. From the 6th day after bromocriptine or vehicle treatment, the size of GH3 Ad-D2S injected xenograft subjected to bromocriptine treatment was significantly smaller than that of other groups, and this significant difference continued till the end of the experiment (Table 1, Fig. 3, P < 0.05). The nude mice were sacrificed and the tumors were removed. The average tumor volume in Ad-D2S + BRC group was 1.02 ± 0.07 cm³. The inhibition rate of tumor growth in Ad-D2S + BRC group was 68.3%, which was significant higher than that in other groups (Table 1, P < 0.05). The average tumor volume in Ad-EGFP + BRC group was 1.70 ± 0.08 cm³ with a growth inhibition rate of 34.4%. The average tumor volumes in Ad-D2S group, Ad-EGFP group and PBS group were 2.24 ± 0.08 cm³, 2.30 ± 0.13 cm³, and 2.35 ± 0.10 cm³ respectively. There were no significant differences among these three groups in their inhibition rate of tumor growth. These findings indicated that Ad-D2S expression played an important role of the therapeutic action of bromocriptine in the GH3 xenograft model and that adenovirus D2S transfection could sensitize GH3 xenograft to bromocriptine therapy.

3.4. Ultrastructural changes after combination treatment of D2S infection and bromocriptine

Electron microscopy showed typical cell apoptotic changes in xenograft tumor in Ad-D2S + BRC group. Organelles and secretory granules in cytoplasm obviously decreased. Changes in mitochondria ultrastructures were found, which included disruption and loss of mitochondrion cristae, mitochondria swelling, and vacuoles formed in cytoplasm (Fig. 4A). Xenograft tumor cells also displayed apoptotic features in nuclear morphology, such as condensation, margination and fragmentation of chromatin to the nuclear membrane and karyopyknosis (Fig. 4B–D). No obvious cell apoptotic change was observed in PBS group, Ad-EGFP group, Ad-D2S group (Fig. 4E and F).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor weight (g)</th>
<th>Tumor volume (cm³)</th>
<th>IRTG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: PBS group</td>
<td>2.40 ± 0.11</td>
<td>2.35 ± 0.10</td>
<td>0</td>
</tr>
<tr>
<td>B: Ad-EGFP group</td>
<td>2.34 ± 0.09</td>
<td>2.30 ± 0.13</td>
<td>–</td>
</tr>
<tr>
<td>C: Ad-D2S group</td>
<td>2.28 ± 0.09</td>
<td>2.24 ± 0.08</td>
<td>–</td>
</tr>
<tr>
<td>D: Ad-EGFP + BRC group</td>
<td>1.74 ± 0.08</td>
<td>1.70 ± 0.08</td>
<td>34.4</td>
</tr>
<tr>
<td>E: Ad-D2S + BRC group</td>
<td>1.05 ± 0.07*</td>
<td>1.02 ± 0.07*</td>
<td>68.3</td>
</tr>
</tbody>
</table>

IRTG: inhibition rate of tumor growth.

* P < 0.05 vs group A, B, C.

** P < 0.05 vs group A, B, C, D.
3.5. Tumor cell apoptosis after combination of D2S infection and bromocriptine treatment

As shown in Fig. 5I-A–C, there were only a few TUNEL positive cells in PBS group, Ad-EGFP group and Ad-D2S group, which displayed relatively low IOD value (PBS group: 660 ± 208; Ad-EGFP group: 695 ± 238; Ad-D2S group: 707 ± 212). In Ad-EGFP + BRC group, TUNEL positive cell was significantly increased (Fig. 5I-D) with greater IOD (5124 ± 1002) than that of PBS group, Ad-EGFP group and Ad-D2S group \((P < 0.05, \text{Fig. 5II})\). IOD in Ad-D2S + BRC group was significantly higher (15,845 ± 1868, \(P < 0.05, \text{Fig. 5I-E}\)) than that of other four groups, indicated that D2S infection significantly promote GH3 cells apoptosis induced by bromocriptine treatment (Fig. 5II).

3.6. Expression of apoptosis-related proteins after combination of D2S transfection and bromocriptine treatment

Expression of Bcl-2 protein was observed in all groups. There were no significant differences in Bcl-2 expression levels among PBS group, Ad-EGFP group, Ad-D2S group and Ad-EGFP + BRC group \((P > 0.05, \text{Fig. 6I})\). Comparing with other four groups, Bcl-2 expression was significantly reduced in Ad-D2S + BRC group \((P < 0.05, \text{Fig. 6I})\). Expression of Bax protein was found in all groups. PBS group, Ad-EGFP group and Ad-D2S group showed no significant differences in Bax expression. On the contrary, significant increase of Bax expression was indicated in Ad-EGFP + BRC group and Ad-D2S + BRC group \((P < 0.05, \text{Fig. 5II})\) as compared to PBS group, Ad-EGFP group and Ad-D2S group. The ratio of Bcl-2 to Bax in Ad-EGFP + BRC group and Ad-D2S + BRC group was significantly lower than that in other three groups \((P < 0.05, \text{Fig. 6III})\). The ratio of Bcl-2 to Bax in Ad-D2S + BRC was further reduced as compared with Ad-EGFP + BRC group \((P < 0.05, \text{Fig. 6III})\).

Expression of active Caspase-3 was not detected in PBS group, Ad-EGFP group and Ad-D2S group. A significant increase of active Caspase-3 expression was found in Ad-EGFP + BRC group and Ad-D2S + BRC group \((P < 0.05, \text{Fig. 6IV})\). In addition, active Caspase-3 was significant higher in Ad-D2S + BRC group than that in Ad-EGFP + BRC group \((P < 0.05, \text{Fig. 6IV})\).
4. Discussion

Bromocriptine, a kind of ergot peptide derivative, is the first-line treatment for high PRL level caused by prolactinomas. Previous studies have found that DA’s curative effect is not only positively correlated with D2R, but also related to the expression ratio of the two isoforms of D2R, especially to the D2S expression (Caccavelli et al., 1994; Wu et al., 2010). The D2R expression level and the ratio of D2S/D2L significantly decreased in drug-resistant adenomas. Sarkar et al. (2005) observed that constitutive expression of D2S in PR1 cells increased these cells ability to respond to DA and to inhibit PRL production and cell proliferation via a D2R and TßRII interaction in vitro. Radl et al. (2011) reported that DA induced apoptosis of PR1 cells through D2S receptor activation in the presence of estradiol in vitro. This study further confirmed that DAs-induced apoptosis in GH3 cells was mediated by D2S in vitro and in vivo. Taken together, these studies have indicated that D2S plays an important role in the therapeutic action of bromocriptine in prolactinomas.

Adenovirus is one of transgenic carriers with high efficiency and reliability. It has used in gene therapy research and clinical trials because of its following advantages: high infection efficiency, high levels of exogenic-gene expression, simple preparation of highly titer recombined virus, suitable capacity for most exogenic genes and possible reconstruction to target carries. Freese and colleagues (1996) identified that adenovirus transfection induced expression of tyrosine hydroxylase increased dopamine production and decreased prolacin secretion in human prolactinoma cultures. Williams et al. (2001) demonstrated that in vivo dopamine enhancement gene therapy using adenovirus may be useful tool for the treatment of prolactinoma. Candolfi et al. (2006) used adenovirus-mediated tumor necrosis factor-α (TNF-α) and FasL to trigger apoptosis in GH3 cells. Lee et al. (1999) used adenovirus-mediated thymidine kinase combined with ganciclovir to treat GH3 adenoma xenograft in nude mice. In the current study, we determine the role of adenovirus-mediated D2S in the therapeutic action of bromocriptine using both in vitro and in vivo approaches.

GH3, a rat pituitary adenoma cell line, lacks or has very low expression of D2R (An et al., 2003). Our present study demonstrated that adenovirus-mediated D2S transfection could sensitize GH3 to bromocriptine in vitro. We established a subcutaneous xenograft-tumor model of the nude mice with GH3 cells by cell-suspension inoculation. The xenograft received adenovirus-mediated D2S gene has a significant increase of D2S expression. Consistent with our in vitro study, bromocriptine treatment remarkably inhibited the tumors’ growth when combined with adenovirus D2S transfection. Beginning at the sixth day after bromocriptine treatment, a significant difference in tumor volumes was observed between the combined-therapy group and all other control groups. By the end of the treatment, the growth inhibition rate of tumors in the combined-therapy group reached 68.3%.

Our electron microscopy showed that typical apoptosis occurred in the xenograft of the Ad-D2S + RBC group, but, not in all other control groups. TUNEL assay showed that there were significantly more apoptosis cells in the Ad-D2S + RBC group than in any other control group. These results have provided the first in vivo evidence that adenovirus D2S transfection could sensitize DA-resistant prolactinomas in vivo via apoptotic mechanism.

Bcl-2 family genes have been well-established as apoptotic regulators consisting of anti-apoptosis genes (Bcl-2, Bcl-xl, Bcl-xY, etc.) and pro-apoptosis genes (Bax, Bix, Bad, etc.). Bcl-2 and Bax play important roles in regulating the mitochondrial apoptosis pathway (Adams and Cory, 1998; Cory and Adams, 2002; Martinou and Youle, 2011). Moreover, the ratio of Bcl-2/Bax is the key factor in this process. The apoptosis effect of bromocriptine on pituitary adenoma cells have been indicated to be mediated through p53 and Bcl-2 (Yin et al., 1993, 1999). Bromocriptine could exert inhibitory effect on lactotrophs in nude mice by increasing Bax protein...
expression (Gruszka et al., 2004), which could eventually activate caspase cascade and apoptosis process (Earnshaw et al., 1999; Philchenkov, 2004). An et al. (2003) established GH3D2L and GH3D2S cell lines, and discovered that dopamine inhibited cell activities via Caspase-3 activation in vitro (An et al., 2003). Our study further demonstrated that, in vivo adenovirus D2S gene transfection increased D2S expression, sensitized the bromocriptine’s therapeutic action in GH3 xenografts potentially by modulation of Bax and Bcl-2 expression and activation of Cascase-3. In summary, the present study demonstrated that adenovirus D2S transfection significantly increased the D2S expression in culture GH3 cells and in vivo GH3 xenograft. Combined with bromocriptine treatment, adenovirus D2S transfection significantly inhibited the growth of GH3 xenograft through Bcl-2 and caspase mediated pro-apoptotic mechanism. Out study suggested that adenovirus-mediated D2S gene transfer combined with bromocriptine may provide a novel treatment for DA-resistant prolactinomas.

Disclaimers

The authors have no conflicts of interest.

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Appendix A. Supplementary material

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